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Pediatric Nephrology

Sixth Edition

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Springer

Pediatric Nephrology

Ellis D. Avner, William E. Harmon, Patrick Niaudet, Norishige Yoshikawa (Eds.)

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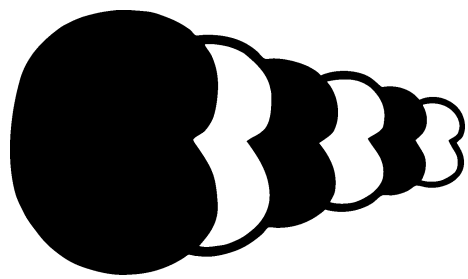
Sixth Completely Revised, Updated and Enlarged Edition

With 632 Figures and 260 Tables



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Preface

Through its past five editions, *Pediatric Nephrology* has become the standard medical reference for health care professionals treating children with kidney disease. This new edition, published only five years since the previous version, reflects the tremendous increase of critical information required to clinically translate molecular and cellular pathophysiology into the prevention, diagnosis, and therapy of childhood renal disorders. This text is particularly targeted to pediatricians, pediatric nephrologists, pediatric urologists, and physicians in training. It is also targeted to the increased number of health care professionals involved in the multidisciplinary team caring for children with kidney disease and their families: geneticists, genetic counselors, nurses, dialysis personnel, nutritionists, social workers, and mental health professionals. Finally, this reference is designed to serve the needs of primary care physicians (internists and family practitioners), as well as internist nephrologists who are increasingly involved in the initial evaluation and/or longitudinal care of children with renal disease under managed health care delivery systems evolving throughout the world.

The new, sixth edition of *Pediatric Nephrology* is organized into twelve main sections. The text begins with an overview of the basic developmental anatomy, biology and physiology of the kidney which provides the critical information necessary to understand the developmental nature of pediatric renal diseases. This is followed by a comprehensive presentation of the evaluation, diagnosis and therapy of specific childhood kidney diseases, including the extensive use of clinical algorithms. Of particular note is a special section on how rapidly-evolving research advances in molecular genetics, cell biology, and evidence-based medicine are being translated into new clinical approaches and therapies for many childhood renal diseases. The final sections focus on comprehensive, state-of-the art reviews of acute and chronic renal failure in childhood. To keep pace with the dramatic changes in pediatric renal medicine since the previous edition, the content of the sixth edition has been extensively revised. More than 40% of the sixth edition has been completely re-written by new authors, all recognized as global authorities in their respective areas. The remainder of the text has been completely revised and updated, often with new junior authors joining senior authors from the previous edition. In addition to the "Around the World Section", which focuses on unique aspects of pediatric nephrology practice and the epidemiology of pediatric renal disease in different regions of the world, all of the chapters have been rewritten to reflect a global, worldwide perspective. **This has led to the official endorsement of the sixth edition of *Pediatric Nephrology* by the International Pediatric Nephrology Association (IPNA) as the standard global reference text in the field of childhood kidney disease. We are proud that the IPNA logo adorns the cover of *Pediatric Nephrology* in recognition of this endorsement.** The Editors look forward to a dynamic interaction with IPNA to take advantage of future opportunities that such a collaboration may provide in the areas of education and outreach activities.

Other significant changes are also present in this new, sixth edition of *Pediatric Nephrology*. The textbook has a new publisher, Springer, which has led to a new "look" of the cover and printing, as well as the welcome expansion to two volumes. In addition, in accord with all previous editions of the text, a new Editor, Professor Norishige Yoshikawa of Wakayama University, Japan, has joined the Editorial Team. This regular change in editors continues to provide a dynamic mixture of continuity, new ideas, new perspectives and globalism. The current editors are internationally recognized leaders in complementary areas of pediatric nephrology, and reflect the global nature of the text and the subspecialty it serves. The Senior Editor, Professor Ellis D. Avner, serves as Associate Dean for Research and Professor of Pediatrics and Physiology of the Medical College of Wisconsin and Director of the Children's Research Institute, and has a focused interest in developmental renal biology, congenital/genetic diseases of the kidney, and research methods in nephrology. Professor William E. Harmon, serves as Director of Pediatric Nephrology and Professor of Pediatrics at the Children's Hospital Boston and Harvard Medical School, and is a leading expert in the field of childhood chronic renal disease and its treatment. Professor Patrick Niaudet, Director of the Pediatric Nephrology Unit at the University of Paris

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and the Hôpital Necker Enfants Malades, is an internationally-recognized educator and clinician-scientist in childhood nephropathy with a particular focus on childhood nephrosis. Professor Norishige Yoshikawa serves as Chairman and Professor of Pediatrics at Wakayama University, and has a focused interest in all aspects of childhood glomerulopathies, particularly IgA nephropathy.

In closing, the Editors wish to thank a number of individuals whose efforts were critical in the success of this project. The book would never have reached this sixth edition without the dedication of our professional colleagues at Springer, Ms. Marion Krämer, Ms. Gabriele Schröder, and particularly Mr. Andrew Spencer, our Managing Development Editor, who served as our "guide for the perplexed" in all aspects of project management. We thank our families, and particularly our wives Jane, Diane, Claire, and Hiro for their support and understanding. In particular, the Senior Editor wishes to recognize his lifetime partner in all endeavors, Jane A. Avner, PhD for her extraordinary editorial assistance. And finally, we thank our mentors, our students, and most importantly, our patients and their families. Without them, our work would lack purpose.

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Development

1 Embryology

Adrian S. Woolf · Jolanta E. Pitera

Introduction

In the last few decades, considerable advances have been made in understanding kidney development (1, 2). In previous years, this process was described in purely anatomic terms, but we can now interpret the anatomy in terms of dynamic morphogenesis, or acquisition of form, driven by the expression of specific genes. Although our long-term aim is to understand human kidney development, most functional studies have been performed in mice and therefore these animal experiments are described in some detail. As is obvious to any Pediatric Nephrologist, developmental disorders account for a wide spectrum of kidney diseases that cause considerable morbidity and mortality in the first years of life (3–5). Renal malformations such as agenesis (absence of the kidney) and dysplasia (failure of normal renal differentiation) represent major defects of development, whereas hypoplasia (too few nephron units) is another, more subtle, developmental defect. Furthermore, enhanced proliferation, a characteristic of undifferentiated cells, occurs in Wilms tumor and cystic kidney diseases. Although these diseases are described in detail elsewhere in this book, they are alluded to here as illustrative examples of "nephrogenesis gone wrong": in some of these disorders, defined aberrations of cell biology and genetics shed light on normal human kidney development.

Anatomy of Kidney Development

Overview

Potter has provided the most complete anatomic description of human kidney development (6). The reader is also referred to recent reviews (1, 2). Three sets of "kidneys" form in mammalian embryos: the pronephros, mesonephros, and metanephros. The metanephros is the direct precursor of the adult kidney, whereas the others essentially involute before birth. The anatomic events of human and mouse nephrogenesis are similar, but the timetable of development differs. Human gestation is 40 weeks but mouse gestation is about 20 days. The human metanephros appears 5 weeks after fertilization and on late embryonic day 10 in mice. The first metanephric vascularized glomeruli form by about 7 weeks in humans and day 14–15 in mice. The final layer of nephrons forms by 36 weeks' gestation in humans, whereas nephrogenesis continues for one postnatal week in mice. In addition, there are some anatomic differences. The human mesonephros contains glomeruli with capillary loops, but mouse mesonephric tubules have rudimentary glomerular tufts. Healthy adult humans have approximately 1–2 $\times$ 10^6 glomeruli in each kidney (7) and murine species (8) have proportionately fewer glomeruli per kidney. Finally, whereas the human renal pelvis has multiple papillae, the murine kidney has one. A cartoon of the early stages of human metanephric development is depicted in *Fig. 1-1*, and a histologic analysis is shown in *Fig. 1-2*. *Fig. 1-3* indicates the major cell lineages derived from the metanephros, and *Fig. 1-4* addresses the formation of blood vessels in the metanephros.

Pronephros and Mesonephros

The mesoderm forms during gastrulation, and embryonic kidneys subsequently develop from nephrogenic cords, masses of intermediate mesoderm located behind the embryonic coelom between the dorsal somites and the lateral plate mesoderm. At the height of their development, the pronephros and the mesonephros extend in series from the cervical to lumbar levels. They develop in a segmental manner as tubules that are induced to differentiate from mesoderm by the adjacent pronephric and mesonephric (or Wolffian) duct. In humans, the pronephros develops from the third embryonic week and contains rudimentary tubules opening into the pronephric duct. The human mesonephros begins to develop in the fourth week of gestation and contains well-developed nephrons comprising vascularized glomeruli connected to proximal and distal type tubules draining into the mesonephric duct, itself a continuation of the pronephric duct. The mesonephric duct extends to fuse with the cloaca, the urinary bladder precursor, at the end of the fourth week. The pronephros and mesonephros can be regarded as a

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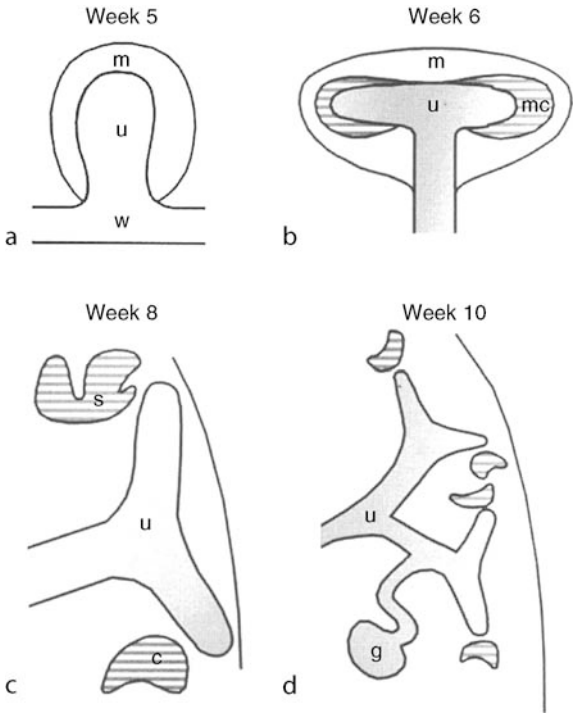
Figure 1-1

Early development of the metanephros. Cross-sectional diagrams of the human metanephros at approximately 5 weeks' (a), 6 weeks' (b), 8 weeks' (c), and 10 weeks' gestation (d). Note that the most primitive structures are located in the periphery of the maturing organ. *u*, Ureteric bud; *m*, mesenchyme; *mc*, mesenchymal condensate; *c* and *s*, comma and S-shaped bodies; *g*, glomerulus; *w*, Wolffian duct.



single unit, and as the wave of differentiation spreads caudally, the cranial end of this organ complex begins to regress. By one-third of the way through human gestation, most cells in these organs have involuted. In the male, mesonephric tubules in the area of the gonad form the efferent ductules, and the mesonephric duct gives rise to the epididymis and ductus deferens. In the female, some mesonephric tubules persist as the epoophoron and para-oophoron.

Metanephros

The metanephros is the last embryonic kidney to develop and is identified in humans and consists of two components (1, 6). These are the ureteric bud epithelium, which branches from the caudal part of the mesonephric duct around 4 weeks of gestation, and the metanephric mesenchyme, which condenses from the intermediate mesoderm around the enlarging tip, or ampulla, of the bud. The metanephric kidney can be identified as an entity around week 5–6 of gestation. The ureteric bud and its branches form epithelia of the collecting ducts, renal pelvis, ureter and bladder trigone, whereas the metanephric mesenchyme differentiates into nephron tubules (glomerular, proximal tubule, and loop of Henle epithelia) and the interstitial fibroblasts. These lineages may be more plastic than previously considered, as discussed later in this chapter. In humans, the renal pelvis and major calyces are apparent by the tenth to 12th week of gestation. The pelvis forms from remodeling of the first

Figure 1-2

WT1 immunostaining in human fetal kidneys. (a) Normal human fetal kidney shows a gradient of WT1 immunoreactivity (*black*) from nephrogenic cortex (*on right*) to maturing nephrons (*on left*). Cells in condensates and vesicles (*arrowheads*) are weakly positive for WT1. Expression increases in the mesenchymal to epithelial transition with high WT1 levels in the proximal limb of S-shaped bodies (*open arrowheads*) and the podocytes of fetal glomeruli (*g*). A ureteric bud branch tip (*u*) is negative. (b) Intense WT1 expression is maintained in the podocytes of maturing glomeruli. Bars are 10 μm. (Pictures courtesy of Dr PJD Winyard, Institute of Child Health, London, UK.)

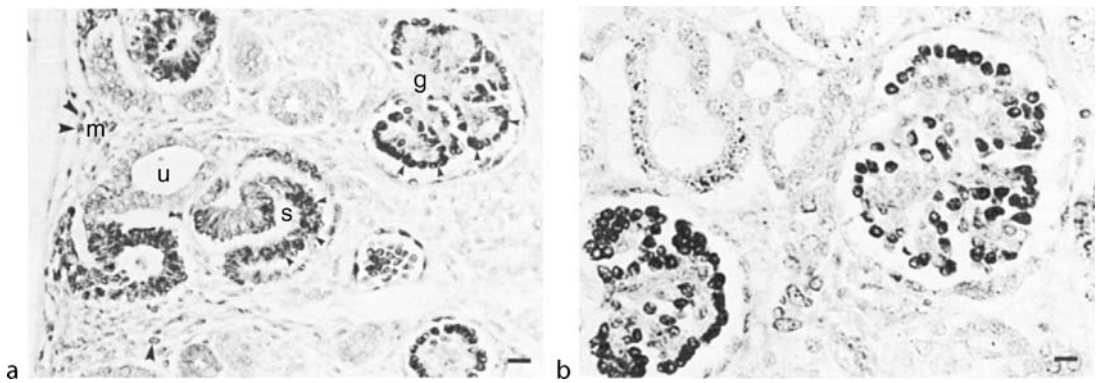


Figure 1-3

Main cell lineages arising in the metanephros. (Modified from Hardman P, Kolatsi M, Winyard PJD et al. Branching out with the ureteric bud. *Exper Nephrol* 1994;2:211–219; with permission from S. Karger, AG Basel.)

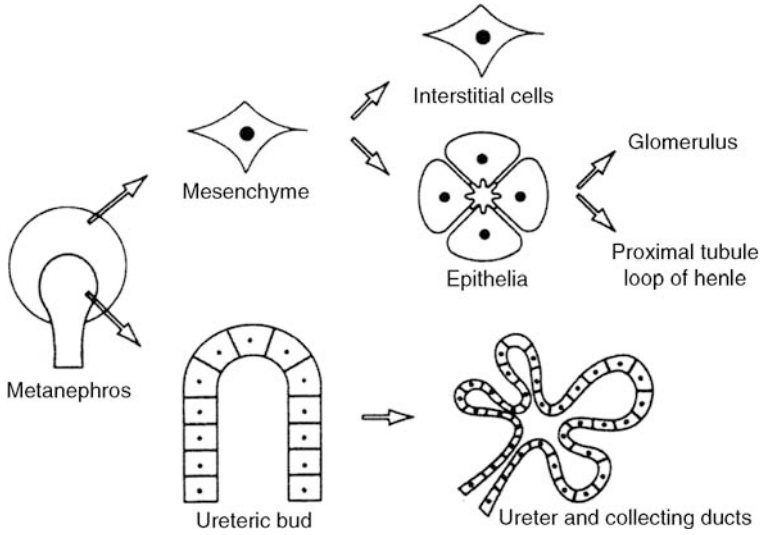
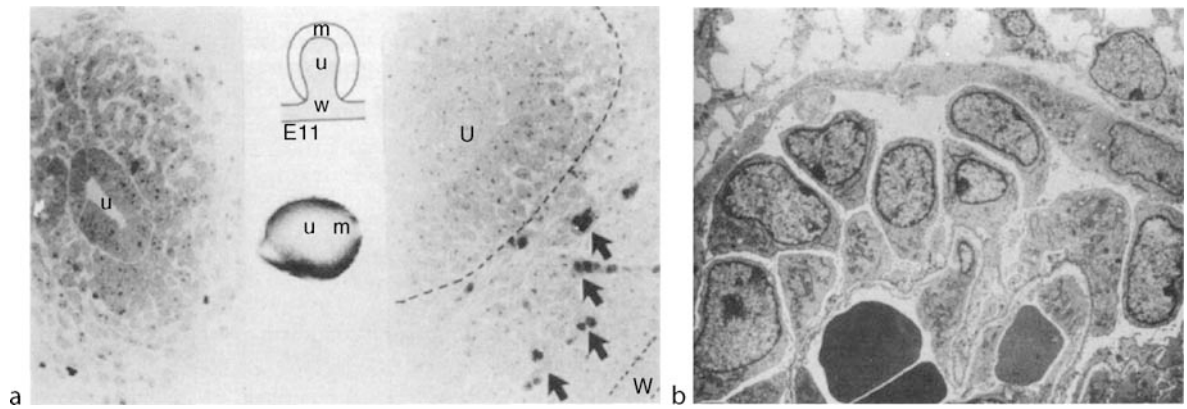


Figure 1-4

Capillary formation in the mouse metanephros. (a) The mouse metanephros at the ureteric bud stage contains no formed capillaries. Center panel shows diagram of the ureteric bud stage above a photograph of the intact organ. Flanking panels are histology sections of the organ in different planes: the right panel shows an area of loose connective tissue and capillaries between the metanephros itself and the Wolffian duct. (b) Electron microscope image of a glomerulus that has formed in the metanephros. *u*, Ureteric bud; *m*, mesenchyme; *w*, Wolffian duct.



six generations of ureteric bud branches, and the minor calyces arise from the next generation of branches. Each minor calyx is associated with 20 ampullae, which will form the papillary collecting ducts: these indent the calyx to form the familiar cup shape seen in intravenous urograms.

Up to 14 weeks' gestation, the formation of each new collecting duct is associated with the induction of a nephron from adjacent metanephric mesenchyme. The differentiation of each nephron starts with mesenchymal cell aggregation around ureteric bud branch tips. Each condensate subsequently forms a lumen (the vesicle stage) and elongates to form a tubule (the comma-shaped body), which then shows regional specialization into primitive glomerular and proximal tubular epithelia (the S-shaped body). The proximal end of each S-shape

becomes the glomerular epithelium while the distal end of each tubule fuses with the adjacent branch of the ureteric bud. With each division of the ureteric bud, a new layer of nephrons is induced from stem cells in the periphery of the organ. Between 14 and 20 weeks, each ampulla induces 3–6 nephrons without dividing. During this process, the connecting tubule of the older, innermost nephron shifts the position of its point of attachment away from the ampulla to the connecting tubule of the next-formed nephron so they are joined together in arcades of 4–7 nephrons. Up to 34–36 weeks' gestation, the ureteric bud branch tips advance further outward and another 4–7 nephrons form and attach separately just behind the ampullary tips. Thereafter, no new nephrons form although each tubule continues to mature even into the postnatal period. These changes include the elongation of the loops of Henle toward the medulla as well as convolution of the proximal tubule.

The adult kidney is highly vascular and receives approximately 20% of the cardiac output. However, at the inception of the metanephros, no mature vessels are present in the renal mesenchyme. The first patent capillaries are evident around the stalk of the ureteric bud, when it has branched once or twice and capillaries later appear in the glomerular crevices of the S-shaped bodies (9). The primitive multilayered visceral glomerular epithelium subsequently forms a monolayer of podocytes, which abut glomerular capillary loops. At 9–10 weeks' human gestation, the most mature nephrons, located toward the center of the metanephros, are the first to acquire capillary loops and a patent Bowman's space. The forming glomerular basement membrane is thought to be synthesized by both the endothelium and epithelium (10). The fusion of the two embryonic membranes and its subsequent biochemical maturation correlates with the progressive restriction of filtration of macromolecules. At its inception, the human metanephros receives its blood supply from the lateral sacral branches of the aorta. As development proceeds, the organ is located at progressively higher levels and is supplied by higher branches of the aorta. By 8 weeks' gestation, the metanephros is located in the lumbar position, and ultimately the definitive renal arteries arise from the aorta at the level of the second lumbar vertebra.

The Ureter and Urinary Bladder

The lower urinary tract forms in synchrony with the metanephric kidney (11). The urogenital sinus is the urinary bladder rudiment, and it separates from the rectum by 4 weeks of human gestation. At this time, its epithelium fuses with that of the mesonephric duct and the ureteric bud arises as a diverticulum from the posteromedial aspect of the mesonephric duct near where it enters the forming bladder. Between 4 and 5 weeks the human ureter is patent and it has been assumed, because the cloaca is imperforate at that time, that mesonephric urine maintains ureteral patency by increasing intraluminal pressure. The mesonephric duct above the ureteric bud becomes the vas deferens males but involutes in the female. At 5 weeks of gestation, the mesonephric duct below the ureteric bud, the "common excretory duct," involutes, allowing the lower end of the ureteric bud stalk to fuse with the nascent bladder; thereafter, ureteric orifices migrate cranially and laterally. Over the next few weeks, the ureter apparently becomes occluded and then recanalizes, the later event perhaps coinciding with urine production from the first metanephric glomeruli. The embryonic "ascent" of the metanephros from the level of the sacral segments to the lumbar vertebrae is partly associated with ureteric elongation. The urinary bladder becomes a recognizable entity by about 6 weeks of gestation, and the urogenital membrane ruptures around week 7, providing a connection between the bladder and outside of the body. The allantois, another potential outflow tract on the anterior of the developing bladder, forms at 3 weeks days of gestation and involutes by 12 weeks. Towards the end of the first trimester, the ureteric urothelium assumes a pseudo-multilayered arrangement and its walls become muscularized; by this time, the bladder wall has differentiated into circular and longitudinal smooth muscle fibers which continue to mature in the second trimester (11).

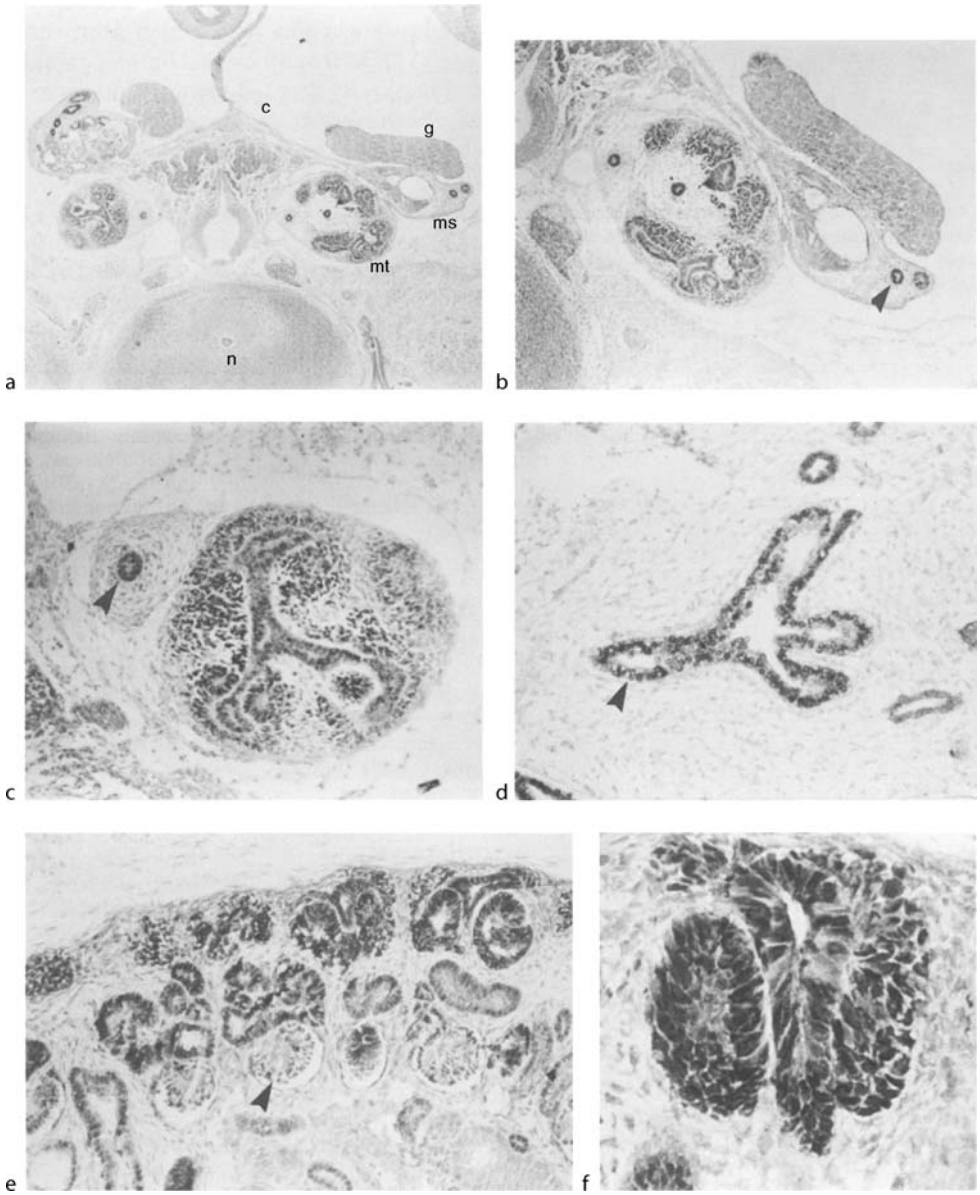
Methods Used to Study the Biology of Nephrogenesis

Descriptive Studies of Gene Expression

Patterns of cell division, death, differentiation, and morphogenesis can be correlated with changes in spatial and temporal patterns of gene expression in terms of messenger ribonucleic acid (mRNA) and protein using by in situ hybridization and immunohistochemistry (*Figs. 1-2* and 5). More recently, microarray "chip" technology has been used to study the spectrum genes expressed during human kidney development (12, 13). These observations provide data for the generation of hypotheses regarding the molecular control of nephrogenesis. These hypotheses can be tested by studying the effects of diverse interventions during normal development. These functional

Figure 1-5

Early human metanephros and the mesonephros. Sections are stained with antibody to PAX2 transcription factor: positive nuclei appear black, whereas others are counterstained with methyl green and appear gray. (a) Transverse section of a 5- to 6-week gestation human embryo showing, on each side of the embryo, a mesonephros (*ms*), metanephros (*mt*), and gonadal ridge (*g*) (×5). Also shown is the central notochord (*n*) in a mass of cartilage that will form the vertebral body, and the coelom (*c*). (b) Enlarged view of (a). Note the mesonephric duct (*arrowhead*) stains for PAX2, as does the flanking paramesonephric duct (x20). (c) High power of metanephros containing the first branches of the ureteric duct with adjacent mesenchymal condensates. The mesonephric duct (*arrowhead*) is nearby (×20). (d) Medulla of an 11-week human kidney shows a major branch of the fetal ureter (*arrowhead*) branching to form collecting ducts: most nuclei in these structures stain for PAX2 (×20). (e) Cortex of an 11-week human fetal kidney shows presence of a nephrogenic outer cortex with increasingly mature nephrons and glomeruli (*arrowhead*) toward the center of the organ. Note that PAX2 is downregulated in more mature elements (×20). (f) High power of (e) Intense staining for PAX2 in the branch tip of a ureteric bud and the flanking renal mesenchymal condensates (×63). (Pictures courtesy of Dr PJD Winyard, Institute of Child Health, London, UK.)



experiments can be performed in the intact animal, in vivo, or "in the test tube," in vitro.

Functional Studies In Vitro

Experiments performed by Grobstein several decades ago are classic examples of in vitro studies (14). Grobstein found that the mouse metanephros would form a small kidney in organ culture over days. *Figure 1-6* shows how the explanted renal tract of a midgestation mouse embryo grows in organ culture over several days. Of note, other branching organs, such as the lung and the salivary gland, can also be grown in the same manner (*Fig. 1-7*). If either the renal mesenchyme or the ureteric bud was cultured in isolation, however, Grobstein noted that they failed to differentiate. This clearly demonstrates that embryonic tissue interactions are critical for kidney development, and we now know that growth factors are important signaling molecules involved in these inductive processes. Growth may be modulated in organ culture with antisense oligonucleotides that impair the transcription of metanephric mRNAs or with antibodies that block the bioactivity of secreted or cell surface proteins (15–17).

Figure 1-6

Whole-mounts mouse embryonic tissues immunostained (black) for the PAX2 transcription factor. (a) Series of lateral views of embryos at embryonic day 9 (E9) to embryonic day 11 (E11); the former time-point approximately anatomically-equivalent to a 3.5 week human gestation, and the latter time point equivalent to 5 week human gestation. Note the pronephric/mesonephric duct (*arrowheads*) expresses PAX2. By E10, the upper part of the mesonephric duct connects to mesonephic tubules (labeled *meso*), while the most caudal part branches to form the ureteric bud which invades a section of PAX2 expressing intermediate mesoderm, to form the metanephros (boxed area, labeled *meta*). (b) These frames depicted the growth and differentiation of a complete E10.5 renal tract (paired mesonephric ducts, mesonephroi and metanephroi). Note that, over 72 h, the mesonephros involutes while the metanephros grows in size and complexity.

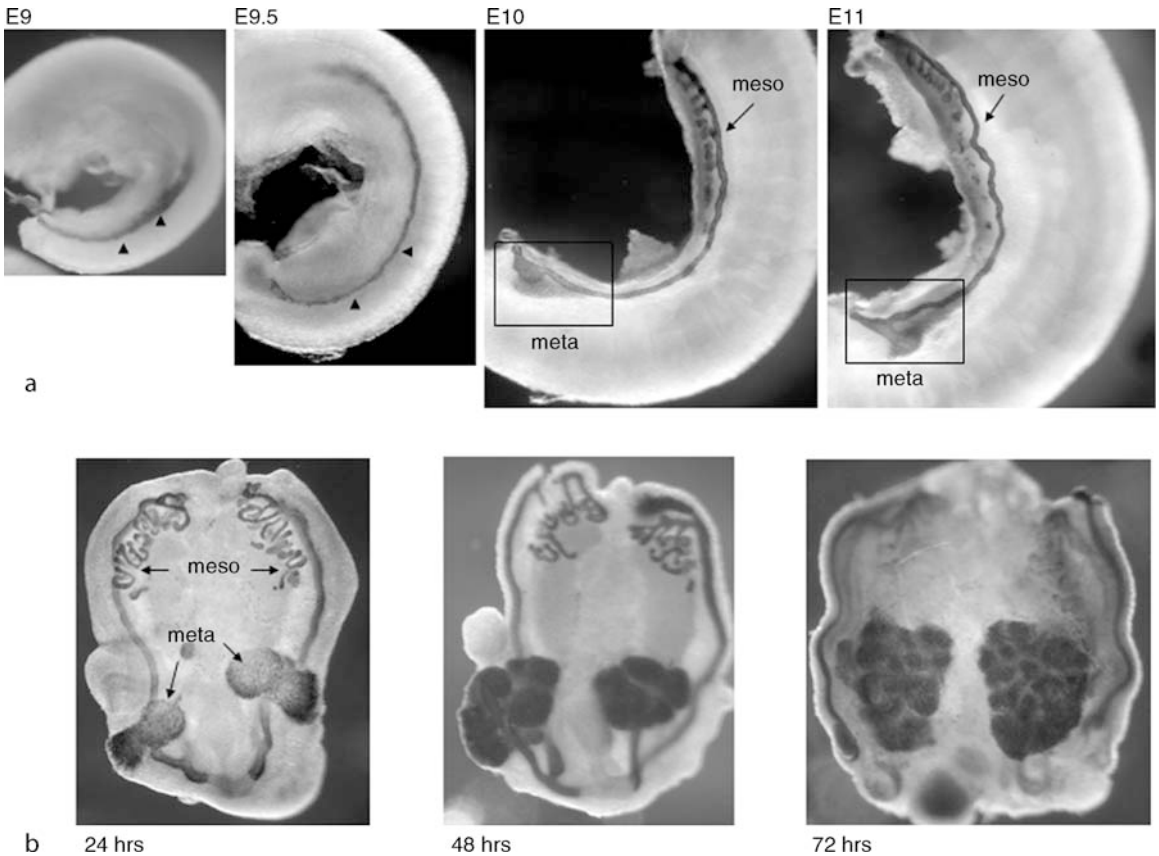
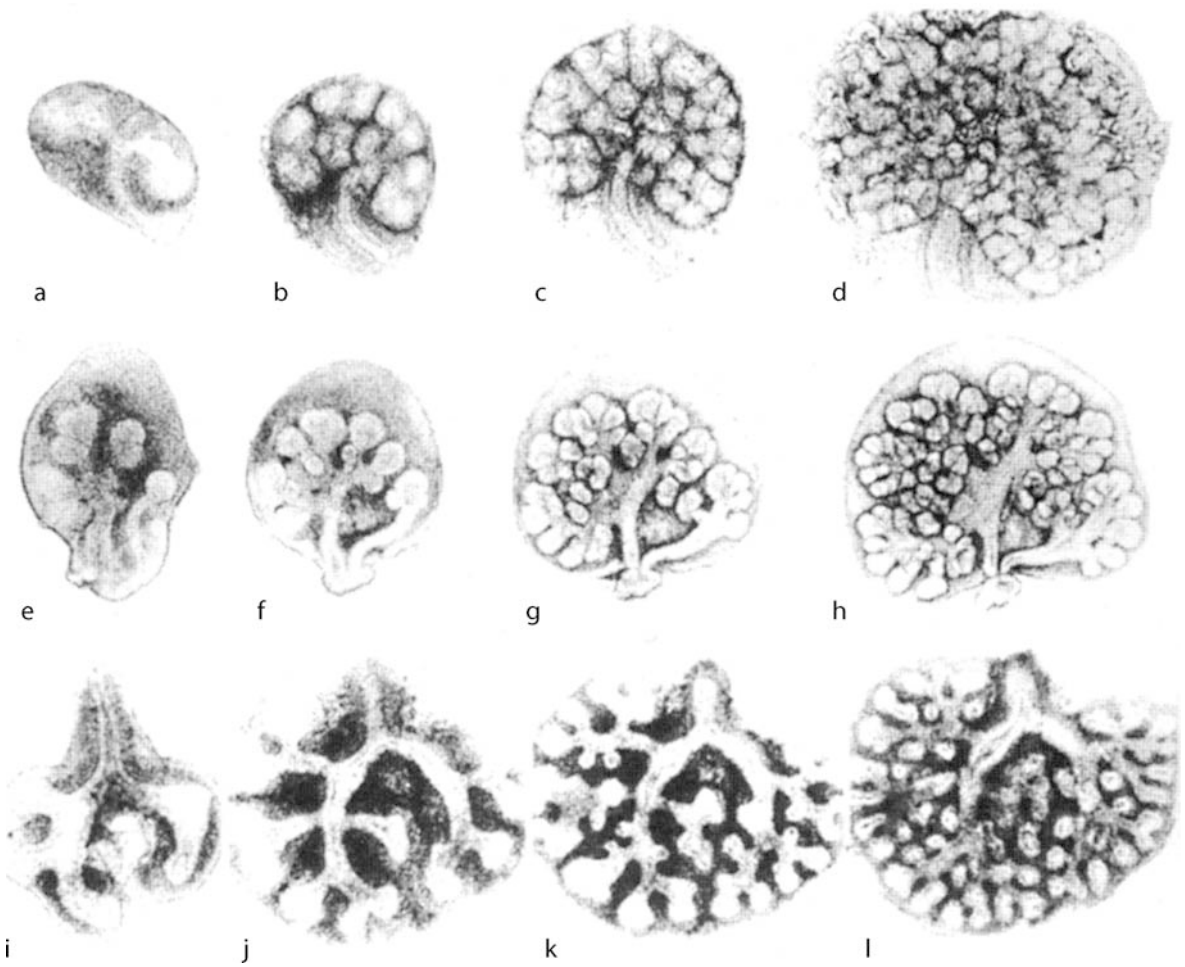


Figure 1-7

Growth of mouse embryonic tissues in organ culture. Note branching morphogenesis occurs over 1 week in organ culture. (a–d) show the metanephros; (e–h) show the salivary gland; and (i–l) show the lung. (Modified from Hardman P, Kolatsi M, Winyard PJD et al. Branching out with the ureteric bud. *Exp Nephrol* 1994;2:211–219; with permission from S. Karger, AG Basel.)



Other technological advances have made it feasible to transfer new genes into the metanephros in vitro (18, 19). Qiao and Herzlinger used retroviral transduction to introduce a reporter gene into renal mesenchymal cells; they subsequently demonstrated that these precursors not only formed nephron tubules, as expected, but also differentiated into a minor proportion of cells within collecting duct epithelia (19). Finally, the generation of metanephric cell lines has made it feasible to study the expression of multiple genes in homogeneous populations of precursors (13, 16) as well as the potential normal and abnormal differentiation of these cells in response to defined stimuli (13, 20).

Functional Studies In Vivo

In vivo experiments on developing kidneys have used physical, teratogenic (e.g., chemical), and genetic strategies. For example, surgical interruption of the avian mesonephric duct prevents the conversion of intermediate mesoderm into mesonephric tubules and also prevents the formation of the metanephros (21). Complete obstruction of the sheep fetal ureter in midgestation generates hydronephrotic kidneys, with generation of cysts and disruption of nephrogenesis resembling human renal dysplasia (22). With regard to teratogenic studies, an example is the generation of urinary tract malformations after

exposure to high doses of vitamin A (23) or elevated levels of glucose (24). Welham et al (8, 25) reported that the imposition of mild (9%) dietary protein restriction during rat pregnancy reduced numbers of glomeruli per kidney measured postnatally when nephrogenesis has finished. This was associated with enhanced apoptotic deletion of renal mesenchymal precursors, and an altered spectrum of gene expression, at the start of metanephrogenesis. In humans, the equivalent developmental timeframe would be 5–7 weeks gestation, and this might represent a critical window when kidney morphogenesis might be affected by dietary influences.

The most powerful in vivo experiments, however, alter the expression of metanephric molecules by genetic engineering using transgenic animal technology. First, levels of a specific protein can be increased by inserting a coding deoxyribonucleic acid (DNA) sequence, linked to a strong promoter, into the genome of early embryos. This is usually done by microinjection into the male pronucleus of fertilized ova. The phenotype of such mice illustrates the effects of an excess of a molecule (26). Even more informative is the technique of homozygous recombination in which the function of a gene can be ablated. Here, mouse embryonic stem cells are genetically engineered in vitro and then incorporated into early embryos that develop into chimeric mice. If the altered cells contribute to the germ-line, animals with homozygous and heterozygous gene deletions can be generated by further breeding (27). The phenotypes of these null mutant or "knock-out" mice, which include the complete absence of metanephric development, have so far suggested that several tens of genes are essential for normal nephrogenesis in vivo: *Table 1-1* lists some of these. Many of these animal models also have defects in nonrenal systems because the same genes are expressed in and critical for the normal development of organs other than the kidney.

Hundreds of molecules are known to be expressed during nephrogenesis (28), and tens of these have been considered to be functionally important based on organ culture studies. However, mice with null mutations of the same genes sometimes have normal kidney development in vivo. Hence, we can speculate that numerous metanephric molecules are of little functional significance or are redundant in the intact embryo. It also follows that organ culture must constitute a relatively stressful milieu in which it is comparatively easy to disrupt development by altering levels of a single molecule. Of note, the genetic background, or strain, of mice with defined mutations can affect the kidney phenotype, suggesting the presence of modifying genes. When two structurally similar molecules are expressed at identical locations in

Table 1-1

Examples of Mutant Mice with Renal Malformations

Transcription factor genes
FOXD1 (small, fused and undifferentiated kidneys)
BRN1 (poorly differentiated loops of Henle)
EYA1 (absent kidneys)
FOXC1(duplex kidneys)
FOXC2 (hypoplastic kidneys)
HOXD11 paralogue compound mutants (small or absent kidneys)
LIM1 (absent kidneys)
LMX1B (poorly formed glomeruli)
PAX2 (small or absent kidneys)
SALL11 (failure of ureteric bud outgrowth)
SIX1 (small or absent kidneys)
SIX2 (accelerated tubulogenesis and small kidneys)
TBX18 (hydronephrosis)
TSHZ3 (hydronephrosis)
WT1 (absent kidneys)
Growth factors and receptor genes
AT1 (poor papillary growth)
AT2 (diverse kidney and lower urinary tract malformations)
BMP4 (kidney and ureter malformations)
BMP7 (undifferentiated kidneys)
FGF7 (small kidneys with fewer glomeruli)
GDF11 (small or absent kidneys)
GDNF or its receptor *RET* (small or absent kidneys)
NOTCH2 (malformed proximal nephron)
PDGFB (absent mesangial cells)
ROBO2 (duplex kidneys)
SHH (hydronephrosis)
WNT4 (undifferentiated kidneys)
Adhesion molecules and receptor genes
ITGA3 (decreased collecting duct branching)
ITGA8 (impaired ureteric bud branching and nephron formation)
FRAS1 (absent kidneys)
FREM2 (absent kidneys)
GPC3 (dysplastic kidneys)
LAMB2 (nephrotic syndrome)
Other genes
BCL2 (small kidneys)
RAR compount mutants (small or absent kidneys)
UPK3A (hydonephrosis and vesicoureteric reflux)

the metanephros, both loci may have to be ablated to generate a renal malformation in vivo (29, 30).

Cell Biology of Nephrogenesis

Cell Proliferation and Cell Death

Proliferation is prominent in the tips of the ureteric bud branches and in the adjacent mesenchymal cells in the nephrogenic cortex of the metanephros (31). Renal mesenchymal stem cells may reside within this area, and such cells are believed to divide to generate a copy of themselves and also another cell. These cells subsequently differentiate into nephron epithelia or interstitial cells. Other evidence, discussed later in this chapter, suggests that cells in the renal mesenchymal compartment can also differentiate into glomerular capillaries and juxtaglomerular cells. Stem cells have previously been considered to be absent from the mature kidney; although if they did exist, they might provide a source of cells for the regeneration of nephron epithelia after nephrotoxicity. Recently, preliminary evidence was provided that a rare subpopopulation of medullary kidney stromal cells may in fact constitute such a population in the adult kidney (32).

Not all cells born in the developing kidney are destined to survive the fetal period. In 1926, Kampmeier reported that the first layers of metanephric nephrons "disappeared" before birth (33), a process likely to be associated with the remodeling of the first divisions of the ureteric bud during formation of the pelvis. In fact, a degree of cell death normally occurs in the mesenchyme adjacent to primitive nephrons, where it may regulate the number of cells in each tubule, the number of nephrons formed, or perhaps the density of adjacent stromal/interstitial cells (8). These cells die by apoptosis, a process accompanied by nuclear condensation and fragmentation. These deaths are sometimes called programmed because they are part of the normal program of development and each cell "commits suicide" by an active program of biochemical events, including digestion of genomic DNA into fragments of about 200 nucleotides by calcium-dependent endonucleases. Apoptosis also occurs in the developing medulla (*Fig. 1-8*) and it has been suggested that this process is implicated in the morphogenesis of the thin ascending limb of the loop of Henle (34) and also in deleting excess β intercalated cells in the collecting duct (35). Furthermore, apoptosis appears to be a normal event in morphogenesis of capillaries in developing glomeruli, critical for lumen formation (36).

Therefore normal nephrogenesis involves a fine balance between cell proliferation and death. Excessive proliferation is associated with the generation of neoplasms (e.g. Wilms tumor) and cysts (e.g., polycystic kidney diseases) (37). Conversely, excessive apoptosis would cause a reduction of kidney growth resulting in an organ with fewer nephrons than normal (e.g., a hypoplastic kidney) or even involution of a metanephric kidney (e.g., some dysplastic kidneys) (38, 39).

Differentiation

As individual renal precursor cells become specialized, they undergo differentiation. For example, some renal mesenchymal cells differentiate into primitive nephron epithelia, while others differentiate into stromal cells, or interstitial fibroblasts (40). Precursor cells later become "terminally differentiated" to enable them to perform specific functions of the adult organ. For example, cells within a nephron precursor form the glomerular parietal and visceral epithelia as well as the cells that comprise the proximal tubule and loop of Henle. The term *lineage* describes the series of phenotypes as a precursor differentiates into a mature cell.

Morphogenesis

Morphogenesis describes the developmental process by which groups of cells acquire complex three-dimensional shapes. Examples include the formation of nephron tubules from renal mesenchymal cells and the serial branching of the ureteric bud to form the collecting duct system. The process of morphogenesis also occurs during angiogenesis and vasculogenesis, modes of renal capillary formation discussed later in this chapter (9). Angiogenesis also involves the fundamental cellular process of directional movement, or migration.

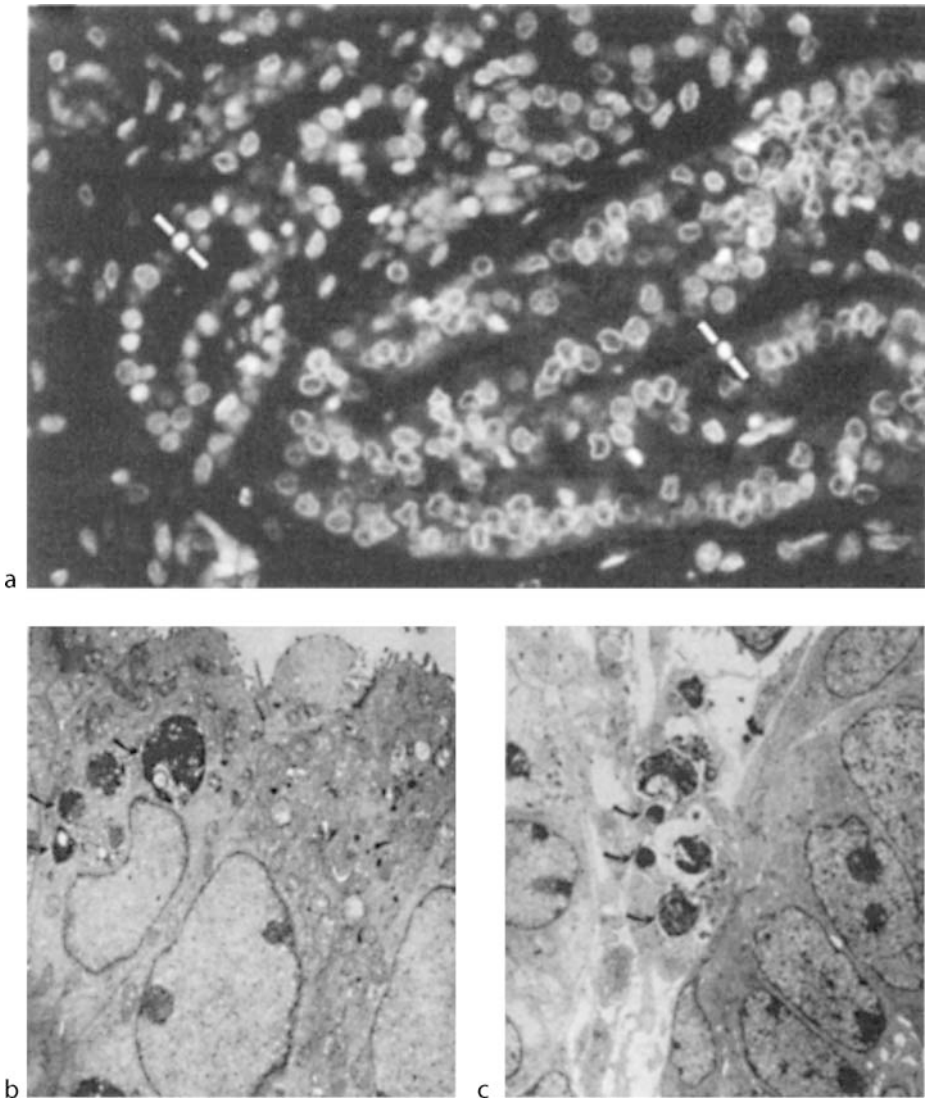
Molecular Control of Nephrogenesis

Overview

Three main classes of molecules are expressed during nephrogenesis: transcription factors, growth/survival factors, and adhesion molecules. Several have been implicated in kidney development, as assessed by studies of mutant mice (see *Table 1-1* for a selective list) and humans with kidney malformations (1–5, 28, 41, 42).

Figure 1-8

Cell death in normal nephrogenesis. (a) Apoptotic cell death is detected in the outer medulla of the human metanephros, as assessed by bright, condensed, propidium-iodide stained nuclei (between the *white bars*) in primitive loops of Henle and other tubules, probably collecting ducts (×63). (Courtesy of Dr PJD Winyard, Institute of Child Health, London, UK.) (b, c) Electron microscope images of the medulla of a mouse metanephros to show apoptotic nuclei (*curved arrows)* being engulfed by epithelial cells (b) and cells within the interstitium (c).



Note that italics are used when referring to genomic sequences, whereas regular typescript is used for gene products e.g., *PAX2* gene and PAX2 protein (*Figs. 1-5, 6, 9–11*). The following criteria should be satisfied for a molecule to be definitively involved in normal nephrogenesis: it must be expressed by the metanephros in an appropriate spatial or temporal manner; functional experiments should demonstrate that its absence perturbs kidney development in organ culture and in vivo; and the molecule should have an appropriate bioactivity on isolated populations of precursor cells. At present, few molecules have been shown to fulfill all three criteria. Expression patterns of several key molecules active in the metanephros are shown in *Figs. 1-9–12*.

Figure 1-9

Gene expression in initiating mouse metanephros; ureteric bud stage. Sections of mouse metanephric kidneys at embryonic day 10.5, the anatomical equivalent to human 4.5 week gestation metanephros. Gene expression (appearing in *black*) has been assessed by either in situ hybridization to detect RNA (HOXD11, GDNF and RET) or immunohistochemistry (SIX1, SIX2, PAX2 and SALL1) to localize protein. The tissue has been lightly counterstained with hematoxylin (appears *grey*). Note expression of the transcription factors HOXD11, SIX1, SIX2 and SALL1 in the metaneprhic mesenchyme (*m*) only. The PAX2 transcription factor is expressed in both the metanephric mesenchyme and the urteric bud (*u*) which is growing into the mesenchyme. The growth factor GDNF is expressed in the mesenchyme and signals via the RET receptor tyrosine kinase in the bud.

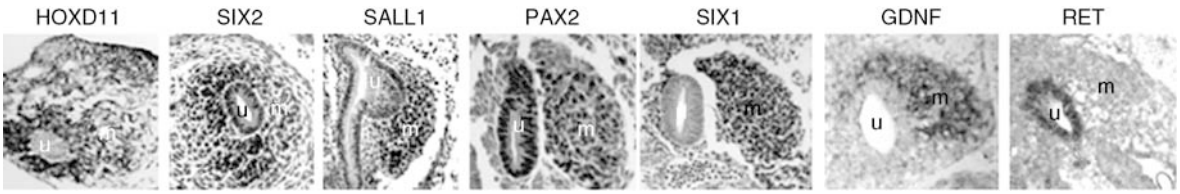


Figure 1-10

Gene expression in initiating mouse metanephros: initiation of branching stage. Sections of mouse metanephric kidneys at embryonic day 11, the anatomical equivalent to human 5 week gestation metanephros. Gene expression (appearing in *black*) has been assessed by either in situ hybridization to detect RNA (EYA1, HOXD11, PAX2, GDF11, GDNF and RET) or immunohistochemistry (SALL1, SIX1, SIX2, ITGA8, LIM1 and BRN1) to localize protein. The tissue has been lightly counterstained with hematoxylin (appears *grey*). The ureteric bud has branched one-two times and that the metanephric mesenchyme is condensing around the branch tips. Note the expression in the mesenchyme-only of the transcription factors EYA1, HOXD11, SALL1, SIX1 and SIX2. The transcription factor PAX2 the growth factor GDF11 are expressed in both the urteric bud branches and the mesenchyme. The growth factor GDNF is expressed in mesenchyme around the RET-expressing tips of the arboring bud branches. The integrin ITGA8 is expressed in the condensed mesenchyme. The transcription factors LIM1 and BRN1are beginning to be expressed in mesenchymal condensates which are nascent nephrons.

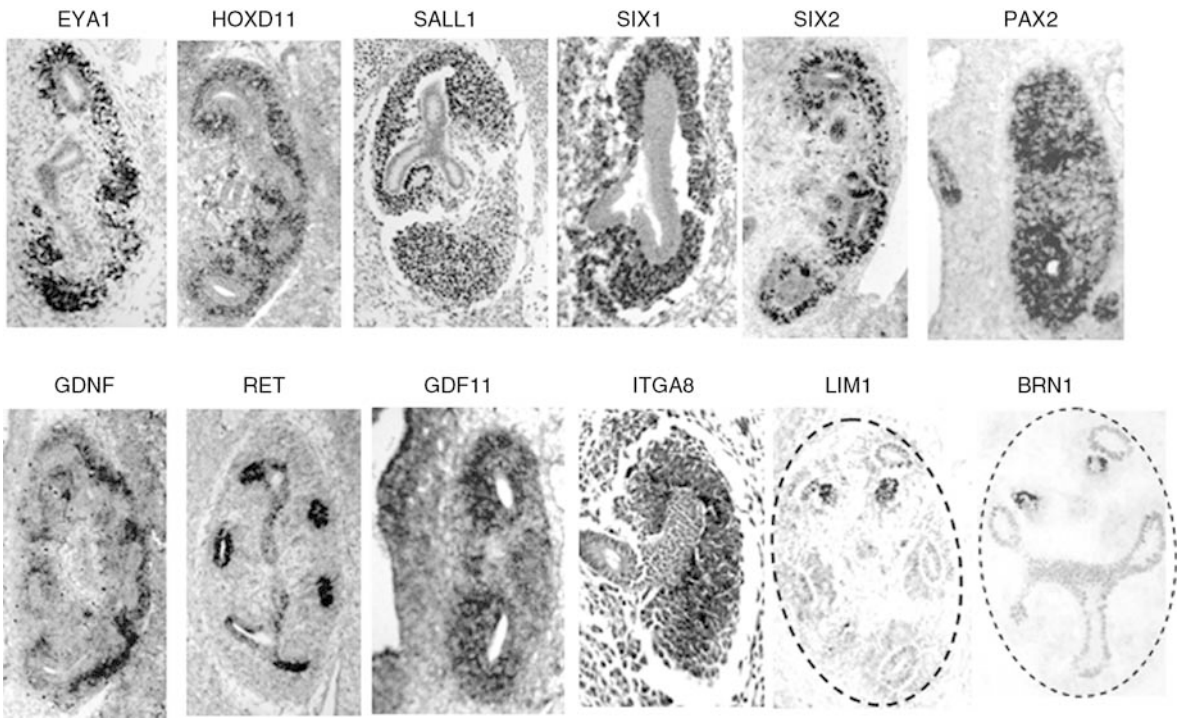
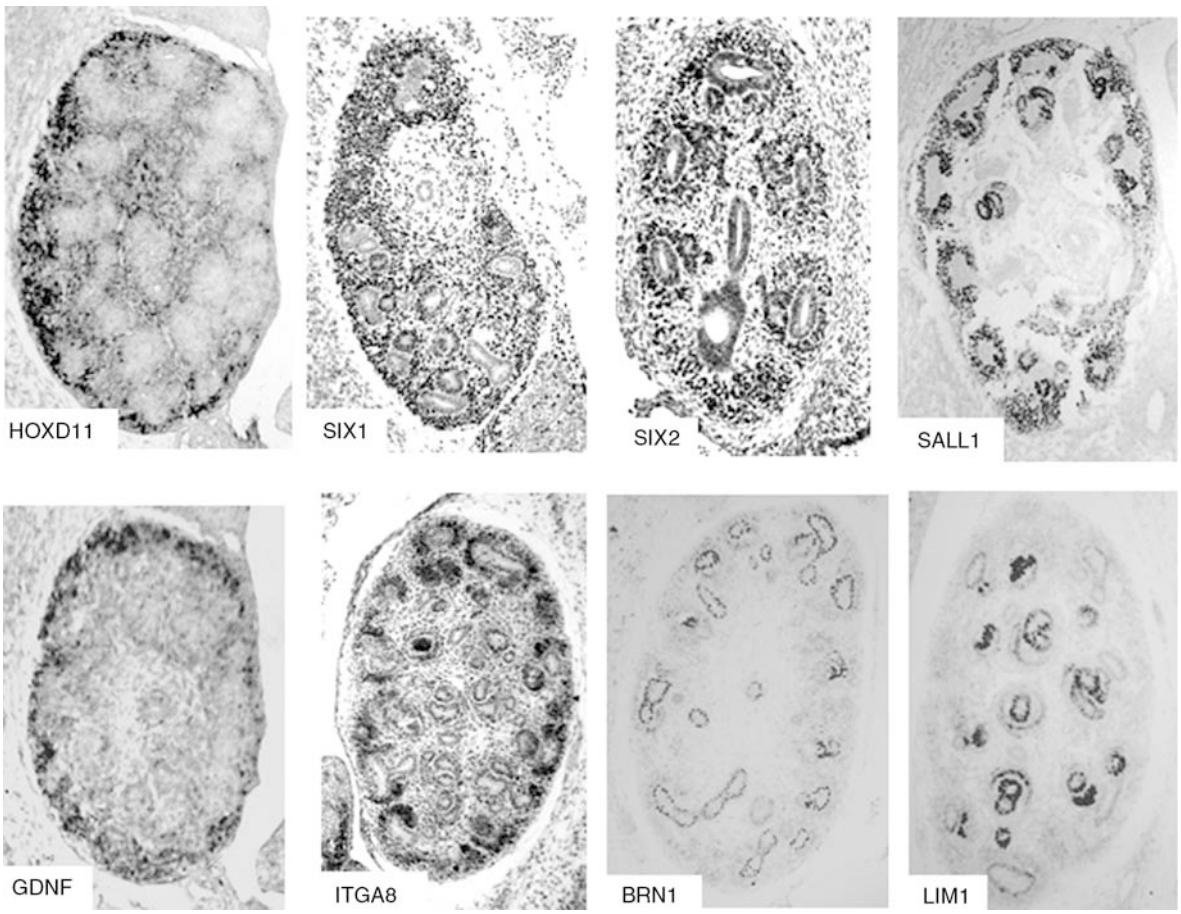


Figure 1-11

Gene expression in initiating mouse metanephros: nephron precursor stage overview. Sections of mouse metanephric kidneys at embryonic day 12, the anatomical equivalent to human 6 week gestation metanephros. Gene expression (appearing in *black*) has been assessed by either in situ hybridization to detect RNA (HOXD11 and GDNF) or immunohistochemistry (SIX1, SIX2, SALL1, ITGA8, BRN1 and LIM1) to localize protein. The tissue has been lightly counterstained with hematoxylin (appears *grey*). By this stage, the bud has undergone multiple rounds of branching and the first nephron precursors have differentiated into vesicles and S-shaped bodies; a rim of mesenchyme remains in the periphery and will continue to generate new nephrons. HOXD11, SIX1, SIX2, SALL1, GDNF and ITGA8 continue to be expressed in the mesenchyme while BRN1 and LIM1 are prominently expressed in various parts of the emerging nephron (see also *Fig. 1-12*).

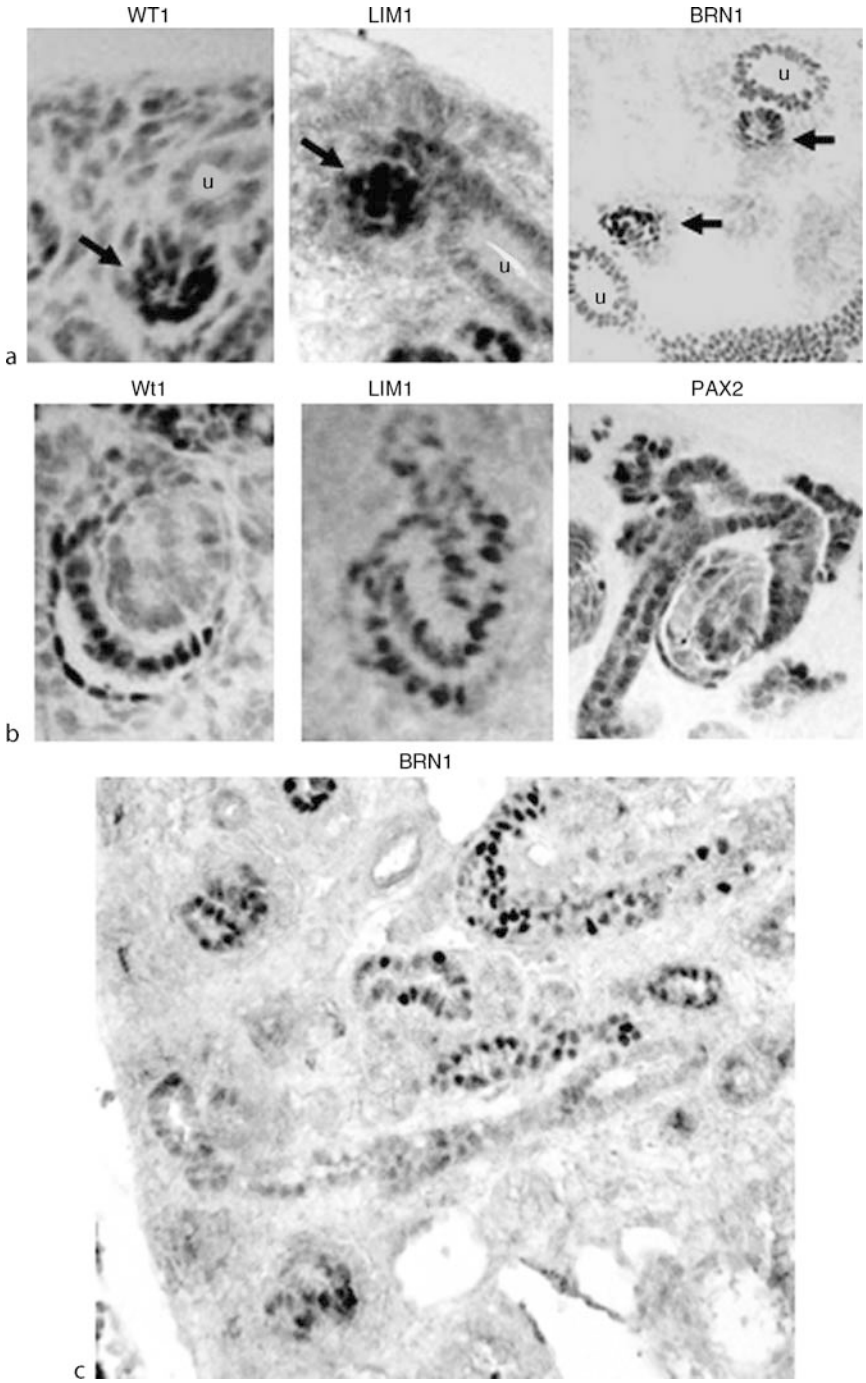


Transcription Factors

Transcription factor proteins bind to DNA and regulate expression of other genes. Because they can enhance or switch-off the transcription of mRNAs, transcription factors have been likened to conductors of an orchestra during normal development. These molecules can be classified into families that share similar DNA-binding protein motifs and domains. One such motif is called the zinc-finger, which describes a projection of the molecule that intercalates with DNA. Examples of transcription factors expressed during nephrogenesis include members of the homeobox (HOX) family (30), that contain DNA-binding homeodomains, as well as the paired-box (PAX) family, which contain DNA-binding paired-domains (27). At present, little is known about the specific targets of many of the transcription factors expressed in the developing kidney. However, it has been reported that levels of integrin α8 (ITGA8), which links metanephric cell membranes with the extracellular environment, are increased by HOXD11 (43), and that this homeobox gene, acting on concert with PAX2 and another molecule called eyes

Figure 1-12

Gene expression in initiating mouse metanephros: nephron precursor stage high power views. (a) in the nephrogenic zone, the transcription factors WT1, LIM1 and BRN1 are upregulated in condensates and vesicles (*arrows*), as the mesenchyme undergoes transition to nephron epithelium. BRN1 is also weakly expressed in cells near ureteric bud branch tips (*u*). (b) In more mature nephronic structures, the S-shaped bodies, WT1 is expressed in nascent glomerular epithelia, Lim1 is expressed in the emerging glomerulus and proximal tubule, while PAX2 is dowregulated but it remains expressed in adjacent ureteric bud branches. (c) BRN1 is also expressed in forming loops of Henle.



absent 1 (EYA1), stimulates the expression of the metanephric growth factor called glial cell line-derived neurotrophic factor (GDNF) (44).

Growth Factors

The metanephros is rich in growth factors that modulate cell survival, proliferation, differentiation and morphogenesis. Factors that have a positive effect on growth include angiopoietin 1 (ANGPT1), bone morphogenetic protein 7 (BMP7), epidermal growth factor (EGF) and its embryonic homologue transforming growth factor α (TGFα), fibroblast growth factors (FGFs), GDNF, growth differentiation factor 11 (GDF11), hepatocyte growth factor (HGF), insulin-like growth factor I and II (IGFI and II), leukemia inhibitory factor (LIF), platelet derived growth factor A and B chains (PDGFA and B), and vascular endothelial growth factor (VEGF). Less studied are those which have negative effects on growth and differentiation include TGFβ and tumor necrosis factor α (TNFα); the importance of these factors may be more apparent in malformed kidneys, where their expression is up-regulated and they may perturb normal growth (45, 46). Some factors, such as BMP4, have positive or negative effects on growth and differentiation depending on the specific stage of renal tract morphogenesis being studied. In some cases, a single factor can have multiple effects by virtue of binding to more than one different receptor; for example, angiotensin II generally acts to promote growth through its type I (AT1) receptor but also stimulates apoptosis through the AT2 receptor (47).

When acting on neighboring cell, growth factors are *paracrine* factors, but when acting on the producing cell, they are *autocrine* factors. Growth factors bind to cell-surface receptors, many of which are receptor tyrosine kinases that, after ligand binding, dimerize and become phosphorylated, thereafter transducing signals into the cell. Factors acting via receptor tyrosine kinases include ANGPTS, EGF, FGFs, HGF, IGFs, TGFα, and VEGF. Others, including BMPs, GDF11 and TGFβ, bind receptors with threonine and serine kinase activity. GDNF is a distant relative of TGFβ but signals through a receptor tyrosine kinase after binding to an accessory receptor. Yet other metanephric growth factors, including angiotensin, LIF and TNFα, signal through different classes of receptor.

Adhesion Molecules

The third major class of molecule comprises the adhesion molecules. Some mediate the attachment of cells to one another while a second group mediates attachment of cells to the surrounding extracellular matrix (ECM). Examples of the former include neural cell adhesion molecule (NCAM), whose adhesive properties are independent of calcium, and E-cadherin, whose adhesive properties depend on calcium. Molecules in the second group include collagens, fibronectin, laminins, nidogen, and tenascin. Many bind to cell surfaces via integrin receptors to provide a physical framework for epithelial tubules and endothelia. Some of these interactions also modulate growth and differentiation in an analogous fashion to the binding of growth factors to their receptors. Proteoglycans, including syndecan and heparan sulfate, constitute another type of adhesion molecule. They also bind growth factors such as FGFs and VEGF, hence sequestering and storing these molecules as well as modulating their binding to receptor tyrosine kinases. Recently, the Fraser syndrome (FRAS1/FREM) gene family has been identified; these encode several proteins form a complex in the basement membrane near the basal surfaces of developing renal epithelia and the complex most likely interacts with both growth factors, modifying their activities, and with other ECM proteins and with integrins: these, and similar, molecules are discussed in detail later in this chapter.

Conversion of Metanephric Mesenchyme into Nephron Epithelia

Uninduced Metanephric Mesenchyme Is Preprogrammed to Form Nephrons

Isolated metanephric mesenchyme can be induced to form nephrons in vitro by recombination with the ureteric bud or by apposition to embryonic spinal cord. However, mesenchyme from other embryonic organs cannot be stimulated to produce nephrons by either the ureteric bud or heterologous inducers (14). Hence, by the time the metanephros can first be detected, the renal mesenchyme has already been programmed to form nephrons, but it requires additional, inductive signals from the ureteric bud to permit its differentiation. There has been some progress regarding the molecules responsible for this programming of the renal mesenchyme. The transcription factor LIM1 (LIM homeobox 1) is expressed in the intermediate mesoderm before it forms the renal mesenchyme, and the metanephros fails to form in mice, which lack LIM1 (48). However, the embryonic expression of this gene is widespread, and diverse nonrenal organs are malformed in null mutants. Another transcription-associated

molecule, EYA1 (Figs. 1-9 and 1-10), was found to be dispensable for formation of the mesonephric duct but it specifies the part of the intermediate mesoderm which will form the metanephric mesenchyme (49). *EYA1* heterozygous mutations in humans can cause a spectrum of renal malformations, including agenesis in the context of the branchio-oto-renal syndrome (50). Of note, another transcription factor called SIX1 is expressed in an overlapping pattern to EYA1 (Figs. 9-11) and both may act in a similar pathway to stimulate expression of other nephrogenic genes (51). Indeed, *SIX1* mutations can also cause branchio-oto-renal syndrome in humans (50).

Uninduced Metanephric Mesenchyme Is Preprogrammed to Die

When cultured in isolation, murine renal mesenchyme fails to survive. In contrast, the recombination with either ureteric bud epithelium or embryonic spinal cord rescues mesenchymal cells from death and induces them to form nephrons. Koseki et al. demonstrated that death of the isolated renal mesenchyme was mediated by apoptosis and that it was an active process, as assessed by a requirement for mRNA and protein synthesis (52). They also reported that isolated renal mesenchyme could be rescued from death by the addition of EGF, the adult homolog of TGFα, or by phorbol ester, a chemical that enhances the activity of protein kinase C. Perantoni et al. reported that FGF2 could also facilitate survival of isolated renal mesenchyme (53), an important observation when taken with the fact that the ureteric bud secretes this factor (54). Barasch et al (55) have reported that metalloproteinase-2 stimulates mesenchymal growth by preventing this cell population from dying. Recent experiments show that HOX11 transcription factors enhance the expression of SIX2 in renal mesenchyme (Figs. 1-9–11) to perpetuate the uninduced, precursor population (30, 56). Furthermore, *SIX2* mutations have been found in humans affected by a spectrum of kidney malformations (57).

The *Wilms tumor 1* (*WT1*) gene produces multiple transcripts, some of which act as transcription factors, while others are likely to affect splicing of mRNA before export from the nucleus (58). WT1 is expressed at low levels in metanephric mesenchyme, and levels are upregulated during differentiation into nephron precursors (31) (Fig. 1-2). In vivo, absence of WT1 protein causes fulminant death of the intermediate mesoderm, which would normally form the metanephric mesenchyme, producing renal agenesis (59). To date, *WT1* human mutations have yet to be associated with renal agenesis, although the importance of this molecule in nephrogenesis is emphasized by the occurrence of mutations in some cases of Wilms tumor, thought to arise from kidney precursor cells (60). It has been reported that WT1 regulates the expression of members of the EGF family which are expressed in the metanephros and which enhance ureteric bud growth (61). A similar final phenotype can be generated in mice that are homozygous null mutants for either *PAX2* or *GDNF* or *RET*, a gene coding for a GDNF receptor on the ureteric bud. In these cases, the primary defect is a failure of outgrowth of the ureteric bud from the mesonephric duct: the defect in the renal mesenchyme is secondary to the loss of its normal inducer tissue in vivo. Nishinakamura et al. (62) have recently reported that mesenchymal expression of the SALL1 (sal-like 1/homologue of Drosophila spalt) transcription factor (Figs. 1-9 and 1-10) is necessary for early inductive events in the murine kidney; the human homologue is mutated in the Townes-Brockes syndrome, a disorder associated with renal tract malformations (63).

Condensation of Renal Mesenchyme

The first morphologic step in nephron formation is the aggregation of renal mesenchymal cells to form a condensate. At the same time, these nephrogenic precursor cells undergo a burst of proliferation and upregulate the expression of the transcription factors WT1 (31) and PAX2 (Figs. 1-5, 6, 9–11) (31). PAX2 prevents kidney cells from undergoing apoptosis (64) and inhibition of PAX2 by antisense oligonucleotides prevents condensation in metanephric organ culture (65). Mice lacking one copy of the *PAX2* gene are born with small kidneys (27, 66) and humans with heterozygous, inactivating mutations of *PAX2* have renal hypoplasia as part of the renal-coloboma syndrome (67). Other genes that are switched on or upregulated as renal mesenchymal cells aggregate include MET (68, 69), ITGA8 (70) (Figs. 1-10 and 1-11), and BCL2 (31). HGF is expressed by renal mesenchyme and it induces the expression of epithelial markers in the same population of cells. Mice deficient in ITGA8 have small, severely malformed kidneys, with defective nephron formation and impaired ureteric bud branching. A ligand for this integrin is expressed on the surface of ureteric bud branches, and therefore this heterodimer most likely coordinates morphogenetic interactions between the mesenchymal condensate and the ureteric bud epithelium (71). BCL2 is located in the nuclear and mitochondrial membranes and prevents apoptosis, perhaps by interfering

with lipid peroxidation. Homozygous null mutant mice have fulminant renal apoptosis during development and are born with renal hypoplasia (38, 72). Thus, this combination of molecules together enhances the survival, growth and differentiation of mesenchymal cells as they begin their journey to becoming nephron epithelia.

Morphogenesis into Nephron Tubules

Next, the mesenchymal condensate forms a lumen and differentiates into an increasingly mature nephron via the vesicle, comma, and S-shaped stages. This process is associated with the replacement of the intermediate filament vimentin by cytokeratin in all segments apart from glomerular podocytes. In addition, there are profound changes of expression of adhesion molecules. Neural cell adhesion molecule (NCAM) becomes downregulated (73) while E-cadherin appears at sites of cell-cell contact (adherens junctions) in the primitive nephron (17). Numerous studies have implicated the latter molecule as playing a part in the genesis of epithelia (74, 75). At the same time, the expression of the extracellular matrix molecules collagen I and fibronectin are downregulated, and primitive tubular epithelia begin to synthesize a basement membrane containing collagen IV, laminin, heparan sulfate, and nidogen (17, 76). Evidence from organ culture experiments supports the concept that the interaction of laminin-1, a cruciform trimeric molecule, with a cell-surface receptor, ITGA6, is essential for lumen formation of the primitive nephron (17, 77).

Other types of molecules have been implicated in the growth of primitive nephrons. WNT (wingless-type MMTV integration site family) glycoproteins are secreted signaling factors. WNT4, a member of this family, is upregulated by PAX2 in renal mesenchymal cells as they differentiate into nephrons and may have an autocrine role in this process (78). Mice with *WNT4* homozygous null mutations have metanephroi in which the renal mesenchyme has become induced but it fails to differentiate passed the condensate stage (79). Furthermore, *WNT4* mutations have been reported in humans who have a syndrome of renal agenesis, Mullerian duct derivative anomalies and virilization (80, 81). BMPs are members of the TGFβ superfamily and transduce growth signals through type I and II receptor serine/ threonine kinases. BMP7 is expressed by the branches of the ureteric bud and is also upregulated in primitive nephrons (82, 83). Nephrogenesis is impaired in *BMP7* null-mutant mice with formation of only a few nephrons and ureteric bud branches. LIF, a member of the interleukin-6 family, is secreted by the ureteric bud and can transform renal mesenchyme into epithelia, including proximal tubules and glomeruli, acting together with FGF2 and TGF family members (84, 85). Other growth factors, such as the IGFs, may play permissive roles in growth at this stage of nephrogenesis (86).

While several recent studies have implicated molecules in construction of glomeruli (see next section), presently little is known about genes which control the differentiation of primitive nephrons into the specialized non-glomerular cells in the mature mammalian nephron i.e. proximal tubule, loop of Henle and distal convoluted tubule. However, this type of detailed analysis has been performed for cells in the Malpighian excretory tubule of embryonic *Drosophila* fruit flies (87). The zebrafish embryonic (pronephric) tubule contain proximal tubule-like segments, and these are abolished upon downregulation of retinoic acid (vitamin A-mediated) signaling (88). The significance of this striking observation with regard to mammalian tubule segmentation is unknown although vitamin A supplements enhance nephrogenesis in metanephric organ culture (89) and very high doses of vitamin A derivatives can impair kidney differentiation in utero (23), suggesting the need for an optimal level of retinoic acid signaling in mammals as well as fish. Indeed, the retinoic acid-upregulated growth factor called midkine is expressed in nephron precurors where it enhances nephrogenesis (90).

The LIM1 transcription factor (*Figs. 1-10–12*) is strongly-expressed in the renal vesicle and subsequently in the proximal section of the S-shaped body in cells destined to form proximal tubule and glomerular epithelia (48). The NOTCH signaling system was first implicated in controlling cell fate in embryonic flies; NOTCH 1 and 2 are expressed in the renal vesicle and Notch 2 is essential for differentiation of proximal tubules and podocytes (91). Whether LIM1 controls expression of NOTCH has not been established. Mutations of genes coding for components of the renin-angiotensin system have been reported in individuals with renal tubular dysgenesis, an autosomal recessive disorder in which kidneys form but lack properly-differentiated proximal tubules (92). The mechanism of this effect is uncertain and may be explained either by direct, differentiating-enhancing effects of angiotensin II on proximal tubules or by an indirect effect of underperfusion of the fetal kidney associated with systemic hypotension. The observation is also interesting in view of the fact that the incidence of kidney malformations may be increased in fetuses of mothers taking angiotensin enzyme converting inhibitors (93). Finally, BRN1 (also known as POU domain, class 3, transcription factor 3), another transcription factor, is expressed in the distal

portion of the nephron vesicle and S-shaped body and eventually in the thick ascending limb of the loop of Henle (*Figs. 1-10–12*); it controls the elongation of the loop of Henle and expression of segment specific molecules such as uromodulin (Tamm-Horsfall protein), the Na^+:K^+:$2Cl^-$ cotransporter (NKCC2) and barttin (BSND) (94).

Formation of the Glomerulus

WT1 transcription factor is essential for the maintenance of the mature podocyte, where it downregulates the transcription of PAX2 (95) (*Fig. 1-12*) and, in mice, transgenic overexpression of PAX2 causes congenital nephrotic syndrome (26). In humans, heterozygous mutations of *WT1* can cause the Denys Drash syndrome in which congenital nephrotic syndrome and propensity to Wilms tumor are key features; affected glomeruli have PAX2 overexpression in podocytes (96). Frasier syndrome, in which glomerular degeneration occurs later in childhood, also features *WT1* mutations and is associated with progressive glomerulopathy, male pseudohermaphroditism, and gonadal dysgenesis with increased risk of gonadoblastoma and malignant germ cell tumors (97). LMX1B (LIM homeobox transcription factor 1) is another transcription factor expressed in podocytes and human heterozygous mutations lead to glomerular proteinuria in the context of the nail patella syndrome (98). In glomeruli of null mutant *LMX1B* mice, the normal switch (99) from immature/embryonic α1 and α2 forms of collagen IV to the mature forms α3 and α4 (mutated in autosomal forms of Alport syndrome) fails to occur; moreover, the transcription factor binds upregulates expression of human COL4A4 (100). Nephrin is a transmembrane protein of the immunoglobulin family of cell adhesion molecules and is a key component of the glomerular slit diaphragm; human mutations of *NPHS1* which codes for this protein lead to congenital and also to later onset steroid-resistant nephritic syndrome (101, 102). Podocin is another important podocyte protein: it appears to link the slit diaphragm with the cytoskeleton and mutations of *NPHS2* which codes podocin are associated with autosomal recessive steroid-resistant nephrotic syndrome (103). Among the molecules which show deregulated expression in mice with *NPHS2* mutation include LMB1B, discussed above, and TGFβ1 encoding a growth factor implicated in scarring (104).

As the glomerular epithelium matures, the basement membrane becomes rich in laminin b2. Mice without the functional gene encoding this protein develop albuminuria and enhanced glomerular basement membrane permeability to ferreting soon after birth, before structural changes such as foot process effacement and loss of slit diaphragms (105). Humans with Pierson syndrome have early onset nephrotic syndrome together with microcoria (nonreactive narrowing of the pupils due to aplasia of the dilatator pupillae muscle) have heterozygous mutations of *LAMB2* (106). Laminin a5 is another protein expressed in the glomerular basement membrane and both nascent podocytes and glomerular endothelia express and insert this protein into the glomerular basement membrane (107). *LAMA5* null mutations in mice are associated with a failed switch from laminin a1 which normally occurs during the invasion by capillaries of the fetal glomerulus (see below); mutant gomeruli lack endothelia and mesangial cells (108). Podocytes express α3β1 integrin dimers and they appear critical for the normal construction and maintenance of intact glomeruli as assessed by lesions in mice with ablations of either the *ITGA3* or the *ITGB1* gene (109, 110).

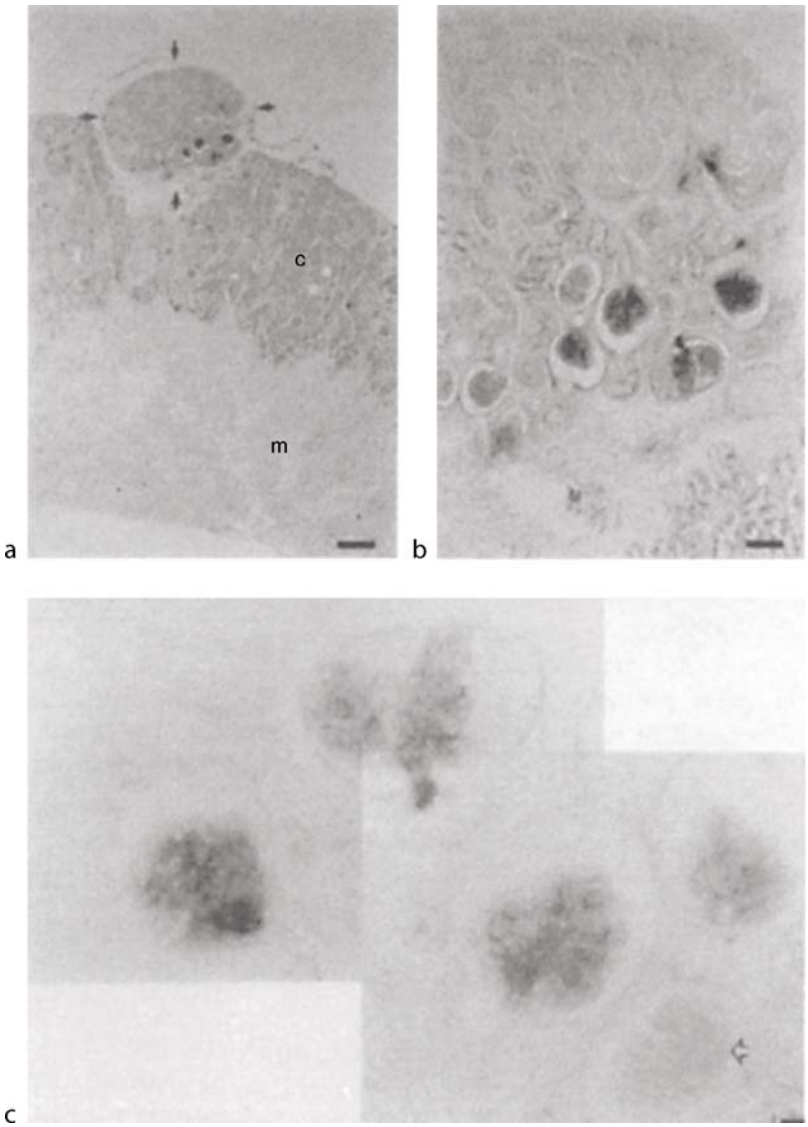
Embryonic blood vessels arise by vasculogenesis or angiogenesis. In vasculogenesis, mesenchyme differentiates in situ to form capillaries. In contrast, angiogenesis involves ingrowth from existing capillaries. The first embryonic vessels of the yolk sac, endocardium, and dorsal aorta arise by vasculogenesis, but thereafter, descriptive studies alone cannot ascertain the origin of embryonic vessels. When avascular murine renal mesenchyme is induced to differentiate in organ culture, the glomeruli that develop lack capillaries as assessed by light and electron microscopy (111), a result used to argue against the possibility of glomerular vasculogenesis. When mouse metanephroi were transplanted onto avian chorioallantoic membranes, forming glomeruli acquired capillary loops but these were of host origin as assessed by a quail-specific nuclear marker and antisera to chick collagen IV, a component of endothelial basement membrane (10, 112). These results were used to argue that glomerular vessels form by angiogenesis in vivo. However, neither organ culture nor chorioallantoic membrane are likely to provide a normal environment for growth of glomerular capillaries.

More recent studies have found that morphologically undifferentiated renal mesenchyme contains subsets of cells which express markers characteristic of endothelial cells, including receptors for the vascular growth factors, VEGF and ANGPT1 (113, 114). When transgenic mice were used, in which an endothelial-specific β-galactosidase reporter gene product could be detected histochemically, endothelial precursors were visualized in intermediate mesoderm condensing around the caudal end of the mesonephric duct, with a similar pattern noted in renal mesenchyme at the ureteric bud stage (113, 115).

Loughna et al. (113) determined whether these precursors could differentiate into endothelia using a model in which metanephroi form filtering glomeruli after transplantation into the neonatal nephrogenic cortex. When transgenic avascular metanephroi were transplanted into wild type hosts, differentiated donor tissue contained transgene-expressing glomerular arterioles and capillary loops (Fig. 1-13). Using other transplantation strategies (e.g. to the anterior eye chamber), others have provided similar evidence that renal capillaries can arise directly from early metanephroi (115, 116). Furthermore, in organ culture, metanephric capillary survival and growth

Figure 1-13

Transplantation of *TIE1/LACZ* metanephroi into wild-type neonates. (a) After 1 week, the E11 transplant differentiated (*arrows*) in the host cortex (*c*). Transgene expression (*black*) was confined to the transplant. No staining was detected in host cortex or medulla (*m*). (b) Donor glomeruli expressed the transgene intensely. (c) Positive capillary loops in glomeruli are visible. Podocytes (*open arrow)* do not express the transgene. Bars: 120 μm in (a) 30 μm in (b) 10 μm in (c). (Loughna S, Hardman P, Landels E et al. A molecular and genetic analysis of renal glomerular capillary development. *Angiogenesis* 1997;1:84–101, with permission from Kluwer Academic Publishers, Dordrecht, The Netherlands.)



are enhanced by addition of VEGF or ANGPT1, or by growth in a hypoxic atmosphere (117–119). Indeed, the contention that the developing kidney is "hypoxic" in vivo is supported by the observation that it expresses high levels of hypoxia-inducible factor proteins, which are normally down-regulated postnatally in healthy kidneys (120). One can speculate that capillary formation within the metanephros is at least in part driven by hypoxia, and that that low oxygen tension increases expression of diverse vascular growth factors and their receptors. Indeed, nascent podocytes express high levels of VEGF, and when this gene is experimentally-downregulated capillaries are severely disrupted in forming glomeruli (121).

Other molecules are implicated in vascular growth within the metanephros. These include the Eph/ephrin family of membrane receptors which appear to be critical in cell-cell recognition (122). Renin is widely expressed in perivascular cells in the arterial system of the metanephros but becomes restricted to juxtaglomerular cells during maturation. Recent evidence suggests that the metanephric mesenchyme contains renin-expressing cells precursor cells which can contribute to the vasculature and perhaps also to tubule epithelia (123, 124). In addition, other molecules required to generate bioactive angiotensin are expressed in the metanephros, as are its AT1 and AT2 receptors. In rats there is evidence that angiotensin II may enhance glomerular endothelial growth in vivo (125, 126). Furthermore, the ANGPTs and TGFB1 also play roles in glomerular capillary morphogenesis, perhaps by controlling endothelial survival (36, 127, 128).

It has been speculated that mesangial cells arise from the same lineage as glomerular capillaries because neither cell forms when the metanephros is cultured under standard organ culture conditions (111). It was assumed that mesangial cells were derived from cells outside the embryonic kidney. However, mesangial cells develop when metanephroi are transplanted and grown in oculo (116), and thus mesangial precursor cells may be present in renal mesenchyme. Whatever their origin, PDGFB is crucial for the differentiation of mesangial cells in vivo. Mice with null mutations of either the gene coding for this growth factor, or its receptor, lack mesangial cells and develop bizarre, malformed glomeruli (129, 130). PDGFB is expressed by primitive nephron epithelia, and mesangial precursors express the receptor, suggesting a paracrine mode of action. As mesangial cells mature, both that ligand and receptor are coexpressed, suggesting an autocrine activity. Other growth factor signaling systems may play roles in development of this lineage: for example, HGF and MET are coexpressed by immature mesangial cells (131).

Branching Morphogenesis of the Ureteric Bud

Growth Factor Control of Ureteric Bud Growth

When the mouse ureteric bud is grown in organ culture with its adjacent renal mesenchyme, it undergoes branching morphogenesis; however, it fails to differentiate when cultured in isolation. It is established that the epithelia of the ureteric bud express various receptors, mostly tyrosine kinases, that transduce differentiation signals by binding to mesenchyme-derived growth factors. The most important signaling system involves RET, a receptor tyrosine kinase expressed in mesonephric duct, ureteric bud, and its branching tips (132–137) (*Figs. 1-9–11*). GDNF causes tyrosine phosphorylation of RET after binding to a membrane-linked accessory receptor called GDNFRα. The ligand is expressed by condensing renal mesenchyme, while GDNFRα is expressed in the same cells as RET. Lower levels of the accessory receptor are found in renal mesenchyme, where it may concentrate the ligand and also prevent its diffusion with initiation of ectopic ureteric bud branches. Mice with homozygous null mutations of *RET* or *GDNF* do not develop kidneys because of deficient outgrowth of the ureteric bud. Experiments using culture of whole metanephric rudiments with blocking antibodies to GDNF demonstrate that this signaling system is critical for stimulation branching after initial outgrowth of the ureteric bud. Equally impressive, the addition of recombinant GDNF to cultured metanephroi induces ectopic ureters. When the ureteric bud is cultured as a monolayer, it dies by apoptosis over a few days but GDNF can partially reverse this process (137). The factor also stimulates survival and morphogenesis of RET-transfected collecting duct cells in vitro (138). Recently heterozygous mutations of *RET* have been identified in humans with bilateral renal agenesis (139) and a rare *RET* polymorphism has been reported in individuals with primary, non-syndromic vesicoureteric reflux (140).

The site of GDNF-induced initiation of the ureteric bud from the mesonephric duct has to be tightly-regulated because too caudal or too cranial buds will not optimally meet the nearby renal mesenchyme, thus risking failure of kidney formation. Furthermore, multiple buds, which might generate duplex ureters and kidneys, are to be avoided. For these reasons, there exists a complex molecular machinery to regulate bud initiation, and to regulate its branching into the collecting duct system. For example, Sprouty-1 prevents aberrant bud initiation as does the ROBO2/SLIT2 signaling system; when either

system is downregulated, bud initiation and branching are abnormal and multiple ureters arise from each mesonephric duct (141–144). Of note, *ROBO2* mis-sense gene variants have been noted in rare families with multiple cases affected by vescio-ureteric reflux and/or duplex kidneys (144). The growth factor BMP4 is expressed in a broad band around the mesonephric duct, but not where the ureteric bud normally emerges: it appears to prevent ectopic branching, although its effects are complex, and it also enhances the elongation of the ureter and is implicated in muscularization of the ureter (145). Recently, BMP4 mutations have been reported in humans with renal tract malformations (57). The actions of BMP4 itself are fine-tuned by the action of another secreted factor, gremlin-1, a BMP4 antagonist (146). Apart from GDNF, other growth factors enhance the growth and branching of this lineage, either directly, such as EGF family members, FGF7 and HGF, or by indirectly upregulating GDNF signaling, such as GDF11 and WNT11 (61, 147–150).

Transcription Factors and Ureteric Bud Growth

As yet, little is known about how the expression of transcription factors within the ureteric bud affects growth of this lineage. One exception is PAX2 which is expressed in the mesonephric duct and in the ureteric bud branch tips and thereafter in maturing collecting ducts (31) (*Fig. 1-6*). The expression of PAX2 in the ureteric bud lineage correlates with growth, and both are downregulated as the ducts mature. In these cells, however, PAX2 appears to act as a survival factor rather than a molecule which directly enhances proliferation. Mice with heterozygous null mutation of *PAX2* show enhanced apoptosis in fetal collecting ducts (64). Moreover, collecting duct cyst apoptosis is increased, and cyst growth slowed, in congenital polycystic kidney (*cpk Cystin* mutant) mice which also have one inactivated allele of *PAX2* (151). In mice genetically engineered to lack both *PAX2* alleles, the ureteric bud fails to branch from the mesonephric duct, producing renal agenesis (27). In this context, ureteric bud initiation appears to fail because of GDNF/RET signaling is disrupted. Indeed, in normal development PAX2 normally upregulates expression of RET within the bud itself (152) and renal mesenchyme also expresses PAX2 where it binds to the *GDNF* promoter and upregulates the transcription of this growth factor (153). In fact, within the mesenchyme, PAX2 co-operates with other transcription factors, such as the HOXD family and EYA1, to enhance expression of GDNF (44) (*Figs. 1-9–11*). β-catenin is a transcription factor-associated molecule expressed in the ureteric bud lineage and is required for branching morphogenesis; in this context it is required to upregulate LIM1, PAX2, RET and WNT11 (154).

Cell Adhesion Molecules and Ureteric Bud Growth

The stalk of the ureteric bud is surrounded by a basement membrane composed of laminins and collagen IV, as well as nidogen/entactin and tenascin. As assessed by electron microscopy, the basement membrane is attenuated around the tips of the ureteric bud (19), and branching epithelia may be exposed to a renal mesenchymal matrix rich in collagen I and fibronectin. There is evidence that matrix molecules affect branching morphogenesis in vitro. In monolayer culture of ureteric buds epithelia, proliferation is enhanced by a fibronectin versus laminin substrate (137), consistent with the observation that cells at the branching tips have a high proliferation rate (31). Collagen I appears permissive for branching of collecting duct cells, whereas some components of the basement membrane are inhibitory (155). HGF-induced branching into collagen I is accompanied by an increase in matrix degrading molecules (e.g., collagenases, such as matrix metalloproteinases, and plasminogen activating proteases, such as urokinase) (156). *LAMA5* mutants sometimes have absent kidneys (108) and mice genetically engineered to lack ITGA3, which forms functional dimers with ITGB1 subunits, have a reduced number of medullary collecting ducts (110). Renal mesenchymal expressed adhesion related molecules may have important indirect effects on growth of the ureteric bud; an example is provided by ITGA8 (*Figs. 1-10* and *11*) which is expressed in a heterodimer with ITGB1 in condensing renal mesenchyme where it, by an unknown mechanism, upregulated GDNF expression and hence bud growth (157).

In humans, a protein called anosmin-1 coats the surface of the ureteric bud and its branches where it is thought to mediate activities of growth factors; mutations of the gene, *KAL1*, which codes for anosmin-1 lead to renal agenesis in the context of X-linked Kallmann syndrome (158, 159). This provides a paradigm for several other basement-membrane associated molecules in the ureteric bud and collecting duct lineage. For example, FRAS1 (Fraser syndrome 1) and FREM2 (FRAS1-related extracellular matrix) are large, multidomain proteins inserted into the basal plasma membrane and which protrude into the extracellular space; the proteins form a complex where they may mediate the expression and

actions of nephrogenic growth factors at the same time interacting with structural molecules such as integrins. Null mutation of either gene in mice and humans are usually associated with renal agenesis in the context of Fraser syndrome, which also features embryonic skin blistering (160–163). Similarly, glypican-3 is a ureteric bud-associated cell surface heparan sulfate proteoglycan which regulates the tempo of arborization in part by regulating activities of BMPs and endostatin (164, 165); in humans, *GPC3* mutations are associated with cystic kidney maldevelopment in the context of the Simpson-Golabi-Behmel syndrome, an X-linked condition characterized by prenatal and postnatal overgrowth with visceral and skeletal abnormalities (166).

Further Maturation of the Collecting Ducts

As the bud branches, the stems mature into the collecting ducts, which contain three types of cell: the potassium-handling principal cells and the proton-handling α and β intercalated cells (167). Corticosteroids enhance collecting duct differentiation, and there is also some plasticity regarding the lineage of these cells based on in vitro studies (168, 169). During this period of maturation, the Na^+-K^+-ATPase becomes relocated from the apical to basal plasma membrane, and this process is perturbed in some polycystic kidney diseases (170). Galectin-3 is a cell adhesion molecule which is prominently expressed by maturing collecting stalks. Here it may regulate epithelial growth by interaction with laminin and other extracellular matrix molecules (171, 172). Furthermore, the protein is also located in primary cilia (173), flow sensors which protrude into the lumen of the tubule (174). Downregulation of galectin-3 enhances cyst formation in a mouse model of recessive congenital polycystic kidney disease (173). The protein is also involved in the genesis of apical epithelial characteristics of collecting duct cells (175) and, on the basal side of collecting duct cells it acts in concert with another extracellular molecule called hensin to enhance epithelial differentiation (176). *Polycystic kidney disease 1* (*PKD1*) the gene most commonly mutated in human autosomal dominant polycystic kidney disease is prominently expressed by fetal collecting ducts (177).

Fetal collecting ducts also express various transcription factors which play roles in maturations. As examples, p53 is associated with collecting duct dilatation (178) and experimental downregulation of hepatocyte nuclear factor 1β (HNF1B) causes polycystic kidneys associated with deregulation of the normal longitudinal arrangement of mitotic spindles found in extending tubules (179, 180). It is notable that *PKD1* is under transcriptional control of p53 (181), and that HNF1B normally up-regulates the transcription of several "anti-cyst genes," including the one mutated in human autosomal recessive polycystic kidney disease (179). In humans, inherited or de novo heterozygous mutations of *HNF1B* cause the renal cysts and diabetes syndrome; here, affected individuals have a predisposition to diabetes mellitus and can also have a spectrum of structural kidney defects from cystic dysplasia to a polycystic phenotype (182–184). The gene may also control tubular physiological functions because mutations are associated with increased plasma levels of uric acid and also with hypomagnesemia (183, 184).

Metanephric Stromal Cells

Little is known about the mechanisms that control the differentiation of renal mesenchyme into stromal cells (40), or interstitial fibroblasts, but there is evidence that stromal cells are essential for epithelial development. For example, the G_{D3} ganglioside is expressed by stromal cells surrounding the stalk of the ureteric bud, and antibodies to this molecule prevent bud morphogenesis (185). Metanephric stromal cells also express the BF2 winged-helix transcription factor, also known as FOXD1 (186). Mice that were homozygous null mutants for this gene have impaired branching of the collecting ducts and also perturbed conversion of renal mesenchyme to nephrons (187). Other evidence emphasizes that metanephric stromal and epithelial cells are involved in complex reciprocal signaling loops, with expression of retinoic acid receptors in the stroma playing important roles (188). A similar example is provided by stromal cells which express the KIT receptor tyrosine kinase for stem cell factor; some of these cells express angiogenic markers and these cells may enhance development of adjacent epithelia (186). Interestingly, other data show that, at the inception of the metanephros, a subset of cells at the periphery of the organ express VEGF receptors and these cells appear to send an unknown signal with upregulates PAX2 and thereafter GDNF expression (189). Some metanephric stromal cells have neuronal characteristics, staining positively with neurofilament markers; the role of these cells is unknown, but their survival can be modulated by neurotrophin 3 (190). Clifford Grobstein found that when an embryonic spinal cord was placed on the opposite side of a filter to metanephric mesenchyme, nephrons were induced to differentiate (14). Further investigations showed that neurons had penetrated the mesenchyme through

the microscopic pores of the filter, and if the neurons in the spinal cord were destroyed, induction did not occur (191). As assessed by antibodies against neurofilaments and neural cell surface gangliosides, neuronal cell bodies can be observed around the ureteric bud in vivo and their terminals surround mesenchymal condensates (192).

Lower Urinary Tract Development

At the same time that the metanpehric kidney, the lower urinary tract develops in harmony, with the ureters and bladder maturing into structures ready to receive urine and to respectively propel it into the bladder which in turn stores urine, intermittently expelling it via the urethra.

The separation of the urogenital sinus from the cloaca is in part mediated by EPH/EPHRIN reciprocal signaling and by sonic hedgehog (SHH), a secreted morphogen (193, 194). A series of cell rearrangements join the embryonic ureter (i.e. the ureteric bud stalk) to the bladder to generate the uretero-vesical junction which prevents reflux of urine upon bladder contraction (195, 196). After the basic anatomy of the lower tract has been established in this manner, the nascent urothelium in the bladder and ureters secretes SHH which acts via the PTCH1 receptor expressed in surrounding mesenchymal cells first to enhance their proliferation and then, via upregulation of BMP4, to induce differentiation of these cells into visceral smooth muscle (11, 197–200). In mice, the T-box 18 (TBX18) and Teashirt-3 (TSHZ3) transcription factors are expressed in ureteric mesenchymal cells and are required for ureteric mulscularization antenatally (201, 202). At later stages of ureteric smooth muscle maturation, angiotensin II appears to become important and, acting through its AT1 receptor, it enhances peristalsis and also may have a direct effect on morphogenesis (203). Mutation of *SHH* or *TBX18* or *TSHZ3* or genetic or pharmacological downregulation of angiotensin signaling all lead to hydronephrosis in the absence of anatomical obstruction (200–204), emphasizing the importance ureteric peristalsis in moving urine from the kidney pelvis into the urinary bladder. At the same time that ureteric smooth muscle is differentiating, the adjacent epithelium differentiates into a water-tight pseudo-stratified urothelium. BMP4 is apparently needed to prompt the ureteric bud epithelium to form urothelium and indeed overexposure of the intrarenal ureteric bud branches induces them to acquire urothelial characteristics (205). FGF7 stimulates fetal urothelial proliferation (206) and terminal differentiation is associated with upregulation of five types of uroplakin proteins which aggregate in plaques at the urothelial apical surface. Not only to they confer water-tight properties to the lower urinary tract but they are somehow also essential in morphogenesis because mice which lack UPK3A and humans with heterozygous mutations of *UPK3A* have malformed ureters, sometimes with vesicoureteric reflux (207–209).

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2 Glomerular Circulation and Function

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The essential function of the kidney is to preserve constancy of body fluid and electrolytes by removing water and potentially harmful metabolic end-products, e.g., uric acids, sulfates, phosphates, while preserving blood pressure, and essential solutes, e.g., sodium, chloride, bicarbonate, sugars, amino acids. The process begins in the renal glomerulus, where plasma is ultrafiltered under pressure through a semipermeable glomerular capillary wall. The ultrafiltration separates plasma water and crystalloids from blood cells and protein macromolecules, which remain in the glomerular circulation. The magnitude of this filtration process is enormous and requires a high rate of renal blood flow (RBF). Indeed, the entire plasma volume is cycled through the glomerular system 20 times per hour. The RBF and glomerular filtration rate (GFR) are interrelated such that maintenance of an adequate RBF is crucially important for optimal GFR while the glomerulus is an active participant in determining the RBF.

Renal Blood Flow

Blood flow to the kidneys comprises 20–30% of cardiac output (CO) and is determined by two factors: renal perfusion pressure (RPP), which is approximately equal to the systemic arterial blood pressure (BP), and renal vascular resistance (RVR), which is determined primarily by the afferent and efferent arterioles. The relationship can be expressed as RBF = BP/RVR. Although renal blood flow is the parameter usually discussed, it is the renal plasma flow (RPF) that is clinically relevant. Thus, at a given level of RBF, RPF may vary with the relative volume of packed red cells. For example, RPF increases with anemia. Lambs bled to decrease their hematocrit from 33 to 14% double their RPF (1). Because RBF is partly determined by the need for oxygen delivery, RPF may be high with severe chronic anemia such as occurs with sickle cell disease. In such circumstances both the RPF and the GFR are elevated because of decreased volume of red blood cells.

Like most organs, the kidneys possess intrinsic autoregulatory mechanisms that adjust local renal vascular resistance when renal perfusion pressure changes. This autoregulation maintains RBF relatively constant in the face of changing BP and RPP under physiologic conditions (2). Many hormonal systems regulate RVR and hence RBF. The nature and magnitude of their effects are often age-specific because anatomic factors (innervation), presence and distribution of receptor subtypes (angiotensin II receptors), and postreceptor signaling events change with development (3–6). In addition, autoregulatory efficiency is impaired in several conditions including extracellular fluid (ECF) depletion, diuretic exposure, congestive heart failure, and renal parenchymal damage (7–10). Each of these conditions makes the organism more susceptible to acute renal failure in the face of relatively minor changes in BP that cause substantial changes in RBF (see below).

Development of Renal Blood Flow

Prenatal Renal Blood Flow

Fetal renal blood flow is low, but increases with gestational age. Doppler ultrasound at 25 weeks of gestation shows the RBF to be 20 ml/min while at 40 weeks the RBF is 60 ml/min (11). Within the kidney, the relative perfusion varies with cortical depth with deeper nephrons of the cortex receiving more blood flow than nephrons in the superficial layers (12). This distribution of blood flow parallels morphologic maturation because deeper nephrons are the first to form and mature; superficial nephrons are not completed until near term (13).

Although the kidneys in the human embryo produce urine by 12 weeks' gestation, the role of kidneys in fetal homeostasis is minor compared to the placenta. The percentage of cardiac output perfusing the kidneys is low during intrauterine life. For example, during late gestation, the kidneys of fetal lambs receive only about 2.5% of the cardiac output while the placenta receives 40%. The kidneys of 10- to 20-week human fetus receive only 3–7% of cardiac output (14). Thus, the clinical relevance of intrauterine RBF and also glomerular filtration is less for the clearance of fetal plasma than for the formation of urine and hence amniotic fluid.

Fetal hemodynamics and urine formation are affected by maternal factors such as the maternal volume status,

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drugs, and vasoactive substances that cross the placenta. For example, acute oral hydration, which is sufficient to decrease plasma osmolality of healthy pregnant women, increases fetal urine production in near-term fetuses (15). Furosemide given to pregnant women induces diuresis in the fetus; likewise, maternally administered nonsteroidal antiinflammatory drugs lessen urine production and may lead to low glomerular filtration even after the baby is born (16, 17). Angiotensin II (Ang II) has distinct effects on the maternal, uteroplacental and fetal hemodynamics. Maternal circulation appears to be particularly sensitive to the vasoconstrictive effects of Ang II, which would tend to preserve uteroplacental and fetal circulations (18, 19). However, this fetoprotective effect disappears with long-term exposure to elevated levels of Ang II. Thus, although the first 4 h of Ang II infusion into pregnant ewes did not compromise uteroplacental/fetal perfusion, more than 20 h of heightened Ang II caused a dramatic decrease in the placental perfusion and compromised fetal gas exchange (20). These observations illustrate the important effect of Ang II on the uteroplacental circulation and by extension on fetal well-being. Ang II actions to maintain BP in utero and at the same time maintain RVR at a high level can be counteracted by inhibitors of its actions. Angiotensin converting enzyme inhibitors (ACEI's), e.g., captopril, lead to a decrease in fetal BP, RVR, and GFR in ewes, resulting in oligoanuria (21), and has been observed to cause anuria in the human fetus and newborn (22, 23). These hemodynamic changes may result from decreased Ang II synthesis and/or accumulation of bradykinin. Angiotensin II receptor antagonism did not affect blood pressure or RVR in fetal piglets or puppies (24, 25) while bradykinin receptor antagonism attenuated renal vasodilation following Ang II receptor antagonism of neonatal rats (26). These findings indicate a role for vasodilatory role of kinins offsetting Ang II-modulated vasoconstriction in the maturing kidney and complement the observations that the developing kidney expresses high levels of immunoreactive bradykinin and receptors. It is interesting that the kinin components localize to the deeper parts of the kidney, which would favor preferential perfusion of the deeper and not the superficial nephrons that characterizes the fetal kidney (27). Nonetheless, prenatal exposure to angiotensin II type 1 receptor blockers (ARBs) is now well documented to cause anuria/renal failure resulting from structural and functional renal impairment. At least in part, this reflects bradykinin generation from increased Ang II interaction with the angiotensin II type 2 receptor (AT2) (28, 29).

Another vasodilator that likely plays an important role in fetal renal hemodynamics is nitric oxide (NO) (30). Basal production of NO in third-trimester fetal sheep maintains baseline RBF; its inhibition increased RVR by 50% and blocked the increase in GFR and the natriuresis that accompany volume loading. The NO effects may be direct or through modulation of AII actions. Overall, the low renal blood flow in utero reflects incomplete renal mass that increases exponentially during fetal life, structural immaturity of resistance vessels (narrower vascular lumina) as well as unique modulation by vasoactive compounds such as Ang II, prostaglandins, kinins and nitric oxide.

Postnatal Renal Blood Flow

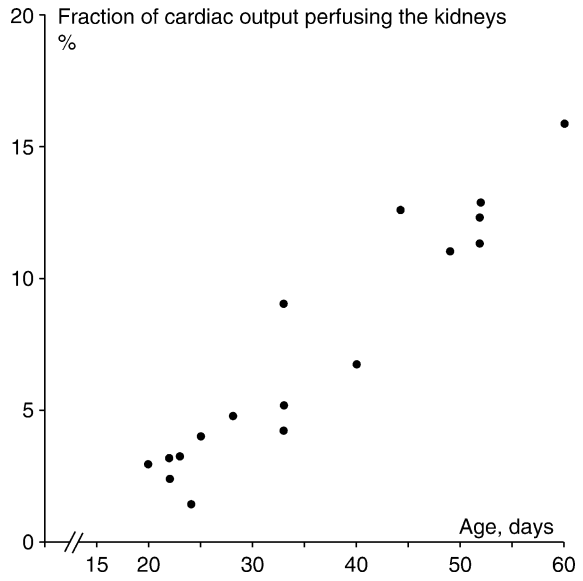
Renal blood flow, measured as a clearance of PAH (CPAH) and corrected for body size, is low in human neonates and correlates with gestational age. For example, CPAH is 10 ml/min/m^2 in babies born at 28 weeks, and 35 ml/min/m^2 in those born at 35 weeks of gestation (31). After birth, RBF increases steadily, doubling by 2 weeks and reaching mature levels by 2 years of age (32). The postnatal change in RBF primarily reflects the considerable increase in the relative RBF to the outer cortex (33–35).

Renal blood flow is governed by two factors: cardiac output (CO) and the ratio of renal to systemic vascular resistance. After birth, both an increase in CO and a decrease in RVR favor an increase in RBF. Furthermore, RVR decreases much more than systemic vascular resistance (12), allowing for a progressive increase in the renal fraction of CO. For example, RBF increases 18-fold in newborn pigs during the first 5 weeks of life, while CO (corrected for body surface area) increases only 7.2 times during the same period (*Fig. 2-1*). Not only is renal vascular resistance a function of the arteriolar resistance offered by the sum of the glomerular vessels, but also by the number of existing vascular channels. New nephron formation increases the number of channels, and hence decreases renal vascular resistance. New nephron formation contributes to the postnatal decrease in renal vascular resistance and increase in RBF only in premature babies born before 36 weeks of gestation (13).

Other factors that control the postnatal decrease in renal vascular resistance are largely those affecting the resistance of glomerular arterioles. In rats, both afferent and efferent arteriolar resistances decrease by a factor of 3 between 40 days of life and maturity (36). This decrease in renal vascular resistance may be linked to a decrease in vasoconstrictors and/or activation of potent vasodilators. Catecholamines, but especially the renin angiotensin

Figure 2-1

Renal blood flow as a percentage of cardiac output, plotted versus age, in growing rats between 17 and 60 days of age (reprinted with permission from (33)).



system, are high in the early postnatal period of premature and term infants (37–41). The role of the renin angiotensin system has been studied most extensively. Angiotensinogen undergoes a dramatic postnatal increase in liver expression before decreasing and settling to the adult level (42); renin production in neonates is robust and expands beyond the juxtaglomerular apparatus to include more proximal segments of the renal arterial tree (43); abundance of renal ACE increases postnatally such that within 2 weeks of birth it surpasses adult levels, as does the level of circulating ACE (44). Both the angiotensin II type 1 receptor (AT1) and AT2 receptor subtypes are expressed in the neonatal kidney (3, 39–46). The AT2 receptor is believed to play an important role in apoptotic processes during organogenesis including that of the kidney and urinary tract and wanes after birth (45, 47). Expression of AT1 receptor peaks postnatally at twice the adult level (46). Overall, the substrate, receptors as well as the enzymes required for production and actions of Ang II are exuberantly expressed in the neonatal kidney and contribute to the vasoconstriction of the neonatal kidney. In addition Ang II has been shown to have important role in the development and function of the renal outflow tract by inducing the development of the renal pelvis by stimulating the proliferation and differentiation of smooth muscle cells around the ureters and by promoting ureteral peristalsis (48). Absence of these Ang II-mediated effects results in renal hydronephrosis.

Locally counteracting these vasoconstrictive effects is the postnatal increase in the activity of prostaglandins, nitric oxide and kinins, that serves to temper renal vasoconstriction and contribute to the maturational increase in RBF. Indomethacin (which inhibits prostaglandin) lowers RBF in newborn rabbits (49) and decreases renal function in infants indicating an important role for vasodilator prostaglandins (49–52). Endothelium-derived nitric oxide release by the renal artery, as well as constitutive nitric oxide synthase activity in the renal microvasculature increases with fetal and postnatal maturation of guinea pigs (53). This increase in nitric oxide production is paralleled by increased sensitivity of vascular smooth muscle to nitric oxide after birth, which contributes to nitric oxide's modulation of postnatal renal blood flow. As in utero, an important contribution of the vasodilators to the maturational increase in RBF is undeniable; however, as in the fetus, it may be linked back to the renin–angiotensin system through the AT2 receptor, which is known to be a potent stimulator of prostaglandin, nitric oxide and kinins (54).

Measurement of Renal Blood Flow

Concept of clearance. Substances reaching the kidney through the circulation may undergo glomerular filtration, tubular reabsorption, or tubular secretion. Most solutes are freely permeable across the glomerular capillary and undergo filtration, followed by tubular reabsorption or secretion along the various nephron segments. Renal clearance of a substance X (C_x) is the volume of plasma from which X is removed or cleared by the kidney within a period of time. Glomerular filtration and tubular secretion facilitate clearance of a solute, while tubular reabsorption impedes it. It is calculated as follows:

$$C_x = (U_x \times V)/P_x$$

where U_x and P_x are the concentrations of X in urine and plasma, respectively, and V is the urine flow. The units of clearance are volume per unit time, usually milliliter per minute. For example, if P_x = 40 mg/ml, U_x = 80 mg/ml, and V = 100 ml/min, then C_x = 200 ml/min. Clearance is a more appropriate concept in describing the renal handling of a certain substance than urinary excretion rate (i.e., $U_x \times V$) because clearance takes into account the plasma level of X.

If a plasma substance is totally excreted on a single passage through the kidneys, it can be used as a marker

of RPF. However, in reality, only about 92% of the total RPF passes through the functioning excretory tissue, a fraction termed effective renal plasma flow (ERPF). Effective renal plasma flow is commonly measured as a clearance of para-amino hippurate (PAH), a weak acid that is almost completely extracted by the renal tubule cells and eliminated in the urine (55). Measurement of ERPF as CPAH requires a constant infusion of PAH and multiple plasma and urine specimens. A simplified modification using a single injection technique, although less accurate, can be used. The use of CPAH as an estimate of RBF has a major limitation in young infants because the renal tubular extraction of PAH is incomplete; it is 65% in infants younger than 3 months of age and reaches adult levels only by 5 months of age (56). Thus, CPAH underestimates RBF in infants younger than 5 months of age.

Indirect Assessment of Renal Blood Flow

Radionuclide markers and radiographic techniques can be used to assess RBF. Radiopharmaceuticals used in imaging of the kidneys can provide estimates of RBF or GFR. They have gained wide use in clinical studies of both children and adults because they do not require biochemical assays. The markers are usually labeled with radioactive iodine or technetium. Because of concern over accumulation of radioactive iodine in the thyroid, noniodine radioactive tags are usually preferred in children. The major usefulness of nuclear methods is the ability to obtain "split" renal function (i.e., separate measurements for each kidney). This information is invaluable when renal function is asymmetric, such as in unilateral renal hypoplasia, scarring, obstruction, or renal vascular lesions.

Radioactive hippuran is another agent used for assessing renal function. It is excreted by glomerular filtration (20%) and tubular secretion (80%), both governed by RPF. After intravenous injection, timed images are obtained and a computer-generated time activity curve is obtained for the region of interest drawn around each kidney. Split renal function is also calculated from the renogram by computer analysis. Other markers include iothalamate (57), orthoiodophippurate (handled by the kidney in a manner similar to PAH and hence a marker of RBF), pentaacetic acid (DTPA), and dimercaptosuccinic acid (DMSA) (58, 59).

Doppler ultrasonography is a radiologic method that assesses blood velocity in the renal vessels. Although limited in its sensitivity and by the position of the vessels, this method can provide screening information regarding the patency and flow through the renal vessels and detect significant arterial stenosis (60). The resistive index (RI), often mistakenly thought of as a measurement of RBF, is a crude index of the resistance of the kidneys to blood flow. It takes into account systolic and diastolic blood flow in the renal vessels as measured by Doppler and may be helpful in the follow-up of some forms of acute renal failure.

Glomerular Filtration

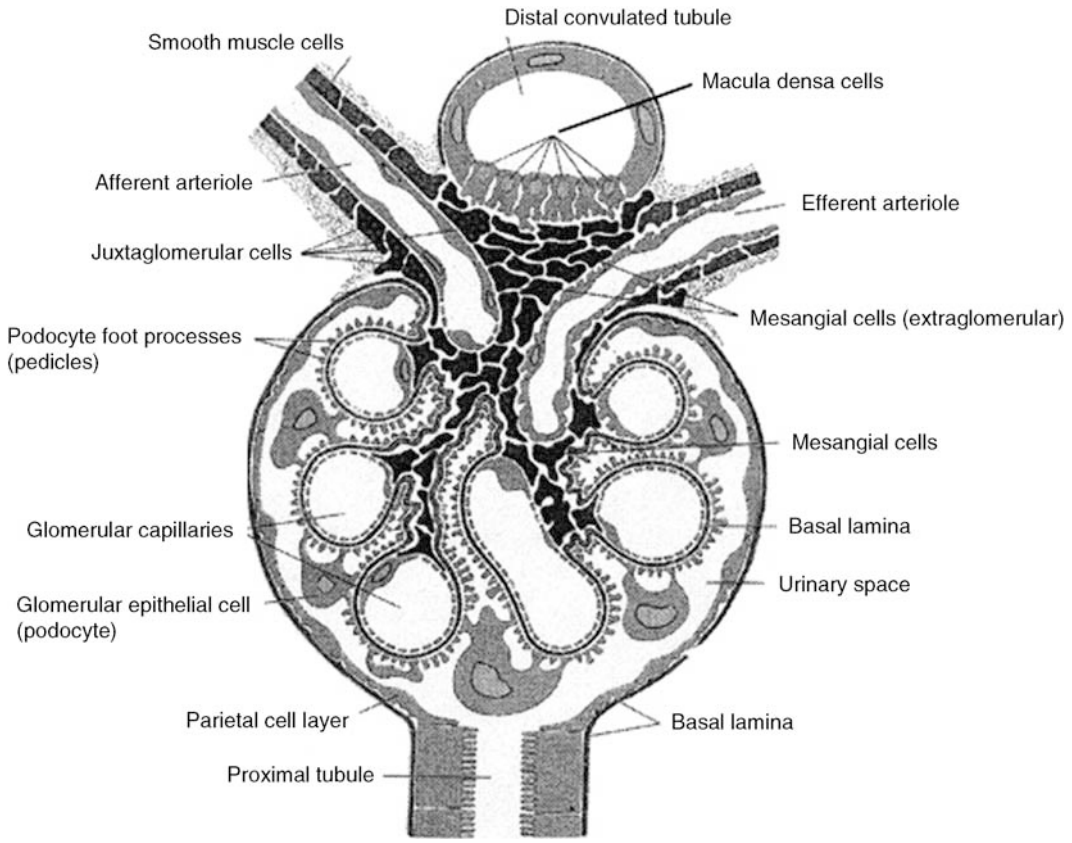
Whole kidney GFR represents filtration occurring in both kidneys and is the product of single-nephron glomerular filtration rate (SNGFR) and the number of filtering nephrons. Formation of new nephrons, nephronogenesis, occurs mainly during intrauterine life and proceeds at different rates in different species. In humans, it is complete by 36 weeks gestation (13). Nephronogenesis continues postnatally in rats until 1 week (33), in dogs until 3 weeks (61), and in guinea pigs until 6 weeks of age (62). However, regardless of the species, once nephronogenesis is complete, it is not reactivated even in the face of reduction in the functional renal mass, i.e., disease or surgical resection. Any increase in GFR after nephronogenesis, therefore, reflects increased filtration in individual residual nephrons. The degree of this compensatory increase correlates with the magnitude of the initial loss of renal mass and is more pronounced in the young (63–72). Apart from lack of new nephron formation after nephron loss, compensatory renal growth reflecting increased tubule length and interstitial expansion can start in utero. For example, in the model of unilateral obstruction in fetal lambs at 60 days, contralateral kidney weight increased by 50%, together with an increase in indices of cell proliferation (hyperplasia), however, there is no increase in glomerular number (66, 67). The increase in single nephron function that follows a loss of other nephrons early in life is greater in glomeruli in the outer cortex; however, when loss occurs later in life, the increase is more evenly distributed among all nephrons (73).

Theoretical Considerations of Glomerular Filtration

As a filtering structure, the glomerulus is essentially a tuft of capillaries, and filtration is transudation of fluid across the capillary wall into Bowman's space (*Fig. 2-2*). Two characteristics distinguish glomerular ultrafiltration

Figure 2-2

Schematic representation of a glomerulus. Blood enters the glomerular capillaries through the afferent arteriole, courses through the capillary tuft, and exits through the efferent arteriole. Filtration takes place across the capillary wall into Bowman's space. Mesangial cells are strategically located to control the filtration surface area. The juxtaglomerular apparatus, one of the sites of tubuloglomerular feedback regulation, is depicted. Terminals from the renal nerve are also shown (reprinted with permission from the artist, Dr. W. Kriz).



from transcapillary exchange in other organs: (1) The glomerular capillary wall exhibits an extraordinarily high net permeability to water and small solutes, with up to 33% of intraglomerular plasma being filtered; and (2) the glomerulus almost completely excludes plasma proteins the size of albumin and larger from its filtrate. The filtration rate is determined by the same Starling forces governing movement of fluid across other capillary walls, that is, imbalance between transcapillary hydraulic and oncotic pressure differences. These can be summarized as follows:

1. Mean glomerular transcapillary hydraulic pressure difference, $\Delta P = (P_{GC} - P_{BS})$
2. Systemic plasma colloid osmotic pressure, π_A
3. Glomerular plasma flow rate, Q_A
4. Glomerular capillary ultrafiltration coefficient, K_f

When the individual pressures are expressed as average values over the entire length of the capillary, SNGFR is given by the equation:

$$\begin{aligned} \text{SNGFR} &= k \times S \times (\Delta P - \Delta\pi) \\ &= k \times S \times P_{UF} \\ &= K_f \times (\Delta P - \Delta\pi) \\ &= K_f \times P_{UF} \end{aligned}$$

where P_{BS} is the hydraulic pressure in Bowman's space; $\Delta P - \Delta\pi$ are the mean glomerular transcapillary hydraulic and colloid osmotic pressure difference, respectively; S is the total surface area available for filtration; K_f the glomerular ultrafiltration coefficient, is the product of k and S. In this equation, average values are used for $\Delta P - \Delta\pi$ because ΔP decreases and $\Delta\pi$ increases

as the plasma flows from the beginning to the end of the capillary within a given glomerulus.

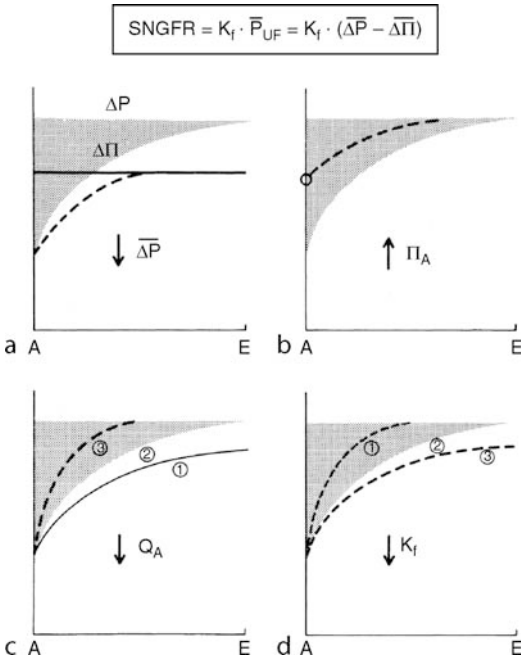
Effect of Perturbation in the Determinants of SNGFR

Mean Glomerular Transcapillary Hydraulic Pressure Difference, ΔP

Changes in ΔP seldom play a significant role in altering SNGFR because the autoregulatory mechanism in the afferent arteriole sustains glomerular capillary pressure despite large changes in systemic BP (*Fig. 2-3*). For example P_{GC} remained unchanged in Munich-Wistar rats despite a drop in renal perfusion pressure from 115 to 80 mm Hg induced by aortic constriction (8). Similarly, GFR in patients with mild to moderate hypertension is usually normal (72). As BP rises, spontaneously hypertensive rats maintain normal P_{GC} by increased afferent arteriolar resistance (73). However, when BP changes outside the autoregulatory range, both P_{GC} and GFR change accordingly. In circulatory collapse, GFR is severely reduced. Significant reductions in P_{GC} and SNGFR are noted in rats when BP falls below 80 mm Hg (8). As ΔP becomes equal to systemic plasma oncotic pressure πA, filtration diminishes to zero. Conversely, when an increase in BP is extreme, P_{GC} and filtration increase despite a marked concurrent increase in preglomerular vascular resistance. During a decrease (or increase, respectively) in renal perfusion pressure, autoregulation of P_{GC} is largely determined by the ability of the afferent arteriole to constrict/dilate and of the efferent arteriole to respond conversely. Importantly, the autoregulatory range is not fixed. For example, renal autoregulation is impaired by volume contraction. In volume-depleted animals, lowering BP leads to a reduction in P_{GC} and GFR at a relatively high renal perfusion pressure, whereas little or no change occurs in euvolemic animals (8). This effect and low circulating volume play significant roles in the reduction in GFR that accompanies hypovolemic shock. Renal autoregulation is also readjusted in severe chronic hypertension in which acute hypotensive treatment may lead to a decrease in GFR and a rise in serum creatinine. Such phenomenon in the setting of chronic hypertension might reflect a structural (i.e., not readily reversible) rather than functional narrowing of arteriolar lumen, as a consequence of long-standing elevation in renal perfusion pressure. Although this change may be beneficial in maintaining P_{GC} and GFR in the steady state, it leads to a loss of responsiveness of the renal vasculature to an acute reduction in RPP.

Figure 2-3

Schematic portrayals of the process of glomerular ultrafiltration. In each panel, the *vertical axis* represents pressure and the horizontal axis represents distance along the glomerular capillary from afferent arteriole (A) to efferent arteriole (E). The *shaded areas* represent normal mean net ultrafiltration pressure (P_{UF}), determined by the hydraulic pressure (ΔP) exceeding oncotic pressure ($\Delta\pi$). P_{UF} decreases along the length of the capillary with the increase in oncotic pressure in the capillary as colloid-free plasma is filtered. In (a), the reduced mean transcapillary hydraulic pressure difference (ΔP) is shown as a lower *horizontal line*, resulting in less ultrafiltration; (b) increased systematic colloid osmotic pressure (π_A) is represented by the *raised interrupted line*, resulting in less ultrafiltration; C, reduced glomerular plasma flow rate (Q_A); D, reduced ultrafiltration coefficient (K_f). The altered ΔP profile as a consequence of each of the above changes is given by an *interrupted curve* in each panel. Curve 1 in C and curve 3 in D represent conditions of filtration pressure disequilibrium, whereas curve 3 in C and curve 1 in D represent equilibrium. The Starling equation is also given and describes the determinants for SNGFR.



Changes in ΔP can also occur because of changes in P_{BS}. These occur with acute urinary tract obstruction and some forms of acute renal failure. Early in acute ureteral

obstruction, whether partial or complete, SNGFR is well maintained despite marked elevation in P_{BS} due to compensatory increases in both P_{GC} and Q_A. Twenty-four hours after complete ligation of a ureter, P_{BS} returns to near normal, yet GFR remains low as a result of a low Q_A secondary to vasoconstriction.

Systemic Plasma Colloid Osmotic Pressure, π_A

Derived primarily from serum proteins, π_A is a function of the number of molecules of protein present per unit volume of solution. At the same concentration, a small protein (e.g., albumin) will contribute more to oncotic pressure than a large protein (e.g., globulin). An isolated change in systemic plasma protein concentration (C_A), and hence in π_A, would theoretically be expected to change SNGFR in an opposite direction (*Fig. 2-3*). However, this does not occur. Acute reductions in C_A from 5.5 g/dl to 3.5 g/dl, induced by infusion of colloid-free solutions into rats, did not increase SNGFR because the fall in π_A and hence the rise in P_{UF} elicited a decrease in K_f through an unknown mechanism; the opposing influences of an increase in P_{UF} and a decrease in K_f maintained SNGFR nearly constant (34). In an experimental rat model of nephrotic syndrome, K_f was markedly low, accounting for the markedly low SNFGR (74). This reduction of K_f observed in the nephrotic syndrome may account for the low or normal GFR rather than higher values that would be predicted when C_A is low and circulation volume is not appreciably affected (75).

Hypoproteinemia in association with severe malnutrition is often accompanied by a decrease in GFR (76, 77). This decrease in GFR may be overlooked because serum creatinine (S_{Cr}) may not be elevated, consequent to a low muscle mass and creatinine production. GFR is reduced in protein malnutrition even when C_A is normal. The reduction in SNGFR is a result of reduced filtering surface area because glomerular size is smaller in protein-malnourished animals.

Glomerular Plasma Flow Rate, Q_A

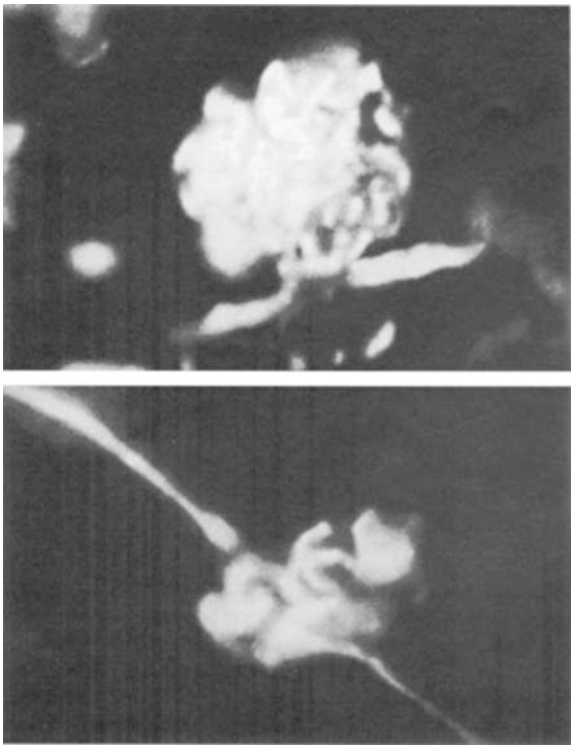
The impact of a change in Q_A on SNGFR will depend on whether the other determinants of SNGFR have been concurrently modified (*Fig. 2-3*). For example, infusion of isooncotic plasma selectively increases Q_A, while constriction of the aorta or the renal artery decreases both Q_A and ΔP (8). Under certain circumstances, filtration does not occur along the entire length of the glomerular capillary, but ceases at some point before its end. This is because plasma oncotic pressure increases progressively from the beginning to the end of the glomerular capillary. This progressive rise in oncotic pressure is accelerated when K_f is high or Q_A is low. Thus, a decrease in Q_A results in cessation of glomerular filtration at an earlier portion of the glomerular capillary tuft and hence a reduction in GFR. Experimental administration of renal vasodilators, such as PGE_1, acetylcholine, bradykinin, or histamine, causes substantial increase in renal blood and plasma flow in humans or in animals, but GFR is unaffected. SNGFR remains constant because of the opposing influences of an increase in Q_A and a decrease in K_f that occurs in response (78). The unexpected decrease in K_f could represent a direct action of these substances on the glomerular capillary, distinct from their known dilatory effects. Conversely, vasoconstrictors, such as AII and norepinephrine, are capable of producing substantial reductions in RPF, but little resultant change in GFR. Again, this is a result of a significant compensatory increase in P_{GC} as a consequence of the pressor-induced increase in efferent arteriolar resistance (79).

Glomerular Capillary Ultrafiltration Coefficient, K_f

Glomerular capillary ultrafiltration coefficient is the product of the glomerular capillary permeability to water (k) and the surface area available for filtration (s). Because changes in K_f inevitably lead to directionally similar changes in $\Delta\pi$ (*Fig. 2-3*), changes in K_f, unless extreme, are not expected to cause major changes in SNGFR. Nevertheless, a profound fall in K_f can affect GFR as demonstrated in rats with various experimental conditions. Many of these are disease models, such as minimal change nephrotic syndrome, acute renal failure, acute and chronic ECF depletion, and congestive heart failure, in which a reduction in K_f is the main factor in decreasing SNGFR (10, 80, 81). A variety of hormones and vasoactive substances, including antidiuretic hormone (ADH), adenosine, AII, endothelin, catecholamines, prostaglandins, acetylcholine, histamine, and vascular endothelial growth factor (VEGF) modulate SNGFR by influencing K_f (10, 78, 82, 83). The mesangial cells are thought to be the main locus of their actions because they appear to have a capillary surface area-regulating function. They possess intracellular contractile myofilaments, bear receptors to vasoactive agents and contract in response to these agents (*Fig. 2-4*) (84). It is speculated that hormones and

Figure 2-4

Structural expression of a reduction in the glomerular capillary ultrafiltration coefficient, K_f. The *top panel* represents the cast of a normal glomerulus; the *bottom panel* represents a glomerulus after a stimulus (renal nerve stimulation known to induce contraction of mesangial cells and a decrease in K_f) is applied. Mesangial cell contraction leads to obliteration of some of the glomerular capillaries, i.e., anatomical reduction in the surface area available for filtration, reflected as a decrease in the functional parameter K_f (from Ichikawa I, Kon V, Fed Proc 1983; 42:3078. With permission).



vasoactive substances regulate glomerular capillary filtering surface area, S, and hence GFR, by affecting mesangial contractility.

Defense of Glomerular Filtration Rate

Nonmammalian vertebrates have effective homeostatic mechanisms to alter GFR, drastically which is critically important to maintain hydration in these species (85). They can afford to alter GFR because toxic nitrogenous wastes are excreted through nonrenal organs such as gills, skin, and cloacae. In contrast, mammals, with their highly and variable fluid intake, have developed a greater capability to conserve and eliminate water from the body, largely through an expanded and highly regulated reabsorptive capacity of the renal tubules. However, because glomeruli are the only route for elimination of metabolic wastes and toxins, the GFR in mammals is remarkably constant, and high relative to other species. Mammals have developed specific mechanisms that maintain GFR stable over a wide range of blood pressure and extracellular fluid (ECF) volume, which ensures an effective removal of large amounts of nitrogenous waste that are constantly produced. The mechanisms that maintain GFR stable depend on adjustments at the glomerular loci, namely the afferent and efferent arterioles, and likely also in the glomerular capillary bed itself (*Fig. 2-5*). Two mechanisms, namely the myogenic reflex and tubuloglomerular feedback, are important for the autoregulation of GFR during changes in blood pressure. In the young, autoregulation of RBF is maintained through the same mechanisms but over a lower range of RPP that reflects the lower prevailing BP in the young (2, 24).

Myogenic Reflex

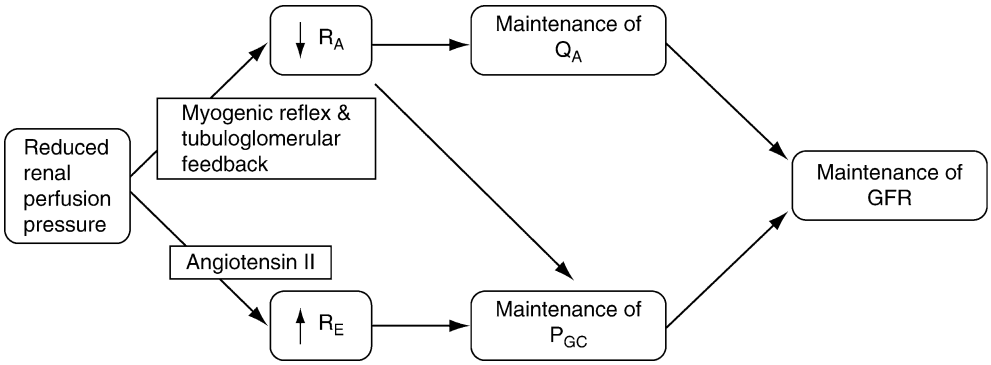
The myogenic reflex describes the theory that an increase in transmural pressure increases vascular tone. In the renal circulation this is particularly important in the afferent arteriole, which dilates in response to a decrease in RPP. This dilation also serves to preserve P_{GC}. At the same time, P_{GC} (and GFR) is also maintained in the adult animal through stimulation of renin release and the selective vasoconstrictor effect of AII on the efferent renal arteriole (10). This reflex is independent of renal nerves or macula densa mechanisms and reflects the inherent characteristics of the vessel (24, 86–88). This response has been demonstrated in isolated perfused renal vessels in which a change in vasomotor tone occurs in response to changes in the perfusion pressure in mature animals. However, a definitive role of the myogenic reflex during gestation and early life has not been defined.

Tubuloglomerular Feedback (TGF)

Constancy of GFR is also determined by the tubuloglomerular feedback system, which describes the coupling of the distal nephron flow and SNGFR. Anatomically, in each nephron, the distal tubule returns to the parent glomerulus and contributes to the formation of the macula densa, which consists of specialized cells of the ascending loop of Henle located between the afferent and efferent arterioles and the glomerulus. In this system, the stimulus to adjust SNGFR is related to the rate of distal

Figure 2-5

Mechanisms contributing to the autoregulatory maintenance of renal blood flow and glomerular filtration rates in the face of a reduction in renal perfusion pressure. R_A, afferent (and R_E, efferent) arteriolar resistance; Q_A, glomerular plasma flow rate P_{GC}, glomerular capillary hydraulic pressure; GFR, glomerular filtration rate. In the young animal, high baseline level of AII, inability to maximally activate the renin–angiotensin system on stimulation under certain circumstances, and low responsiveness of the vasculature to the constrictor action of AII may limit ability to autoregulate GFR (reprinted with permission from (10)).



tubular flow and also to the composition of the tubular fluid, particularly the chloride concentration and the tubule fluid osmolality (80, 81, 89–91). The signal is perceived in the macula densa and transmitted to the vascular structures of the nephron, particularly the afferent arteriole, but also to the efferent arteriole and the glomerular capillaries, which in concert adjust the rate of filtration. This feedback system is well suited to adjust the rate of filtration and maintain constancy of salt and water delivery to the distal nephron where tubular reabsorption is precisely regulated. Thus, an inverse relationship between filtration and tubular flow is established such that a decrease in tubular flow increases the rate of SNGFR and vice versa.

TGF is triggered by activation of the apical Na,K,2Cl cotransporter (NKCC2) in the macula densa cells by luminal NaCl (92). There are two NKCC2 isoforms. Targeted disruption of the A isoform of NKCC2 results in diminished blunting of GFR in response to increasing perfusion rate or NaCl concentrations (93). Mice with targeted disruption of the B isoform of NKCC2 have reduced TGF sensitivity at low flow rates (94). These findings indicate that the two isoforms are necessary for normal TGF response across the physiologic ranges of tubular flow and NaCl concentration. Increases in luminal NaCl have been shown to induce dramatic swelling in the macula dense cells that is accompanied by swelling and contraction of the afferent arteriole, particularly the intraglomerular segment (95, 96). This is accompanied by release of ATP into the juxtaglomerular interstitium, where enzymatic degradation to adenosine occurs. The smooth muscle cells within the afferent arterioles express receptors for ATP and adenosine and perfusion of isolated afferent arterioles with adenosine results in vasoconstriction (97). Purinergic receptor knockout mice have markedly impaired TGF as do mice lacking the A1 adenosine receptors. (98, 99) Likewise, interruption of ecto-5′-nucleotidase/CD 73 (ecto-5′-NT), which catalyzes the extracellular dephosphorylation of AMP to adenosine in the juxtaglomerular interstitium, dramatically impairs TGF (100). TGF is also impaired in mice lacking capacity for juxtaglomerular dephosphorylation of ATP/ADP to AMP (101). Regulation of the ATP to adenosine conversion, is modulatable. Thus, high ambient sodium chloride concentration increases ecto-5′-NT enzymatic activity while activity is decreased in the presence of a nitric oxide donor compound (102). Notably, provision of exogenous ecto-5′-NT improved impairment of renal autoregulation in experimental anti Thy-1 nephritis (103).

The constituents of the juxtaglomerular apparatus (macula densa tubular epithelium, mesangial cells, afferent arteriolar myocytes, and endothelium) are tightly linked by gap junctions enabling rapid intercellular communication. (104–106) The TGF-mediated increase in cytosolic calcium within the JGA mesangial cells is propagated through the gap junctions to the proximal afferent arteriole and as far as the podocytes (107). Gap junction proteins, connexin 37 and connexin 40, appear crucial in this signal transmission (108). Ang II is an important cofactor in the adenosine mediated vasoconstriction of the afferent arteriole. A1 adenosine receptor knockout mice have decreased afferent arteriolar vasoconstriction in response to Ang II (109). Conversely, TGF is impaired in experimental animals with genetic disruption of ACE or AT1A, a circumstance where the AngII signal is interrupted (110–112).

The interaction between adenosine and Ang II is complex. Repeated exposure to Ang II causes progressive reduction in vasoconstriction of isolated perfused afferent arterioles. However, adenosine pretreatment prevents diminution of this vasoconstrictive response by increasing the calcium sensitivity of the smooth muscle contractile apparatus (myosin light chain kinase) that involves post receptor interaction, particularly through G proteins (92, 113).

Recent observations suggest that connecting tubules also regulate glomerular arteriolar tone, through a connecting tubule glomerular feedback (CTGF) (114). In superficial cortical nephrons, the connecting tubule (CNT) is observed to return to the glomerulus in close proximity to the afferent arteriole. Rabbit CNT perfused with increasing sodium chloride concentrations demonstrate afferent arteriolar dilatation. The vasodilatation is blocked when CNT is infused with amiloride (but not hydrochlorothiazide), indicating that ENaC mediates this novel crosstalk between tubule segment and the glomerulus. In contrast to TGF, which functions to preserve whole organism sodium, CTGF favors sodium excretion. Identification of CTGF adds another level of understanding to aldosterone's role in sodium and potassium homeostasis, given its similar dependence on ENaC (115).

The existence of a tubuloglomerular feedback mechanism has been established in the superficial nephrons of young (30-day-old) rats (116). Its sensitivity (i.e., the change of SNGFR induced by a given change in tubule flow rate) is maximal around the values of SNGFR and tubule flow rate prevailing under normal undisturbed conditions. As SNGFR and tubule flow rates increase with growth, adjustments in the TGF mechanism take place to maintain this relationship, and the relative sensitivity of the system remains unaltered.

As noted earlier, afferent arteriolar dilation and RBF adjust to decreasing renal perfusion pressure in both adults and immature animals. However, one study found that although decreasing the renal perfusion pressure by ~ 30% from baseline was accompanied by a minimal fall in the GFR in adult rats, in young rats, GFR plummeted by more than 80% (117). Micropuncture experiments revealed that the profound hypofiltration in the young rats reflected decreased glomerular capillary pressure. Because glomerular capillary pressure, in large part, reflects efferent arteriolar vasoconstriction maintained by AII, the autoregulatory decompensation observed in the young animals likely reflects incompetence in the AII-mediated vasoconstriction of the efferent vessels. In this connection a similar degree of water deprivation causes a greater increase in the plasma renin activity in adult animals than in immature animals and a higher dose of AII is required in immature than in adult animals to effect a similar increase in glomerular capillary pressure (117). Taken together, it appears that the young have limited ability to activate AII and that the immature efferent arteriole has a limited responsiveness to AII. Thus, even in the face of afferent vasodilation following decreasing renal perfusion pressure, young animals develop hypofiltration. These observations provide a mechanism for dissociation between renal blood flow and the GFR in that dilation in the afferent arteriole without sufficient vasoconstriction in the efferent arteriole is insufficient to maintain a transcapillary pressure that promotes glomerular hypofiltration in the young (*Fig. 2-5*).

As noted above, of the two currently recognized receptors for AII, AT1 and AT2, the AT1 is most abundantly expressed and transduces the bulk of the recognized actions of AII including efferent arteriolar constriction (3, 118). Glomerular AII hyporesponsiveness in the neonatal kidney does not appear to reflect inadequate AT1 receptor density, as kidney AT1 expression peaks postnatally at twice the adult level (46). Further, as noted above, AII availability is also maximized reflecting an abundance of renal angiotensinogen, renin, angiotensin converting enzyme (ACE) (42–44). The observed hyporesponsiveness of the neonatal kidney, therefore, appears to reflect inadequate postnatal maturation of postreceptor processes. It is possible, however, that the blunted vasoconstriction of the neonatal efferent arteriole in response to AII reflects the vasodilatory contribution of the AT2 receptor, which is abundant during development but wanes with maturation. This may occur though a direct effect of the AT2 receptor or though AT2-mediated stimulation of NO and bradykinin (54).

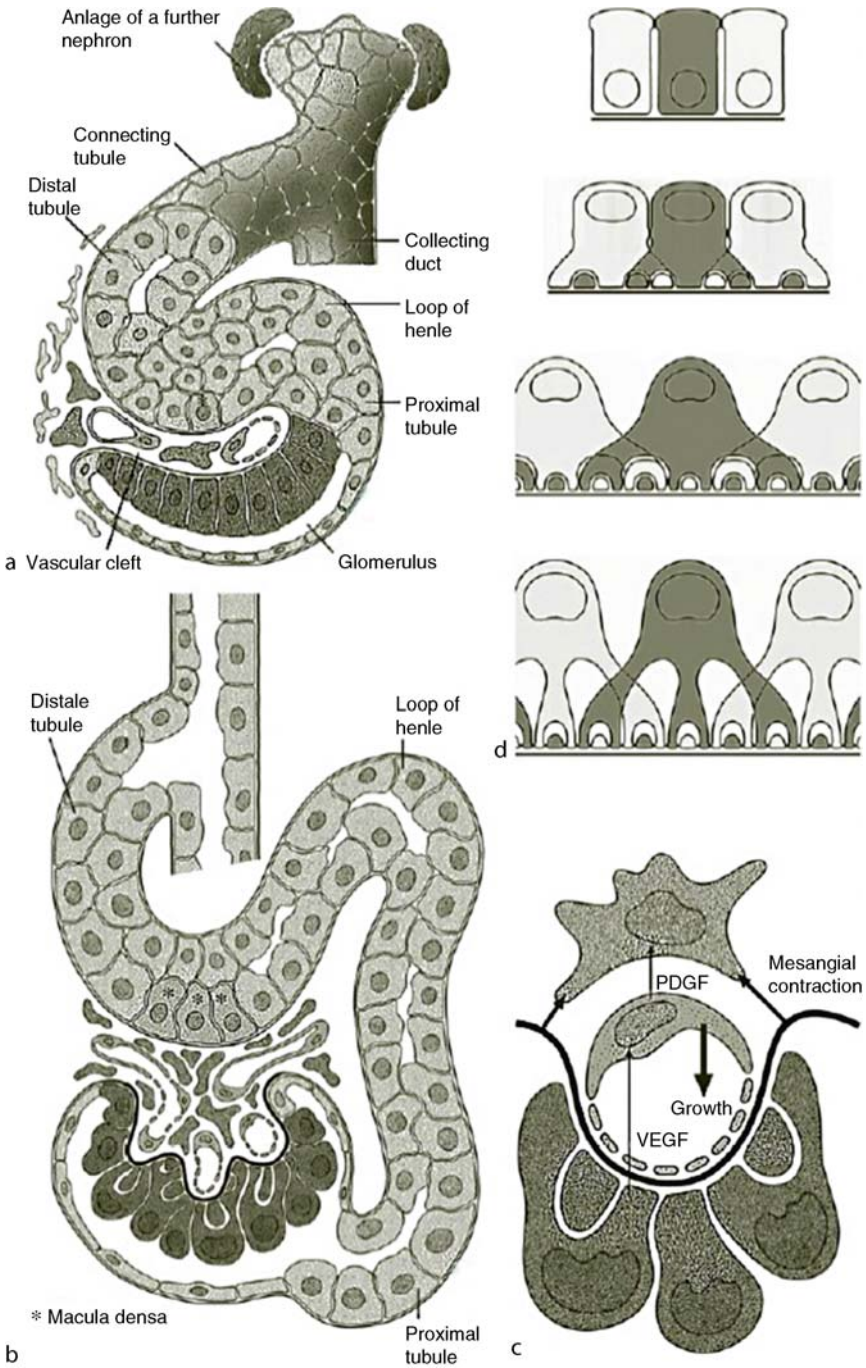
Development of Glomerular Filtration Rate

Ontogenic Development of Glomerular Capillaries

Glomerulogenesis in humans begins at 5 weeks gestation with reciprocal induction of the ureteral bud and nearby metanephric blastema (condensing mesenchyme) (119). At the point of contact between these primordial structures, epithelial cells are progressively generated as the renal tubule precursor, and eventually as the glomerular epithelial cell layers whose earliest precursor is the comma shaped body (120). A further indention of the comma shaped body creates a distal vascular cleft, defining the glomerular precursor as an S shaped body (*Fig. 2-6*). The proportion of developing nephrons in the S shaped body stage peaks in the second trimester

Figure 2-6

Stages in the development of a glomerulus and the filtration barrier. (a) The S-shaped body stage after formation of a distal vascular cleft. The cleft separates the presumptive podocytes (distally) from the tubule cell precursors of macula densa cells (proximally). In the cleft are infiltrating endothelial and mesangial cells. (b) The capillary loop stage with early formation of a glomerular tuft. (c) The podocyte – endothelial cell – mesangial cell paracrine axis. (d) Consecutive stages of podocyte development, finishing as complex cells with interdigitating foot processes connected by a slit membrane. (Reprinted with permission from (120)).



of human gestation (121). On the distal surface of this distal vascular cleft, podocytes develop under the influence of WT1 (122).

Under the influence of podocyte-derived vascular endothelial growth factor A (VEGF-A), endothelial cells migrate into the distal cleft of the S shaped body, interposing between podocytes and macula densa cells (120, 123). The developing glomeruli are not invaded by intact capillary loops. Instead, the migrating and proliferating endothelial cells form capillary cords that undergo central apoptosis to form a lumen, a process regulated by TGF beta (124). A critical role of podocyte VEGF-A generation to glomerular ontogeny has been highlighted by studies of podocyte haploinsufficiency of the VEGF gene (125). Thus, a single VEGF-A allele results in abnormally swollen glomerular endothelial cells and proteinuria. Glomeruli in which podocytes have but a single hypofunctional VEGF A allele show complete disappearance of endothelial cells a few days after glomerular maturation, with the affected animals dying at 3 weeks of age. Absence of a functional podocyte VEGF-A allele results in small glomeruli with no endothelial cells and no filtration barrier. Taken together, these observations underscore the importance of VEGF in development as well as maintenance of the normal endothelial cell integrity.

Endothelial cells, recruited and maintained by podocyte-derived VEGF A, in turn release PDFG (126). PDGF, in turn, induces migration of mesangial cells from nearby mesenchyme into the vascular distal cleft where they proliferate alongside the developing endothelial cell capillary (127). Infiltration by the mesangial cells has the effect to split the capillary in loops that results in a glomerular capillary tuft with multiple parallel branches. PDGFR-beta receptor gene null mice have glomeruli with a single balloon-like capillary loop that is devoid of mesangial cells (128). Similarly, impairment of mesangial cell anchoring to the basement membrane surrounding the endothelial capillary, as seen in mice with mutation in laminin alpha 5, leads to reduced capillary loop formation (129, 130).

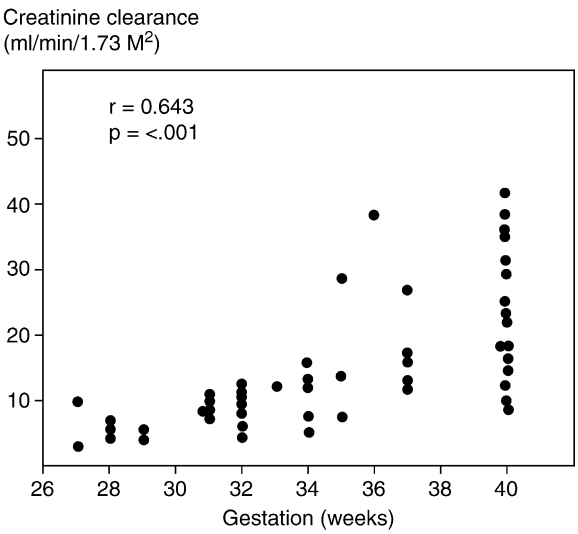
A variety of factors appear important for podocyte survival and differentiation in the S shaped body and later stages. WT-1 is essential (122, 131–133). Thus, WT-1 expression peaks in podocyte precursors where it regulates expression of podocyte membrane protein podocalyxin as well as VEGF-A, which are central to normal glomerular development. Interruption in the signaling of bone morphogenic proteins (BMP)-2, -4, and -7 results in abnormal localization of podocytes, such that parietal epithelial cells have podocyte markers, express VEGF, and have even been observed to give rise to ectopic glomerular capillaries (134). Mesangial cell density in these mutants is also dramatically decreased, resulting in collapsed glomerular tufts. That dosage of BMP in normal glomerular development is tightly controlled is evidenced by observations that transgenic mice selectively overexpressing BMP4 in podocytes have decreased podocyte VEGF together with inadequate numbers of endothelial cells and mesangial cells. Notably, nephrin, thought to be the sine qua non protein of the mature podocyte and essential to formation of the slit diaphragm between podocyte foot processes, does not appear to be essential to podocyte development or viability (135).

Prenatal GFR

Glomerular filtration rate in the fetus correlates with gestational age and body weight and parallels the increase in renal mass (136, 137). However, even corrected for body weight, prenatal GFR at every stage of development is much lower than in adults. For example, creatinine clearance measured within 24–40 h of birth in 30-week and younger premature infants is less than 10 ml/min/1.73 m^2 body surface area; at 34 weeks it is <15 ml/min/1.73 m^2; while at 40 weeks, ranges between 10–40 ml/min/1.73 m^2 (*Fig. 2-7*) (137, 138). Direct measurement of intrauterine glomerular function is obviously limited and creatinine is not an ideal indicator of fetal renal function because it freely crosses the placenta such that the fetal level actually reflects maternal levels. Endogenous low molecular weight proteins such as Cystatin C and b2-microglobulin have been

Figure 2-7

Creatinine clearance measured within 24–40 h of birth in premature and full term infants (reprinted with permission from (138)).



shown to be useful in assessing renal function of adults, children, and infants and have been used to assess prenatal renal function, see below (139, 140). Cordocenteses measurements of Cystatin C and β2-microglobulin have generated reference values in fetuses with normal amniotic fluid volume, normal chromosomes and absence of sonographic evidence of renal/extrarenal abnormalities as well as fetuses with abnormalities in these parameters and/or postnatal evidence of renal dysfunction (139).

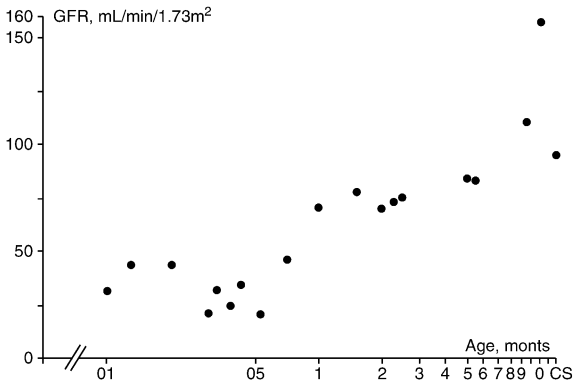
Postnatal GFR

At birth, the placental function of regulating fetal homeostasis becomes shifted to the kidneys. Compared to the adult, the GFR of a term newborn baby is less than 10% of the adult level whether expressed per gram of kidney weight, body weight or surface area and correlates closely with the gestational age (*Figs. 2-7*, *2-8*, *2-9*) (137, 138, 141–145). However, during the first 2 weeks of life, the GFR doubles and continues to increase, reaching adult levels by 2 years of age (146). This increase lags in premature babies (142, 144–146). As with RBF, development of GFR proceeds centrifugally within the kidney and the maturational increase in whole kidney GFR reflects primarily an increase in the single nephron GFR within superficial nephrons and less in the juxtamedullary nephrons (33, 147, 148).

All four determinants of SNGFR, ΔP, π_A, Q_A, and K_f contribute to the maturational increase in GFR to varying degrees. In early stages, the systemic blood pressure in humans averages 40 to 70 mmHg, which is below the autoregulatory range (148, 149) and likely contributes to a low P_{GC} and ΔP that has been shown in immature animals (36). Indeed, the blood pressure in babies born at 28–43 weeks of gestation predicted their creatinine clearance (33, 38). Plasma protein concentration, therefore the resultant π_A, is lower in newborns than in older children (5–6 vs. 6–8 g/dl) and is a factor that would increase ultrafiltration. However, the maturational increase in π_A that would hinder ultrafiltration is offset by a more profound increase in P_{GC} having the net effect on P_{UF} to promote ultrafiltration. Experimental studies during later postnatal maturation indicate that π_A and P_{GC} are at adult levels and remain constant (36). The further increase in SNGFR is attributable to an increasing plasma flow rate, Q_A that reflects an increasing caliber of afferent and efferent arterioles and decreasing resistances in these arterioles. Experimental and human observations support the parallel increase in plasma flow and GFR. Thus, increasing circulating blood volume by delayed clamping of the umbilical cord or intravenous fluid infusion increases inulin clearance (150, 151). Finally, rising hydraulic conductivity as well as surface area of the glomerular capillaries likely contribute to maturational increase in the capillary ultrafiltration coefficient, K_f. Glomerular size, glomerular capillary basement membrane surface area, and capillary permeability to macromolecules all increase from neonatal period to adulthood (152, 153). However, neither human nor animal data can provide the precise contribution of such changes to the increasing GFR.

Figure 2-8

GFR during the first year of life (reprinted with permission from (143)).

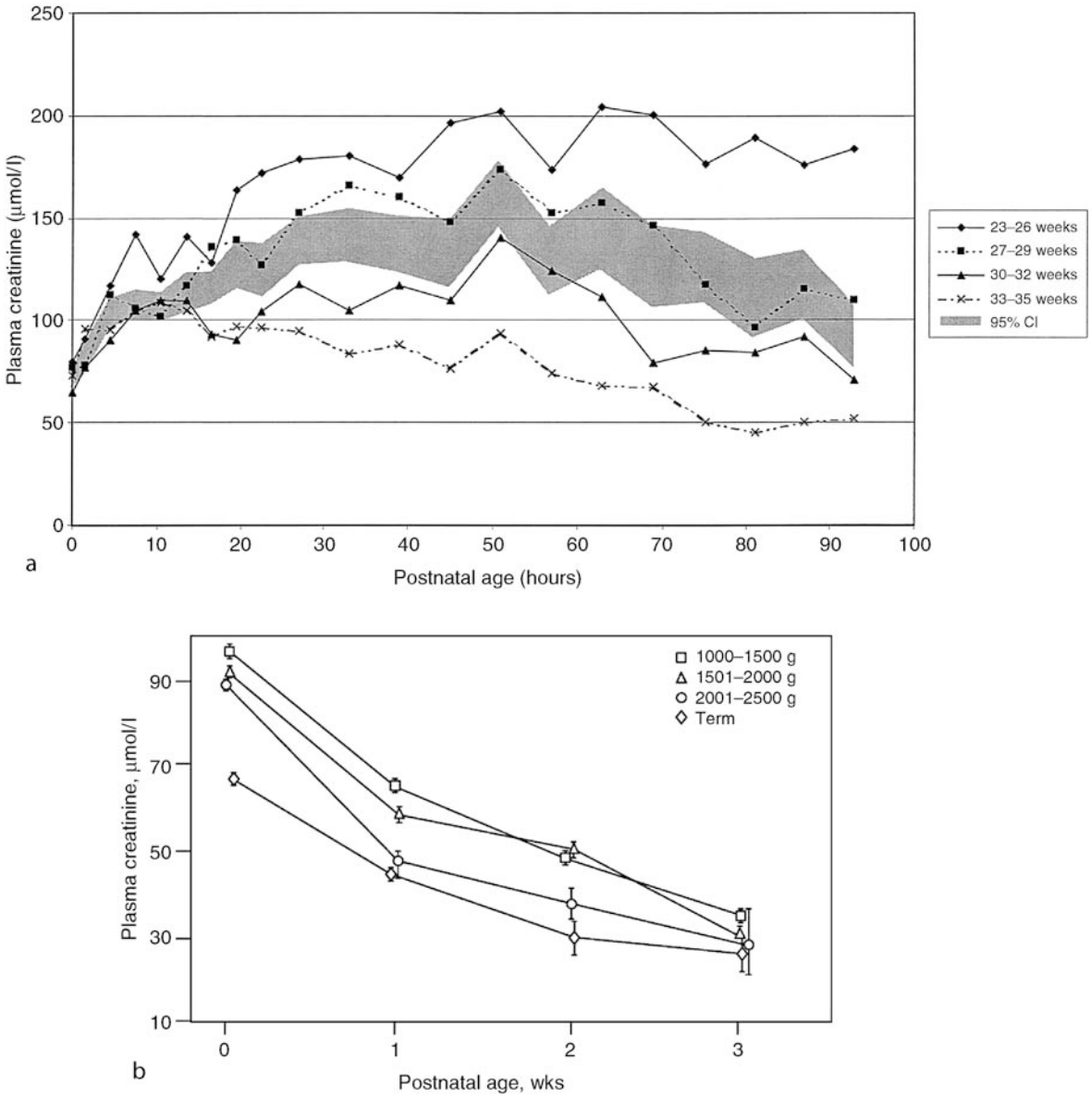


Impaired Fetal Glomerular Development and Adult Disease

As noted above, total GFR is the product of single nephron GFR and the number of filtering nephrons. Thus, failure to attain sufficient nephrons during glomerulogenesis may negatively impact the whole kidney GFR at birth. The number of glomeruli in healthy humans has been estimated at ~1 million in each kidney (154, 155). Recent observations, however, indicate considerable interindividual variability in the final number of nephrons, which is impacted by an assortment of prenatal factors. For example, low birth weight, especially fetal growth retardation, protein malnutrition, vitamin A deficiency, drugs such as aminoglycosides, cyclosporine A and glucocorticoids as well as metabolic disorders such as maternal hyperglycemia have all been shown to cause a significant nephron deficit in the fetus (155–162). In addition, recent evidence suggests that a reduced number of nephron units leads to adverse cardiovascular and renal consequences in adulthood. Thus, individuals who have even a modest decrease in the number of nephrons are at an increased risk of developing hypertension, cardiovascular disease and progressive chronic renal dysfunction (154, 163).

Figure 2-9

(a) Plasma creatinine values during the first 4 days of life in preterm infants. The shaded area represents 95% CIs for the mean plasma creatinine of all infants (reprinted with permission from (144)). (b) Plasma creatinine values during the first 3 weeks of life in preterm infants. The plasma creatinine inversely correlates with body weight and gestational age in the first weeks of life (reprinted with permission from (145)).



Low Birth Weight

Assessments of nephron number in autopsies of intrauterine growth retarded infants have observed significant decrease in the nephron complement compared to normal weight control infants (156). A study on autopsy kidneys from all ages revealed a direct correlation of nephron number with birth weight (164). Indeed, among patients younger than 18 years (who have minimal aging-related nephron loss), regression analysis estimated a gain of 518,000 nephrons for every kilogram increase in body weight. The ramifications of decreased nephron mass become detectable even by the time a child reaches school age. Thus, youngsters who were growth retarded as newborns

were found to have higher blood pressures, though not overt hypertension (165, 166). Adults with hypertension have been found to have fewer nephrons compared to nonhypertensive controls (167–169). Hypertensive patients also have larger glomeruli, more than twice the size of nonhypertensive controls, a finding that is indicative of glomerular environment at heightened risk for sclerosis (170). A large cohort of former premature infants in the Netherlands assessed at age 19 years showed a correlation between lower birthweight and lower GFR in adulthood, though all were still in the normal range. Attesting to the presence of incipient renal disease in these individuals was the finding of a more than twofold increase in microalbuminuria in those who were formerly small for gestational age. (171). Among a very large cohort in Norway, those with birth weight less than 2 standard deviations below the mean had an odds ratio of 2.4 (95%CI 1.46–3.94) for GFR $<$ 92 ml/min/1.73 m^2 in men aged 20–30. In women of similar birth weight, the odds ratio of GFR $<$ 86 ml/min/1.73 m^2 was 2.0 (95% CI 1.21–3.29) (172). The more robust association of deleterious renal consequences of low birth weight in men in this Norwegian study is reiterated by the recent observation of increased chronic kidney disease in American men (but not women) with birthweight $<$ 2,500 g. (173). It is worth noting that serum creatinine may be insufficiently sensitive to detect small differences in GFR attributable to decreased nephron mass early in life. In this regard, a study of children at age 8–13 years found no correlation between GFR (estimated by serum creatinine) and birth weight whereas the more accessible assessment of systolic blood pressure documented an inverse relationship with birth weight. Interestingly, GFR measurement, estimated by serum Cystatin C, was significantly lower in the lower quartiles of birthweight even by the age 8–13 years, with an estimated GFR of 73–92 ml/min/1.73 m^2 for those born weighing less than 2,500 g compared to GFR 89–109 for those born weighing greater than 3,000 g (174). (See discussion of clinical assessment of GFR below).

Extreme Prematurity

Nephronogenesis continues through postconceptional age of 36 weeks. Previously, it was believed that nephronogenesis continued unimpeded in infants born before this gestation. However, recent findings reveal a significantly reduced glomerular count among premature infants who lived past the end of nephronogenesis (175) Thus, the radial glomerular count (RGC) of whole kidneys in infants born at 27 weeks of gestation obtained at autopsy examination at age 63 weeks post conception was 8 compared to RGC of 6 in infants at 25 week of gestation dying within a week. These findings confirm that there is postnatal nephronogenesis in preterm infants. However, RGC of 8 already represents impairment of nephronogenesis when compared to term infants in whom RGC was 10.4, suggesting that the extrauterine environment for ill premature infants is hostile to nephron development. Among infants with even transient elevations in serum creatinine, the RGC was further reduced, to 6.5, indicating the marked deleterious consequences of renal impairment during nephronogenesis (175).

Maternal Factors

A variety of maternal factors can negatively impact nephronogenesis in the infant. These include maternal malnutrition, placental insufficiency, Vitamin A deficiency, corticosteroid exposure, and maternal hyperglycemia. Maternal malnutrition and placental insufficiency are two major factors that contribute to programming a subsequent increase in risk of adult cardiorenal diseases (176). Notably, improvement in a deficient intrauterine environment even late in nephronogenesis can benefit the ultimate nephron number. Thus, placental restriction in rat pups (through uterine vessel ligation) that is followed by postnatal fostering by inadequately lactating mothers caused a nephron deficit and hypertension at 20 weeks of age (177). By contrast, similarly placentally-restricted rat pups nursed by mothers with intact lactation from postnatal day 1 to weaning showed improved nephron number (~25% higher) and no adult hypertension. These improvements likely reflected restored nephronogenesis during the last stages, which in the rat, continues for the first week of postnatal life. (177).

The effect of protein malnutrition on nephron number appears to be mediated, at least in part, by an increase in apoptosis of mesenchymal cells of the very early metanephros and through reduced expression of periureteric genes, prox-1 and cofilin-1. (178, 179). Retinoic acid, derived from dietary vitamin A, regulates the c-Ret receptor for GDNF, a crucial mediator for ureteric bud branching. (155, 157, 168). Moreover, recent evidence suggests that retinoic acid supplementation may improve nephron endowment in rats exposed to low protein diet in utero (180). Dexamethasone exposure of cultured metanephroi down regulated GDNF and upregulated BMP-4 culminating in impaired renal branching morphogenesis. (181). Even near physiologic levels of natural corticosterone administered to pregnant rats during gestation has

resulted in nephron deficit, pointing to potential deleterious developmental effects of maternal stress during gestation (182). Lastly, experimental induction of diabetes in pregnant mice resulted in smaller, less numerous glomeruli in the offspring. Further studies reveal that hyperglycemia in these mice results in apoptosis of developing glomerular podocytes and tubular cells in association with dramatic upregulation of NF-κB as well as the renal renin angiotensin system (which also exhibits ectopic expression) (183).

Clinical Assessment of Glomerular Filtration Rate

Inulin Clearance

Assessment of glomerular filtration rate is the single most important measurement of renal function. Substances reaching the kidney may undergo one of several processes including glomerular filtration, tubule reabsorption, tubule secretion, and intrarenal metabolism. These considerations necessitated the search for an "ideal GFR marker." Among various substances considered, inulin emerged and has remained the standard against which all other techniques of measuring GFR are compared to validate their accuracy (184, 185). Inulin is a polymer of fructose, containing, on average, 32 fructose residues and has a molecular weight of about 5,700 Da. Natural inulin is derived from plant tubers such as dahlias, chicory, and Jerusalem artichokes. Although the molecular configuration of inulin varies depending on the source, the Stokes-Einstein radius that affects filtration is constant at about 5.0 nm. Inulin fulfills the following criteria:

1. It is freely and completely filterable at the glomerulus.
2. It is neither secreted nor reabsorbed by tubules.
3. It is neither metabolized nor synthesized by the kidney.
4. It is not bound to plasma proteins, or if it is, the free unbound as well as the bound components can be measured separately.
5. It is physiologically inert.

Because of these characteristics of inulin, the rate of inulin filtered into Bowman's space equals the urinary excretion of inulin. Moreover, inulin concentration in Bowman's space equals that of plasma. Thus, the flow rate of the fluid filtered into Bowman's space: GFR = C_{inulin} = U_{inulin} × V/P_{inulin}. For example, if P_{inulin} = 0.5 mg/ml, U_{inulin} = 50 mg/ml, and V = 1.1 ml/min, then GFR = C_{inulin} = 110 ml/min. Although this is a straightforward relationship, there are several points worth emphasizing. It is plasma, not urine that is being cleared of inulin. In the above example, all inulin is removed from 110 ml of plasma each minute. The inulin clearance is independent of the rate of urinary flow rate. Thus, the concentration of inulin in the urine increases as the volume decreases and vice versa at a given GFR. The inulin clearance is also independent of the concentration of inulin in the plasma; thus, as plasma inulin concentration increases, its appearance in the urine increases as more is filtered. Although inulin clearance remains the most accurate method of assessing GFR, it is cumbersome in routine clinical settings. The drawbacks include difficulty in obtaining inulin, the preparation of inulin and requirement for continuous intravenous infusion to maintain constancy in its plasma concentration. Moreover, measurements of inulin levels are not routinely available in hospital clinical laboratories. These drawbacks have led to the development of other methods to estimate GFR.

Modifications of the Standard Clearance Method

Because of the difficulty in maintaining intravenous access and obtaining urine samples in newborn infants and young children, various modifications of the standard clearance tests have been used to yield indirect assessments of GFR. One earlier modification used an intravenous infusion of a GFR marker, without urine collection (186). Because at steady state the amount of marker infused per unit time (I) equals the amount excreted in the urine, as well as the amount filtered,

$$U_x \times V = I$$

$$= GFR \times P_x,$$

hence

$$GFR = I/Px$$

Thus, only blood sampling is required, together with accurate knowledge of infusion parameters (infusion rate and marker concentration), and certainty that a steady state has been attained. One such marker used for clearance determination is either unlabeled or ^{125}I-iothalamate infused subcutaneously via a portable minipump (187). After steady state (8–24 h), the marker clearance can be calculated. Significant tubular secretion of iothalamate, however, has curtailed its use as a marker of GFR (188).

Clearance of a marker has also been measured by a single injection of that marker, followed by analysis

of its disappearance rate, as assessed from repeated plasma samples. This method bypasses the necessity of both continuous intravenous infusion and urine collection. Radiolabeled markers such as ^{51}Cr-EDTA (147), ^{125}I-iothalamate (57), and ^{99m}-Tc-DTPA have all been used for GFR measurement after single injection. Beyond radioactivity, use of these agents has been limited by availability, tubular excretion and overestimation of GFR, and variability among commercial sources, respectively (188). Renewed interest in ioxehol as a GFR marker has been stimulated by need for precise but manageable GFR measurement for a multicenter study of chronic kidney disease (CKD) in pediatric patients (189). Iohexol is a nonionic, low osmolarity contrast agent (Omnipaque™) with a molecular weight of 821 Da, long-used as a marker for GFR in Scandinavia (190–192). It fulfills several criteria of an ideal GFR marker, in that it is cleared only by glomerular filtration without being absorbed, secreted, or metabolized by the kidney (189–193). Additionally, iohexol has negligible extrarenal clearance (even with reduced renal function), is <2% protein bound, and is readily measurable by high performance liquid chromatography (HPLC)/ mass spectrometry. Importantly, iohexol clearance after single injection shows excellent agreement with traditional inulin clearance (193). Schwartz and coworkers have demonstrated in children with Chronic Kidney Disease that as few as two venous blood samplings 120 min and 300 min after iohexol injection are sufficient for GFR determination (189). Moreover, accurate GFR has been obtained after iohexol injection with HPLC/mass spectrometry measurement of finger prick blood spots collected on filter paper, raising the possibility of reduced clinic time for patients (194). Formal clearance studies remain costly and are still reserved for selected cases when accurate estimation of renal function is necessary.

Creatinine as a Marker of GFR

In clinical practice, GFR is most often estimated from measurements of serum creatinine concentrations or the clearance of endogenously produced creatinine. These require only collections of blood and/or urine samples. Most commonly, renal function is estimated simply by obtaining a serum creatinine measurement as the initial screening assessment, and in monitoring the increase as an estimate of the rate of deterioration of renal function. In the steady state, U_{creat} V (creatinine excretion rate) equals creatinine production rate which is constant. GFR is inversely proportional to plasma creatinine concentration because GFR = (U_{creat} V)/P_{creat}. Thus, an increase in plasma creatinine from 1 to 2 mg/dl or from 4 to 8 mg/dl represents a functional loss of 50% of GFR between measurements, although the absolute decline in function is less in the latter as is the fractional residual function. The utility of serum creatinine measurement stems from the relatively constant production of daily creatinine, and is independent of diet, protein catabolism, and physical activity. Creatinine production is a function of muscle mass. Approximately 1 g creatinine is derived from 20 kg muscle mass in 1 day (i.e., 50 mg/kg muscle) (195). Thus, if GFR completely ceases, e.g., acute renal failure, the plasma creatinine concentration is expected to increase by approximately 1.5 mg/dl/24 h. In individuals of average proportions, creatinine production is 15–20 mg/kg per day in boys and 10–15 mg/kg per day in girls and infants. This is the amount that is expected to be found in a complete 24-h urine collection, although more recent data indicate somewhat higher levels of urinary creatinine excretion. Thus, females < 10 years excreted 15.8 mg/kg/day urinary creatinine (SD 3.1), whereas boys of the same age excreted 17.7 mg/kg/day (SD 4.1). Females aged 10–14 and 14–21 years had urinary creatinine excretion of 17.8 (SD 2.9) and 17.1 (SD 4.7) mg/kg/day, respectively. Males aged 10–14 and 14–21,with the impact of increasing muscle mass, had urinary creatinine excretion of 18.4 (SD 3.4) and 22.3 (SD 5.4) mg/kg/day, respectively (196). Notably, even with precise clinic urine collection techniques, daily creatinine excretion variability may be as high as 20% (197). Nevertheless, the above ranges are helpful in detecting incomplete 24 h urine collections. It is also notable that dietary supplementation with creatine (a myocyte component taken by athletes for improved muscle function) can lead to elevated serum creatinine. Such creatine supplementation can lead to the erroneous diagnosis of decreased kidney function based on serum creatinine level (198).

Creatinine clearance (C_{Cr}) is a well accepted estimate of GFR (as measured by C_{In}) in patients who are older than 1 month of age (199) who have normal or have only moderately decreased renal function. However, in some settings, C_{Cr} is an unreliable estimate of GFR. C_{Cr} overestimates GFR with severely decreased renal function; when GFR falls below 20 ml/min per 1.73 m^2, C_{Cr} overestimates Cin by approximately 20%. C_{Cr} has been shown to overestimate C_{In} in adults with nephrotic syndrome (200) and in kidney transplant donors and recipients (201, 202). Both C_{Cr} and C_{In} underestimate GFR in some forms of acute renal failure in which the functional integrity of the renal tubule is disrupted. Under these conditions, filtered creatinine and inulin can be reabsorbed by passive back diffusion. In acute tubular

necrosis following cardiac surgery, 50% of filtered inulin undergoes tubular backleak (203). Thus, measurements of GFR in the setting of acute renal failure with tubule injury are invalid for assessing glomerular filtration rate. Nevertheless, in practice, changes in S_{Cr} have sufficient accuracy to follow the course of patients with acute renal failure.

Values for S_{Cr} in human newborns approximate the maternal S_{Cr} level, reflecting fetal–maternal equilibration of creatinine during the second half of gestation and dependence on placental/maternal clearance (204). Over the first few weeks of life, S_{Cr} decreases, and represents GFR maturation, which eliminates both the maternal creatinine and the newborn's endogenous creatinine production (*Fig. 2-9*). However, in preterm infants, a significant increase in S_{Cr} has been noted in the first few days of life, most pronounced in the most premature (144, 205) (*Fig. 2-9a*). The rise in creatinine in these infants has been attributed to delayed establishment of glomerular filtration, possible tubular resorption, or passive back diffusion of creatinine by the immature renal tubule. In this regard, although mature rabbits have creatinine clearance that exceeds inulin clearance ($C_{Cr}/C_{In} = 1.21$), reflective of tubular secretion of creatinine, newborn rabbits have creatinine clearance that falls short of inulin clearance (C_{Cr}/C_{In} 0.84), pointing to creatinine back diffusion(206). Low-birth weight infants have daily urinary creatinine excretion rates during the first 2 weeks of life that correlate with birth weight, gestational age, and body length (207). Serum creatinine concentration in normal infants and children increases with age and is slightly higher at any age in males than in females (*Fig. 2-10*) (208).

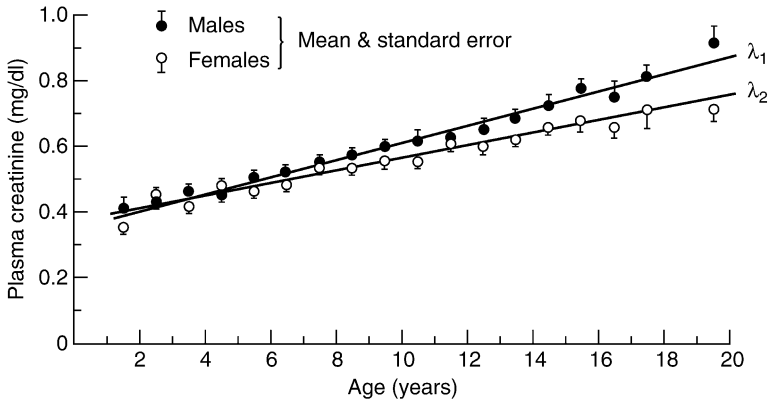
Estimation of GFR by C_{Cr} entails obtaining an accurately timed urine collection over a long period and is thus impossible in children who are not toilet trained (without the use of an indwelling urinary catheter) and remains a challenging exercise for most children. To ease GFR determination, investigators have attempted to derive formulas to estimate creatinine clearance from serum creatinine level, in conjunction with anthropometric measurements such as height, weight, or BSA. The following formula, derived by Schwartz and others, yields values of GFR that correlate with those obtained from C_{Cr} and C_{in} (209):

$$GFR = \delta L/S_{Cr}$$

where GFR is expressed in ml/min per 1.73 m^2; L represents body length in centimeters; S_{Cr} is serum creatinine in milligrams per deciliter; and δ, a constant of proportionality is age and sex dependent. Since the emergence of the Schwartz formula, the prevailing method of creatinine measurement has changed from the Jaffe reaction to newer enzymatic methods, which produce lower serum creatinine levels (210, 211). For this reason, altered δ values have been proposed. Thus, rather than $\delta = 0.55$ for most children and adolescent females, $\delta = 0.47$ has been suggested (188, 212). This need for systematic alteration in δ in light of changes in creatinine measurement is in accordance with significant overestimation of GFR by the Schwartz formula noted in recent studies (189, 213). Equations have been developed from the Modification of Diet in Renal Disease (MDRD) study which more accurately estimate GFR in adults (214). The equations take into account the individual's serum creatinine, blood urea

Figure 2-10

Mean plasma creatinine concentration (mg/dL) plotted against age for both sexes. The regression equations are for males: y = 0.35 + 0.025 × age, and for females: y = 0.37 + 0.018 × age (reprinted with permission from (208)).



nitrogen, serum albumin, age, gender, race and body size. In children, however, the MDRD equation significantly overestimates GFR. (213). Cockcroft-Gault, an equation developed in adults to estimate GFR, has been considered unsuitable for children, though some recent studies indicate its closer approximation in children to C_{In} than the Schwartz formula. (213, 215). Although these formulae are useful in day-to-day management, a more precise measurement of GFR should be obtained whenever a high accuracy is required.

Cystatin C as a Marker of GFR

While inulin clearance remains the gold standard and serum creatinine concentration and creatinine clearance are currently most widely used to estimate GFR, Cystatin C is another endogenous marker which may improve upon limitations of the creatinine-based estimates of GFR. (216, 217) Cystatin C may be particularly useful in detecting changes in GFR between 60 and 90 ml/min/1.73 m^2, the "creatinine-blind range" of GFR, where significant decline in GFR may occur without change in serum creatinine (218, 219). Cystatin C is a 13 kDa protease inhibitor that is constitutively synthesized by all nucleated cells and is freely filtered through the glomerulus and completely reabsorbed and catabolized by tubular cells, so that none returns to the blood flow (217, 218, 220). There are two commercially available methods for measuring Cystatin C in serum: immuno-nephelopmetric and immuno-turbidimetric assays, though availability is still restricted to reference laboratories and associated with high turn around time in clinical practice (218). Nephelometric Cystatin C has shown greater correlation with measured GFR and there remains disagreement about the interchangeability of Cystatin C levels from various methodologies (216, 221, 222).

A Cystatin C reference range has been established for adults (223–225). A pediatric reference range for Cystatin C together with levels of serum creatinine was published based on measurements in 291 children aged 1 day to 17 years, including preterm infants (*Fig. 2-11*) (225). These data reiterate the high creatinine values at birth and during the first week of life (see above) and the fall that occurs over the first month. Creatinine levels then remain constant until 2 years of age when they rise to adolescent values. By contrast, Cystatin C values more closely parallel functional clearance studies. Preterm babies have the highest levels of Cystatin C followed by infants < 1 year reflecting kidney immaturity. By 1 year of age Cystatin C levels approximate those in adults. Recent studies also find that Cystatin C may be a more accurate serum marker than creatinine in individuals with impaired renal function (226, 227). Notably, fetal serum Cystatin C levels do not show any relationship to maternal serum Cystatin C (228). For this reason, fetal serum concentrations of Cystatin C appear to be useful predictors of postnatal renal function (139).

Clinical factors which may confound Cystatin C interpretation have been described. Hyperthyroidism elevates and hypothyroidism decreases Cystatin C levels (218). Glucocorticoids increase and cyclosporine A can lower Cystatin C levels which may complicate GFR estimation after transplantation and obscure early cyclosporine nephrotoxic effects (229). High blood ketone levels have been associated with reduced Cystatin C levels. Gender and race have some impact on Cystatin C levels; women have levels that are 9% lower while blacks have levels that are 6% higher for a given GFR (230). Thus, for a GFR of 60 ml/min/1.73, the Cystatin C level of a white woman and black man would differ by 13%, less than the 50% difference in serum creatinine predicted on the basis of the same variables by the MDRD equation (231).

Given that its production is not limited to muscle cells, Cystatin C may be preferred for determination of renal function in groups at the extremes of muscle mass. In a study of healthy adults with differing physical activity, serum and urinary creatinine correlated directly with fat-free body mass while Cystatin C did not (232). Thus, serum creatinine levels were 0.95 ± 0.17, 0.96 ± 0.13, and 1.04 ± 0.12 mg/dl for those with sedentary, mild, or moderate/intense physical activity. In the same groups, serum Cystatin C levels were identical (0.82 ± 0.14, 0.80 ± 0.14, and 0.79 ± 0.14 mg/L), evidencing normal renal function in all groups, without the confounding impact of muscle mass on serum creatinine level (232). By contrast, patients with spina bifida have reduced muscle mass, and GFR estimated by Cystatin C shows superior correlation with ^{99}Tc-DTPA-measured GFR; the Schwartz formula does not correlate well and markedly overestimates GFR (233, 234).

As with creatinine, equations have been derived utilizing serum Cystatin C levels in an attempt to improve accuracy of GFR estimation (*Table 2-1*). Because of the inverse relationship of serum Cystatin C and GFR, the basis of most of these equations is GFR = 1/Cystatin C or GFR = $[\text{Cystatin C}]^{-1}$ with different factors and coefficients derived by linear regression from relatively small, often single center populations. Differences may also be attributable to the different Cystatin C assays and GFR methodologies (235–243). Recently, equations have emerged which incorporate both serum Cystatin C and

Table 2-1

Cystatin C-based equations for estimation of GFR

Ref	Equation	Cystatin C Assay	GFR Method	Population
Equations for adults				
(235)	$GFR = 78 \times CyC^{-1} + 4$	1	^{51}Cr-EDTA	Renal transplants (n = 25)
(236)	$GFR = 87.1 \times CyC^{-1} - 6.87$	2	Iohexol	Adults (n = 40; 29 diabetics)
(237)	$GFR = 80.35 \times CyC^{-1} - 4.32$	1	^{125}I-iothalamate	CKD patients (n = 123)
(238)	$GFR = 77.239 \times CyC^{-1.2623}$	1	Iohexol	Adults (n = 100)
	$GFR = 99.434 \times CyC^{-1.5837}$	2	Iohexol	Adults (n = 100)
(239)	$GFR = 87.62 \times CyC^{-1.693} \times 0.94$ (female)	2	Iohexol	Adults (n = 451)
(240)	$GFR = 66.8 \times CyC^{-1.30}$	1	Iothalamate	CKD patients (n = 357)
	$GFR = 76.6 \times CyC^{-1.16}$	1	Iothalamate	Renal transplants (n = 103)
(241)	$GFR = 169 \times CyC^{-0.63} \times S_{creat}(mg/dL)^{-0.608} \times Age^{-0.157}$	1	^{99m}-Tc-DTPA ^{51}Cr-EDTA	CKD, Chinese (n = 376)
(230)	$GFR = 177.6 \times CyC^{-0.57} \times S_{creat}(mg/dL)^{-0.65} \times Age^{-0.20} \times 0.82$ (female) $\times 1.11$ (if black)	1	^{125}I-iothalamate ^{51}Cr-EDTA	CKD patients (n = 3418)
Equations for children				
(140)	$GFR = 162 \times CyC^{-1} - 30$	2	Inulin	CKD patients (n = 184)
(242)	$\log GFR = 1.962 + 1.123 \times \log (1/CyC)$	1	^{99m}-Tc-DTPA	CKD patients (n = 536)
(239)	$GFR = 87.62 \times CyC^{-1.693} \times 1.376$ (if < 14 years) $\times 0.94$ (female)	2	Iohexol	Children (n = 85)
(212)	$GFR = 75.94 \times Cy^{-1.17} \times 1.2$(renal transplant) $GFR = 43.82 \times e^{0.003Ht} / [CyC^{0.635} \times S_{creat}(mg/dL)^{0.547}$	1	Iothalamate	Pediatric renal patients (n = 103)
(243)	$GFR = 63.2 \times [CyC/1.2]^{-0.56} \times [S_{creat}(\mu M)/96]^{-0.35} \times [BW(kg)/45]^{0.30} \times [Age\ (Y)/14]^{0.40}$	1	^{51}Cr-EDTA	Pediatric renal patients(n = 100)

Adapted from (218); Cy C [Cystatin C, mg/L]; 1-immuno-nephelometric assay; 2-immuno-turbidimetric assay

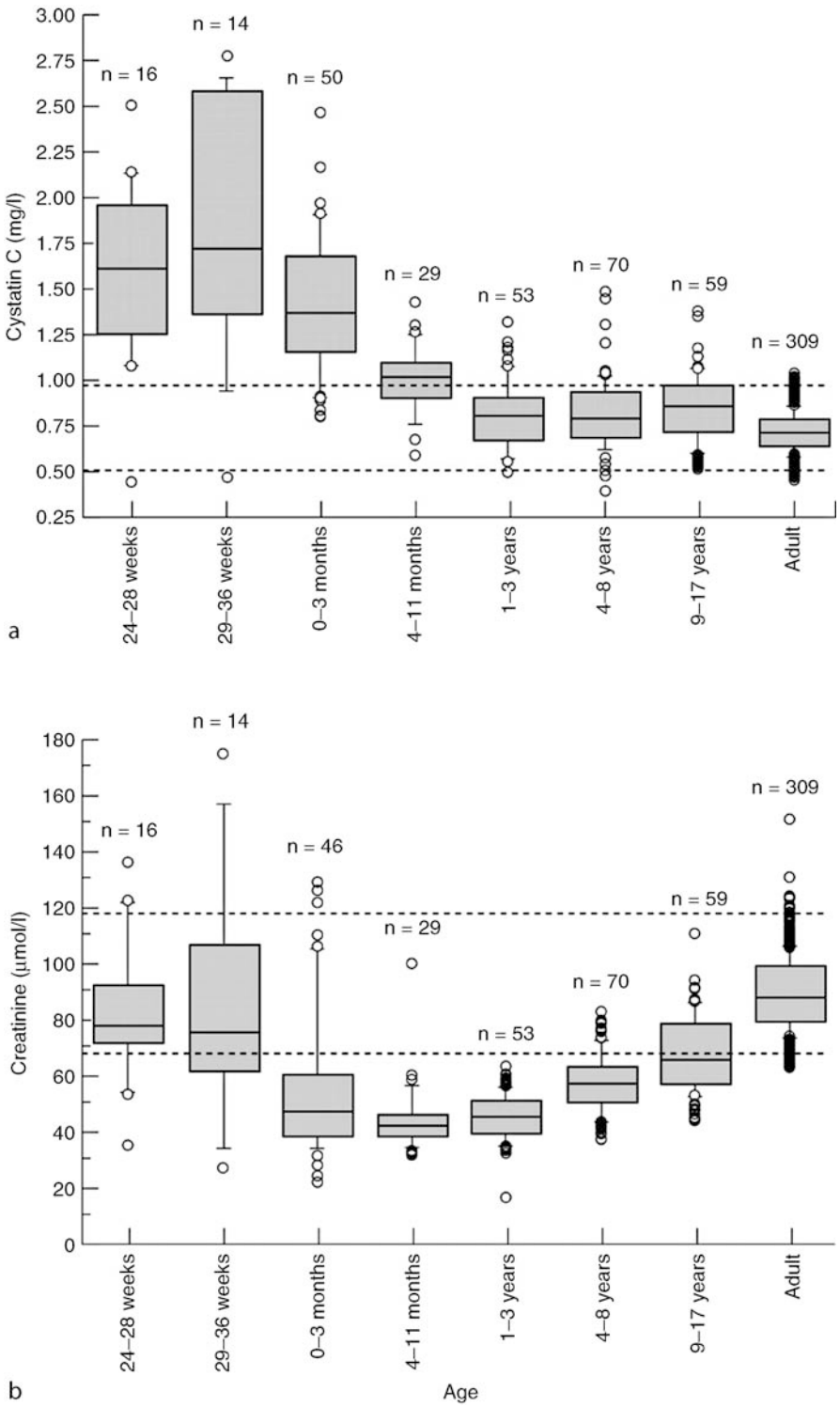
creatinine which more closely correspond with measured GFR than either creatinine or Cystatin C alone. Whereas pediatric equations are derived from relatively small sample sizes, a GFR-estimating equation for use in adults has recently been published which may prove more robust by including both Cystatin C and creatinine as well as being derived from a large, multicenter cohort of CKD patients (230).

Other Endogenous Markers of GFR

Variability of current endogenous markers of GFR with certain pathophysiologic conditions, e.g., Cystatin C derangement with corticosteroid therapy, has prompted an ongoing search for additional markers of GFR. β trace protein (β-TP) is one such marker (244). β trace protein (also known as lipocalin prostaglandin D2 synthase) is a 24 kDa protein freely filtered at the glomerulus which originates in the central nervous system, synthesized by glial cells. It is a major component of cerebrospinal fluid where its conversion of prostaglandin H2 to D2 contributes to nociception, temperature and sleep regulation. A recent pediatric reference range for β trace protein (age 2–20) shows stable serum levels from at 0.43–1.04 mg/L without gender effects. In patients with reduced renal function, β-TP increases. In pediatric patients with reduced renal function, β-TP showed higher percentage

Figure 2-11

Range for cystatin C and creatinine measured in 291 children aged 1 day to 17 years (225).



increase at GFR < 30 ml/min/1.73 m^2, than did Cystatin C. In children with neuromuscular disorders, however, significant deviation above and below the β-TP reference range was seen, likely reflecting neuromuscular pathology rather than renal dysfunction (244). Similarly, although Cystatin C is a useful marker of GFR in spina bifida patients, β-TP does not correlate with ^{99m}Tc-DTPA clearance, likely due to the obscuring impact of meningomyelocoele on β-TP production (234). Lastly, β-TP also appears to be significantly impacted by corticosteroids so that it does not improve in this regard over Cystatin C (245).

Glomerular Sieving of Macromolecules

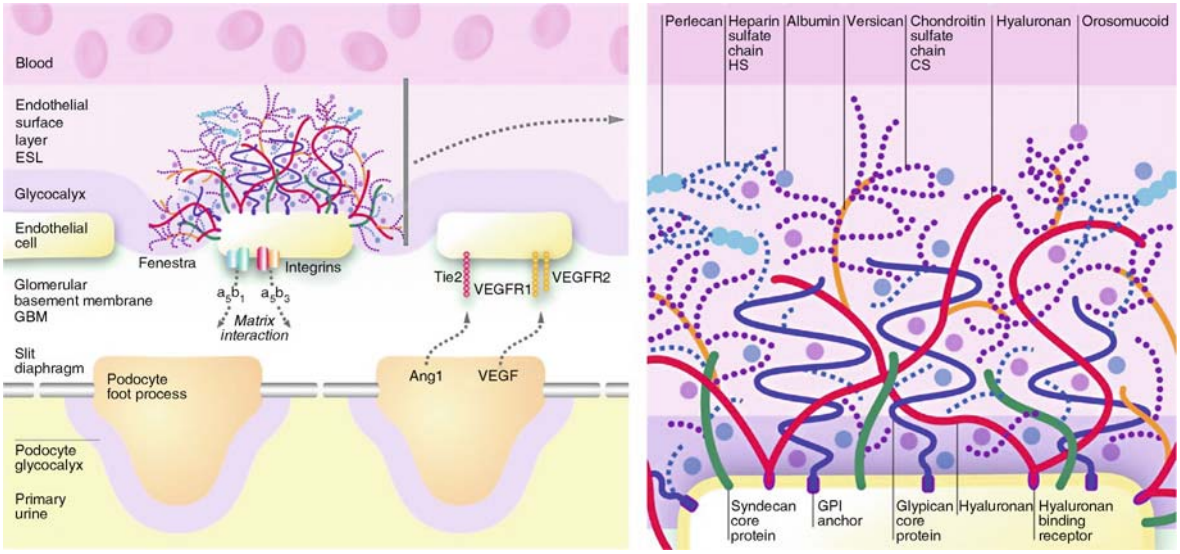
The enormity in the quantity of filtration generated by the glomerulus underscores specific features of the glomerular capillary bed that allows high permeability to water and small molecules while at the same time providing efficient selectivity that bars cells, proteins larger than albumin, and charged molecules (246). This barrier function of the glomerular capillaries is influenced by the size, shape, and charge of the macromolecules. Micropuncture studies and urinary clearance analyses that compare concentration of a given macromolecule in Bowman's space/urine to plasma have been used to obtain the glomerular sieving coefficient for a variety of macromolecules. Sieving coefficients are inversely correlated with the effective radius of the macromolecules. Thus, clearance of the larger proteins, such as albumin and globulin, is markedly less than that of smaller proteins such as monomeric immunoglobulin light chains (247, 248). Molecules without charge, including dextran and polyvinylpyrolidone, that are neither reabsorbed nor secreted (unlike proteins), have been used extensively to study glomerular capillary size selectivity both in experimental settings and in human diseases (247–253). Greater restriction of anionic than of neutral or cationic molecules suggests an electrostatic barrier that is charge selective. The observation that for a given chromatographic radius and charge density, the protein sieving coefficient is smaller than that of neutral dextrans suggests that the glomerular capillary barrier is also shape-selective. Thus, proteins are believed to behave as rigid spheres, whereas dextrans are more compliant so as to have smaller effective radii (248). It appears that each of the three major components of the glomerular capillary wall (endothelial cells, basement membrane and epithelial cells with their podocytes and slit diaphragms) provide impedance to macromolecular filtration. That endothelial cells and/or epithelial cells are important in this barrier function is illustrated by the observation that permeability of the isolated glomerular basement membrane is much higher than in the intact glomeruli (254). Indeed, native anionic ferritin particles accumulate in the endothelial fenestrae and in the lamina rara externa of the basement membrane.

Role of Endothelial Cells

Until recently, glomerular endothelial cells, with 60–80 nm fenestrations comprising 20% of their surface area, have not been thought to contribute significantly to the filtration barrier, aside from cellular exclusion from the urinary space (255). However, new observations clearly show that the glomerular endothelium contributes significantly to both size selectivity and charge selectivity of the filtration barrier. Only 5–10% of vascular albumin passes through the glomerular basement membrane (256, 257). Newer techniques allow visualization of a continuous glycocalyx which lines the endothelium and fenestrations, extending 10–20 nm into the vessel lumen. Additionally, a delicate endothelial surface layer (ESL), has been observed to extend~200 nm above the glomerular endothelium (*Fig. 2-12*) (258). Whereas the glycocalyx is composed of membrane-bound proteoglycans, the most abundant of which is heparan sulfate proteoglycan, the ESL additionally contains secreted proteoglycans and adsorbed plasma proteins (259). One group found proteinuria in mice transgenic for human heparanase, potentially related to degradation of glycocalyx (260). In vitro enzymatic degradation of the glycocalyx, without disruption of underlying endothelial cells (achieved by using neuraminidase, heparinase III, and human heparanase), resulted in several fold increase in albumin flux across a cultured endothelial cell monolayer (261). Infusion of glycocalyx-degrading enzymes into mice (hyaluronidase, heparinase III, and chondroitinase) increased by 2.5-fold the penetration of circulating lipids to within 50 nm of the endothelium. Isolated perfused kidneys from chondroitinase-treated mice showed fivefold increase in the fractional clearance of albumin (262). Moreover, clearance of Ficoll 35.5 A (similar to albumin in size but neutral rather than anionic) did not increase, suggesting that the endothelial glycocalyx contributes substantially to the charge selectivity of the glomerular filtration barrier. Similar results were obtained in experimental mice, in which 40 weeks of diabetes caused a threefold increase in glomerular albumin clearance but no change in clearance of neutral Ficoll 35.5 A. This increase in albumin clearance was associated with

Figure 2-12

Molecular model of the glomerular filtration barrier. The three layers of the barrier are the fenestrated endothelial cells lining the inside of the glomerular capillary, the glomerular basement membrane and the podocyte foot processes with their intervening slit diaphragm. Components of the endothelial surface layer are shown in detail in the right panel (reprinted with permission from (259)).



down regulation of ESL components versican and the chondroitan sulfate proteoglycan decorin (263).

Role of Glomerular Basement Membrane

The glomerular basement membrane, five times as thick as basement membranes of other vascular beds, is recognized as providing an important barrier to macromolecular passage (259). The glomerular basement membrane is composed of a fibrous network of type IV collagen, laminin, and proteoglycans (including perlecan and agrin, containing high levels of negatively charged heparin sulfate moieties) that provide size and charge-selective restriction to glomerular filtration. (264–266). Type IV collagen is a trimer formed from three of six chains (alpha 1 to alpha 6) encoded on three chromosomes. The trimer α1:α1:α2 constitutes Type IV collagen in embryonic GBM, whereas α3:α4:α5 is expressed postnatally (267, 268). Mutations of the alpha 5 chain, encoded on the X chromosome, leads to defects in the adult collagen IV and to thinning and distortion of the glomerular basement membrane that characterizes X-linked Alport's hereditary nephritis. Heterozygous mutations of the alpha 3 and alpha 4 chains underlie thin basement membrane nephropathy, whereas homozygous mutations lead to autosomal Alport's hereditary nephritis (268). Laminin plays an increasingly recognized role in basement membrane structure and sieving impedance. Mice deficient in laminin-2 chain develop nephrotic syndrome (269, 270). In humans, truncating mutations in the gene for laminin β2 (LAMB2) produce Pierson syndrome consisting of congenital nephrosis with eye abnormalities and mental retardation (271, 272). Nonsyndromic congenital nephrosis is described with missense mutations of LAMB2 (273, 274). Prevailing opinion for some time has been that negatively-charged heparan sulfate in the GBM constitutes the main charge selective filter of the glomerulus, although recent evidence is challenging this notion (275). In vivo removal of GBM sulfated glycosaminoglycans in rats by heparinase III does not result in proteinuria; neuraminidase treatment causes proteinuria while leaving heparan sulfate intact in the GBM (276). Similarly, mice transgenic for human heparanase show fivefold reduction in glycosaminoglycan anionic sites in GBM, but lack proteinuria (277). A function proposed for heparin, is that by attracting and holding water molecules, it enables the GBM to function as a gel (278), a description which may be eroding the older concept of GBM as a sieve (279).

Role of Podocytes

The epithelial cell layer of the glomerular capillary network furnishes the restrictive size selection of macromolecular filtration, preventing passage of proteins the size of albumin or larger. Glomerular epithelial cells (podocytes) consist of a large cell body, major processes, and long, interdigitating foot processes separated by a filtration slit diaphragm that is ~30–50 nm wide and possesses numerous pore-like structures measuring 40 × 140 A. Recent studies have clarified the molecular structure and functional implications of the slit diaphragms. The gene mutated in congenital nephrotic syndrome of the Finnish type, NPHS1, was found to encode a slit diaphragm protein, nephrin. Nephrin is a transmembrane glycoprotein similar to immunoglobulin-like cell adhesion molecules, which participates in an interdigitating homophillic interaction from opposing podocytes, forming a zipper-like sheet between them (280–284). A variety of other proteins are now known to contribute to the slit diaphragm linking podocyte foot processes, including NEPH-1 and NEPH-2, FAT1, P-Cadherin, and podocin (285). Besides comprising the physical barrier which impedes the flow of albumin into the urinary space, each of these slit diaphragm components is linked through their intracellular domains to other proteins connecting them to the actin cytoskeleton of the podocytes (*Fig. 2-13*). Intracellularly, nephrin interacts with CD2-associated protein (CD2AP) and other proteins such as Nck that connect with actin (286). Mice deficient in CD2AP have massive proteinuria and early death (287). Likewise, selective deletion of Nck from podocytes of transgenic mice results in defects in the formation of foot processes and in congenital nephrotic syndrome (288). Nephrin phosphorylation sites Y1204 and Y1228 appear to be the sites of Nck binding. Induction of puromycin nephrosis in rats decreased phosphorylation of nephrin's Nck binding sites as well as loss of filamentous actin and increase in globular actin. Loss of phosphorylation, thus, appears to sever the linkage of nephrin to actin leading to dramatic perturbations in podocyte structure and function. Preliminary data also indicate that phosphorylated nephrin is decreased in human minimal change nephrotic syndrome as well (289) These studies underscore the appreciation for the pivotal role of the podocyte and slit diaphragm in glomerular filtration barrier function.

VEGF appears to be critical for normal function and maintenance of the glomerular filtration barrier, beyond its role in glomerulogenesis. Thus, VEGF functions in an autocrine manner on podocytes and reduces podocyte apoptosis, doing so in concert with nephrin (290, 291). Autocrine VEGF upregulates podocin in podocytes and increases its interaction with CD2AP (292). Downregulation of a podocyte VEGF receptor, VEGFR2 (by infusion of semaphorin 3a) disrupts podocytes leading to acute nephrotic-range proteinuria, foot process effacement, and down regulation of slit diaphragm proteins nephrin and podocin, as well as CD2AP. Endothelial cells were also impaired with swelling and loss of fenestrations as well as detachment from the GBM (293). The importance of intact VEGF signaling to maintenance of glomerular endothelium is further underscored by the observation of renal thrombotic microangiopathy in patients after treatment for neoplasia with monoclonal antibodies to VEGF (294). Similarly, a soluble placental-derived inhibitor of VEGF (sflt1) is elevated in the maternal circulation in preeclampsia, the renal lesion of which is characterized by microangiopathy (295, 296).

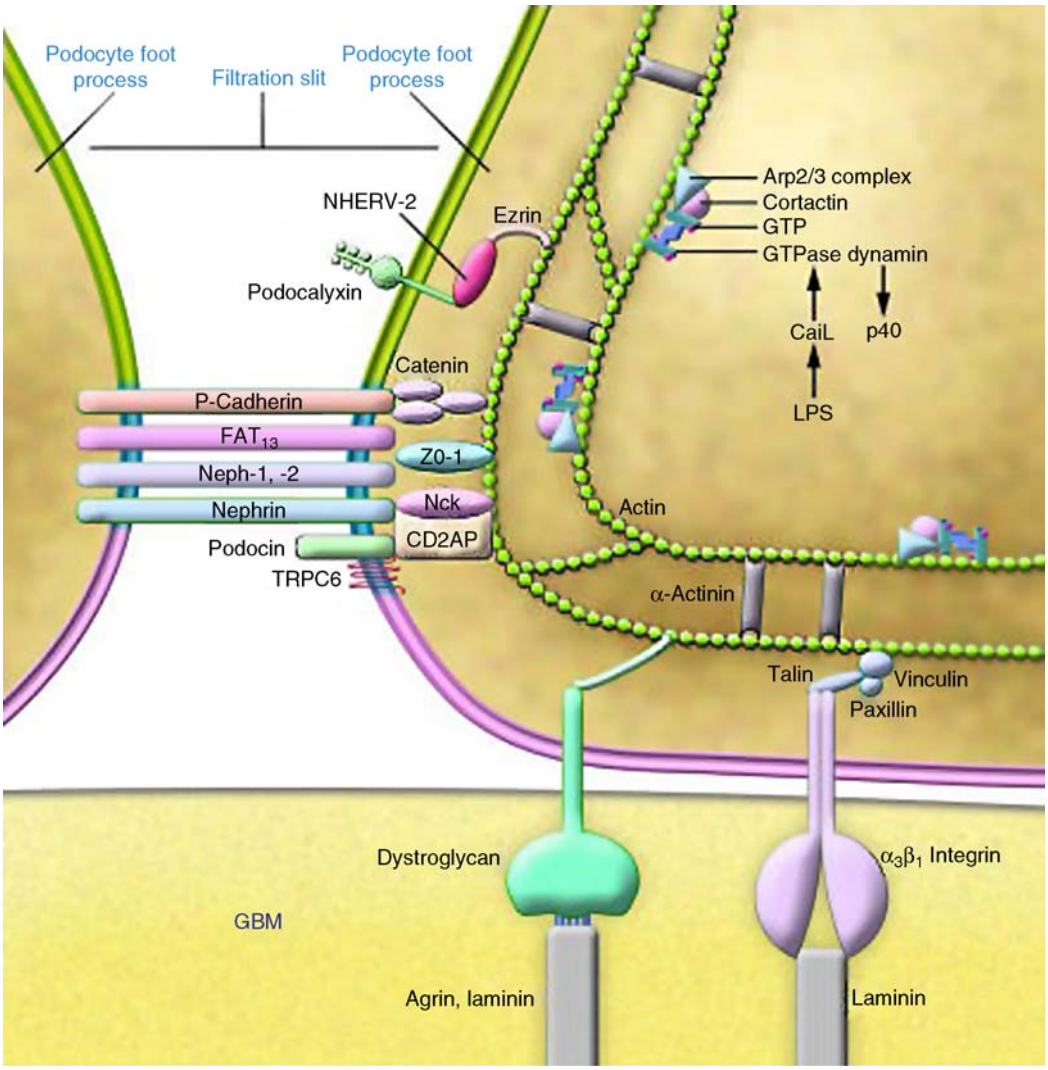
A newly appreciated component of the filtration barrier is the space bounded below by the GBM and foot processes and above by the underside of the podocyte cell bodies, the subpodocyte space (SPS) (297). The subpodocyte space (including the overlying and defining podocyte cell bodies) covers 60% of the total filtration barrier but is not readily imaged by either scanning electron microscopy or transmission electron microscopy which has limited its examination (298). In SPS-covered areas, glomerular filtrate cannot enter directly into the urinary space without traversing the long and tortuous subpodocyte space, exited by a very limited number of pores. In fact, the area available for efflux from the SPS appears to be several hundredfold lower than the area for influx, increasing glomerular flow resistance by 1.3–26 times that posed by areas of uncovered glomerular filtration barrier (299). Stated another way, glomerular filtration through SPS-covered areas may be only 4–75% (mean 29%) that of uncovered areas. Beyond this, evidence supports retention of 10 kDa dextran–rhodamine in this space (but not 450 Da lucifer yellow or 3 kDa dextran), suggesting the SPS makes a size selective contribution to the glomerular filtration barrier as well (300).That the contractile apparatus of podocytes (defining the dimensions of the SPS and exit pores) is readily functional, raises the possibility for particular relevance of the SPS in certain pathophysiologic settings.

Role of the Renin Angiotensin System

In addition to the structural characteristics of the capillary wall components that determine its permeability, individual determinants of SNGFR also impact filtration of macromolecules. Glomerular capillary flow rate, but particularly the glomerular capillary pressure, is thought to modulate membrane characteristics such that an in-

Figure 2-13

Schematic drawing of the basolateral portion of podocytes. The filtration slit is composed of nephrin and other structural molecules between podocytes. Many of these filtration slit components are themselves linked to the intracellular actin cytoskeleton of the podocyte. Podocytes are also linked to the glomerular basement membrane through their actin cytoskeleton (reprinted with permission from (280)).



crease in glomerular pressure augments proteinuria. Infusion of Ang II, or endogenous stimulation of Ang II activity, increases the fractional excretion of protein, whereas decreasing the glomerular capillary pressure has the opposite effect (301–303). These studies underscore that glomerular hemodynamic changes can allow macromolecules to escape into the urinary space. It is of interest that acute exercise-induced proteinuria, in which the sieving defect is believed to be linked to increased intraglomerular pressure, is lessened by pretreatment with ACEI (304). Conversely, antagonism of Ang II actions by ACEI or ARB acutely lessens proteinuria and is well documented to decrease protein excretion in many different chronic settings (305–307). The mechanism for this antiproteinuric effect is in part related to decreased efferent arteriolar resistance and therefore glomerular capillary pressure, attributable to both reduction of Ang II effect and augmentation of bradykinin effect (308).

Independent of hemodynamic changes, RAS-inhibition enhances glomerular barrier size selectivity, underscoring the deleterious impact of Ang II. Thus, infusion of neutral dextrans into patients with chronic proteinuric

nephropathies produces a pattern of dextran clearance that is significantly lessened by treatment with enalapril, ramipril, or valsartan, particularly at high molecular radius (251, 309, 310). Mechanisms of Ang II-induced barrier dysfunction appear to include direct podocyte effects. Thus, Ang II (through AT1) increases albumin flux across podocyte monolayers in vitro that is associated with F-actin disorganization and zonula occludens-1 fragmentation (311). Injured podocytes in vitro have diminished ability to stimulate glomerular endothelial cell growth, which is restored by AT1 antagonism, in part by restoring VEGF signaling (312). Ang II also appears to decrease podocyte expression of slit diaphragm components nephrin and podocin through AT1, whereas AT2 appears to have a salutary effect on slit diaphragm components (313). Interestingly, in nonglomerular vascular tissues, evidence supports a direct role for VEGF stimulation through the AT2 receptor. These latter studies raise AT2 as a potential therapeutic target for stimulation in order to enhance glomerular filtration barrier function, even beyond AT1 receptor blockade (314, 315).

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3 Renal Tubular Development

Michel Baum

Organization of the Nephron

The kidney is faced with the enormous task of maintaining a constant composition and volume of the extracellular fluid. The adult ingests nutrients and water and generates waste products that must be eliminated to maintain this balance. In other words, the amount of electrolytes that are ingested and absorbed must be eliminated and the waste products from metabolism must also be excreted. This challenge is all the more complex as our dietary intake is quite variable from day to day. Despite this variable intake, there is virtually no change in the volume or composition of the extracellular fluid volume from day to day.

There are two possible ways that our kidney could balance ingestion and excretion. The kidney could be a secretory organ, from where all the excess solutes and water would be excreted by tubular secretion. This would be very inefficient and require an enormous amount of energy. In addition, in times of a disturbance in the extracellular fluid volume such as a high salt intake or volume loss from diarrhea, for example, the regulatory systems necessary to maintain a constant extracellular fluid volume and composition while excreting waste products would be very complex. On the other hand, the kidney could filter an enormous quantity of extracellular fluid, which would be very efficient in removing waste products, and reclaim the desired salt, organic solutes and water. The mammalian kidney actually uses both mechanisms to perform its job, which is necessary for our survival on land. The adult kidney filters ~150 l of isotonic fluid a day and reclaims most of it, leaving the waste products to be excreted. In addition, there are secretory processes for solutes such as organic anions and cations in the proximal tubule and secretory mechanisms to excrete the excess acid generated from metabolism in the distal nephrons, which aid in maintaining homeostasis.

To accomplish the remarkable task of reclamation of the necessary solutes and water in the filtered load, the mammalian kidney has evolved into a highly specialized organ with one million units called nephrons. Each nephron is a tube consisting of epithelial cells and is divided into 12 specialized segments as shown in *Fig. 3-1*. The epithelial cells allow the vectorial transport of solutes. The proximal tubule is responsible for the bulk reclamation of solutes and for the secretion of organic cations and anions. Approximately two-thirds of the glomerular filtrate is reabsorbed by the proximal tubule in an isotonic fashion. Virtually all of the organic solutes, as well as the majority of bicarbonates, phosphates, and chlorides, are reabsorbed in this segment. The proximal tubule is divided into S_1, S_2, and S_3 segments, on the basis of the rates of transport of some solutes, and morphological changes that occur down the proximal tubule. The nephron makes a hairpin turn, which aids in the generation of concentrated urine. The length of the thin descending and ascending limb is variable among species, with desert rodents having very long thin limbs as they need to conserve water and excrete very concentrated urine. The length of the thin ascending and descending limbs increases as one goes from the superficial cortex down to the medulla. The thin descending limb expresses aquaporin 1 on the apical and basolateral membranes and is very permeable to water (255), but is impermeable to solutes. This results in a concentrated fluid in the medulla with a very high sodium chloride content, providing a passive driving force for sodium chloride diffusion in the thin ascending limb. The thin ascending limb is impermeable to water but has a high permeability to NaCl (145). The chloride channel (CLC-K1) in the thin ascending limb is developmentally regulated (160). There is no expression in the fetus and until the end of the first week of life, in rats. There is a correlation between CLC-K1 and urinary osmolality, suggesting the important role of this channel in generating a hypertonic medulla (160). Diffusion of NaCl causes a high interstitial osmolality. This loop structure, along with the thick ascending limb, generates the countercurrent multiplication system that results in a medullary osmolality far greater than that of blood (162, 258). The importance of the passive properties of the thin limbs in this counter current system is exemplified in mice which are deficient in the water channel designated aquaporin 1, and do not express aquaporin 1 in the thin descending limb (182). The urine osmolality of aquaporin 1 deficient mice is greater than plasma, but far less than control mice expressing

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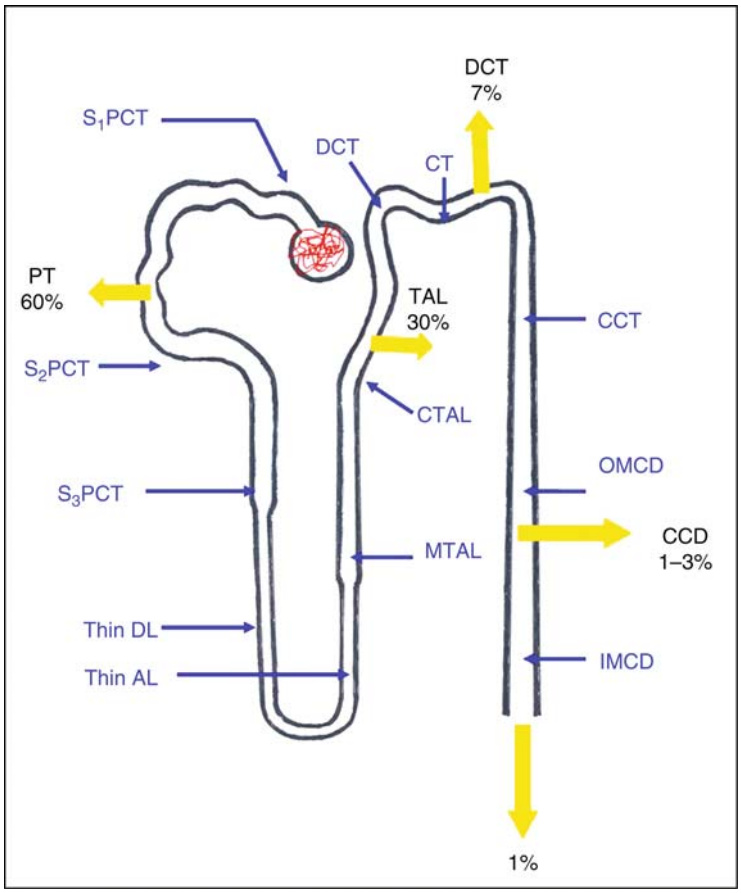
Figure 3-1

This cartoon depicts the nephron with its 12 segments. Shown in blue are the nephron segments. S_1, S_2, S_3 PCT depict the three segments of the proximal tubule. The loop of Henle consists of the thin descending limb (thin DL) and thin ascending limb (thin AL), the medullary (MTAL) and cortical thick ascending limb (CTAL). The distal convoluted tubule is comprised of the (DCT), connecting tubule (CT) and the initial cortial collecting tubule. The collecting duct is made up of the cortical collecting tubule (CCT), outer medullary (OMCD) and inner medullary collecting duct (IMCD). Shown in yellow is the percentage of sodium reabsorbed by the proximal tubule (PT), thick ascending limb (TAL), distal convoluted tubule (DCT) and cortical collecting duct (CCD). One percent of the filtered sodium is excreted.



aquaporin 1 (182). Unlike control mice, aquaporin 1 knock-out mice cannot increase their urine osmolality in response to water deprivation.

The thick ascending limb is the segment responsible for ~30% of sodium chloride transport and has a vital role in generating a concentrated medulla. Apical sodium chloride absorption is mediated by the bumetanide sensitive cotransporter. One of the unique features of this segment is that it is impermeable to water and thus the fluid leaving this segment is hypotonic to blood. In addition, this segment has a very high paracellular permeability to cations and is responsible for much of calcium and magnesium transport. The distal convoluted tubule is responsible for ~5–10% of NaCl transport. NaCl transport in this segment is mediated by the thiazide sensitive cotransporter. Active transcellular calcium and magnesium transport also occurs in this segment.

The rest of the distal tubule is separated into the connecting tubule and the cortical, outer and inner medullary collecting tubule. These segments are responsible for potassium secretion, final urinary acidification and water absorption, the latter mediated by the action of vasopressin. While the fraction of salt transport and renal acidification is only a fraction of that in other

nephron segments, the collecting tubule is responsible for the final modulation of the tubular fluid. Thus, the final composition of urine and significant regulation of transport occur in this segment.

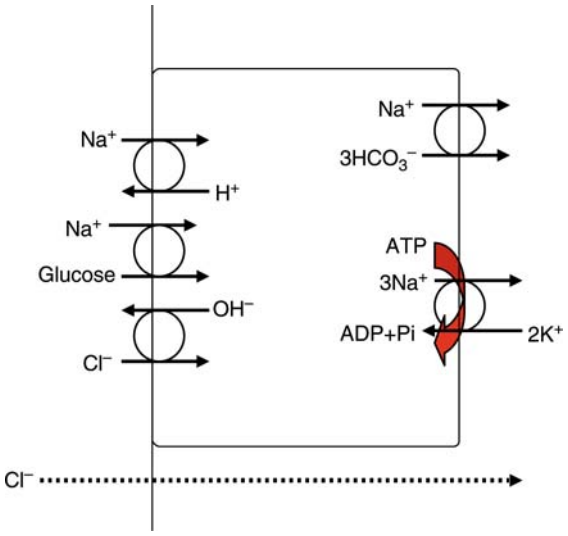
Principals of Membrane Transport

The cells along the nephron are quite different in the various nephron segments, as will be discussed in the subsequent sections. The cells in each nephron segment are poised for vectorial transport. The apical and basolateral membranes are, by and large, a lipid bilayer which would be impermeable to water and solutes if there were not specific proteins to facilitate transport across the apical and basolateral membranes. In addition, many transporters are regulated to adjust their rate of transport to meet the physiologic changes in volume status or concentration of solutes in the extracellular milieux.

The reabsorption of solutes along the nephron is characterized by active and passive transport processes. A typical cell is shown in *Fig. 3-2.* It should be appreciated that if all active transport was inhibited along the nephron, we would excrete urine with the composition and volume of the glomerular ultrafiltrate. Passive transepithelial transport is, by and large, the result of gradients generated by active transport. Most active transport is the result of the basolateral Na^+-K^+-ATPase. This transporter pumps three sodiums out of the cell in exchange for two potassium ions. The pump utilizes ATP and it is an example of primary active transport. This pump is vital to the generation of the low intracellular sodium and high intracellular potassium concentration as well as the negative intracellular potential difference across the apical and basolateral membranes. Both the low intracellular sodium and this potential difference can provide a driving force for secondary active transport. For example, in *Fig. 3-2*, the reabsorption of glucose via a sodium-dependent transporter utilizes both the sodium gradient and the relative negative cell potential to bring glucose to the cell. The Na^+/H^+ exchanger on this cell is electroneutral and utilizes the sodium gradient to secrete protons and reabsorb sodium. Both the sodium glucose and the Na^+/H^+ exchanger are secondary active transport processes dependent on the basolateral Na^+-K^+-ATPase. The secretion of protons will cause the luminal pH to drop, providing a favorable driving force for the Cl^-/OH^- exchanger, an example of tertiary active transport. Thus, in secondary and tertiary active transport, the transporters do not utilize ATP directly; however, inhibition of the ATP-dependent Na^+-K^+-ATPase would bring these transport processes to a halt.

Figure 3-2

A proximal tubule cell which shows the Na^+-K^+ ATPase on the basolateral membrane, an example of primary active transport. Na^+-K^+ ATPase decreases the intracellular sodium to about 10 mEq/l and increases the intracellular potassium to approximately 140 mEq/l. The pump is electrogenic with a cell negative potential of about 60 mV. The sodium gradient provides the driving force for the apical Na^+/H^+ exchanger and both the sodium gradient and the potential difference provide the driving force for the apical sodium glucose transporter. The secretion of protons via the Na^+/H^+ exchanger results in the driving force for the Cl^-/OH^- exchanger which is an example of tertiary active transport. Chloride is shown transversing the paracellular pathway. Bicarbonate is exiting the basolateral membrane via a sodium bicarbonate cotransporter.



In addition to active transport, a substantive amount of passive transport occurs between the cells across the tight junction. Active transport along the nephron will generate ion and solute gradients between the lumen and peritubular fluid. Depending on the permeability properties of the tight junction, passive absorption or secretion can occur. In the cell depicted in *Fig. 3-2*, there is passive chloride transport across the paracellular pathway. It has become apparent that the characteristics of the tight junction vary along the nephron. The tight junction creates the primary permeability barrier to the diffusion of solutes across the paracellular pathway. Occludin and claudin proteins are localized to junctional fibrils and are transmembrane components of tight junctions (4, 199, 200). These tight junction fibrils or strands are a major factor determining the permeability properties of the

paracellular pathway (4, 75, 75, 76, 76). The claudin family of tight junction proteins now numbers 24. Occludin has a ubiquitous distribution and is not responsible for the differential permeability properties in the various nephron segments. The claudin isoforms present at the tight junction of various epithelia determine the resistance and the permeability properties of the epithelia (4, 75, 75, 76, 76). The distribution of claudin isoforms varies along the nephron and is responsible for the unique permeability properties of each nephron segment.

The final form of passive transport is called solvent drag. Solvent drag has been postulated to be responsible for a small fraction of transport in the proximal tubule. The reabsorption of solutes could result in water movement that could entrain or carry solutes with it. For this to occur, the solute should have a low reflection coefficient or high sieving coefficient (sieving coefficient = 1/refection coefficient). In other words when a solute is entrained in fluid and hits a membrane or tight junction, it could pass through it and be transported or bounce off and not be transported. Direct measurements of solute drag in the proximal tubule of neonates and adults have shown that it contributes to a negligible fraction of transport (79, 146, 230, 233).

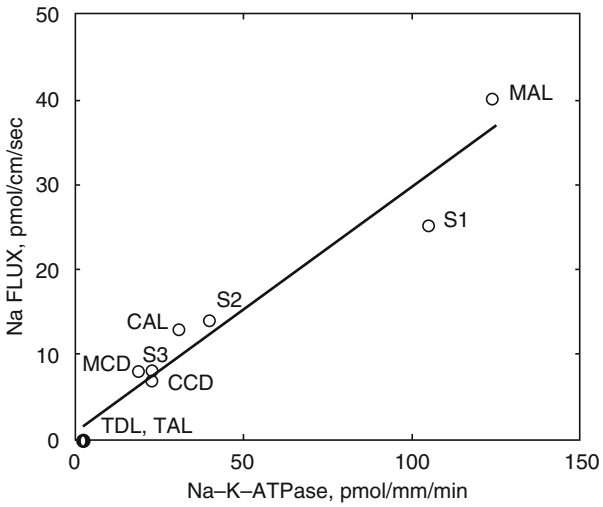
Maturation of Na^+-K^+-ATPase along the Nephron

The Na^+-K^+-ATPase is located on the basolateral membrane of most tubules in the kidney. It is a heterodimer composed of an α and β subunit. There are four different α subunits and 3 β subunits on mammalian cells which have different functional properties (47) There is also evidence for a small γ subunit that is not required for Na^+-K^+-ATPase activity (47, 244). The γ subunit binds to the α subunit, stabilizes the enzyme, and plays a regulatory role in enzyme activity (47, 244). The adult kidney expresses the α1 and ß1 isoforms of the Na^+-K^+-ATPase (100, 214). The α subunit is the catalytic subunit and has the cation, ATP and ouabain binding sites (47). The β subunit is the regulatory subunit and is essential for the function of the enzyme (47, 189). Several hormones that regulate sodium transport along the nephron act, at least in part, by regulating Na^+-K^+-ATPase activity (42, 47, 99, 109, 154, 188, 208, 264–268, 311).

The Na^+-K^+-ATPase is responsible for lowering the intracellular sodium concentration and establishing the negative cell potential difference. Thus, the Na^+-K^+-ATPase provides the driving force for sodium transport across the nephron. As shown in *Fig. 3-3*, there is a direct relationship between sodium transport and Na^+-K^+-ATPase activity factored per millimeter of tubule along the nephron (111). Neonates have a lower renal Na^+-K^+-ATPase activity than adults (10, 11, 109, 271, 274, 325). As will be discussed, there is a developmental increase in sodium transport in each nephron segment, which is paralleled by an increase in Na^+-K^+-ATPase activity as shown in *Fig. 3-4*.

Figure 3-3

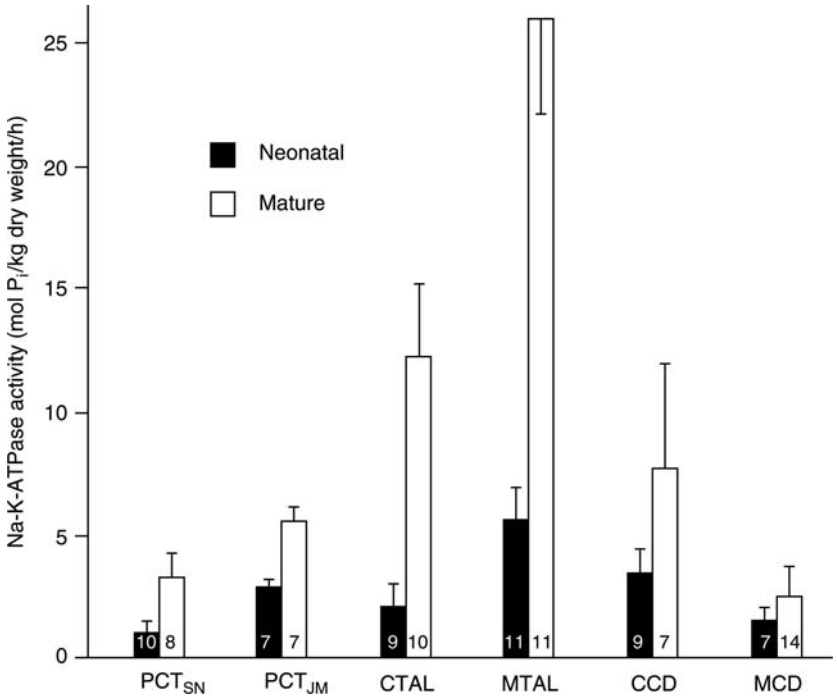
Sodium transport is plotted against Na^+-K^+ ATPase activity. As is shown, the rate of sodium transport in various nephron segments parallels Na^+-K^+ ATPase activity (111).



The parallel maturational increase in sodium transport with Na^+-K^+-ATPase activity (274) along with the striking relationship between sodium transport and Na^+-K^+-ATPase activity (111), suggests that the maturational increase in apical sodium transport may contribute to the postnatal increase in Na^+-K^+-ATPase activity. In cell culture studies an increase in intracellular sodium caused a stimulation in Na^+-K^+-ATPase activity (126, 166) as well as an increase in the α subunit mRNA and membrane pump density (80). In addition to in vitro studies, there is evidence that a chronic increase in Na^+/H^+ exchanger activity induced by metabolic acidosis, increased Na^+-K^+-ATPase activity, an effect that was blocked by coadministration of the Na^+/H^+ exchange inhibitor, amiloride (108). Finally, there is a postnatal increase in both serum thyroid hormone and glucocorticoid levels with age (28, 131, 132, 323). Both glucocorticoids and thyroid hormone have been shown to increase Na^+-K^+-ATPase activity (11, 68, 70, 71, 112, 208).

Figure 3-4

Na^+-K^+ ATPase activity is shown in nephron segments of neonates and adults. As is demonstrated, there is a maturational increase in Na^+-K^+ ATPase activity in every nephron segment (271).



Proximal Tubule Transport

Proximal tubule transport is characterized by a phenomenon called threshold, which is depicted in *Fig. 3-5.* It is the threshold that keeps our serum bicarbonate at 25 mEq/l. If we were to ingest bicarbonate and try to raise the serum bicarbonate level, we would have a bicarbonaturia and our serum levels would return to 25 mEq/l as long as we were euvolemic. Our serum glucose is set by other factors well below the threshold level. As shown in *Fig. 3-5*, if we increased the serum glucose level, we would reabsorb more glucose until the load of glucose delivered to the proximal tubule exceeded its ability to reabsorb glucose and we would have glucosuria.

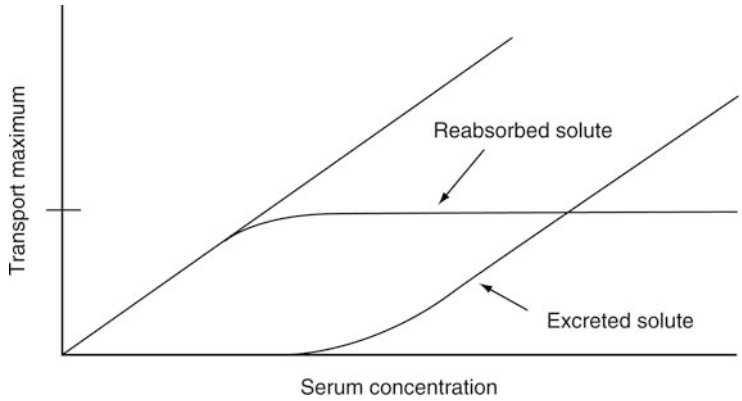
In the adult kidney there is a parallel change in proximal tubule transport with alterations in glomerular filtration rate. This phenomenon has been designated glomerular tubular balance. If this did not occur, an increase in glomerular filtration rate would swamp the distal nephron with solutes and water and there would be a huge natruresis and diuresis. A similar phenomenon must occur during postnatal development. There must be a parallel increase in proximal tubule transport with the maturational increase in glomerular filtration rate. If this did not occur the neonate would die of dehydration when the glomerular filtration rate increased after birth.

In the neonate there is a concomitant increase in tubular transport to accommodate or balance the increase in glomerular filtration rate (140, 163, 305). However, glomerular tubular balance is not present in the fetus (198). Renal development is characterized by centrifugal maturation. The surface nephrons are relatively immature compared to the juxtamedullary nephrons. These immature nephrons with short proximal tubules have glomerular tubular imbalance (198). This is clinically relevant as neonates born before 34 weeks of gestation can have glucosuria and very premature neonates can have significant salt wasting (12).

The proximal tubule reabsorbs 60% of the glomerular filtrate in an isoosmotic fashion. Due to the fact that the proximal tubule has a relatively high permeability to many ions, even solutes which are not actively transported by this segment get absorbed by the paracellular pathway. The luminal fluid concentration of magnesium, which is not actively transported, would rise over two-fold by the end of the proximal tubule as over half of the fluid is reabsorbed. This does not happen because magnesium is passively reabsorbed across the paracellular pathway as

Figure 3-5

This figure depicts the concept of renal threshold. As the delivered load increases to the tubule either by an increase in the serum concentration or an increase in glomerular filtration rate, the amount of solute absorbed increases. At some point, the renal tubular absorption reaches a maximum, called the threshold for that solute, and any further increase in the filtered load is excreted.



the magnesium concentration rises above that in the peritubular capillaries.

Glucose Transport

Glucose is reabsorbed solely by the proximal tubule. Physiologic studies have demonstrated that the S_1 proximal tubule reabsorbs glucose through a high capacity–low-affinity transporter, while in the late proximal tubule (S_3) glucose transport is via a low capacity–high affinity transporter (22). Similar axial heterogeneity of glucose transport kinetics was validated using cortical brush border membrane vesicles to measure apical membrane transport and outer medullary brush border membrane vesicles which contain vesicles from the S_3 segment (316). The high capacity–low affinity sodium dependent glucose transporter on the apical membrane is designated as SGLT-2 (332). This removes the bulk of the glucose from the glomerular ultrafiltrate. The low capacity–high affinity transporter is designated SGLT-1 (130, 143). The glucose that is transported by the tubule exits across the basolateral membrane by facilitative diffusion. As shown in *Fig. 3-6*, SGLT-2 transports one sodium with one glucose molecule while SGLT-1 transports two sodium with each glucose molecule. A defect in SGLT-1 causes glucose-galactose malabsorption as this transporter is also present in the intestine (95, 96, 193, 195). Some patients with familial glucosuria, a benign condition, have a mutation in SGLT2 (63, 184). This axial arrangement of glucose transporters results in reabsorption of virtually all the filtered glucose.

Sodium-dependent glucose reabsorption results in a positive charge entering the proximal tubule cell. This charge leaves a lumen negative transepithelial potential difference. This negative potential provides a driving force for the absorption of an anion or the back diffusion of a cation (sodium) across the paracellular pathway. Thus glucose transport can result in a net absorption of sodium chloride with sodium moving into the cell with glucose and chloride across the paracellular pathway. Whether sodium is recycled or chloride is reabsorbed is dependent on the relative sodium/chloride permeability of the paracellular pathway.

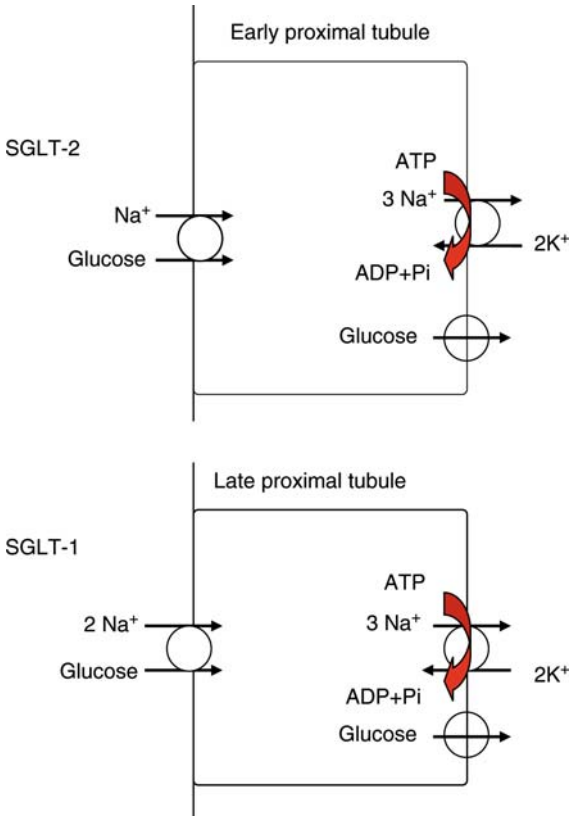
Numerous studies using various techniques and animal species have shown that the fetus and neonate transport glucose at a slower rate than the adult (13, 36, 106, 198, 273). These studies are of clinical relevance as premature neonates can have glucosuria (12, 127, 317). Despite the fact that the glomerular filtration rate is about 100th of that of the adult and the filtered load delivered to the neonatal nephron is also about 100th of that of the adult, the filtered load exceeds the reabsorptive capacity for glucose transport in the premature neonate. Thus there is a time during development when glomerular tubular balance is not present.

Amino Acid Transport

All amino acid transport occurs in the proximal tubule. Unlike most cells, which have amino acid transporters to provide substrates for protein synthesis, the proximal tubule mediates the vectorial transport of amino acids

Figure 3-6

Diagram of glucose transport in the proximal tubule. The early proximal tubule has SGLT-2 on the apical membrane which is a high capacity, low affinity transporter, while in the late proximal tubule the low capacity high affinity transporter, SGLT1 is on the apical membrane. Glucose exits the cell across the basolateral membrane by passive diffusion.



from the filtrate to the blood. The basic principal for transport is similar to glucose transport. The uptake of amino acids is sodium-dependent and electrogenic, with the basolateral exit mediated by facilitated passive diffusion. While there are 20 amino acids that are utilized in the synthesis of proteins, there are not 20 different amino acid transporters on the apical and basolateral membranes. Since some amino acids are similar in structure or charge, there is promiscuity among the classes of transporters. We will briefly discuss the three major classes of amino acid transporters.

The neutral amino acids include leucine, valine, isoleucine, methionine, phenylalanine, tyrosine, cysteine, glutamine, alanine, glycine, serine, histidine, tryptophan and proline. Transport of these amino acids is electrogenic with one sodium being transported with the amino acid across the apical membrane. B^0 AT1 (SLC6A19) has recently been cloned (158, 283). This transporter is expressed on the proximal tubule (250), and transports all neutral amino acids, though there is greater affinity for valine, leucine, isoleucine, and methionine (49). Mutation of SLC6A19 causes Hartnup disease which is an autosomal recessive disorder of variable expression, characterized by a pellagra-like rash, cerebellar ataxia, psychological and neurological disturbances (60, 158, 283). There are other neutral amino acid transporters but their transport properties have been less well characterized (60, 243) (250).

The acidic amino acids are aspartate and glutamate. They are transported across the apical membrane of the proximal tubule in an electrogenic fashion where two sodium ions are transported for each of these negatively charged amino acids (150, 257). Brush border membrane vesical studies have shown that there are at least two apical transporters for glutamate, one with a high substrate affinity and one with a low affinity (330). The high affinity transporter has been cloned and designated EAAC1 (149). EAAC1 is expressed on the apical membrane of the proximal tubule (288). Eaac-1 knockout mice have a dicarboxylic aciduria proving the importance of this transporter in acidic amino acid transport (218). The basolateral transport of glutamate is via a sodium dependent cotransporter, indicating that the intracellular glutamate levels must be very high in the proximal tubule to provide an adequate driving force for sodium exit across the basolateral membrane (256). In addition there is a sodium independent aspartate/glutamate transporter which is localized to the basolateral membrane designated AGT1 (186).

The basic amino acids lysine and arginine utilize the same amino acid transporter as cystine. There are a number of basic amino acid transporters (280). rBAT is a cystine/dibasic amino acid transporter expressed along the proximal tubule but predominantly in the S_3 segment (43, 103). rBAT protein is undetectable in the fetal kidney and is expressed at very low levels even after weaning (110). Mutations in SLC3A1, the gene encoding rBat, results in type I cystinuria (65, 66, 222, 223). B^{0+} AT is a recently cloned cystine transporter/dibasic amino acid transporter (102, 105). Unlike rBAT, B^{0+} AT is predominantly expressed in the early proximal convoluted tubule but it overlaps with the expression of rBAT (103, 201). While both rBAT and B^{0+} AT can function as cystine/dibasic amino acid transporters, they likely function in vivo as a heterodimer (219) (103).

Neonates have a generalized aminoaciduria which is more pronounced in premature neonates (59, 89, 279). While there have been numerous studies examining amino acid transport using a number of species, most

have utilized kidney slices or slurries of renal tubules. These studies are complicated by the fact that one is looking simultaneously at cellular uptake, often from a collapsed tubule, metabolism of the amino acid and basolateral exit. Examinations of glycine transport in tubule suspensions and kidney slices of neonates and adults have provided disparate results. Glycine uptake has been shown to be the same in neonates and adults in some studies (241, 252) and lower in neonates than adults in others (19, 191). The few studies looking at brush border membrane during development, a more direct way of studying uptake transport across the apical membrane, have shown lower rates of transport in the neonate, compared to the adult (73, 192).

Organic Acid Transport

In addition to filtration and reclamation, the kidney has the ability to secrete some substances through organic anion transporters. There are five organic anion transporters on the basolateral membrane (OATS) of the proximal tubule (20, 167, 282, 339, 344). Different OATS have different substrate specificities. Organic acids transported by OATS include prostaglandins, uric acid, nonsteroidal anti-inflammatory drugs, β lactam antibiotics, antiviral medications, para-amino hippurate, probenecid, uric acid, bumetanide, salicylates, methotrexate and many others (167, 282, 339). Many of these substances are protein bound in the blood which limits their excretion by glomerular filtration. The basolateral uptake of OATS is an example of tertiary active transport (167, 282). OATS take up organic anions into the cell predominantly in exchange for α-ketoglutarate. The α-ketoglutarate that is exchanged for the organic acid enters the cell via an α-ketoglutarate transporter. The energy for this whole process is the basolateral Na^+-K^+-ATPase. The organic acid transported inside the cell must exit across the apical membrane to enter the primordial urine. The mechanism for this is less well understood but includes members of the multidrug resistance protein family (167, 282, 339), which has been localized to the apical membrane of the proximal tubule (318).

Para-amino hippurate is almost totally removed from the blood with one pass through the kidney and is used as a measure of renal blood flow. Para-amino hippurate has been used to assess the maturation of renal blood flow in humans and to determine the maturational changes in organic anion transport. Studies in humans have shown that there is a maturational increase in para-amino hippurate secretion with adult values being attained at about 2 years of age (64, 253). Para-amino hippurate secretion is less in premature than term neonates (101).

There are a number of factors that could contribute to the maturational increase in organic anion secretion. Since organic anion secretion requires an organic anion transporter (OAT), a sodium dependent organic acid cotransporter and the Na^+-K^+-ATPase to mediate intracellular transport of the organic acid and an apical secretory mechanism to secrete the organic acid, a developmental paucity in any of these transporters, compared to the adult, could be the rate limiting step. OAT1 and OAT2 have been shown to be present in the late gestation fetus and mRNA and protein expression increase during postnatal development (207, 217).

One of the unique features of organic anion transport is that it can be induced prematurely during renal development by itself or another organic anion (134–136, 276). This is not true of adult animals where organic anions do not cause a stimulation in transport (134). In vitro microperfusion studies demonstrated that there was an intrinsic increase in the rate of transport with postnatal age in rabbits and that pretreatment with penicillin increased the rate of para-amino hippurate secretion in vitro (276). The mechanism of this induction of organic anion transport by organic acids is unclear.

Phosphate Transport

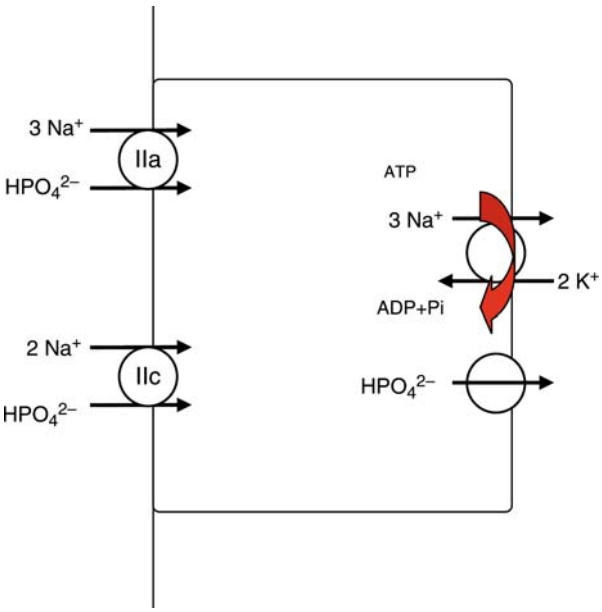
The adult ingests approximately 1–1.5 g of phosphorus a day and 80% of that is absorbed. The adult must be in neutral phosphorus balance: the amount of phosphorus absorbed from day to day has to equal that excreted. The phosphorus in our body is predominantly in the form of phosphate. At a pH of 7.4 there is a 4:1 ratio of HPO_4^{-2}/$H_2PO_4^{-1}$. The kidney maintains phosphate balance by its ability to regulate phosphate transport, which occurs predominantly in the proximal tubule. The main factors that regulate renal phosphate transport are dietary intake itself, and a number of hormones including parathyroid hormone, FGF-23, and growth hormone. Phosphate is essential for bone growth and 85% of our phosphate is in the bones. In addition, phosphate is involved in a myriad of enzymatic reactions and is present in nucleotides, phospholipids, and proteins. Unlike the adult, the neonate must be in positive phosphate balance for growth. The serum phosphate level is higher in the neonate than in the adult. This section will review phosphate transport and then discuss developmental changes which occur in transport and its regulation, which allow the neonate to be in positive phosphate balance.

The transporters involved in the regulation of phosphate transport are shown in *Fig. 3-7*. The first phosphate transporter cloned, designated NaPi-1, did not have the characteristics previously identified in physiologic studies and its function is still not clear (333) (44). There are two sodium-dependent phosphate transporters on the apical membrane of the proximal tubule, one designated NaPi-IIa (183), and the other NaPi-IIc (281). NaPi-IIa is an electrogenic transporter that transports three sodium ions with one phosphate HPO_4^{-2}, while NaPi-IIc transports two sodium ions with every phosphate and is electroneutral (107, 281). NaPi-IIa is regulated by PTH and dietary phosphate intake (155, 172, 173, 310). Phosphate exits the proximal tubule by a transporter which has not yet been identified and characterized. NaPi-IIb is the phosphate transporter on the intestinal apical membrane responsible for absorption of dietary phosphate (133).

The serum phosphate levels are higher in neonates than adults (77, 137). Since the glomerular filtration rate in the neonate is only a fraction of that of the adult, it is possible that this is the factor that is responsible for the relative hyperphosphatemia in neonates. However, early studies in human neonates found that the fraction of phosphate reabsorbed compared to the glomerular filtration rate was higher in neonates and infants than that in adults (77, 83, 137, 242). In the first 24 h of life, human neonates reabsorb over 95% of the filtered phosphate (137). This level drops to 90% later in the first week of life (77, 137). This fractional reabsorption of phosphate meets or exceeds the reabsorptive capacity of adults and older children eating a normal phosphate diet (242).

Figure 3-7

A proximal tubule cell reabsorbing phosphate is shown. The top apical phosphate transporter NaPi-IIa is the predominant phosphate transporter in adult rodents. NaPi-IIc is the predominant phosphate transporter on the apical membrane of neonatal rodents. NaPi-IIa is electrogenic while NaPi-IIc is electroneutral.



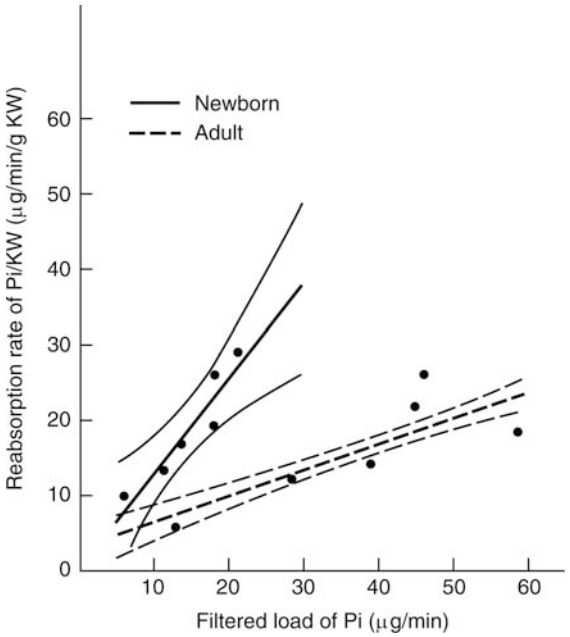
Animal studies demonstrated that young rats had a greater rate of phosphate reabsorption than adult rats (69). This was seen in rats that received parathyroidectomy, indicating that an altered response to parathyroid hormone was not the factor that caused the disparity in renal phosphate uptake (69). Finally, both young and adult rats responded to a low phosphate diet with an increase in the fractional reabsorption of phosphate; the magnitude of phosphate absorption was again higher in young animals (69).

While the above studies are consistent with an enhanced tubular reabsorptive capacity in neonates, this could be due to a higher reabsorptive capacity of the neonatal tubule, a diminished response to a phosphaturic factor or the result of an increased response to a substance which increases phosphate transport. To determine if there was an inherent increase in tubular phosphate transport, Johnson and Spitzer examined phosphate absorption in neonatal and adult kidneys perfused in vitro (147). As shown in *Fig. 3-8*, neonatal kidneys had a higher phosphate reabsorptive rate at any filtered load of phosphate which can only be due to an increased inherent rate of phosphate transport. In addition, they found that while addition of parathyroid hormone to the perfusate caused a phosphaturia in adult kidneys, there was no increase in phosphate excretion in neonatal kidneys. These studies directly demonstrate that neonates also have an attenuated effect of the parathyroid hormone, the primary factor regulating phosphate transport. A blunted effect of parathyroid hormone on phosphate reabsorption has also been demonstrated in young rats compared to adult rats in vivo (125, 328).

Micropuncture studies of neonatal and adult guinea pigs and rats have also demonstrated that there is a higher intrinsic rate of phosphate transport in young animals (153) (336). Studies using brush border membrane vesicles have demonstrated that the maximal rate (Vmax) of phosphate transport was several-fold higher in neonates than in adults, while there was no difference in the Km, the phosphate concentration at half maximal velocity (209). A low phosphate diet increased the Vmax of the

Figure 3-8

The rate of phosphate absorption by isolated perfused kidney preparation. As the filtered load increases, there is an increase in the reabsorptive rate in both the neonate and the adult kidney. At all filtered loads the rate of phosphate reabsorption per gram of kidney weight was higher in the neonate. From (147), with permission.



sodium phosphate transporter in adult guinea pigs, while a high phosphate diet had the opposite effect. There was no significant difference in Vmax in brush border membranes from neonates gavaged with different phosphate containing diets (209). As shown in *Fig. 3-9*, the 3-week-old rat has a maximal rate of phosphate absorption that was higher than that of adult rats (205). At all ages, there was an augmentation in the maximal capacity of phosphate reabsorption with a low phosphate diet (205). A greater effect of phosphate deprivation on brush border membrane vesicle phosphate uptake and NaP-IIa protein abundance was demonstrated in 4-week-old rats compared to older adult rats (336). Finally, the driving force for phosphate entry across the apical membrane may be greater in neonates. The intracellular phosphate concentration was almost 40% lower in kidneys measured using NMR (21).

Maturational studies examining the changes in NaPi-IIa expression have revealed that NaPi-IIa mRNA and protein are not detected in developing nephrons until there is a distinct brush border membrane (314). NaPi-IIa protein abundance was greater in brush border membranes from 13-day-old rats compared to 22-day-old rats (314). Others however have found that brush border membrane vesicle from suckling and adult rats had a slower rate of phosphate uptake than weanling rats (21 days old) (312). There was no change in NaPi-IIa mRNA abundance, but NaPi-IIa protein abundance from brush border membrane vesicles confirmed the transport findings that 21-day-old rats had the highest NaPi-IIa protein expression (312). Studies comparing 28-day-old rats to adult rats have also demonstrated greater brush border membrane NaPi-IIa protein abundance in young rats than in adults (336).

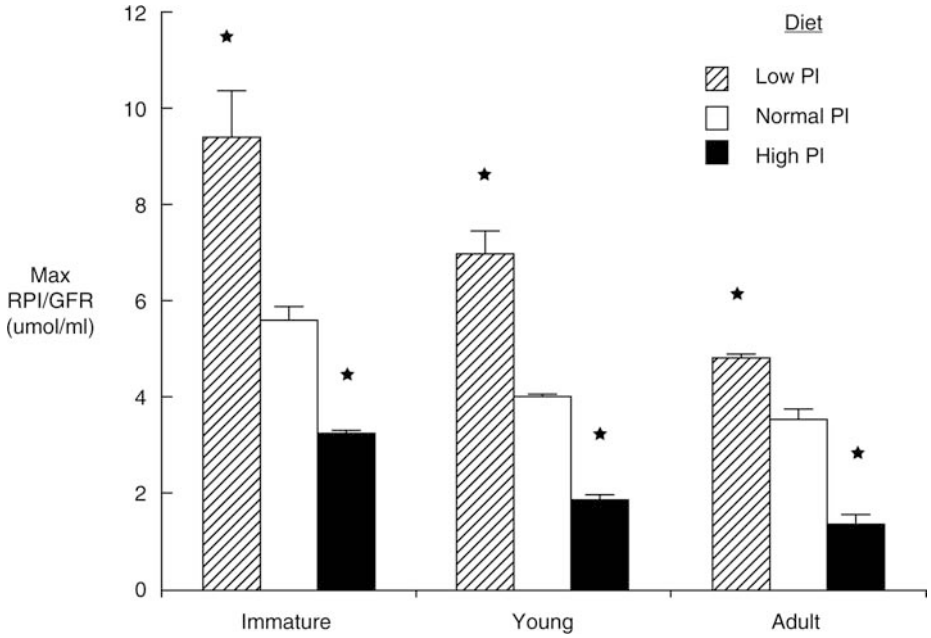
The high rates of phosphate transport in weanling rodents suggested that there may be a developmentally regulated transporter with greater expression at that of development (304). Indeed, NaPi-IIc was recently cloned and is a brush border phosphate transporter with its highest expression at the time of weanling in the rat (281). NaPi-IIc, like NaPi-IIa, is regulated by dietary phosphate uptake (281). The relative importance time of NaPi-IIa and NaPi-IIc may be quite different in humans. Patients with hereditary hypophosphatemic rickets with hypercalciuria, a rare autosomal recessive characterized by hypophosphatemia secondary to renal phosphate wasting and high vitamin D levels, have a mutation in the gene encoding NaPi-IIc, suggesting that NaPi-IIc may be the predominant renal phosphate transporter in humans and that NaPi-IIa cannot compensate for the loss of NaPi-IIc (40).

Growth hormone increases phosphate transport via stimulation of IGF-1 in the proximal tubule (228). Brush border membrane vesicle phosphate transport increased in dogs that were administered growth hormone compared to vehicle treated controls (122). While growth hormone is not a significant regulator of phosphate transport in the adult, this may not be the case in the growing animal. Administration of a growth hormone-releasing factor antagonist, which suppresses growth hormone secretion, has no effect on phosphate transport in adult rats, but significantly reduces phosphate absorption in young growing rats (124, 206, 338).

There are a number of hormones that have been shown to regulate phosphate transport including insulin (84), fibroblast growth factor-23 (34, 57, 291), frizzled-related protein 4 (41) and klotho (164, 227). These hormones may have differential effects on phosphate transport in the neonate and adult, but this is yet to be determined.

Figure 3-9

Age dependent maximal phosphate reabsorption is seen in 3–4 week old rats (immature), 6–7 week old rats (young), 12–13 week old rats (adult). All rats were parathyroidectomized. A low dietary phosphate intake stimulated phosphate absorption in all age groups. The maximal capacity for phosphate absorption was in the immature groups which need phosphate for growth. From (205), with permission.



Proximal Tubule Acidification

The proximal tubule reabsorbs 80% of the filtered bicarbonate. Luminal proton secretion is via the Na^+/H^+ exchanger and the H^+-ATPase. In the adult, one-third of proton secretion is via the luminal H^+-ATPase, and two-thirds is mediated by the luminal Na^+/H^+ exchanger that is designated NHE3 (24, 225). The secreted proton titrates the filtered HCO_3^- to generate H_2CO_3 which is converted to CO_2 and H_2O by luminal carbonic anhydrase (Carbonic anhydrase IV). CO_2 diffuses into the cell and combines with H_2O, which is facilitated by intracellular carbonic anhydrase (Carbonic anhydrase II) to regenerate H_2CO_3. H_2CO_3 dissociates into a proton that can be secreted across the apical membrane, and bicarbonate that exits the basolateral membrane via the basolateral $Na(HCO_3)_3$ symporter. The sodium which enters the cell via the Na^+/H^+ exchanger exits the basolateral membrane by the Na^+-K^+-ATPase, which provides the driving force for luminal proton secretion by the Na^+/H^+ exchanger. There are maturational changes in most of these processes that will be described below.

Neonates have a lower serum bicarbonate concentration than adults, which is the result of a lower threshold for bicarbonate (92). Premature neonates can have physiologic bicarbonate concentrations as low as 15 mEq/l (277). The lower bicarbonate threshold is mediated in large part by the lower rate of proximal tubule acidification. The fine tuning of renal acidification is mediated in the distal nephron which, by and large, is responsible for the secretion of acid from metabolism and new bone formation. Studies have shown that there is a maturational increase in bicarbonate absorption during postnatal development (30, 273), which accounts for the mutational increase in the bicarbonate threshold.

The greatest developmental changes in proximal tubule acidification occur on the apical membrane. There is a four-fold increase in Na^+/H^+ exchanger activity during postnatal maturation and an even greater increase in apical H^+-ATPase activity (23, 24, 284). Low levels of Na^+/H^+ exchanger activity have been measured in the fetus as well (37). Despite the fact that there was Na^+/H^+ exchanger activity on the apical membrane of the

neonatal rat at 1–2 weeks of age, as shown in *Fig. 3-10*, there was virtually no NHE3 on the brush border membrane (38, 284), the Na^+/H^+ exchanger on the apical membrane of the adult proximal tubule (45, 340).

NHE3 knock-out mice have been shown to have substantive proximal tubule apical membrane Na^+/H^+ exchanger activity (74). The apical Na^+/H^+ exchanger likely responsible for this non NHE3 Na^+/H^+ exchanger activity is NHE8, a recently discovered NHE isoform (116, 117). NHE8 has sodium dependent proton extrusion capabilities, which made it a potential candidate for the developmental isoform (345). As shown in *Fig. 3-10*, apical NHE8 was predominantly present in neonatal proximal tubules at a time when NHE3 was almost undetectable (38). Thus there is a developmental Na^+/H^+ exchanger isoform.

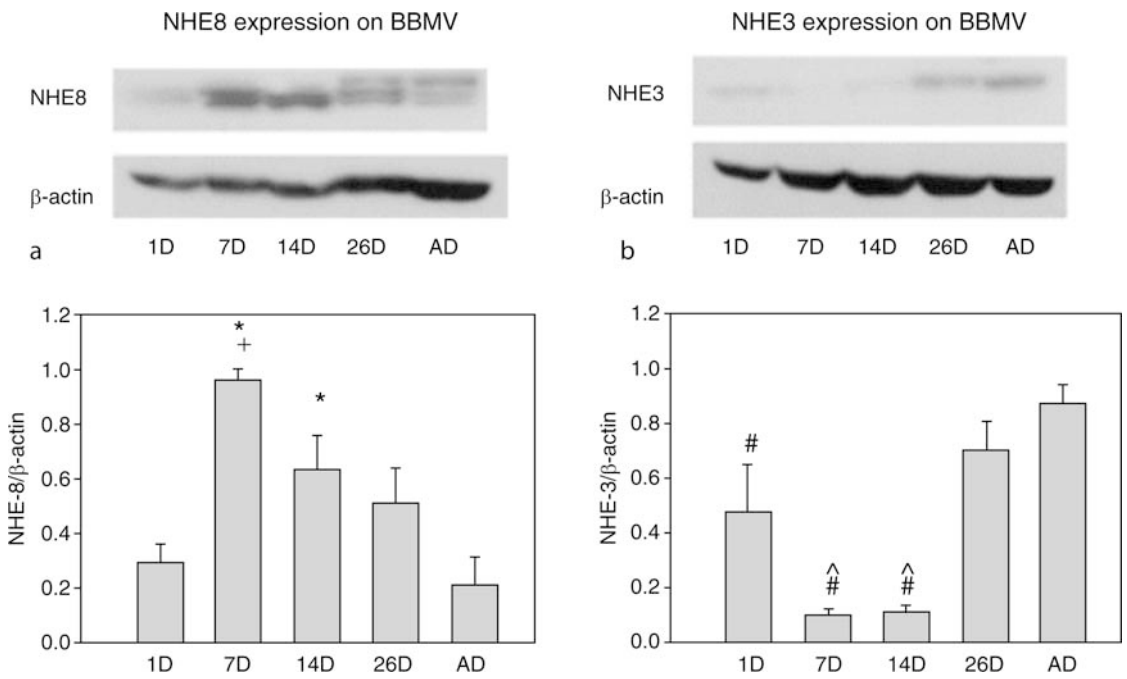
The $Na(HCO_3)_3$ symporter mediates bicarbonate exit in both the neonatal and adult proximal tubule. While there is a maturational increase in basolateral membrane $Na(HCO_3)_3$ symporter activity, it is relatively small compared to the magnitude of the change in Na^+/H^+ exchanger activity on the apical membrane (31). In addition to bicarbonate exit, the basolateral membrane Na $(HCO_3)_3$ symporter plays an important role in pH regulation of the proximal tubule cell (31).

Carbonic anhydrase which increases the rate of the interconversion of CO_2 and H_2O to Carbonic anhydrase II is located intracellularly in proximal and distal tubule acidifying cells and comprises ~95% of cell carbonic anhydrase activity. Carbonic anhydrase IV is on the apical and basolateral membrane of renal acidifying cells and comprises ~5% of carbonic anhydrase activity (275). Both carbonic anhydrase II and IV increase during maturation of proximal and distal acidification, but neither is likely a limiting factor causing the maturational increase in renal acidification in the proximal or distal tubule (335) (152, 278).

Since the developmental increase in renal acidification is due primarily to apical proton secretion, many studies have examined the cause for the increase in Na^+/H^+

Figure 3-10

Immunoblots of rat brush border membrane vesicles depict the changes in NHE8 (a) and NHE3 (b) protein abundance. As is seen, there is higher expression of NHE8 in the neonate than in the adult brush border membrane. The expression of NHE3 is highest in the adult. The higher NHE3 protein abundance at 1 day of age is likely the result of the surge of glucocorticoids at the time of birth. From (38), with permission.



exchanger activity. There is a substantive increase in both thyroid hormone and glucocorticoids during postnatal development and both hormones increase in parallel with the increase in proximal tubule Na^+/H^+ exchanger activity (28, 131, 132, 323). Administration of either glucocorticoids or thyroid hormone prior to the maturational increase in either hormone results in a precocious increase in exchanger Na^+/H^+ activity and NHE3 protein abundance (28–30). Both thyroid hormone and glucocorticoids increase NHE3 activity by increasing transcription (25, 67). Glucocorticoids have also recently been shown to increase the insertion of NHE3 into the apical membrane of proximal tubular cells by a posttranscriptional mechanism (48).

Interestingly, neither prevention of the maturational increase in glucocorticoids or thyroid hormone alone can totally prevent the postnatal increase in Na^+/H^+ exchanger activity and NHE3 mRNA and protein abundance (27, 28, 30, 287). Thus, there appears to be some redundancy in the postnatal maturational triggers for NHE3. As demonstrated in *Fig. 3-11*, prevention of the maturational increase in both hormones results in the total prevention of the postnatal increase in NHE3 mRNA, protein abundance and Na^+/H^+ exchanger activity (119).

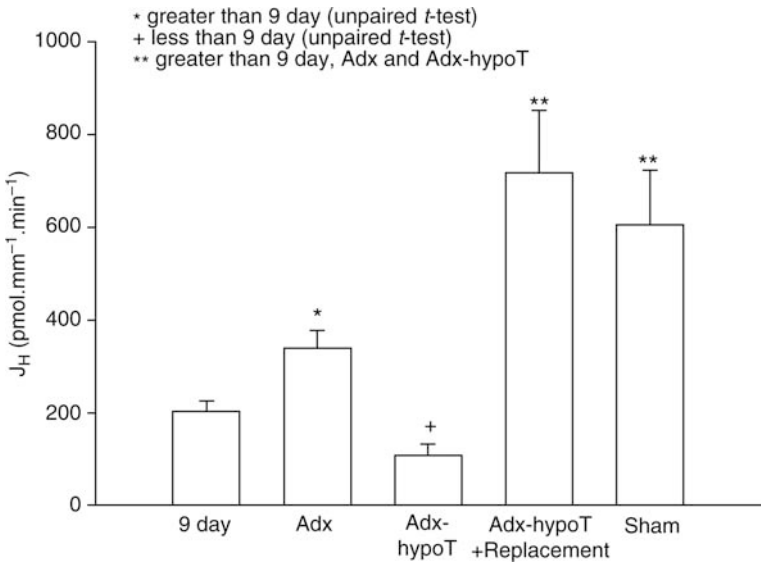
Proximal Tubule NaCl Transport

The glomerulus receives an ultrafiltrate of plasma and as shown in *Fig. 3-12*, immediately changes the luminal composition of the tubular fluid. The early proximal tubule reabsorbs glucose, amino acids and bicarbonate in preference to chloride (176, 239). The reabsorption of sodium with glucose and amino acids results in a lumen negative potential difference. This leaves the luminal fluid in the late proximal tubule with a higher chloride and lower bicarbonate concentration than that of the peritubular fluid.

The axial changes in luminal fluid create the potential for passive chloride diffusion across the paracellular pathway. The lumen to peritubular chloride gradient provides a driving force for the passive diffusion of chloride across the paracellular pathway. In the early proximal tubule, the transcellular reabsorption of sodium with glucose and

Figure 3-11

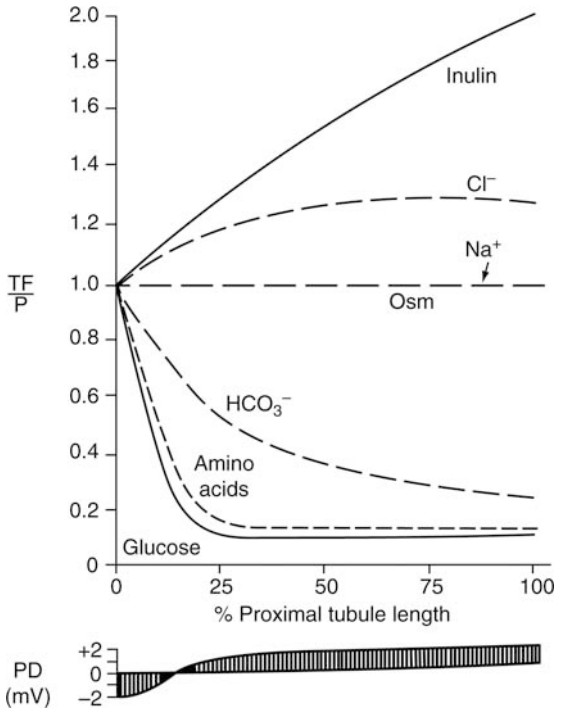
Apical Na^+/H^+ exchanger activity in perfused tubules in 9 day old, and adults that were adrenalectomized as neonates, adults that were adrenalectomized and hypothyroid since the neonatal period, adults that were adrenalectomized and hypothyroid (adx-HypoT) since the neonatal period but given thyroid and glucocorticoid replacement before study and sham controls. The 9 day old rats had a lower rate of Na^+/H^+ exchanger activity than the sham adult. Neonatal adrenalectomy did not totally prevent the maturational increase in Na^+/H^+ exchanger activity, but the maturational increase in Na^+/H^+ exchanger activity was abrogated in the adrenalectomized hypothyroid group. From (119), with permission.



amino acids results in a lumen negative potential difference as depicted in *Fig. 3-12*. This lumen negative potential provides a driving force for either chloride absorption or sodium secretion across the paracellular pathway. Whether chloride is absorbed or sodium is secreted is dependent on the relative permeabilities of chloride to sodium in the proximal tubule. The relative chloride to sodium permeability is higher in the adult than the neonate, so passive chloride transport secondary to the lumen negative potential difference in the early proximal tubule does not occur to a significant degree in the neonate (33, 230). As shown in *Fig. 3-12*, the luminal chloride gradient in the late proximal tubule results in a driving force for chloride diffusion across the paracellular pathway (33, 230). The diffusion of chloride across the paracellular pathway results in a lumen positive potential difference, and a driving force for the paracellular transport of sodium.

Figure 3-12

The axial changes in proximal tubular transport are depicted in this figure. The early proximal tubule preferentially reabsorbs glucose, amino acids and bicarbonate, leaving the luminal chloride solution higher than the blood in the peritubular capillaries. The early proximal tubule has a lumen negative transepithelial potential difference due to sodium dependent glucose and amino acid reabsorption. The higher luminal chloride concentration in the late proximal tubule provides a driving force for passive chloride absorption across the paracellular pathway, causing a lumen positive potential difference. From (239), with permission.



In the adult proximal tubule, approximately half of the sodium chloride is active and transcellular and half is passive and paracellular (3, 26, 287). Active chloride transport is mediated by parallel action of the Na^+/H^+ and Cl^-/base exchangers on the apical membrane (17, 286, 287, 290). It is still somewhat unclear what the nature of the base is as there is evidence for chloride exchange for hydroxyl, formate, and oxalate ions (14–16, 18, 165, 287). The rate of active transcellular chloride transport is lower in the neonate than in the adult. This is due to the lower rate of the apical Cl^-/base exchanger (285, 287), and the Na^+/H^+ exchanger discussed above (23, 24, 284). The driving force for active transcellular NaCl transport is the basolateral Na^+/K^+-ATPase that has lower activity in the proximal tubule (139, 271, 274). There are a number of factors that regulate proximal tubule NaCl transport, including renal nerves, dopamine, and angiotensin II, which are shown in *Table 3-1*. The serum levels of most hormones that regulate sodium absorption are equal or higher in the neonate than in the adult, but in general there is a blunted response to the action of most regulator hormones.

There are also changes in the properties of the paracellular pathway during postnatal development (1, 33, 230, 289). Most importantly, the permeability of the proximal tubule to chloride ions is less in the neonate than in the adult (33, 230, 289). The low permeability to chloride ions results in almost no passive paracellular chloride transport in the neonate (33, 230). As discussed, the permeability properties of an epithelium are determined by the expression of a family of proteins called claudins. The claudin proteins in the tight junction change during postnatal development. Claudins 6, 9, and 13 are present in the neonatal proximal tubule but not in the adult (1). The claudin isoform responsible for the low paracellular chloride permeability in the neonate as well as the factors that cause the claudin isoform changes during development are yet to be determined.

Of the potential factors that cause the maturational changes in paracellular chloride transport, only the thyroid hormone has been examined (32). Administration of thyroid hormone prior to the normal maturational increase results in an increase in chloride permeability. On the other hand, maintaining a hypothyroid state into adulthood prevents the maturational increase in chloride

Table 3-1

Comparison of the serum levels of hormones and their effect on sodium transport in neonates compared to adults

Hormone	Effect on urinary sodium excretion in adult	Neonatal serum level compared to adult	Effect of hormone on sodium in neonatal sodium excretion compared to adult	References
Renin		↑	Level responds appropriately in neonate	(88, 104, 114, 309)
Aldosterone	Increases distal sodium absorption	↑	↓	(8, 39, 104, 293, 307, 309, 319)
Atrial natriuretic peptide	Increases sodium excretion	↑	Probably (93) ↓	(58, 245, 251, 329)
Dopamine	Decreases proximal tubule sodium absorption	↑	↓	(9, 109, 151, 174)
PGE-2	Causes natriuresis and diuresis	Blunted synthesis in the medullary and cortical collecting tubule (269)	↓	(185, 215)

permeability. It is yet to be determined if thyroid hormone is the factor that causes the proximal tubule claudin isoform changes during postnatal development.

Proximal Tubule Water Transport

The proximal tubule reabsorbs most of the glomerular filtrate without a significant change in the luminal osmolality. For this to occur, the proximal tubule must be very permeable to water. Water movement is predominantly through the cell and not across the paracellular pathway (224, 231). The constitutively water permeable proximal tubule and thin descending limb have water channels on the apical and basolateral membranes (255). The isoform of this water channel was previously designated CHIP-28 but has been renamed aquaporin 1 (210–212, 226). Direct evidence of transcellular water transport comes from aquaporin 1 knock-out mice which have a marked decrease in proximal tubule sodium absorption (272). There is a paucity of water channels in the fetal kidney and an increase in expression of aquaporin 1 does not occur until birth (50, 303).

Direct measurements of water permeability have demonstrated that the neonatal rabbit proximal tubule has a higher water permeability that that of the adult (229). To determine the mechanism for the higher water permeability in neonatal rabbit tubules, studies were performed examining the water permeability of apical and basolateral membrane vesicles (202, 235, 234). Despite the higher transepithelial water permeability, the water permeability of the apical and basolateral membrane were lower in the neonate than the adult and there was less aquaporin 1 expression on both the apical and basolateral membranes of the proximal tubule of the neonate (234, 235). The apparent paradox between the higher water permeability in the neonatal proximal tubule and the lower water permeability of the apical and basolateral membranes was resolved with measurements of the contribution of the intracellular compartment to water movement in the neonatal and adult proximal tubule. The intracellular compartment was found to cause a large resistance to water flow and the neonatal proximal tubule intracellular compartment was less of a constraint to transcellular water movement than that of the adult (231).

The postnatal increase in glucocorticoids is a likely factor in mediating the above maturational changes in water permeability and aquaporin 1 expression. Administration of glucocorticoids to neonatal rabbits resulted in an increase in brush border membrane water permeability and aquaporin 1 expression (203). However, the developmental increase in thyroid hormone was shown not to be a factor mediating these postnatal maturational changes in water transport (204).

Thick Ascending Limb

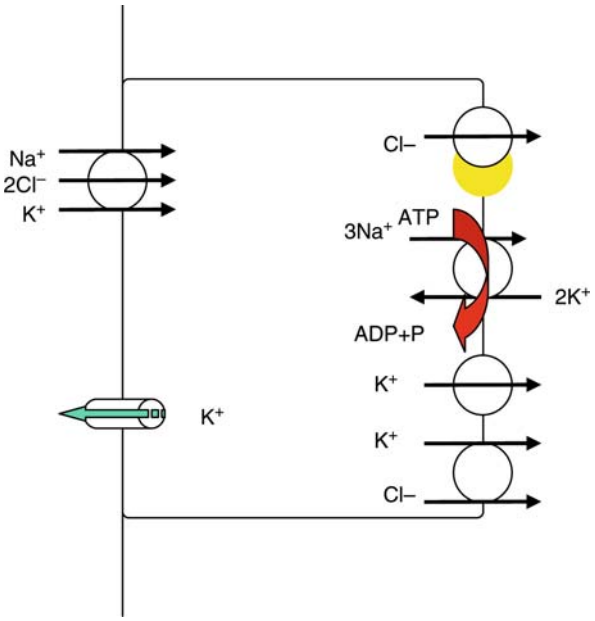
The thick ascending limb is responsible for the reabsorption of 30% of filtered NaCl. The thick ascending limb is

impermeable to water. The reabsorption of NaCl without water by the cortical and medullary TAL along with the distal convoluted tubule generates a luminal fluid with an osmolality of 50 mOsm/kg water. In the absence of ADH action on the collecting tubule, this dilute urine will be excreted. The medullary interstitial hypertonicity is largely generated by active NaCl reabsorption without water by that segment.

The reabsorption of NaCl is due to secondary active transport with the basolateral Na^+-K^+-ATPase generating a low intracellular sodium to provide a driving force for luminal sodium entry. A cartoon of a thick ascending limb cell is shown in *Fig. 3-13.* Sodium enters the cell via the furosemide or bumetanide sensitive $Na^+/K^+/2Cl^-$ cotransporter (128, 129). The $Na^+/K^+/2Cl^-$ cotransporter is electroneutral, yet there is a lumen positive transepithelial potential difference in the thick ascending limb (118). The positive luminal potential is generated by apical potassium recycling via the potassium channel ROMK1 (196, 341). With recycling of potassium back into the lumen, there is the net reabsorption of one sodium and two chloride ions which exit the cell across the basolateral membrane. Sodium exits the cell via the Na^+-K^+-ATPase. Chloride predominantly exits the cell via a chloride channel designated ClC-Kb (2, 156, 161). A subunit of this chloride channel designated barttin is important in the function of ClC-Kb (97). The lumen positive potential difference, generated by potassium secretion into the lumen, generates a driving force for the passive reabsorption of cations including Mg^{++} and Ca^{++}. The thick ascending limb has a very high permeability to magnesium and calcium ions, which results in a substantial fraction of filtered calcium and magnesium being reabsorbed passively across the paracellular pathway in this segment (56, 61, 62, 144, 248). The unique permeability properties of the thick ascending limb are due to the expression of claudin 16 which is mutated in familial hypomagnesemia with hypercalciuria and nephrocalcinosis where there is renal magnesium and calcium wasting (301).

Figure 3-13

The figure depicts a thick ascending limb cell. On the apical membrane is the $Na^+/K^+/2Cl^-$ cotransporter that is sensitive to loop diuretics and the potassium channel designated ROMK that is depicted as the green arrow. The basolateral membrane has a Na^+-K^+ ATPase, a KCl cotransporter, a chloride channel designated ClC-Kb, with its accessory channel designated barttin in yellow and a potassium channel. A loss of function mutation in the $Na^+/K^+/2Cl^-$ cotransporter, ROMK, ClC-Kb, or barttin results in Bartter's syndrome.



Mutations of the $Na^+/K^+/2Cl^-$ cotransporter (297), ROMK (179, 298), ClC-Kb (296) and barttin (46, 97) all cause Bartter's syndrome. Barttin is also expressed in the ear where it is a subunit of ClC-Ka and ClC-Kb. Mutations in barttin cause sensory neural hearing loss (46, 97).

Micropuncture studies of fluid from the early distal tubule showed that the osmolality of the fluid was significantly lower in the adult than in the neonatal rat (347). This is consistent with lower rates of sodium transport in the thick ascending limb but this study did not examine the latter parts of the diluting segment. Human neonates are able to dilute their urine to the same level as an adult (50 mOsm/kg water). This is vital as neonates ingest a hypotonic fluid, mother's milk. Sodium transport in the thick ascending limb has been directly examined in vitro and shown to be five-fold lower in the neonate than in the adult (138).

The $Na^+/K^+/2Cl^-$ cotransporter can first be detected in the mid-late gestation rat's thick ascending limb and macula densa (170). In the rat, there is a postnatal maturational increase in $Na^+/K^+/2Cl^-$ cotransporter, ROMK, Na^+-K^+-ATPase mRNA and protein abundance, but no change in ClC-K mRNA abundance (308). Administration of dexamethasone before the normal maturational increase at the time of weaning resulted in a premature increase in urinary concentrating ability and increase in $Na^+/K^+/2Cl^-$ cotransporter, Na^+-K^+-ATPase mRNA and protein abundance, but no change in ROMK protein abundance (308). There is also a postnatal maturational increase in thick ascending limb Na^+-K^+-ATPase activity

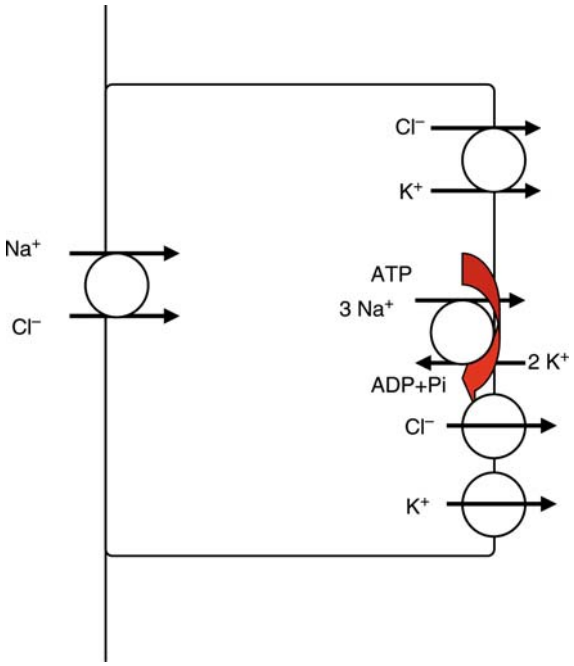
(238, 271) (87). The maturational increase in thick ascending limb Na^+-K^+-ATPase activity could be accelerated precociously by glucocorticoids and prevented by neonatal adrenalectomy (87, 237).

Distal Convoluted Tubule

The distal convoluted tubule reabsorbs approximately 7% of the filtered sodium. This segment is water impermeable and further reabsorption of salt without water occurs in this segment resulting in the nadir of luminal fluid osmolality. The transporters responsible for NaCl transport in the distal convoluted tubule are shown in *Fig. 3-14.* Sodium entry is via the thiazide sensitive cotransporter (94, 213, 292, 322). Mutations in the thiazide sensitive cotransporter cause Gitelman's syndrome (299, 300, 302). There is also evidence for parallel Na^+/H^+ and Cl^-/base exchange mediating sodium entry in the rat (324). The isoform of this Na^+/H^+ exchanger is NHE2 (72). Chloride exits the cell via a basolateral chloride channel ClC-K (178) and a KCl cotransporter KCNQ1 (175, 321). There is also a basolateral potassium channel (346). There is active transcelluar calcium and magnesium transport in the distal convoluted tubule but there is no information about the developmental expression and regulation of these transporters (81, 197).

Figure 3-14

Distal convoluted cell showing the transporters involved in active sodium reabsorption. The apical NaCl transporter is the thiazide sensitive cotransporter. Inactivating mutations in the thiazide sensitive cotransporter lead to Gitelman's syndrome.



The distal convoluted tubule is very difficult to study in vitro and in vivo. No studies have been performed to date, examining the relative abundance of the thiazide sensitive cotransporter in the neonate and adult. A study has been preformed that suggests that there is increased sodium absorption in 24- compared to 40-day-old rats (6, 115). Before detailing this study, it is important to know that studies have shown that the neonate is less able to excrete a salt load compared to the adult (5, 82, 115). For example, administration of isotonic saline equal to 10% of the dogs weight to an adult dog resulted in excretion of 50% of the salt load over 8 h but only 10% of the salt load was excreted in the 1–2 week old dog (115). This limited ability to excrete a sodium load was not due to the lower glomerular filtration rate in the neonate (115). Because sodium transport is less in the proximal tubule, thick ascending limb and collecting tubule, the nephron segment where there was avid neonatal sodium reabsorption was unclear. It must be stated that it was never considered that the volume of distribution of the saline infusion was greater in the neonate than the adult to account for these findings.

A micropuncture study suggested that the sodium retention in the neonate was the result of avid sodium reabsorption in the distal convoluted tubule (6). The tubular fluid to plasma Na/inulin (a volume marker) was greater in the early distal tubule in the 24-day-old neonate compared to the 40-day-old neonate due to decreased proximal and loop sodium absorption in the younger rat. This difference disappeared by the late distal tubule indicating that the 24-day-old rat had a higher rate of sodium absorption in that distal convoluted tubule. Volume expansion resulted in a higher distal delivery of sodium from the early proximal tubule of the 24-day-old rat, and there was less sodium remaining by the end of the distal nephron consistent with the distal convoluted tubule being the segment responsible for neonates' failure to excrete a sodium load compared to adults.

Urinary Concentration and Dilution

The urine exiting the distal convoluted tubule and entering the collecting duct has an osmolality of ~50 mOsm/kg water. Whether the urine will be of this osmolality or

maximally concentrated will depend on the presence or absence of vasopressin (ADH). Neonates can dilute their urine to nearly the adult level of nearly 50 mosm/kg water (7, 187, 249). Thus the neonates which imbibe mother's milk can excrete free water (169, 187, 249). However, unlike the normal adult where it is almost impossible to drink enough water to cause hyponatremia (30 l/day), neonates have a rather limited ability to generate and excrete free water and improperly mixed and dilute formula can result in hyponatremia (187).

The maximum urine osmolality of the human neonate is ~400–600 mOsm/kg water (123, 220, 334). The adult urine osmolality can be achieved at 1.5–2 years of age (334). There are many potential developmental factors that could limit the ability of the neonate to generate a maximally concentrated urine. The factors involved in urine concentration include:

1. The ability to sense an increase in serum osmolality or decrease in extracellular volume and secrete vasopressin.
2. There must be vasopressin receptors on the basolateral membrane of the collecting duct.
3. The collecting duct must be able to generate cAMP.
4. The loop of Henle must have generated a concentrated interstitium.
5. The architecture of the medulla must not limit the concentrating ability.
6. The collecting tubule must have aquaporins on the apical and basolateral membrane.
7. There must not be extracellular or intracellular mechanisms upregulated in the neonate, which limit urinary concentrating abilities.

During prenatal and postnatal maturation, there are anatomical changes which occur that likely affect urinary concentrating ability. There is an increase in the medullary capillary density, a decrease in medullary intersitial connective tissue, an increased presence and length of the thin limbs and the tubules become more tightly packed with cells in the loop decreasing in height with maturation (55, 315). The length of the papilla increased linearly from day 10 to day 40 in the rat (238). All these developmental anatomical changes are necessary for the counter current multiplication system to be maximally efficient. Accompanied by these anatomical changes are concomitant increases in the medullary sodium and urea concentration (238, 306). Administration of a high protein diet or urea to human neonates results in an increased ability to concentrate urine, implying that the ability of urea to accumulate in the medulla can be limited by dietary intake (90, 91). Adrenalectomy in neonatal rats prevented the maturational increase in urine osmolality while administration of glucocorticoids prior to the maturational increase caused a premature increase in urinary concentrating ability (238).

Principal cells in the collecting tubule and the cells in the medullary collecting duct express aquaporin 2 on the apical membrane and aquaporins 3 and 4 on the basolateral membrane (35, 52, 159, 313, 326). Aquaporin three null mice have diabetes insipidus whereas aquaporin 4 null mice only have a small concentrating defect after water deprivation indicating that basolateral water movement is primarily via aquaporin 3 (180, 181). Vasopressin causes the intracellular vesicles containing aquaporin 2 to fuse with the apical membrane resulting in the insertion of aquaporin 2 into the apical membrane (326). There is a developmental increase in aquaporin 2 expression (35, 52, 254, 342, 343). The maturational increase in aquaporin 2 is accelerated by administration of synthetic glucocorticoids prior to the normal postnatal increase in plasma glucocorticoids (343). Neither aquaporin 3 nor aquaporin 4 are factors impairing urinary concentration in the neonate (35, 157).

The fetus and neonate respond to increases in serum osmolality, stress and hypovolemia with appropriate increases in plasma vasopressin levels. The fetal sheep has an increase in plasma vasopressin with an infusion of hypertonic saline and increase in plasma osmolality (168, 294, 331). Plasma vasopressin also increases in fetal sheep in response to volume depletion induced by hemorrhage or diuretics (85, 168, 247) Despite the fact that the fetal lamb can increase serum vasopressin levels, infusion of vasopressin resulted in a blunted increase in urine osmolality compared to adult sheep (246). It is hard to assess these issues in humans. However, comparison between a relatively stressful vaginal delivery compared to a cesarian section has consistently demonstrated higher vasopressin levels in neonates born vaginally (86, 121, 221, 240). However, there was no correlation with vasopressin levels and the degree of perinatal asphyxia in one study (221), while there was a correlation in another (86). In total, it appears that the fetus and neonate can respond to appropriate stimuli with vasopressin secretion.

There is a developmental increase in vasopressin receptors in the kidney, however vasopressin receptor abundance does not appear to be a limiting factor in urinary concentration in the neonate (216, 236). Exogenous administration of vasopressin has been show to increase the urine osmolality in fetal sheep showing that vasopressin action on the collecting tubule is a prenatal event (246). Vasopressin acts in the collecting tubule by increasing cAMP. There is some discrepancy about the

extent of cAMP production during postnatal maturation in comparison to adults. The sum of the data indicate that while vasopressin stimulates cAMP production in the neonate, the amount of cAMP generated is attenuated compared to the adult (113, 148, 236, 270).

The response of the collecting tubule to vasopressin has been examined in vitro (54, 141, 232, 295). The neonatal water permeability did not increase in response to vasopressin comparably to that seen in the adult (54, 141, 232, 295). Studies have shown that the phosphodiesterase activity is greater in the neonatal collecting tubule (232). The water permeability of the neonatal collecting tubule was identical to the adult in the presence of a phosphodiesterase inhibitor demonstrating that the predominant factor limiting the action of vasopressin in the collecting tubule was the enhanced degradation of cAMP generated by vasopressin in the neonatal collecting tubule (232). Vasopressin increases prostaglandin production in the collecting tubule which attenuates the vasopressin mediated increase in cAMP production (51, 53). Vasopressin mediated cAMP production, while less in the neonatal collecting tubule, increases to adult levels in the presence of indomethacin, a prostaglandin synthesis inhibitor (53). Thus, prostaglandin production by the collecting tubule may attenuate the effect of vasopressin in the neonatal tubule to a greater extent than in the adult tubule (53).

Distal Tubule Acidification

The distal nephron makes the final adjustment in distal acidification. The distal nephron, under normal circumstances, secretes the protons equal to that generated from metabolism and in children the protons liberated from the formation of bone. The cortical collecting tubule has two types of cells that are involved in renal acidification. The α-intercalated cell is responsible for proton secretion and is shown in *Fig. 3-15*. There is also a β-intercalated cell, which can secrete bicarbonate when the animal is alkalotic or eats a diet with alkali content (190). This is unusual for humans but not for some mammals which can ingest an alkali diet (194). The β-intercalated cell has the reverse polarity of the α-intercalated cell but the Cl^-/HCO_3^- exchanger on the apical membrane of the β-intercalated cell is different from that on the apical membrane of the α-intercalated cell.

The neonatal cortical collecting tubule has fewer and less differentiated intercalated cells than that of the adult segment (98, 260). The rates of both bicarbonate secretion and luminal acidification are less in the neonate than in the adult (194, 260, 263). The intercalated cells in the inner medullary collecting duct were much more differentiated in appearance and secreted protons at a rate comparable to the adult (194, 263). The collecting tubule also has a luminal H^+-K^+ ATPase which causes proton secretion and potassium absorption from the luminal fluid. It is activated under states of metabolic acidosis and hypokalemia (120). The H^+-K^+ ATPase can function at comparable rates in the neonatal cortical collecting tubule to that of the adult (78).

Cortical Collecting Tubule Sodium Transport

While relatively little sodium is reabsorbed by the cortical collecting tubule compared to the proximal nephron segments, it is the final nephron segment responsible for regulating sodium absorption and thus is vitally important for the regulation of sodium homeostasis. Sodium absorption occurs in the principal cell of the collecting tubule which is shown in *Fig. 3-15*. Sodium transport is through the epithelial sodium channel designated ENaC which has three subunits. The driving force for sodium entry across ENaC is the low intracellular sodium concentration and the potential difference across the luminal membrane generated by the basolateral Na^+-K^+ ATPase. The Na^+-K^+ ATPase undergoes a maturational increase in this segment but is not the limiting factor for the maturational increase in sodium absorption (271). The abundance of ENaC on the apical membrane is by and large determined by aldosterone in the adult, but aldosterone has a blunted effect in the neonate, despite the fact that there are higher serum levels in the neonate and ample aldosterone receptors (8, 39, 104, 293, 307, 309, 319).

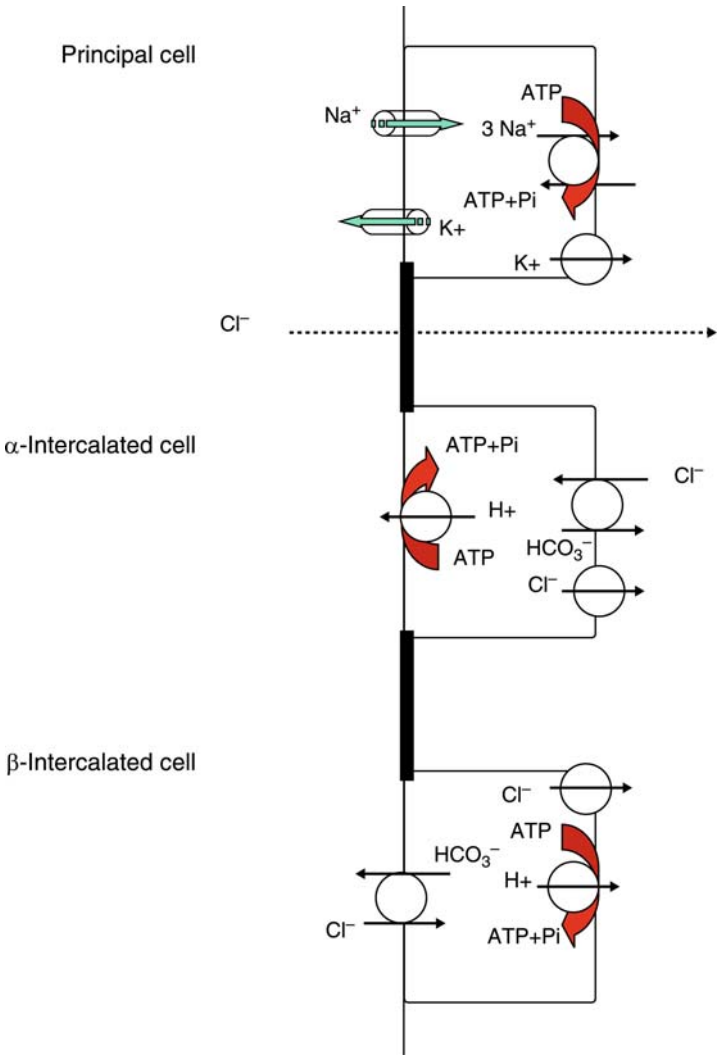
Sodium transport in the cortical collecting tubule increases with postnatal development (259, 319). This increase in sodium transport is paralleled by an increase in the apical expression of ENaC, as ENaC expression is the limiting factor in sodium absorption in this segment (261). ENaC is composed of α-, ß-, and γ-subunits and there is a developmental increase in mRNA and protein abundance of each during postnatal maturation (142, 261, 320, 327, 348).

Potassium Transport

Neonates have higher serum potassium levels than adults. Potassium is the predominant intracellular cation and neonates must be in positive potassium balance for

Figure 3-15

Three cells of the cortical collecting tubule are demonstrated. The principal cell is shown above with sodium entering the cell down its electrochemical gradient via a channel designated ENaC. This generates a lumen negative transepithelial potential difference. Potassium is secreted into the lumen down its electrochemical gradient via ROMK. Chloride is moving through the paracellular pathway down the electrical gradient generated by the lumen negative potential difference. The cell below is an alpha intercalated cell which secretes protons into the cell lumen via a H^+-ATPase. The bicarbonate generated in this process is secreted across the basolateral membrane via a Cl^-/HCO_3^- exchanger. The third cell is a beta intercalated cell which secretes bicarbonate ions in the face of metabolic alkalosis. This cell is the reverse of an alpha intercalated cell but the Cl^-/HCO_3^- exchanger is a different isoform.



growth, unlike adults, which excrete the quantity of potassium absorbed from their diet in their urine (120). Adult animals are more readily able to excrete an exogenous potassium load than is a neonate (177). Approximately half of the filtered potassium is reabsorbed in the proximal tubule of the adult and neonate by passive diffusion across the paracellular pathway (171). The loop of Henle reabsorbs 80% of the delivered potassium in the adult and only 45% in the neonate (171). Thus, the adult delivers approximately 10% of the filtered

potassium to the distal nephron while the neonate delivers 25%. The distal convoluted tubule, connecting tubule and cortical collecting duct are the sites of potassium secretion and final modulation of urine potassium excretion.

Potassium secretion in a principal cell is depicted in *Fig. 3-15*. Sodium enters the principal cell through the apical sodium channel down the favorable electrochemical gradient generated by the Na^+-K^+ ATPase on the basolateral membrane. This results in a lumen negative potential difference and a favorable electrochemical gradient for potassium secretion. While there is a maturational increase in the sodium channel and the Na^+-K^+ ATPase, these are not the limiting factors for potassium secretion. There are two potassium channels in the collecting tubule, one channel designated ROMK is on the apical membrane of principal cells and a flow-dependent channel that is activated by stretch designated maxi-K channel (120). There is a maturational increase in potassium secretion in cortical collecting tubules perfused in vitro (259). The secretion of potassium by principal cells is paralleled by the maturational increase in potassium channels (ROMK) on the apical membrane of the principal cell (262, 348). There is also a developmental increase in the maxi-K channel (337). As noted above and in *Table 3-1*, potassium secretion is regulated by aldosterone and there is resistance to the action of aldosterone despite higher serum levels in the neonate (8, 39, 104, 293, 307, 309, 319).

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4 Perinatal Urology

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The increased use of maternal-fetal ultrasound has led to the development of the field of perinatal urology. Antenatal hydronephrosis (ANH) is identified in approximately 1–3% of all pregnancies and is one of the most common birth defects detected (1–4). Other urologic abnormalities have been diagnosed prenatally as well, including renal cystic disease, renal agenesis, stones and tumors. For the pediatric urologist, these prenatal findings have created numerous clinical dilemmas that challenge our understanding of normal and abnormal renal embryology and physiology. In this chapter, we discuss the diagnosis of prenatal urologic abnormalities and the postnatal implications, the rationale behind prenatal intervention, and our clinical experience in managing children with prenatal urologic abnormalities.

Diagnosis

In a large prospective Swedish, study, the incidence of prenatally detected renal anomalies was 0.28% in which two-thirds (0.18%) were hydronephrosis (5). A British study, in which 99% of the pregnant population in Stoke-on-Trent were scanned at 28 weeks' gestation, demonstrated hydronephrosis prenatally in 1.40% of cases, which was confirmed postnatally in 0.65% (3). These authors defined prenatal hydronephrosis as an anteroposterior (A–P) diameter of the renal pelvis greater than 5 mm but noted the lack of consensus on the definition of antenatal hydronephrosis (6–8). Many variations in the definition and management of ANH exist in the literature and clinical practice, including method and frequency of in utero testing, radiographic documentation, classification, or postnatal management (9–15).

When an abnormality of the urinary tract is determined by maternal-fetal ultrasound, several questions should be raised by the ultrasonographer and consulting urologist. Combinations of specific findings direct the differential diagnosis and permit more accurate prognosis and tailoring of postnatal evaluation. The principal findings and their implications are listed in *Table 4-1*.

Diagnostic Accuracy

As both ultrasound and MRI technology improve, more accurate radiographic information is obtainable (16). However, predicting accurate postnatal diagnosis and outcome, regardless of the prenatal information, still remains challenging. The importance of accurate diagnosis is particularly critical in cases where fetal intervention is considered, such as posterior urethral valves (PUV). In other cases, the ability to identify some degree of ANH is adequate to permit a postnatal evaluation if deemed clinically appropriate.

A recent systematic review of the ANH literature attempted to determine the risk of a pathological diagnosis for patients with varying severity of ANH (15). In this review of 1,308 patients with any ANH and postnatal radiographic follow-up, 36% had a postnatal pathological diagnosis. The degree of ANH was defined by the anterior posterior diameter (APD) identified in a particular trimester (*Table 4-2*) (15). The overall risk for any pathology increased with the degree of hydronephrosis; except for vesicoureteral reflux which remained consistent regardless of the degree of ANH (*Table 4-3*) (15).

Although the risk of pathology with degrees of ANH appears to be increased, accurately determining the diagnosis remains difficult (15). An early report by Hobbins et al. suggested that the correct prenatal identification of the site of obstruction could be confirmed postnatally in 88% of cases (17). Subsequent studies reported fairly high false-positive rates ranging from 9 to 22% (6). The majority of false positives in these studies were nonobstructive causes of hydronephrosis, such as high-grade reflux, large, nonobstructed, extrarenal pelves, or transient hydronephrosis. Similarly, the diagnosis of vesicoureteral reflux is extremely challenging to make in-utero, as evidenced by the fact that the risk for vesicoureteral reflux is the same regardless of the degree of ANH (15).

As another example, the accurate diagnosis of posterior urethral valves (PUV), in which intervention might be considered, has proven difficult. In one series, the false-positive rate was as high as 58%, but the criteria for

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Table 4–1

Major diagnostic findings in prenatal imaging

	Finding	Comment
Kidney	Hydronephrosis	Assess degree
	Unilateral & bilateral	May be different degrees
	Parenchymal echogenicity	Should be less than spleen or liver; if increased and organ enlarged, suggests autosomal recessive polycystic kidney disease
	Duplication	Often with dilation of upper pole; may be lower pole dilation
	Cysts	Small cysts associated with dysplasia; simple cyst of upper pole suggests duplication with ureterocele or ectopic ureter; genetic cystic disease
	Urinoma	Perinephric or subcapsular
Ureter	Dilation/tortuosity	Obstruction or reflux
Bladder	Distended	Variation with time
	Wall thickness	In relation to filling status
	Intravesical cystic structure	Ureterocele
	"Keyhole" pattern	Dilated posterior urethra; PUV
	Not visible	Exstrophy
Amniotic fluid	Absence; oligohydramnios	Impaired urine output
	Polyhydramnios	May be seen with mild-moderate hydronephrosis
Gender	Penis/scrotum/ testes	Sex-associated conditions (e.g., PUV)
Spine	Meningocele	Neural tube defect

PUV, posterior urethral valves

Table 4–2

Classification of antenatal hydronephrosis (ANH) by anterior posterior diameter (APD)

ANH Classification	APD	
	2nd Trimester (mm)	3rd Trimester (mm)
1. Mild	≤7	≤9
2. Mild/Moderate	<10	<15
3. Moderate	7–10	9–15
4. Moderate/Severe	≥7	≥9
5. Severe	≥10	≥15

diagnosing valves were quite liberal and perhaps inappropriate (18). In another population-based series, the sensitivity in detecting valves was as low as 23% (6). Increased renal echogenicity and decreased amniotic fluid have been suggested to be indicative of obstructive conditions (19). Although the hallmark signs of an in-utero diagnosis of posterior urethral valves have been described (oligohydramnios, dilated posterior urethra, thickened bladder, and hydroureteronephrosis), there are very few studies that have prospectively examined the clinical urologic implications of these findings alone or in combination (15).

Ureteropelvic Junction Obstruction

The basic features of ureteropelvic junction obstruction (UPJO) in the fetus include dilation of the renal pelvis and collecting system with no evidence of ureteral dilation (*Fig. 4-1*). The best way to detect ureteral dilaion is at the level of the bladder, preferably in transverse view. The threshold for recommending postnatal follow-up is largely arbitrary and currently there are no long-term prospective studies to best determine the degree of postnatal evaluation. Lee et al. demonstrated that increasing severity of ANH increased the chance of identifying postnatal UPJO (15). Others have recommended that unilateral APD over 7 or 8 mm in the third trimester

Table 4-3

Risk of pathology by degree of antenatal hydronephrosis

Postnatal Pathology [% (95% CI)/[a]]	Degree of Antenatal Hydronephrosis					
	Mild (*N* = 587)	Mild-Moderate (*N* = 213)	Moderate (*N* = 235)	Moderate-Severe (*N* = 179)	Severe (*N* = 94)	Trend P-value[b]
Any Pathology	11.9 (4.5, 28.0)	39.0 (32.6, 45.7)	45.1 (25.3, 66.6)	72.1 (47.6, 88.0)	88.3 (53.7, 98.0)	<0.001
UPJ	4.9 (2.0, 11.9)	13.6 (9.6, 18.9)	17.0 (7.6, 33.9)	36.9 (17.9, 61.0)	54.3 (21.7, 83.6)	<0.001
VUR	4.4 (1.5, 12.1)	10.8 (7.3, 15.7)	14.0 (7.1, 25.9)	12.3 (8.4, 17.7)	8.5 (4.7, 15.0)	0.10
PUV	0.2 (0.0, 1.4)	0.9 (0.2, 3.7)	0.9 (0.2, 2.9)	6.7 (2.5, 16.6)	5.3 (1.2, 21.0)	<0.001
Ureteral Obstruction	1.2 (0.2, 8.0)	11.7 (8.1, 16.8)	9.8 (6.3, 14.9)	10.6 (7.4, 15.0)	5.3 (1.4, 18.2)	0.025
Other[c]	1.2 (0.3, 4.0)	1.9 (0.7, 4.9)	3.4 (0.5, 19.4)	5.6 (3.0, 10.2)	14.9 (3.6, 44.9)	0.002

[a]Pointwise 95% confidence intervals were estimated by logistic regression with robust standard errors based on generalized estimating equations with a working independence correlation structure to adjust for clustering by study for all degrees of antenatal hydronephrosis except mild-moderate. Because only one study had subjects with mild-moderate antenatal hydronephrosis, the pointwise 95% confidence intervals had to be estimated using logistic regression with unadjusted standard errors

[b]Testing for trend in risks with increasing degree of antenatal hydronephrosis using logistic regression with robust standard errors based on generalized estimating equations with a working independence correlation structure

[c]Includes prune belly syndrome, VATER syndrome, solitary kidney, renal mass, and unclassified

(5–6 mm with bilateral dilation) warrants postnatal follow-up (20). Some have recommended that all children with any ANH should be investigated postnatally (21). Nevertheless, in the case of significant unilateral hydronephrosis, there is little rationale for in utero intervention. In a few cases with massive dilation, therapeutic aspiration has been recommended for dystocia. In the case of bilateral UPJO, the efficacy of in utero intervention is difficult to assess.

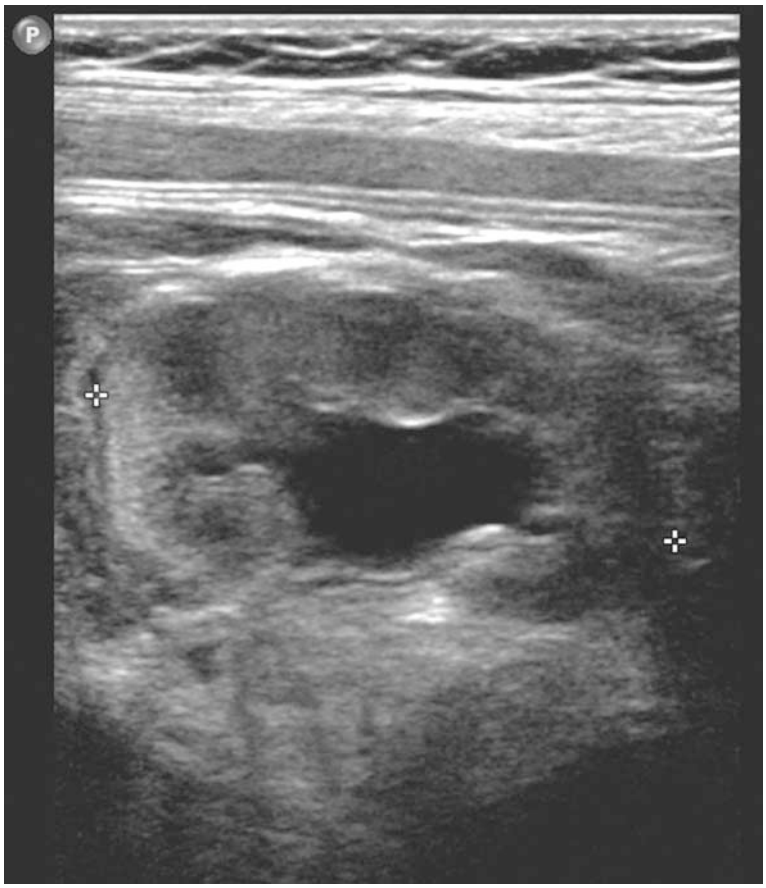
Attempts to correlate prenatal ultrasound appearance with postnatal outcomes have been complicated by the long-standing controversy regarding postnatal evaluation and management of UPJO. Grignon et al. developed a system of grading hydronephrosis secondary to UPJO based on the APD and degree of calyceal dilatation (22, 23). Mandell et al. attempted to correlate the degree of APD relative to gestational age with subsequent need for postnatal surgical intervention (24). They found the "at risk" diameter to be greater than or equal to 5 mm at 15–20 weeks' gestation, greater than or equal to 8 mm at 20–30 weeks' gestation, and greater than 1 cm at over 30 weeks' gestation. An alternative system proposed by Kleiner et al. defined hydronephrosis as the ratio of APD to A-P diameter of the kidney as being greater than 0.5 cm (25); caliectasis was later added as an additional indicator of significant hydronephrosis. Mild degrees of renal pelvic dilatation may resolve in utero. Mandell et al. noted this occurred in 23% of cases, with 66% remaining stable and 9% worsening over the course of the pregnancy (24). Similarly, Lee et al. noted that 88.1% of mild ANH were found to have no postnatal pathology (15). Severe forms of UPJO may be associated with urinary ascites or perinephric urinomas, which often precede nonfunction of the kidney (26).

Cystic Kidneys

The distinction between severe unilateral hydronephrosis and a multicystic dysplastic kidney may occasionally be unclear. The findings of multiple noncommunicating cysts, minimal or absent renal parenchyma, and the absence of a central large cyst are diagnostic of a multicystic dysplastic kidney (MCDK) (*Fig. 4-2*). Bilaterally enlarged echogenic kidneys, particularly if associated with hepatobiliary dilatation or oligohydramnios, suggests autosomal recessive polycystic kidney disease (*Fig. 4-3*). A more challenging finding is normal-sized, diffusely echogenic kidneys that are not associated with other urologic

Figure 4–1

Unilateral renal pelvis dilation with no ureteral dilation in 37-week old fetus.



lesions. Estroff et al. described 19 cases (14 bilateral), including 10 with normal function who survived and 4 with autosomal recessive polycystic kidneys who died (14).

Ureterovesical Junction Obstruction

Less common than UPJO, ureterovesical obstruction (UVJ) is characterized by ureteral dilation along with varying degrees of renal pelvic and calyceal dilation (*Fig. 4-4*). More extreme cases may be confused with single system ectopic ureters, particularly in males. In general, the differentiation is made postnatally.

Duplication Anomalies

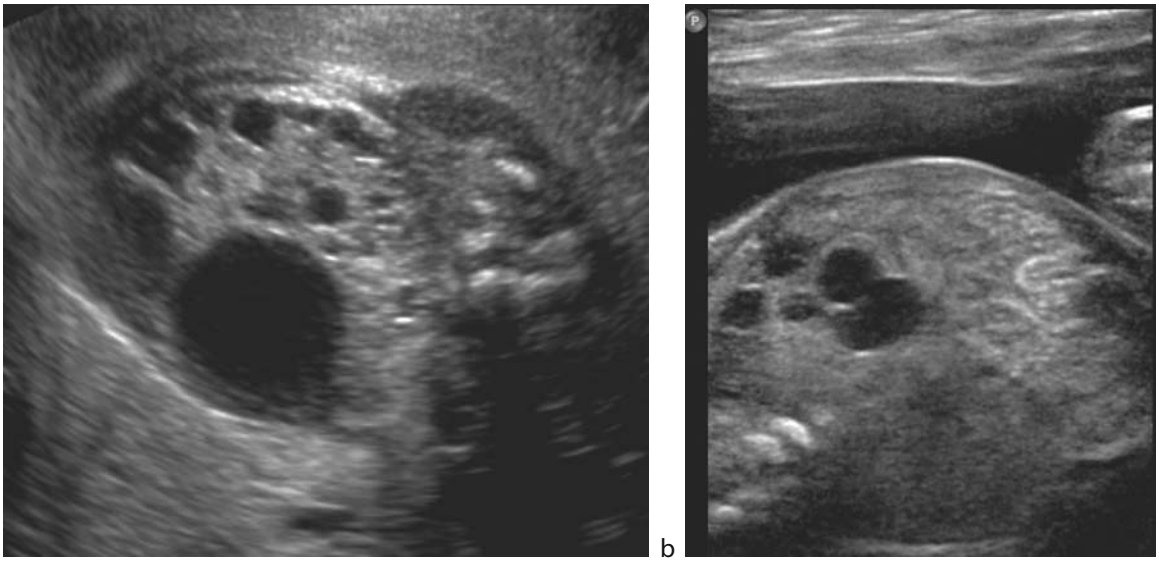
Among the most interesting prenatal urologic findings are duplication anomalies. These are often recognized on the basis of upper pole hydroureteronephrosis, associated with either an obstructing ureterocele within the bladder, or ectopic ureter inserting outside of the bladder (27) (*Fig. 4-5*). Lower pole hydronephrosis may be present as a result of vesicoureteral reflux or more rarely a lower pole UPJO. Occasionally, lower pole dilation is due to obstruction of both the upper and lower pole ureter by the large ureterocele. In some cases of a very large ureterocele, the ureterocele may be mistaken for the bladder.

Vesicoureteral Reflux

One cannot make a firm diagnosis of vesicoureteral reflux (VUR) based on prenatal ultrasound, although intermittent hydronephrosis or hydroureter is highly suggestive. Vesicoureteral reflux may be present in as many as 38% of children with prenatal hydronephrosis (28). Reflux occurred in 42% of children in whom postnatal imaging revealed persistent upper tract abnormalities and in 25%

Figure 4–2

Two different fetal images (a) at 19 weeks) and (b) at 26 weeks of a multicystic dysplastic kidney with multiple noncommunicating cysts.



of those with normal findings on postnatal ultrasound but having a history of prenatal dilation. Tibballs and Debrun reported that in patients with prenatal dilation and postnatally normal renal units by ultrasound, 25% had grade III-V reflux (29). The incidence of high-grade reflux was greater in males than in females as noted in previous studies. In two systemic reviews of the ANH literature a 10–15% incidence of VUR was identified regardless of the degree of ANH (15, 30) indicating that ANH is not indicative of VUR and may not be the appropriate trigger for postnatal evaluation. In a neonate with prenatally detected hydronephrosis, the importance of diagnosing vesicoureteral reflux remains controversial. While, several studies have demonstrated that a high incidence of reflux is associated with prenatally detected hydronephrosis, its clinical significance is unclear.

Posterior Urethral Valves

Perhaps the most important diagnosis to be made prenatally is that of PUV in the male fetus. PUV, at the very least, mandates prompt postnatal intervention and in some cases, prenatal intervention may be warranted. Fetal sonographic findings of PUV include bilateral hydroureteronephrosis, a thick-walled bladder with dilated posterior urethra, and, in more severe cases, dysplastic renal parenchymal changes with perinephric urinomas and urinary ascites (*Fig. 4-6*) (31). When characteristic sonographic findings are present, the differential diagnosis includes prune belly syndrome (with or without urethral atresia), massive vesicoureteral reflux, and certain cloacal anomalies (in genetic females) (32, 33). Prenatal diagnostic accuracy for PUV is far from perfect but is probably better than the 40% figure previously reported (18).

Rationale for Prenatal Intervention

The scientific rationale for prenatal treatment of hydronephrosis is to maximize normal development of renal and pulmonary function. These two aspects of fetal development are closely linked because urine comprises 90% of amniotic fluid volume, and oligohydramnios during the third trimester has been causally related to pulmonary hypoplasia.

Before embarking on prenatal surgical intervention for obstructive uropathy, it is critical to assess the risk-benefit ratio. The most widely accepted indicator of salvageable renal function is analysis of fetal urine. When the urinary sodium is less than 100 mg/dL and urine osmolarity less than 200 mOsm/dL, renal function appears to be salvageable with in utero intervention (*Table 4-4*) (34). The accuracy of these predictors has been challenged (35, 36). More recently, serial aspirations of fetal urine have been reported to yield more valuable results (37). Guez et al. reported ten fetuses who underwent multiple urine samplings and in whom severe obstruction reduced sodium and calcium reabsorption (38).

Figure 4–3

Bilateral markedly enlarged echogenic (“bright”) kidneys (a) with a small cystic lesion (b) in a fetus with oligohydramnios, consistent with autosomal recessive polycystic kidney disease.

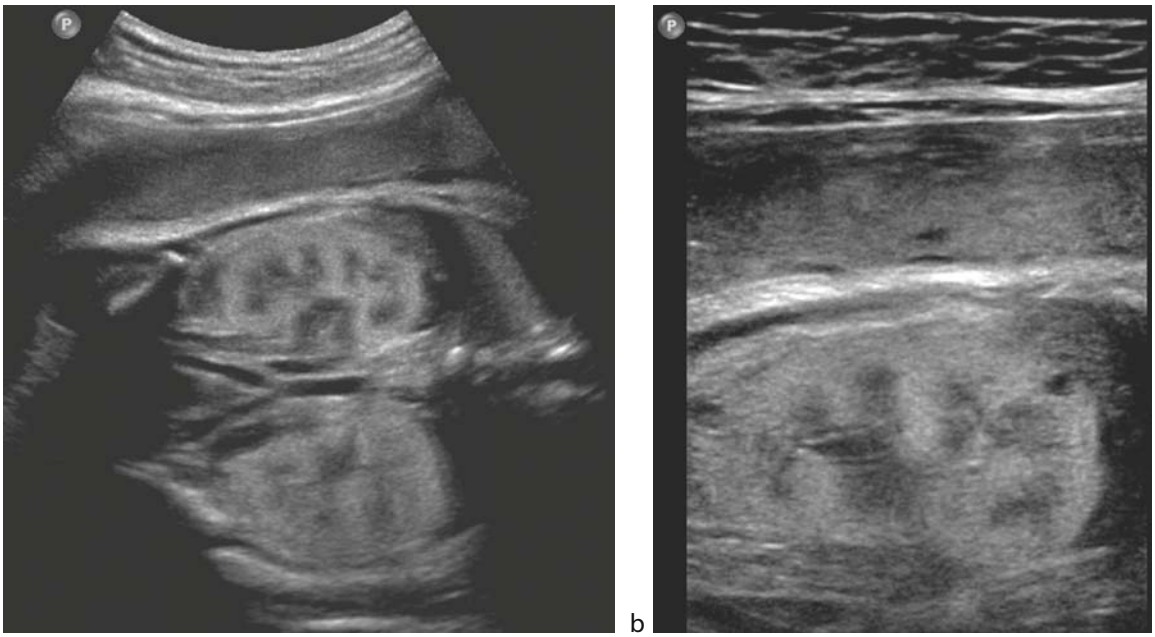
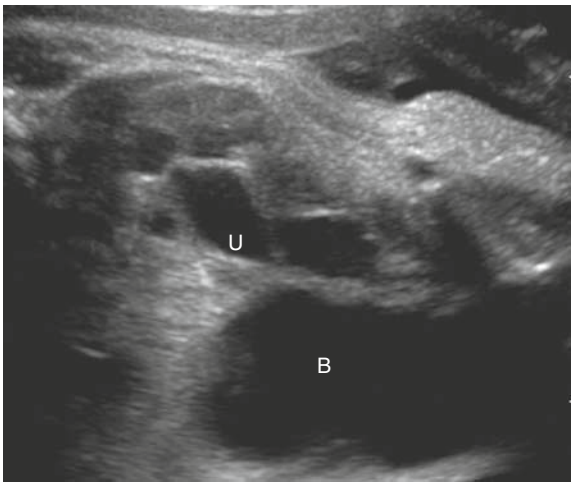


Figure 4–4

Unilateral hydroureteronephrosis at 35 weeks. Note the dilated ureter (U) and bladder (B). Postnatal imaging confirmed this to be a ureterovesical junction obstruction.



They concluded that fetal urinary chemistries were reasonably predictive of severe but not moderate postnatal renal impairment. Other investigators have suggested the use of fetal urinary beta-$\tilde{}_{22}$ microglobulin as an indicator of tubular damage. Using this parameter, poor renal-outcome has been predicted with a specificity of 83% and sensitivity of 80% (39).

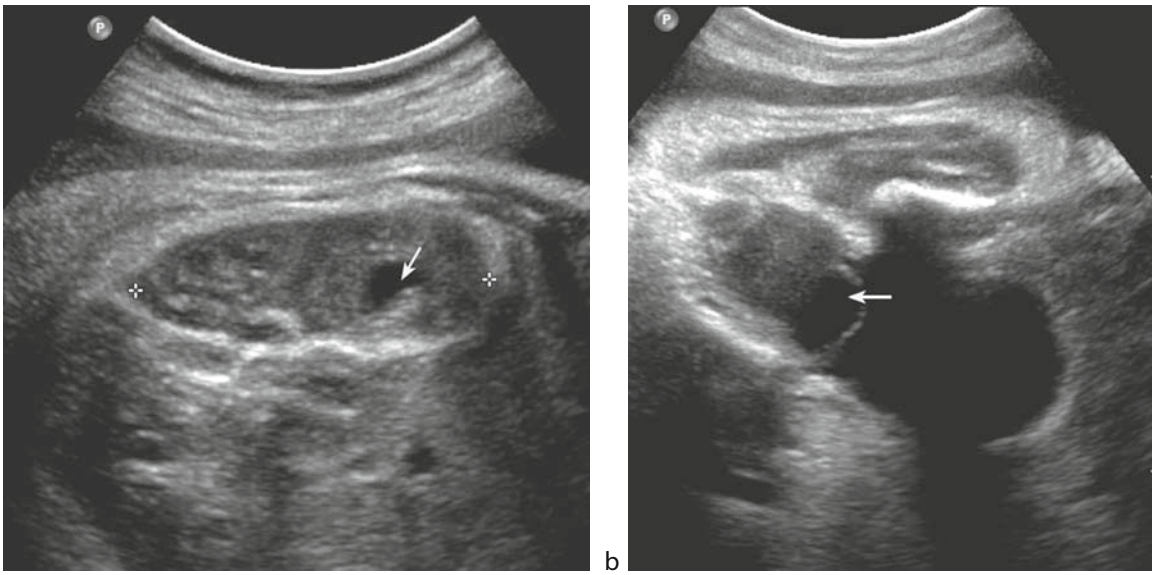
The time of onset of oligohydiamnios has been shown to be an important determinant of outcome (40, 41). In fetuses in which adequate amniotic fluid was documented at up to 30 weeks’ gestation in association with a urologic abnormality, pulmonary outcomes were satisfactory, and postnatal clinical problems were related to renal disease. It seems inappropriate to recommend late urinary tract decompression from a pulmonary or renal basis. It is unclear whether early delivery, to permit earlier postnatal urinary decompression, is beneficial.

Clinical Experience with Intervention for Prenatal Hydronephrosis

The ability to diagnose severe prenatal hydronephrosis and advances in fetal intervention helped develop prenatal surgery for obstructive uropathy. In 1982, Harrison et al. described the initial report of fetal surgery in a 21-week-old fetus with bilateral hydroureteronephrosis secondary to PUV (42). After the 1986 report of the International Fetal Surgery Registry in which outcomes did not seem to justify risk, a de facto moratorium on in utero urinary tract shunting evolved (43). More recently, with improved technology and renewed interest in fetal shunting, most

Figure 4–5

Fetal image of duplex kidney with marked upper pole hydronephrosis (arrow) in contrast to a normal lower pole (a). Associated with this image is the finding of a ureterocele (arrow) within the bladder (b).



cases have been referred to a small number of highly specialized centers actively engaged in prenatal surgery. The initial method of decompression with open surgery has largely been replaced by in utero shunt placement, although this has been complicated by technical problems of shunt dislodgement and, in the case of the double-J shunt, bowel herniation (44). Some investigators have explored the use of fetoscopic methods for direct intervention to provide prolonged bladder drainage, whereas others have attempted direct endoscopic valve ablation (45–49).

Harrison et al. have clearly outlined the indications and contraindications of intervention for prenatal obstructive uropathy (*Table 4-5*) (50). Additionally, serial bladder sampling over 3 days has been used to help determine if the fetus is a viable candidate. The serial nature of the procedure allows one to see the response of the fetal kidneys to bladder decompression (37). The principal reason for considering vesicoamniotic shunting is to prevent early neonatal pulmonary insufficiency and death. The risks that one accepts with intervention include induction of premature labor, perforation of fetal bowel and bladder, and fetal and/or mother hemorrhage and infection.

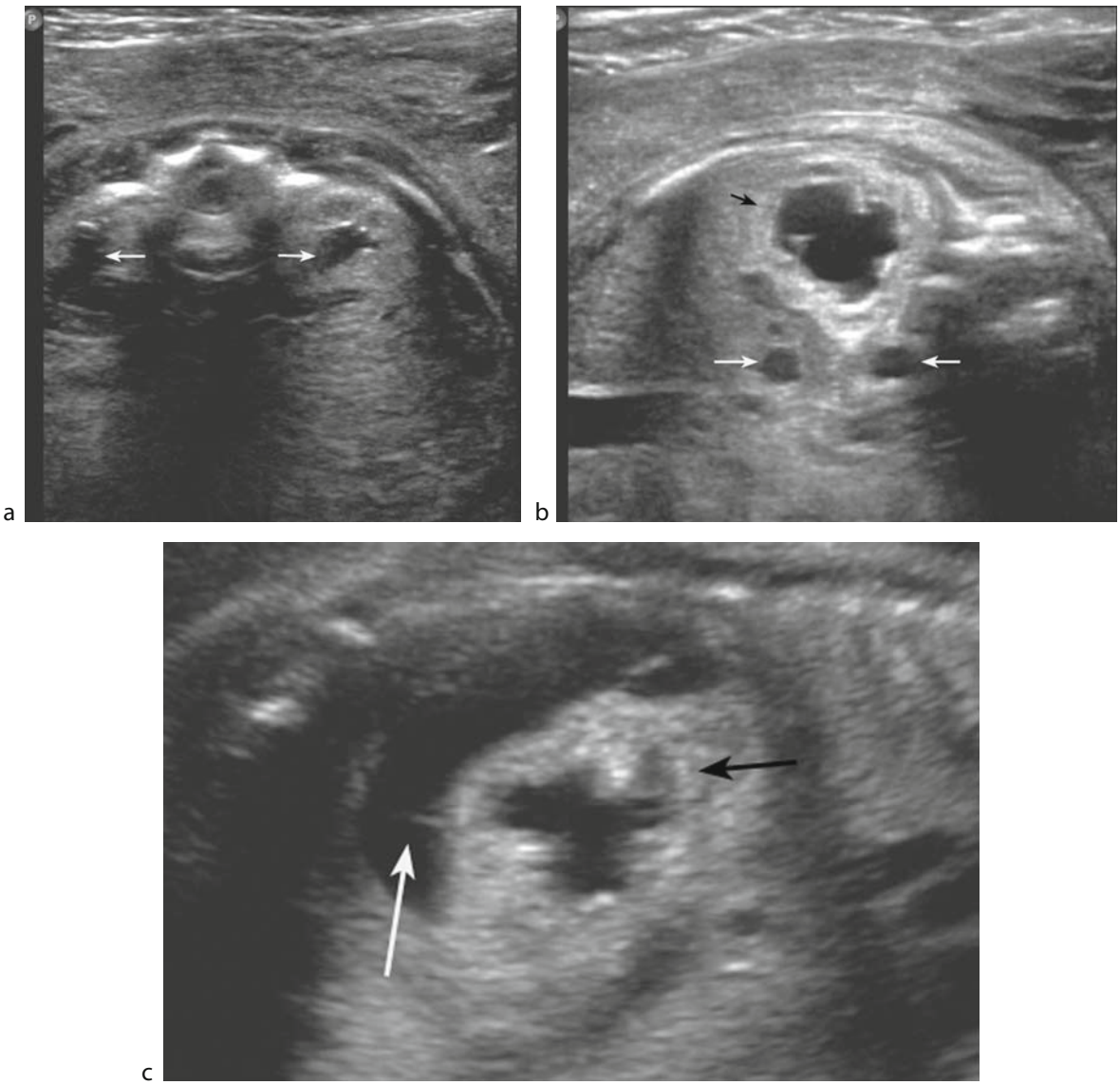
More recently, the ability to influence renal outcome in male patients with PUV but without oligohydramnios has been suggested as a possible indication for in utero intervention. The principal goal of intervention is not to prevent pulmonary hypoplasia and deaths but to prevent or delay end-stage renal failure. Although some reports have shown promise in the ability to distinguish those fetuses with likely early renal failure from those with later-onset failure, the specificity and accuracy of methods using a combination of ultrasound and urinary chemistries (sodium, beta$_2^{\sim}$ microglobulin, and calcium) has not been well defined (51–53). In summary, precise identification of those situations in which intervention may benefit the fetus with obstructive uropathy remains unclear.

Overall, the need to consider in utero intervention for obstruction is not common. In one study, only 9 of 177 fetuses with a diagnosis of hydronephrosis were considered to have PUV and only 3 warranted serious consideration for intervention (24).

To date, the reported long term outcomes of antenatal intervention for severe obstructive uropathy (e.g. PUV, prune belly syndrome, urethral atresia) are mixed (54–62). Variability in patient selection and assessment of outcome within these studies has limited the ability to determine if prenatal intervention has altered the postnatal course. A large systematic review of the prenatal intervention for obstructive uropathy showed a statistically significant perinatal survival advantage with shunting (60). Of the studies that have reported long term outcomes of in-utero vescioamniotic shunting, many of the children have renal insufficiency (57%), and growth impairment (86%) (54, 56, 57). Recently, Baird et al., reported on long-term follow-up (5.8 years) of patients who have survived in-utero shunting (54). They noted

Figure 4–6

Images of a fetus with posterior urethral valves. (a) depicts bilateral echogenic kidneys with hydroureteronephrosis (arrow). (b) demonstrates the associated thickened bladder wall (black arrow) and hydroureter (white arrow). (c) further imaging revealed a perinephric urinoma (white arrow) surrounding the hydronephrotic kidney (black arrow).



acceptable renal function in 44%, mild impairment in 22%, and renal failure in 33%. Prune belly patients had the best renal outcome (57%), followed by PUV (43%), then urethral atresia (25%). Overall, it appears that in-utero intervention for the appropriate patient may reduce the risk of neonatal mortality and may potentially improve renal function. To further improve outcomes, more sensitive and specific markers to better identify which fetus will benefit from in-utero shunting need to be defined.

Postnatal Management of Infants with Prenatally Diagnosed Urologic Renal Abnormality

A child with a prenatal diagnosis of a urologic renal abnormality such as ANH should be carefully evaluated and followed by a pediatric urologist from birth. The vast majority of these children appear entirely healthy and, in the absence of prenatal ultrasound findings, would not have

Table 4–4

Prenatal assessment of renal functional prognosis

	Good		Poor	
Amniotic fluid	Normal to moderately decreased		Moderate to severely decreased	
Sonographic appearance of kidneys	Normal to echogenic		Echogenic to cystic	
Fetal urine	Glick et al. (21)	Johnson et al. (24)	Glick et al. (21)	Johnson et al. (24)
Sodium (mEq/L)	<100	<100	>100	>100
Chloride (mEq/L)	<90	–	>90	–
Osmolarity (mOsm/L)	<210	<200	>210	>200
Calcium (mg/dL)	–	<8	–	>8
β_2-Microglobulin (mg/L)	–	<4	–	>4
Total protein (mg/dL)	–	<20	–	>20
Output (mL/h)	>2	–	<2	–
Sequential improvement in urinary values	–	X	–	–

X, only in this series was the criterion used

Table 4–5

Prenatal intervention for hydronephrosis

Indications (prerequisites)	Contraindications
Presumed obstructive hydronephrosis, persistent or progressive, bilateral or insolitary unit	Unilateral hydronephrosis with an adequately functioning contralateral kidney
Otherwise healthy fetus	Chromosomal abnormalities or presence of associated severe anomalies
Oligohydramnios	Bilateral hydronephrosis without oligohydramnios
No overt renal dysplasia	Severely dysplastic kidneys
Adequate renal functional potential based on urinary indices (see text)	Evidence or uretheral atresia
Informed consent	Presence of a normal twin

any indications for regular urologic follow-up. Parental anxiety is common and should be addressed directly with prenatal counseling and education.

Unilateral Hydronephrosis

The presence of unilateral dilation of the kidney warrants postnatal evaluation in a timely but non urgent fashion (3–8 weeks of life) with an ultrasound (51). The most common diagnoses associated with this finding are UPJO, VUR and UVJO/megaureter. Early ultrasound is unlikely to miss a significant abnormality. A normal postnatal ultrasound indicates that obstructive uropathy is not present; however, it does not determine whether or not the child has VUR (29).

The decision to perform a voiding cystourethrogram (VCUG) or initiate prophylactic antibiotics in the newborn period is unclear. Although some groups advocate postnatal VCUG in any child with a history of prenatal hydronephrosis, others have questioned the value of this approach (63). Infants with severe ANH should be placed on prophylactic antibiotics (amoxicillin, 10 mg/kg/day or 50 mg/day) and undergo a VCUG. Severe ANH may be associated with an increased risk of febrile urinary tract infection (64). As for mild ANH, a recent prospective study of 192 infants with ANH noted that the majority of patients with mild ANH had no significant events during infancy (65). In another study, female infants with a history of ANH and postnatal uropathy had a higher risk of febrile urinary tract infection (66). Regardless, no appropriate prospective studies with coordinated and comprehensive postnatal follow-up have examined this question in a rigorous fashion to provide consensus guidelines (15, 30).

At our institution, children with moderate or severe ANH are placed on prophylactic antibiotics at birth. They undergo renal bladder ultrasound and VCUG postnatally. Diuretic renography is reserved for those with persistent moderate or severe postnatal hydronephrosis not related to VUR. Infants with persistent mild on serial US or no postnatal hydronephrosis are observed and followed clinically. Infants with any degree of antenatal or postnatal

ureteral dilation undergo ultrasound, VCUG and possibly diuretic renography (after 3 months) if clinically indicated.

Perhaps the most challenging aspect of managing prenatal hydronephrosis is determining if and when postnatal surgical correction for obstruction is appropriate. Some have suggested that regardless of the degree of ANH, moderate or severe postnatal hydronephrosis with evidence of decreased renal function should be the indications for surgical intervention (67). Despite the improved anatomic detail afforded by real-time ultrasound and the increasing experience with functional nuclear medicine studies (mercaptotriglycylglycine) no radiographic or clinical gold standard for physiologically significant obstruction exists. Over time, some kidneys have been seen to improve, whereas others appear to lose function. The natural history of prenatal hydronephrosis is not clearly defined.

The debate over the appropriate management of infants with unilateral ANH continues and may ultimately be determined by a combination of epidemiologic, radiographic, and new innovative biomarker discoveries. More accurate and reproducible prenatal and postnatal radiographic documentation of the degree of hydronephrosis and function combined with appropriate natural history data are needed to better categorize the infants. Finally, new serum or urine biomarkers indicative of ongoing renal damage will be critical in helping to further define which infants are truly at risk.

Bilateral Hydronephrosis

Infants with bilateral hydroureteronephrosis may have PUV, bilateral VUR, bilateral UPJO or UVJO, or a combination of the above. For the child with bilateral hydroureteronephrosis suggestive of bladder outlet obstruction, an ultrasound and VCUG should be performed promptly. In boys, PUV is the most important diagnosis to be ruled out. In girls, an obstructing ectopic ureterocele would be the most likely cause for bladder outlet obstruction. In the event that an obstructive lesion is discovered, it should be corrected promptly. For children with suspected lower urinary tract obstruction (e.g. PUV), prompt bladder decompression and antibiotic prophylaxis (amoxicillin 10 mg/kg/day or 50 mg/day) should be initiated prior to radiographic intervention.

Renal Agenesis, Renal Ectopia and Unilateral Multicystic Dysplastic Kidney

Infants born with solitary kidneys (renal agenesis), renal ectopia or unilateral multicystic dysplasia should be evaluated postnatally by US and VCUG. Functional studies such as a dimercaptosuccinic acid study (DMSA) are occasionally needed to confirm the diagnosis. The need for further screening is controversial. It has been reported that among infants with a solitary kidney, 30% have VUR, 11% UPJO, and 7% UVJO (68, 69). Similarly, those with renal ectopia (simple or crossed fused ectopia) may also be at risk for VUR in the ectopic or contralateral kidney (30%) (70–72). However, there are others that report a very low incidence of associated urologic anomalies and do not recommend screening (73).

Multicystic dysplastic kidneys (MCDK) are primarily unilateral, isolated and associated with a good prognosis. If at birth the US findings are not absolutely diagnostic of a classic MCDK, a DMSA study can be used to confirm the diagnosis with the absence of uptake. Patients with MCDK are often thought to be similar to those born with a solitary kidney. Additionally, patients with MCDK have an increased frequency of VUR and UPJO in the contralateral normal kidney (74, 75).

Summary

With the increased use of maternal-fetal ultrasound, more genitourinary abnormalities are being detected prenatally. Although advances in imaging have increased the detection and characterization of these abnormalities, further work is needed to identify which abnormalities are clinically significant. Research directives should focus on identifying which infants require postnatal diagnostic imaging and intervention. Developments in the fields of imaging, proteomics, and genomics may provide the necessary information to not only detect the abnormality, but to also prognosticate which ones require further testing and medical intervention.

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5 Renal Dysplasia/Hypoplasia

Paul Goodyer

Introduction

During normal kidney development, interactions between the branching ureteric bud (UB) and the metanephric mesenchyme (M) generate all the nephrons of each kidney by about the 8th month of gestation. Nephrons are attached like apples to branches of the arborized collecting system. At birth, this crop of nephrons constitutes the individual's nephron endowment for life and ranges widely among normal humans from 0.3 to 1.1 million nephrons per kidney (1).

Normal human renal development begins at about 4 weeks gestation when the ureteric bud grows out from the nephric duct and begins to arborize within the adjacent metanephric mesenchyme. Failure of this initial process leads to bilateral renal agenesis. The fetus is unable to generate amniotic fluid and develops characteristic features of facial compression and pulmonary hypoplasia (Potter syndrome). At the other end of the spectrum, children with subtle renal hypoplasia appear normal but may be at increased risk for hypertension and renal insufficiency following an acquired renal insult later in life (2, 3). Between these extremes are infants in whom kidney development is initiated, but not completed according to plan, leading to small, dysfunctional kidneys (*Fig. 5-1*).

Antenatal ultrasound screening suggests that about 1 in 400 neonates are born with at least one hypoplastic kidney (4). However, postnatal compensatory hypertrophy tends to mask subtle renal hypoplasia and ultrasound screening in school-age children identifies one or more hypoplastic kidneys in only 1 per thousand (5). When renal hypoplasia is bilateral, nephron number may be insufficient for normal extrauterine life, and as somatic growth outstrips nephron endowment, these children develop progressive renal insufficiency and require renal replacement therapy (6).

While pure renal hypoplasia may be caused by a simple paucity of nephrons (e.g., oligomeganephronia), it is more often associated with histopathologic evidence of aberrant developmental fates of the metanephric mesenchyme and profound disturbance of the normal patterning of renal tissue (renal dysplasia). Normal renal architecture is disrupted by isolated tubules surrounded by mesenchymal cuffs (*Fig. 5-2*), fibrotic interstitial zones and even islands of cartilage (*Fig. 5-3*) scattered amid fairly normal-appearing renal tubules.

In the 2007 NAPTRCS report, about 15% of the 11,874 children in the transplant and dialysis databases had a primary diagnosis of renal aplasia/dysplasia/hypoplasia (https://web.emmes.com/study/ped). An additional 20% have primary diagnoses associated with renal hypoplasia/dysplasia (urinary tract obstruction, vesico-ureteral reflux, prune belly syndrome). In the past, clinicians have been advised to perform renal ultrasonography to screen for renal malformation in infants with minor ear malformations (low-set ears, misshapen pinnae, preauricular tags or preauricular sinuses) but the cost-effectiveness of this approach is now questionable because the background prevalence of minor ear malformations is fairly high (7.5 per thousand) in the normal population (7, 8).

Current approaches to classification of human kidney hypoplasia/dysplasia must meld recent advances in the understanding of nephrogenesis with clinico-pathologic observations. In this chapter, we first consider isolated renal agenesis, renal dysplasia and pure hypoplasia as defects in primary initiation, nephron differentiation or ureteric bud branching, respectively. We then review the evidence that renal hypoplasia/dysplasia may be a feature of vesico-ureteral reflux and obstructive uropathy. Syndromes involving renal hypoplasia/dysplasia are many and will be tabulated elsewhere, but we will touch on a few examples of renal dysplasia which can be attributed to mutant genes. Finally, we will consider the genetic and environmental factors which are thought to account for subtle renal hypoplasia in a significant fraction of the normal population.

Renal Agenesis

Bilateral Renal Agenesis (BRA)

Bilateral failure of primary nephrogenesis during fetal life causes a characteristic pattern of facial compression and pulmonary hypoplasia (Potter syndrome) due to the absence of amniotic fluid. Most cases of the Potter syndrome are associated with obstruction of the urinary tract or severe bilateral renal hypoplasia, but primary

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bilateral renal agenesis (BRA) is estimated to occur in about 1 per 5,000 fetuses (9). If screening is performed in the second trimester by transvaginal ultrasound, the incidence appears to be even higher (1 per 705 fetuses) (10). Severe oligohydramnios, evident clinically or by ultrasonography in the second trimester (21–23 weeks), raises difficult issues for parents and the medical team about postnatal viability of the infant (11). High-resolution color Doppler ultrasonography is helpful to detect the fetal renal arteries, distinguishing severe renal hypoplasia from renal agenesis (12). Antenatal MRI has been used to detect the full range of renal malformations (13). BRA is often found in association with unilateral umbilical artery (14). Complete absence of renal parenchyma (renal agenesis) and amniotic fluid predicts that pulmonary hypoplasia will be extreme, often causing pneumothorax and/or inability to oxygenate without ventilatory support in the newborn period (*Fig. 5-4*).

In those who initially survive, the decision about whether or not to embark on chronic peritoneal dialysis is usually dominated by several key issues: A) whether lung development is sufficient to allow oxygenation without respiratory support beyond the first few perinatal days; B) whether there is any functional renal parenchyma (identifiable by MAG3 imaging and/or ultrasonography) allowing sufficient urine volume to permit minimal long-term oral nutrition (100 Kcal/kg/day); C) whether family/institutional resources can sustain dialytic therapy long enough to achieve growth to a body weight which allows renal transplantation. While once considerable hopeless, recent data suggest that aggressive renal replacement therapy is now at least an option. Klassen has reported a 70% survival rate among 23 infants with antenatal oligohydramnios and pulmonary hypoplasia, despite a requirement for ventilation in 16/23 for 1–60 days (15). Thus, there appears to be gradual improvement in lung function during the postnatal period. Among 193 NAPRTCS infants who began peritoneal dialysis within the first

Figure 5-1

Hypoplastic kidney bisected to show presence of ureter and symmetric collecting system derived from initial branches of the ureteric bud. The thin outer rim of renal cortex has normal architecture but nephron number is dramatically reduced. (See color plate 1)

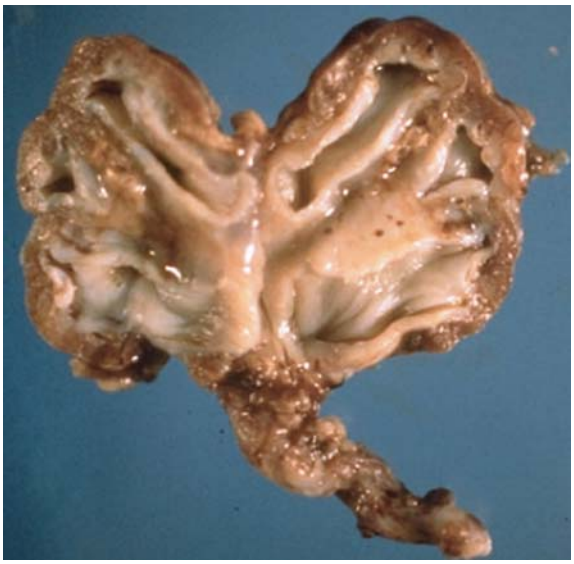


Figure 5-2

Renal dysplasia: peritubular fibrous cuff. (See color plate 2)

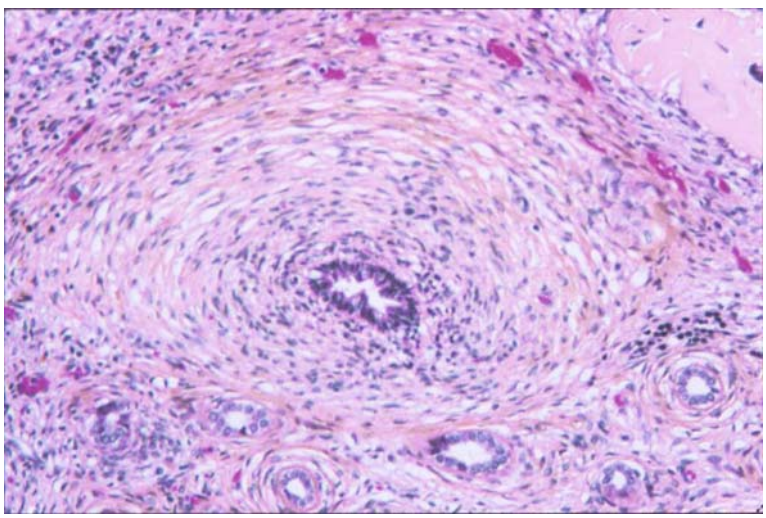


Figure 5-3

Renal dysplasia: island of cartilage amid dysplastic tissue represents aberrant cell fate of metanephric mesenchyme. (See color plate 3)

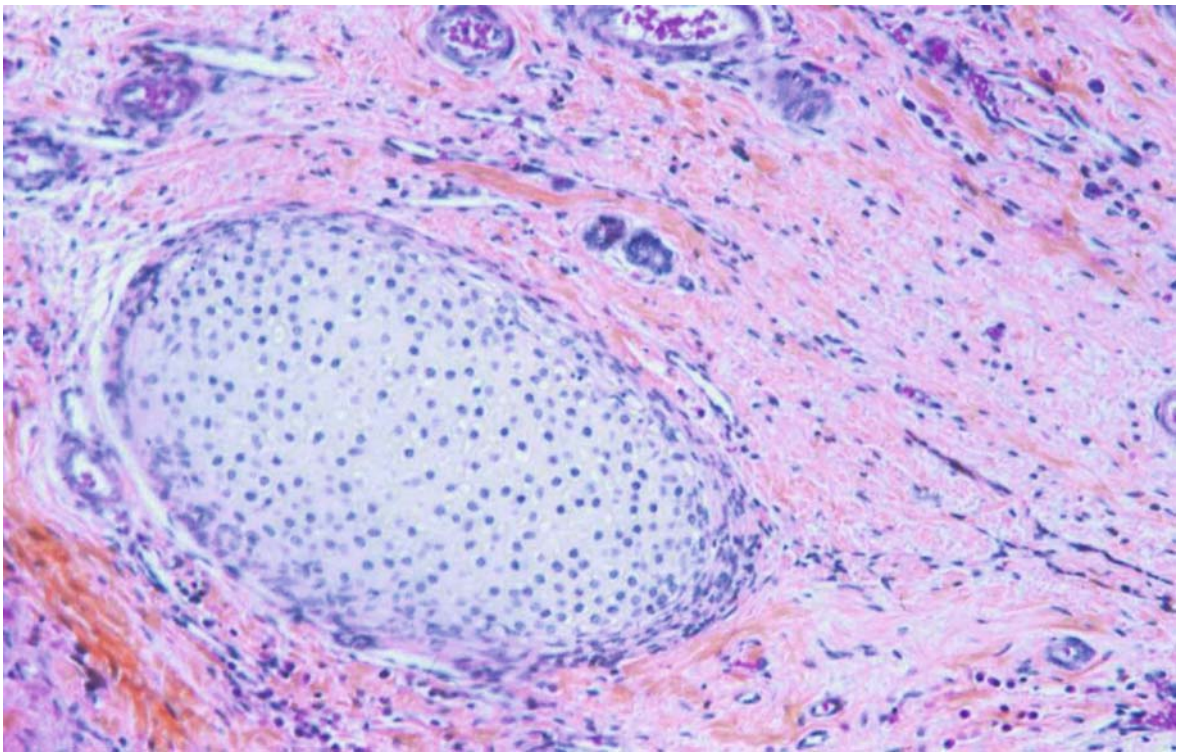
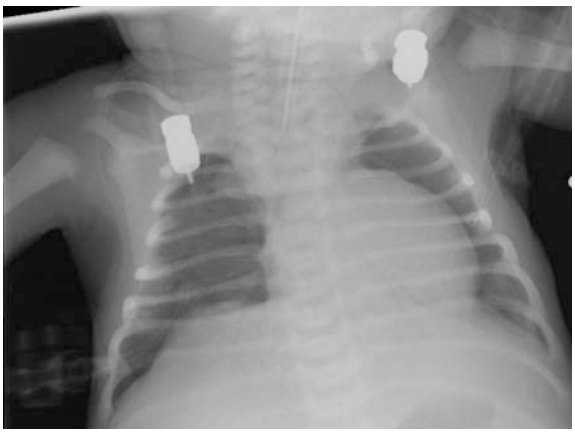


Figure 5-4

Chest X-ray from newborn with Potter syndrome showing pulmonary hypoplasia and right-sided pneumothorax.



month of life, 10% died during the initial dialytic period and another 15% died in a later phase – in some cases after renal transplantation (16). In the cohort from 1999–2005, 80% were transplanted within 3 years (16).

The causes of renal agenesis in humans are not well understood, but reports of absent renal arteries during the second trimester suggest arrest of kidney development at a very early stage. Study of homozygous "knockout" mice has identified a number of critical developmental genes causing experimental bilateral renal agenesis. For example, inactivation of GDNF (a growth factor expressed in the undifferentiated mesenchyme) or RET (the GDNF receptor which is expressed at the surface of ureteric bud cells) or its co-receptor, GFRα results in failure of primary ureteric bud outgrowth (17, 18). Knockout mice lacking key transcription factor genes such as PAX2 or WT1 are also anephric (19, 20). Presumably, homozygous mutations for any one of these genes could account for occasional "sporadic" cases of complete renal agensis in humans. Recently, Skinner et al. identified RET mutations in seven of nineteen stillborn fetuses with bilateral renal agenesis; no causative mutations of GDNF or GFRα were noted (21).

Unilateral Renal Agenesis (URA)

Estimates of the incidence of URA vary: 1 per 750 by transvaginal antenatal ultrasound screen (10); 1 per

1,000 in autopsy series (22); 1 per 1,300 by ultrasound screening of Taiwanese school children (5); 1 per 2,900 by ultrasound screening of North American infants (23). URA is more common in infants of diabetic mothers and among Afro-American mothers than in others (adjusted odds ratio of 4.98 and 2.19, respectively) (23). In a screening study of normal Japanese newborns, 52 of 4,000 newborns (1.3%) had evidence of unilateral hypoplastic/dysplastic kidneys; during follow-up of three of these cases, the abnormal renal tissue involuted in early postnatal life so that eventually no detectable kidney was evident (4). Thus, adults with "congenital" absence of one kidney may often be born with at least some partial, though abortive, renal tissue which regresses at an early stage.

URA is associated with developmental abnormalities of other tissues, particularly the inner ear, genital tract and axial skeleton. Among 40 girls with unilateral renal agenesis, 4/40 (10%) had an ipsilateral mild-moderate sensorineural hearing deficit and 14 (35%) had mullerian duct abnormalities (24). In a retrospective study of patients with mullerian duct abnormalities, 30% had unilateral renal agenesis (25); this association was particularly strong for girls with uterus didelphys (13/16 cases), uterine agenesis (2/5 cases) and unicornuate uterus (2/7 cases). URA was seen in all 11 cases of obstructed uterus didelphys, ipsilateral to the side of the obstructing transverse hemivaginal septum. The incidence of genital tract anomalies in boys with URA is reported to range from 12 to 77%; ipsilateral cystic dysplasia of seminal vesicles and bilateral absence of the vas deferens (with ipsilateral syndactyly) have all been described (26–28). In a prospective study, 202 patients with congenital vertebral abnormalities 54 (26.7%) had at least one genitourinary abnormality detected by intravenous pyelography or ultrasonography; the most frequent being unilateral renal agenesis (29). Although the pathogenetic mechanisms are not well understood, it is evident that defective developmental pathways or genes may disturb morphogenesis of mesenchyme in the ear, genital tract and skeleton, and it is not unreasonable to screen children with URA for these associated anomalies.

The mechanisms which might lead to complete absence of one kidney while sparing the other kidney are not obvious, but again there are lessons to be learned from knockout mice. While most mice lacking both copies of the Ret gene are anephric, a small percentage manage to achieve outgrowth of the ureteric bud and generate a small, though suboptimal, kidney on one side (17). Although counter-intuitive, this suggests that some cases of URA could in fact be caused by inherited mutant genes which affect the two kidneys unequally. In a study of 4,099 fetal/infant autopsies, dysplastic elements were found in 4% of kidneys overall, but, in patients with URA, dysplastic elements were found in 45% of contralateral kidneys. Occasional cases of autosomal dominant URA have been reported (30) and recently, Skinner and colleagues identified RET mutations in two of ten stillborn infants with URA (21). First degree relatives of patients with URA have considerably increased risk of either URA (5%) or BRA (0.8%) (9).

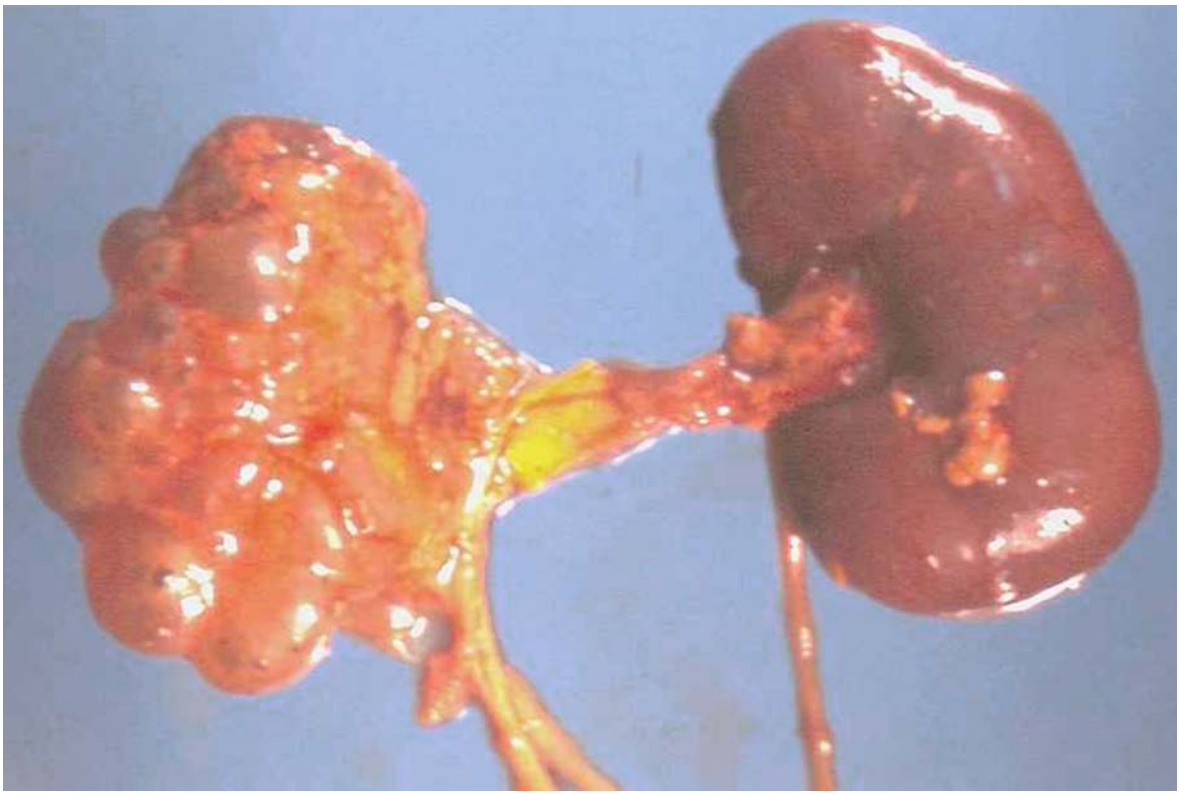
At birth, nephron number is suboptimal in children with URA and the contralateral kidney is stimulated to undergo compensatory hypertrophy. In most cases, plots of renal length or volume versus body length demonstrate gradual compensatory hypertrophy of the unaffected contralateral kidney over the first 3–4 months, crossing percentiles established for normal populations. Failure to undergo compensatory hypertrophy usually indicates contralateral renal dysplasia and may predict progressive renal insufficiency. Evidence of patchy dysplasia detectable by ultrasonography or renal scans is predictive of increased risk of hypertension in childhood. In a study of 29 cases of URA, the hypertrophied contralateral kidney had normal contour in 14 and "scarring" in 15 (31). Mild hypertension was detected by ambulatory blood pressure monitoring in 1/14 and 8/15 subjects, respectively (31). Finally, nuclear DMSA or MAG3 scanning may identify residual hypoplastic/dysplastic tissue instead of complete URA on the affected side. This is sometimes associated with hypertension which responds to excision of the dysplastic tissue.

Multicystic/Dysplastic Kidney (MCDK)

While URA implies a disruption of primary renal development, many infants are born with a unilateral multicystic/dysplastic kidney (MCDK) attached to an atretic ureter (*Fig. 5-5*). This suggests that initial ingrowth of the fetal ureteric bud may have been successful, but that renal development was disrupted at an later stage. MCDKs are recognized as clusters of multiloculated thin-walled cysts which do not appear to connect, distinguishing them from hydronephrotic kidneys. Nuclear scans often show little or no functional parenchyma and ureters are not usually patent, indicating little or no urine formation from an early stage in fetal life. The cystic mass usually lacks a reniform shape; the scanty tissue between cysts is hyperechoic and there is usually no detectable renal artery by Doppler ultrasonography. Microscopic analysis reveals disorganized renal architecture with islands of undifferentiated mesenchymal cells,

Figure 5-5

Non-functional multicystic/dysplastic right kidney and grossly normal-appearing left kidney from an infant who died in the perinatal period of non-renal causes. (See color plate 4)



occasional bizarre differentiation (e.g., cartilage) and few if any normal-appearing nephrons. Cysts are often rimmed by collars of fibromuscular cells. The incidence of unilateral MCDK is about 1 in 4,000 live births (32). Very rarely, bilateral MCDK has been reported and is fatal in the newborn period (33).

More recently, antenatal detection and long term follow-up studies of unresected MCKDs have suggested that the pathogenesis and prognosis for this entity is more complex than was initially appreciated. In about 15% of unilateral cases, postnatal nuclear scans show some minimal functional renal tissue amid the dysplastic areas, so complete absence of renal function is no longer the *sine qua non* (34). Numerous cases of localized (restricted to one pole) cystic dysplasia have been reported (35). More importantly, the contralateral kidney often (20–30% of cases) exhibits some form of limited dysplasia (36–38). About one quarter of contralateral kidneys exhibit vesico-ureteral reflux and this may be associated with recurrent urinary tract infections and progressive renal insufficiency (39). Careful evaluation of contralateral renal growth by serial postnatal ultrasonography, DMSA nuclear scans to detect foci of dysfunctional parenchyma and voiding cystourography to identify contralateral vesico-ureteral reflux may be considered to identify cases with significant contralateral dysplasia (40).

Because experimental obstruction may produce cystic dysplasia, it has been proposed that first trimester urinary tract obstruction might account for MCKD (32, 40). However, the putative obstruction would have to be intrarenal since MCKD ureters are atretic and lower tract obstruction could not explain cases of localized dysplasia. Furthermore, there are reports of autosomal dominant MCKD and chromosomal anomalies (41, 42) suggesting that failure of key genes can lead to MCDK by perturbing the normal pattern of nephrogenesis. A high incidence of subtle genital and other non-renal abnormalities suggest aberrations in shared developmental programs rather than urinary tract obstruction as the primary etiology (37, 38).

About 3% of children with unilateral MCKD develop hypertension (39); in some cases, hypertension resolves

when the dysplastic tissue is resected (43). Ectopic renin gene expression has been documented in macrophage-like interstitial cells (44). Although hypertension is not commonly identified in children with unilateral MCDK (<5%), its prevalence may be underestimated. Subtle abnormalities of blood pressure were found in 5 of 25 such children when studied by ambulatory blood pressure monitoring (45).

It has long been appreciated that MCKDs may undergo complete or partial involution, but the significance of this observation is not yet clear (46). Involution of MCKD tissue has been observed during fetal life and 20–25% of MCDKs involute completely in the first 1–2 years of postnatal life (47–49). When involution does not occur, the spectre of rare malignant transformation is raised. Multiple cases of Wilms tumor (about 3–10 fold increased incidence over the normal population) (50, 51) and renal cell carcinoma (52–55) arising from within the cystic tissue have been reported. On the other hand, because Wilms tumors grow rapidly, effective ultrasound screening would have to be done about every 3 months for 8 eight years (56); renal carcinomas may arise in adulthood. Several recent reports advise conservative management and routine early excision of MCDKs has become much less common than in the past (56, 57). However, the approach to MCKD tissue which does not involute is still made on an individual basis after discussion with the family.

Abnormalities of Kidney Position and Patterning

Variations in the gross architecture of the kidney are fairly common. Nearly 1% of patients undergoing ultrasonography are found to have duplex kidneys; of these 80% have ureteral duplication as well (58). However, there is no evidence that duplication of the collecting system by itself carries any associated morbidity. Very rarely, ectopic kidneys have been reported in the thorax, but these are usually functional and are identified when the unidentified chest mass is spotted incidentally (59).

Horseshoe kidneys represent the most common renal fusion abnormality and occur in every 400–800 individuals (60). They are unusually common (30%) in Turner syndrome (61). Horseshoe kidneys are usually found by ultrasonography but other imaging modalities are needed to identify the anomaly in about one third of cases. In one series, vesicoureteral reflux was noted in 32% and ureteropelvic junction obstruction obstruction in 23% (62). The risk of Wilms tumor is slightly increased in children with horseshoe kidney and there are over 40 reports of this association in the literature (63). However, regular screening for this complication is probably unwarranted. Although 0.5% of Wilms tumors arise within a horseshoe kidney, this is only about 3 times the expected incidence. The arterial supply to the horseshoe kidney is highly variable, presenting a special technical challenge for transplantation. Nevertheless, horseshoe kidneys can be transplanted into recipients en bloc or after division of the isthmus and success rate is equivalent to normal cadaveric transplantation with either approach (60).

Primary Renal Hypoplasia

Renal Coloboma Syndrome

Normal mature kidneys contain about 617,000 glomeruli with mean glomerular size of about 6 μ^3 (64). In the 1970's, pediatric nephrologists recognized a familial form of "oligomeganephronia" in which renal hypoplasia and progressive proteinuric renal failure in childhood were associated with unusually large but otherwise normal-appearing glomeruli (65). During the same period, an autosomal dominant syndrome of renal hypoplasia associated with colobomas of the optic nerve (Renal-Coloboma Syndrome) was also characterized (66). In 1995, Eccles showed that Renal-Coloboma syndrome (RCS) was caused by heterozygous mutations of the PAX2 gene and drew attention to the high frequency of vesico-ureteral reflux in affected families (67–69). When patients with oligomeganephronia were subsequently re-investigated, these patients also proved to have heterozygous PAX2 mutations, though the optic nerve abnormality is often barely detectable (70–72). About 20 different inactivating mutations of the PAX2 gene have been described and the most common (a single base-pair insertion in the second exon of PAX2) also causes RCS in the inbred *1Neu* mouse strain (73). Studies of fetal kidney development in *1Neu* mice have led to the hypothesis that a critical function of the PAX2 gene is to prevent apoptosis of cells in the branching ureteric bud during kidney development (74, 75). Absence of one PAX2 gene is sufficient to permit increased ureteric bud cell apoptosis, compromising nephron number by slowing the rate of branching nephrogenesis. Presumably, PAX2 function also influences structure of the uretero-vesical junction. In this form of primary renal hypoplasia, there is little evidence of dysplasia and glomerular hypertrophy is presumably the compensatory response to the deficit in nephron number.

Branchio-Oto-Renal Syndrome

Renal hypoplasia is seen in another autosomal dominant disorder, the branchio-oto-renal (BOR) syndrome (OMIM 113,650). The BOR syndrome combines variable degrees of renal hypoplasia with branchial arch defects (lateral cervical fistulas or cysts) and a hearing disorder associated with malformed auricle, atresia of the ear canal, anomalies of the middle ear and hypoplasia of the cochlea or semicircular canals) (76). The otic defects can usually be delineated by computerized axial tomography and/or nuclear magnetic resonance imaging to help with diagnosis (77). Most commonly, the BOR syndrome is caused by mutations of the EYA1 gene on chromosome 18q13.3 (78). About 80 different EYA1 mutations have been reported and each tends to be unique to a particular family (79). In a screen of 435 families with features of BOR syndrome, about 40% are associated with EYA1 mutations (79, 80). Renal hypoplasia may be very subtle or may cause end-stage renal failure between 12 and 36 years of age. EYA1 is the human homolog (chromosome 18q13.3) of a small family of transcription factors originally identified in Drosophila because of their importance to eye development, but in humans the eye is unaffected. Within the same family, heterozygous mutations of EYA1 may cause renal defects ranging from bilateral renal agenesis to mild unilateral hypoplasia (81). Although renal hypoplasia can be quite variable in BOR, children with branchial arch defects and normal kidneys (BO syndrome) are probably due to mutations of a different gene on chromosome 1q31 (82).

During normal renal development EYA1 is expressed in the metanephric mesenchyme and homozygous EYA1 knockout mice lack primary outgrowth of the ureteric bud with subsequent failure of nephron induction (83). The developmental pathway regulated by EYA1 also involves members of the SIX family of transcription factors (84); some families with BOR syndrome (<5%) are caused by mutations of the SIX1 or SIX5 genes (80, 85).

Renal Tubular Dysgenesis (RTD)

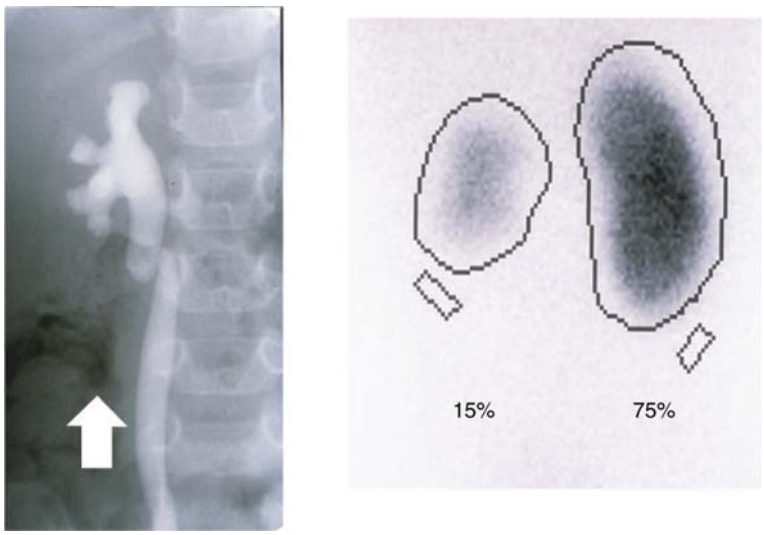
Renal hypoplasia can occasionally be restricted to specific segments of the nephron. Fetuses with autosomal recessive mutations of genes in the renin-angiotensin pathway are born with severe hypotension and Potter syndrome from failure to develop proximal tubules (86). Although glomeruli are relatively normal, the hypoplastic kidneys lack markers of differentiated proximal tubular cells; fetal oligo-anuria is accompanied by cranial hypoplasia (cranial suture diastasis) and survival past the neonatal period is unusual (87). However, it has been reported that infusions of fresh frozen plasma are effective in maintaining blood pressure and improving renal perfusion at birth (87). In one survivor treated with continuous infusion of FFP (1 ml/kg/hour) for 9 days and peritoneal dialysis from day 3, there was spontaneous recovery of urination at one month of age, allowing discontinuation of dialysis after two months (87). The syndrome is genetically heterogeneous and has been associated with autosomal recessive mutations of renin, angiotensin, angiotensin converting enzyme or type I angiotensin II receptor (86). Similar pathophysiology may be operative in children born to mothers taking high dose ACE inhibitors or AT II receprtor therapy for maternal hypertension.

Renal Dysplasia/Hypoplasia and Vesico-Ureteral Reflux

In 1973, Bailey first drew attention to children with urinary tract infection and vesico-ureteral reflux (VUR), who displayed "reflux nephropathy" (*Fig. 5-6*) involving distortion of renal calyces, patchy loss of renal parenchyma and, in some cases, progressive proteinuric renal disease leading to chronic renal failure (88, 89). Children with reflux nephropathy constitute 5% of pediatric renal transplant patients and 3.5% of pediatric dialysis patients in the NAPRTCS 2007 database. Since Bailey's original description, there has been an extensive and confusing literature concerning the pathogenesis of reflux nephropathy; the debate has focused on whether renal scars are acquired lesions due to VUR itself (and preventable by antireflux surgery) or from the aftermath of pyelonephritis (and preventable by prophylactic or early antibiotic therapy) or whether they are a congenital form of renal hypoplasia/dysplasia. It is generally agreed that VUR is associated with urinary tract infection, and that upper tract infection can occasionally lead to the appearance of new renal scars and that the severity of parenchymal loss correlates with the severity of VUR (90). However, reflux nephropathy is strongly familial (62, 91) and most renal "scars" are evident at the time of the first imaging studies (92, 93). Parenchymal defects are associated with VUR in utero (4) and most are recognizable prior to any proven urinary tract infection, particularly in male infants (94). Renal size estimated by magnetic resonance imaging is significantly reduced in children with unilateral or bilateral VUR compared to control children without VUR and correlates with severity of reflux (95). Interestingly, the presence of

Figure 5-6

Renal hypoplasia associated with ipsilateral vesico-ureteral reflux in a 2 year-old boy who presented with his first lower urinary tract infection. DMSA scintigraphy demonstrates normal reniform appearance of the hypoplastic parenchyma which contributes only about 10% of total renal function.



unilateral VUR predicts reduced renal size in both the refluxing and non-refluxing kidney (95).

A mechanistic link between abnormalities of the vesico-ureteral junction and dysplasia of the renal parenchyma is evident from the developmental biology of the urinary tract. During early fetal life, the two nephric (Wolffian) ducts migrate caudally toward the cloaca and, in response to a trophic signal (glial derived neurotrophic factor) from the adjacent mesenchyme, give rise to bilateral ureteric buds at sites corresponding to the future vesico-ureteral junctions. The ureteric buds arborize within the lateral metanephric mesenchyme, inducing nephrons at the tip of each branch. In 1975, Mackie and Stephens observed (by ureteroscopy) that many children born with duplex collecting systems and/or vesico-ureteral reflux also had malpositioned ureteric orifices (96). Recent animal studies have shown that the site of GDNF expression within metanephric mesenchyme and, thus, the site of ureteric bud outgrowth, is under complex regulation by various developmental genes including Pax2, Gdnf, Foxc2, Robo2 and Slt which are crucial for kidney development. In mutant mice, homozygous inactivation of the transcription factor gene Foxc2 causes anatomic abnormalities of the ureters associated with renal hypoplasia in about 60% of offspring (97). Foxc2 is normally expressed in the metanephric mesenchyme and is thought to be involved in guiding outgrowth of the ureteric bud (97). Interestingly the gross abnormalities were often unilateral (70–85% of offspring) and were highly dependent on the genetic background of the mice (97).

VUR and renal hypoplasia are seen in infants bearing heterozygous PAX2 mutations and renal-coloboma syndrome (98). However, this is rare and does not account for most cases of reflux nephropathy (98). More recently, Lu et al. identified a *de novo* translocation and two missense mutations affecting ROBO2 in several patients with renal anomalies and VUR (99). A subsequent study demonstrated ROBO2 missense mutations in about 5% of families with VUR and various congenital anomalies of the kidney and urinary tract (CAKUT) (100).

Renal Hypoplasia/Dysplasia and Fetal Urinary Tract Obstruction

Numerous observations indicate that antenatal obstruction of the urinary tract (UTO) may be associated with disturbances of normal nephrogenesis. Most commonly, renal hypoplasia/dysplasia is reported in males with posterior urethral valves but is also evident in other UTO settings such as prune-belly syndrome and urethral atresia (101–103). Dysplastic areas containing cysts, fibrotic interstitial zones and even islands of cartilage may be scattered amid fairly normal-appearing renal tubules. In fetal monkeys, obstruction produced by injection of agaral

beads into the collecting system at mid-gestation causes progressive diminution in renal size associated with evidence of apoptosis (particularly of collecting duct and glomerular cells), cysts with pericystic fibrotic collars and a defect in branching of the ureteric bud (104). Thus, although the molecular mechanisms are not yet well-understood, fetal UTO appears to modify survival of selected cell lineages and affect signals regulating developmental cell fates. Timing of the fetal obstruction may be important; very early (50 days gestation) obstruction of the urinary tract in lambs causes severe hypoplasia and interstitial fibrosis, whereas obstruction at 60 days results in a larger, cystic kidney with less fibrosis (105). In humans, striking obstruction has sometimes been reported in association with "normal" renal histology, prompting the speculation that UTO late in fetal life may have less effect on determinants of developmental cell fate (106).

As in other forms of renal hypoplasia/dysplasia, the risk of progressive renal insufficiency due to congenital UTO is highly influenced by the number of functioning nephrons at birth. The likelihood of developing end-stage renal disease ranges from 22% to 70% in various studies of boys with posterior-urethral valves and a key predictor of long-term outcome is serum creatinine level one year after relief of obstruction (103, 107). In 29 patients with the prune belly syndrome (deficient abdominal musculature, evidence of UTO, and cryptorchidism), about 1/3 die in the perinatal period from the Potter syndrome; among smaller portions of the renal parenchyma were dysplastic, but most develop end-stage renal disease (108).

Syndromic Renal Hypoplasia/Dysplasia

Renal dysplasia is associated with many different recognizable patterns of malformation, presumably involving genes or developmental pathways shared by multiple organs. Although a more complete list appears elsewhere, several syndromes of interest will be mentioned briefly. Mutations in the hepatocyte nuclear factor (HNF)-1beta gene (TCF2) are responsible for an autosomal dominant syndrome characterized by maturity-onset diabetes of the young, nondiabetic progressive nephropathy, genital malformations, and liver dysfunction (109). The HNF1beta gene is normally expressed in the Wolffian duct, metanephric tubules and Mullerian during fetal life; its absence results in a form of cystic renal dysplasia in which glomerular number is significantly reduced (110, 111). Interestingly, early onset progressive nephropathy may be seen without evidence of diabetes mellitus in some cases and renal dysplasia is quite variable, producing renal hypoplasia on one side with cystic dysplasia in the contralateral kidney (111).

Renal dysplasia is also part of the autosomal dominant HDR syndrome (hypoparathyroidism, deafness and renal anomalies) caused by mutations of another transcription factor gene, GATA3 (112) and is part of the Bardet Biedl syndrome caused by mutations at six different loci including the MKKS gene (113). Other monogenic syndromes with renal dysplasia include, Townes-Brock syndrome (SALL1 gene) (114) and nail-patella syndrome (LMX1B gene) (115).

Variable degrees of renal dysplasia have been described in numerous syndromes for which the etiology is unknown. The causes of the VATER syndrome (vertebral defects, anal atresia, tracheoesophageal fistula and renal dysplasia) are thought to be heterogeneous but presumably identify a common developmental pathway in these tissues (116). In the VACTERL syndrome (with additional limb and cardiac defects), 93% of patients have either vesicoureteral reflux in 39%, unilateral aplasia (23%) or unilateral multicystic dysplasia (7%) (117). Similarly renal dysplasia may be seen in association with developmental defects in the pancreas and liver (Ivemark syndrome) (118), jejunal atresia (119), encephalocoele (Meckel syndrome) (120) and with pancreatic fibrosis, liver dysgenesis and situs inversus (121). In Perlman syndrome, renal dysplasia and Wilm's tumor are associated with fetal gigantism and multiple congenital anomalies (122, 123).

Genetic and Environmental Causes of Subtle Renal Hypoplasia

Genetic influences on kidney size. Among "normal" humans, nephron number varies five-fold (1, 64, 124). While once dismissed as a benign reflection of human diversity, Brenner et al. proposed that individuals born at the low end of the nephron endowment spectrum may have increased risk of developing "essential" hypertension and renal insufficiency later in life (125, 126). They hypothesized that signals driving compensatory hypertrophy of overworked nephrons cause glomerulosclerosis and a cycle of subtle, slowly progressive renal dysfunction (127).

Recent evidence supports this theory; an autopsy study by Keller et al. showed that German adults with essential hypertension had 47% fewer nephrons per kidney than well-matched normotensive controls (128). As predicted, hypertensive subjects had hypertrophic glomeruli (glomerular volume = 233% of controls) and increased glomerulosclerosis (5.5% of glomeruli vs. 0%

in controls). Other evidence suggests that racial differences in congenital nephron number might also explain the relatively high incidence of end-stage renal disease in Aboriginal vs. Caucasian populations (129). At autopsy, Aboriginal subjects had 23% fewer glomeruli (683,174/kidney) than Caucasians (885,318/kidney) ($p < 0.04$).

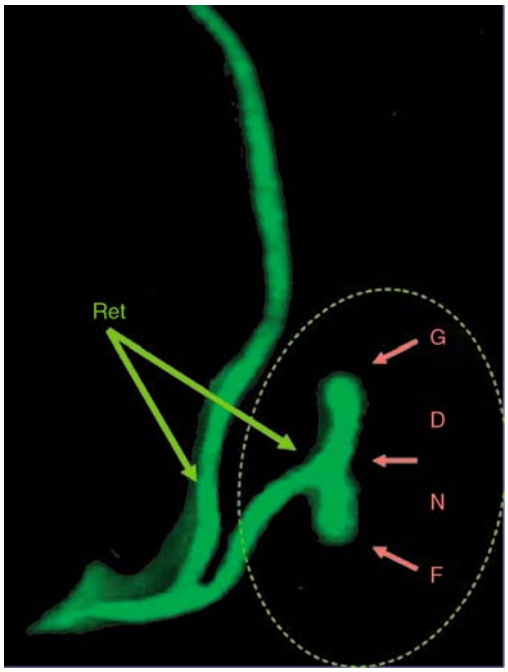
Little is known about the factors which set nephron number, but the GDNF/RET signaling pathway appears to play a central role. GDNF released from metanephric mesenchyme elicits outgrowth of ureteric bud cells bearing its receptor (RET) (130); homozygous *Ret* knockout mice are anephric (17). Both GDNF and RET are expressed during kidney development (*Fig. 5-7*) under the control of the transcription factor, PAX2 (131). Recently, it was found that 15% of human neonates bearing genetic variants of the *PAX2* and *RET* genes are born with kidneys which are 25% smaller than those with the more common alleles (132). It is not yet known whether these genes or the modest reduction in kidney size affect risk for hypertension or susceptibility to renal injury later in life.

Environmental influences on kidney size. Fifty years ago, Wilson reported that severe vitamin A (retinol) deficiency causes renal agenesis (133, 134). More recent observations indicate that even modest maternal retinol deficiency (50% reduction in circulating levels of retinol) can cause significant renal hypoplasia in rodents; postnatal kidney weight is decreased by 24% and nephron number is reduced by 20% (135). Normally, the fetus acquires retinol from the maternal circulation and converts it to an active metabolite, all-trans retinoic acid (atRA), in the kidney and other peripheral tissues (136). In fetal rat kidneys cultured ex vivo, all-trans retinoic acid (0.1–1 μM), accelerates new nephron formation by 2–3 fold (137, 138). In many developing countries, Vitamin A deficiency is widespread but and in North America most pregnant women (99%) have retinol levels in the normal range. Interestingly, newborn kidney size (factored for body size) is 40% smaller in Bangalore, India (where 40% of women were found to have gestational vitamin A deficiency) compared to newborns from Montreal (139). It is not yet known whether this form of subtle renal hypoplasia is directly due to maternal vitamin A deficiency or some other factor.

As discussed above, neonates with mutations of genes in the renin-angiotensin system pathway develop Potter Syndrome and proximal tubular agenesis (86). Similarly, infants born to mothers who receive angiotensin converting enzyme inhibitors during pregnancy have high risk of oligohydramnios and renal insufficiency in the newborn period (140).

Figure 5-7

Initiation of kidney development in a fetal mouse bearing a HoxB7/GFP transgene to identify nephric duct and early ureteric bud. Gdnf production by the metanephric mesenchyme and expression of Ret receptors in the ureteric bud lineage are indicated.



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6 Syndromes and Malformations of the Urinary Tract

Chanin Limwongse

Introduction

Birth defects involving the kidney and urinary system are often encountered and frequently occur in association with other structural abnormalities. A congenital urinary tract anomaly may provide the first clue to the recognition of multiorgan developmental abnormalities. Nevertheless many renal anomalies remain asymptomatic and undiagnosed. Therefore it is critical, not only for pediatric nephrologists but also for pediatricians in general, to be familiar with the common anomalies involving the kidney and urinary system and the more complex disorders with which they may be associated.

The kidney is a pivotal organ in dysmorphology. Although the number of single malformations involving the kidney is limited, combinations of these malformations in conjunction with anomalies involving other organ systems are found in more than 500 syndromes. In addition, many well-known sequences and associations involve the kidney and urinary tract. This chapter discusses common malformations, sequences and associations involving the kidney and urinary tract, and provides a summary of conditions that have these anomalies as one of their features. In addition, *Tables 6-1–6-3* summarize more detailed information about a large number of disorders, including their phenotypic features, reported urinary tract anomalies, pattern of inheritance, causative genes and related references. These tables can be used both to provide readily available information about potential urinary tract anomalies for patients with a diagnosed genetic syndrome and to suggest a differential diagnosis when anomalies are identified. Readers interested in additional details about a specific syndrome are referred to standard reference textbooks and databases about syndromes and malformations for further reading (e.g., (5–7)).

To understand the pathophysiologic basis of structural abnormalities, it is important to be familiar with the meaning of certain terms as they are used in describing malformations and syndromes.

Malformation refers to a single structural anomaly that arises from an error in organogenesis. Such an error may be due to the failure of cells or tissues to form, to die (programmed cell death), or to induce others. Examples include renal agenesis, horseshoe kidney, and bladder exstrophy.

Deformation refers to a single structural anomaly that arises from mechanical forces, such as intrauterine constraint. Examples include many cases of metatarsus adductus, torticollis, and congenital scoliosis. The underlying tissue may be normal or abnormal, and sometimes a malformation (e.g., renal agenesis) can predispose patients to a deformation (e.g., Potter's sequence from oligohydramnios).

Disruption refers to a single structural anomaly, that results from a destructive event after normal morphogenesis. Such events can be caused by lack of vascular supply, an infectious process, or mechanical factors. Examples include limb amputation from amniotic bands and abdominal wall defect from vascular insufficiency related to maternal cocaine use.

Sequence refers to a cascade of abnormalities that result from a single initiating anomaly. Sequences can be malformational, deformational, or disruptive, and they sometimes represent more than one of these categories. Obstruction of urine flow at the level of the ureter during early gestation, for example, can cause malformation of the kidneys, intestines, and abdominal wall – a malformation sequence. At the same time, decreased urine flow will produce oligohydramnios, fetal compression, and multiple deformities of the face, limbs, and chest wall – a deformation sequence.

Syndrome refers to a consistently observed pattern of anomalies found in an individual, whether malformation, deformation, or disruption. Anomalies comprising a syndrome are thought to have a single cause, although in many cases, their causes are still unknown. Examples include Turner syndrome and fetal alcohol syndrome.

Association refers to a constellation of anomalies that occur together more often than expected by chance alone but cannot be explained by a single cause or sequence of events, and so do not represent a syndrome or sequence. VATER association, which is discussed later in this chapter, is a common example.

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Table 6-1

Syndromes and disorders that have urinary tract anomalies as a frequent feature

Syndromes	Urinary tract abnormalities											Other associated anomalies	Other associated anomalies	Gene(s)	Ref.
	Renal agenesis	Ectopia/ horseshoe	Cystic/dysplasia	Duplication	Hypoplasia	Hydronephrosis/ ureter	Diverticulae	Atresia/stenosis	Reflux	Nephritis/ sclerosis	Tumor/ nephromegaly				
Abruzzo-Erickson		H										Coloboma, cleft palate, hypospadias, deafness, short stature	XR	Unk	(48)
Acrocephalopolydactylous dysplasia (Elejalde syndrome)			+		+	+						Acrodactyly, hand hexadactyly, overgrowth, visceromegaly, globular body, redundant neck skin	AR	Unk	(49)
Acrorenal (Dieker)	1	E		+	+	+	Ua					Ectrodactyly, oligodactyly, hypoplastic carpal/tarsal bones	Sporadic	Unk	(50)
Acrorenal (Johnson-Munson)	1, 2					U, Ua						Aphalangy, hemivertebrae, genital/ intestinal/anal dysgenesis	AR	Unk	(50)
Acrorenal (Siegler)		E				+		U	+			Short stature, hypoplastic radii/ ulnae/humeri, oligodactyly	Uncertain	Unk	(50)
Acro-renal-mandibular	1		+									Ectrodactyly, hypoplastic mandible	AR	Unk	(51)
Acro-renal-ocular	1	E			+		B					Hypoplastic thumb, optic coloboma, cleft lip/palate	AD	SALL4	(52)
Adrenoleukodystrophy, neonatal												See Pseudo-Zellweger			
Aglossia-adactylia	1											Micrognathia, cranial nerve palsy	Sporadic	Unk	(53)
Agnathia-holoprosencephaly		H				+						Arrhinencephaly, situs inversus, midline defects	Sporadic	Unk	(54)
Alagille	1		+	+						+		Cholestasis, peripheral pulmonic stenosis, characteristic face	AD	JAG1	(55)
Alport										+		Nephritis, proteinuria, deafness	AD, AR, XL	COL4	(56)
Alsing										+		Nephritis, nephronophthisis, optic coloboma, hip dislocation	AR	Unk	(57)
Alstrom										+		Diabetes mellitus, retinopathy, short stature, deafness	AR	ALMS1	(58)

Amelogenesis imperfecta type IG										+		Enamel hypoplasia, nephrolithiasis, enuresis	AR	Unk	(59)
Amyloidosis type 5										+		Nephropathy, proteinuria, cranial nerve palsy, cutis laxa	AD	GELSOLIN	(60)
Angiotensin converting enzyme (ACE) inhibitor, maternal use										+		IUGR, oligohydramnios, patent ductus arteriosus, limb anomalies, renal artery stenosis, progressive renal failure	Sporadic	none	(61)
Aniridia-Wilms tumor (WAGR)											+	Aniridia, ambiguous genitalia, hypospadia, short stature	AD	WT1	(62)
Axial mesodermal dysplasia	1											Bladder exstrophy, vertebral anomalies, Goldenhar-like	Uncertain	Unk	(63)
Baldellou	1											Hypoparathyroidism, ocular coloboma, MR, seizures	Uncertain	Unk	(64)
Baller-Gerold	+	E	+		+							Craniosynostosis, radial aplasia, malformed ear, anal atresia	AR	RECQL4	(65)
Barakat										+		Mesangial sclerosis, MR, optic atrophy, nystagmus	AD	GATA3	(66)
Bardet-Biedl			+	+		+	+		+	+		Obesity,polysyndactyly, MR, retinopathy, hypogonadism	AR	BBS1-12	(67, 68)
Beckwith-Wiedemann		E	+	+		+					+	Overgrowth, macroglossia, omphalocele, embryonal tumors	Sporadic, AD	CDKN1C, NSD1, H19	(69–72)
Berardinelli						+					+	Insulin resistance, lipodystrophy, acanthosis nigricans, MR	AR	BSCL2	(73)
Brachymesomelia-renal			+									Micrognathia, corneal opacity, craniofacial dysmorphism	Uncertain	Unk	(74)
Branchio-oculo-facial	1											Philtrum hypertrophy, cleft lip/palate, branchial remnant	AD	TFAP2A	(75)
Branchio-oto-renal	1, 2	E	+	+	+	+			+			Branchial remnant, preauricular pit/tag, microtia, deafness	AD	EYA1, SIX5	(76)
Braun-Bayer				+				U		+		Deafness, bifid uvula, digital anomalies	Uncertain	Unk	(77)
Cat eye												See *Table 6-3*	Chromosomal		
Caudal duplication	1, 2	E		+		+			+			Duplication of colon, sacrum, genitalia, vertebral defects	Sporadic	AXIN1	(78)
Caudal regression	1, 2	E, H			+	+	Ua		+			Atresia of colon, anus, genitalia, vertebral defects, TE fistula	AD, AR	VANGL1	(79–80)

Table 6-1 (Continued)

Syndromes	Urinary tract abnormalities											Other associated anomalies	Other associated anomalies	Gene(s)	Ref.
	Renal agenesis	Ectopia/ horseshoe	Cystic/dysplasia	Duplication	Hypoplasia	Hydronephrosis/ ureter	Diverticulae	Atresia/stenosis	Reflux	Nephritis/ sclerosis	Tumor/ nephromegaly				
Cerebro-hepato-renal (Passarge)			+									See Zellweger syndrome	AR	PEX	(81)
Cerebro-oculo-hepato-renal			+									Cerebellar hypoplasia, hepatic fibrosis, Leber amaurosis	AR	Unk	(82)
Cerebro-osteo-nephro dysplasia			+							+		Rhizomelic limb shortening, cerebral atrophy, MR, seizures	AR	Unk	(83)
CHARGE	+	E	+		+	+	+	U	+			See text for details	Sporadic, AD	CHD7	(33–37)
Chondroectodermal dysplasia (Ellis van Creveld)	1		+			+				+		Acromelic dwarfism, polydactyly, nail dystrophy, tooth hypoplasia narrow thorax, CHD	AR	EVC, EVC2	(84–85)
Cocaine, maternal use	1, 2				+	+	Ua		+			Vascular disruption anomalies affecting multiple organs	Sporadic	none	(86)
Cornelia de Lange	1		+		+	+					+	SS,microcephaly, limb defect, hirsuitism, synophrys	Sporadic	NIPBL, SMC1L1	(87)
Crossed renal ectopia-pelvic lipomatosis		E				+		U				Clubbing of fingers, gynecomastia	Uncertain	Unk	(88)
Czeizel						+		U				Ectrodactyly, spina bifida, megacystis	AD	Unk	(89)
Diabetic mother, infant of	1, 2	E, H	+	+	+	+	Ua		+			Neural tube defect, cardiac/limb anomalies, sacral agenesis	Sporadic	none	(90–91)
DiGeorge/velocardiofacial	1, 3	E		+	+	+		+	+			See *Table 6-3*	Chromosomal		
Denys-Drash		E		+						+	+	Pseudohermaphroditism, Wilms tumor, proteinuria	Sporadic	WT1	(92)
Down												See *Table 6-3*	Chromosomal		
Ectrodactyly-ectodermal dysplasia-clefting (EEC)	1		+	+		+			+			Ectrodactyly, hypohidrosis, sparse hair, cleft lip/palate	AD	Unk	(93)

Elejalde												See acrocephalopolydactylous dysplasia			
Epstein										+		Thrombocytopenia, nerve deafness, cataract	AD	MYH9	(94)
Facio-cardio-renal		H				+		U				Cardiomyopathy, conduction defect, MR, typical face	AR	Unk	(95)
Fanconi anemia	1	E, H	+	+	+	+						Pancytopenia, limb defects, leukemia, lymphoma	AR	FANCA-N	(96–97)
Fetal alcohol	1	E, H	+	+	+	+						IUGR, DD, microcephaly, short palpebral fissure	Sporadic	none	(98)
Fibromatosis, infantile					+						+	Multiple myofibromatosis, myositis ossificans	AR	Unk	(99)
Fraser cryptophthalmos	1, 2		+					U				Fused eyelids, ear/genital anomalies, syndactyly	AR	FRAS1	(100)
Frasier					+					+		Male pseudohermaphrodite, amenorrhea, ovarian cysts	AD	WT1	(101)
Goeminne			+							+		Congenital torticollis, keloids, cryptorchidism	XR	Unk	(102)
Goldenhar (oculo-auriculo-vertebral)	1	E	+	+	+	+			+			Hemifacial microsomia, ear anomalies, vertebral defects	Sporadic, AD	Unk	(103)
Goldston			+									Dandy-Walker malformation, cerebellar malformation	Uncertain	NPHP3	(104)
Graham			+								+	Cystic hamartoma of lung and kidney	Sporadic	Unk	(105)
Hemifacial microsomia (oculo-auriculo-vertebral)												See Goldenhar syndrome			
Hemihyperplasia			+								+	Asymmetry, vascular malformation, embryonal tumors	Sporadic	LIT1, H19	(106)
Hepatic fibrosis			+									Congenital hepatic fibrosis	Sporadic	PKHD1	(107)
Holzgreve	2											Potter sequence, cardiac defect, polydactyly, cleft palate	Uncertain	Unk	(108)
Hypertelorism-microtia-clefting		E		+								Microcephaly, cleft lip/palate, MR	AR	Unk	(109)
Ivemark	1		+		+	+						Poly/asplenia, complex CHD, laterality defects	Sporadic, AR	Unk	(110)
Jeune			+									Narrow chest, short limbs, polydactyly, glomerulosclerosis	AR	EVC, EVC2	(84–85)

Table 6-1 (Continued)

Syndromes	Urinary tract abnormalities											Other associated anomalies	Other associated anomalies	Gene(s)	Ref.
	Renal agenesis	Ectopia/ horseshoe	Cystic/dysplasia	Duplication	Hypoplasia	Hydronephrosis/ ureter	Diverticulae	Atresia/stenosis	Reflux	Nephritis/ sclerosis	Tumor/ nephromegaly				
Joubert			+									Vermis aplasia, apnea, jerky eyes, retinopathy, ataxia	AR	JBTS1-7	(111)
Juberg-Hayward		H			+							Microcephaly, cleft lip/palate, abnormal thumbs/toes	AR	Unk	(112)
Kabuki		H		+		+		U	+			MR, characteristic Kabuki-like face, large ears, cleft palate	Uncertain	Unk	(113)
Kallmann	1				+							Anosmia, cleft lip/palate, hypogonadotrophic hypogonadism	AD, AR, XR	KAL1-4	(114)
Kaufman-McKusick		E	+	+		+		Ua				Hydrometrocolpos, polydactyly, anal/urogenital sinus defect	AR	GLI3	(115)
Kivlin		E		+								Short stature, Peters' anomaly, MR, genital/cardiac defects	AR	B3GALTL	(116)
Klippel-Feil	1	E		+								Short neck, cervical vertebral fusion, low posterior hairline	Sporadic, AD	Unk	(117)
Kousseff	1											Sacral meningocele, hydrocephalus, cardiac defects	AR	Unk	(118)
Leprechaunism (Donohue)			+								+	Insulin resistance, lipodystrophy, hirsutism	AR	INSR	(119)
Limb-body wall complex	1	E	+			U, Ua						Lateral body wall defect, limb reduction, CHD	Sporadic	Unk	(120)
Mammo-renal				+								Ipsilateral supernumerary breasts/ nipples	Sporadic	Unk	(121)
Marden-Walker			+		+							Microcephaly, blepharophimosis, micrognathia, contractures	AR	Unk	(122)
Meckel-Gruber			+		+	U, Ua						Encephalocele, cardiac defects, cleft lip/palate, polydactyly	AR	MKS1-4	(123)
Megacystis-microcolon						+		U				Large bladder, intestinal hypoperistalsis, oligohydramnios	AR	Unk	(124)

Melnick-Needles osteodysplasty						+		U				Bowing long bones, short upper limbs, micrognathia	XR	FLNA	(125)
Mendelhall											+	Insulin resistance, acanthosis nigricans	AR	INSR	(126)
Microgastria-upper limb anomaly	1	E			+							Hypoplastic spleen, limb reduction defects	Uncertain	Unk	(127)
Miranda	+		+									Brain malformation, liver dysplasia	Uncertain	Unk	(128)
Moerman	1		+	+	+	+						Short limbs, brain malformation, cleft palate	Uncertain	Unk	(129)
MURCS association	1	E	+					U				See text for details	Sporadic	Unk	
Nager acrofacial dysostosis	+			+		+			+			Facial bone hypoplasia, cleft eyelid, radial ray defect	AD	Unk	(130)
Nail-patella										+		Absent/hypoplastic nails and patellae	AD	LMX1B	(131)
Neu-Laxova	1											IUGR,lissencephaly, CHD, pterygia, ichthyosis	AR,sporadic	Unk	(132)
Neuro-facio-digito-renal	1											Prominent forehead with vertical groove, MR, ear anomaly	Uncertain	Unk	(133)
Neurofibromatosis type I	1							Ua			+	renal artery stenosis, hypernephroma, cafe-au-lait spots	AD	NeUnkro fibromin	(134)
Nezelof			+							+		Arthrogryposis, hepatic impairment, hypotonia, club feet	AR	Unk	(135)
Noonan			+	+		+						Webbed neck, short stature, MR, pulmonic stenosis	AD	NS1	(136)
Occipital horn						+	B	Ua				Cranial exostosis, hyperextensibility, cutis laxa	XR	cUnkATPase	(137)
Ochoa				+		+	B U, Ua +					Facial grimacing with lateral displacement of mouth	Uncertain	Unk	(138)
Oculo-auriculo-vertebral												See Goldenhar syndrome			
Oculo-hepato-encephalo-renal			+		+							Encephalocele, hepatic fibrosis, coloboma	AR	Unk	(82)
Oculo-renal-cerebellar										+		MR, spastic diplegia, choreoathetosis, retinopathy	AR	Unk	(139)
Oculorenal												See Pierson syndrome			
Oculorenal (Karcher)	1				+					+		Optic nerve coloboma	AD	Unk	(140)

Table 6-1 (Continued)

Syndromes	Urinary tract abnormalities											Other associated anomalies	Other associated anomalies	Gene(s)	Ref.
	Renal agenesis	Ectopia/ horseshoe	Cystic/dysplasia	Duplication	Hypoplasia	Hydronephrosis/ ureter	Diverticulae	Atresia/stenosis	Reflux	Nephritis/ sclerosis	Tumor/ nephromegaly				
OEIS (omphalocoele-exstrophy of bladder-imperforate anus-spinal dysraphism) complex			+			+		Ua	+			Malrotation of colon, sacral defect, tethered spinal cord, pelvic bone abnormalities	Sporadic	Unk	
Otorenal			+			+	+	U				Renal pelvis diverticulae, nerve deafness	AD	Unk	(141)
Pallister-Hall	1, 2	E, H	+			+						Hypothalamic hamartoblastoma, polydactyly	AD	GLI3	(142–143)
Pierson			+									Hypoplastic retina, cataract, anterior chamber anomalies	AR	LAMB2	(144)
Penoscrotal transposition		E	+		+	+	B	U				Abnormal placement of external genitalia	Sporadic	Unk	(145)
Perlman			+	+		+				+	+	Early overgrowth, typical face, nephroblastomatosis	AR	Unk	(146)
Polydactyly-obstructive uropathy						+	Ua		+			Post-axial polydactyly of hands and feet	Uncertain	Unk	(147)
Potter (oligohydramnios)	1, 2		+		+			Ua				See text for details	Sporadic	Unk	
Prune belly			+	+		+	Ua		+			See text for details	Sporadic	Unk	
Pseudo-Zellweger			+								+	Hypotonia, seizures, MR, typical face, FTT, hepatomegaly	AR	PTS1	(148–150)
Pyloric stenosis		H	+	+	+	+						Cystic kidney	Sporadic	Unk	(151)
Rass-Rothschild	+					+						Klippel-Feil anomaly, sacral agenesis, cryptorchidism	Uncertain	Unk	(152)
RAPADILINO						+						Radial/patella hypoplasia, diarrhea, short stature, long nose	AR	RECQL4	(153)
Renal/Mullerian hypoplasia		H			+				+			Absent uterus, broad forehead, DD, large fontanel	AR	Unk	(154)

Renal dysplasia or adysplasia	1	E	+		+	+	Ua		+			Abnormal uterus in some patients	AD	RET, UnkPK3A	(155)
Renal-hepatic-pancreatic dysplasia			+									Pancreatic cysts, extrahepatic biliary atresia, Caroli disease	AR	EVC, EVC2	(84–85)
Retinoic acid, maternal use					+	+		U				Ear anomalies, CHD, cleft palate, neural tube defect	Sporadic	none	(156–157)
Roberts	1	H	+			+						Limb reduction, oligo/syndactyly, CHD, dysmorphic face	AR	ESCO2	(158)
Robson										+		MR,macrocephaly, deafness, proteinuria, Alport-like	XR	COL4A	(159)
Rokitansky-Mayer-Kuster-Hauser	1	E	+	U	+							Absence of vagina, uterine anomalies, amenorrhea	Sporadic	Unk	(160)
Rubella, congenital	1		+	+								CHD, MR, deafness, cataract, growth retardation	Sporadic	none	(161)
Rubinstein-Taybi	1	E	+	+	+			Ua				SS, MR, broad thumbs and great toes, typical face	Sporadic	CREBBP	(162–163)
Russell-Silver						+		Ua				SS, triangular face, asymmetry, clinodactyly, hypoglycemia	Sporadic,AD	Unk	(164)
Santos	1											Hirschsprung disease, hearing loss, postaxial polydactyly	AR	Unk	(165)
Say			+									SS,microcephaly, micrognathia, large ear, cleft palate	AD	Unk	(166)
Schimke										+		SS,spondyloepiphyseal dysplasia, immunodeficiency	AR	SMARCAL1	(167)
Schinzel-Giedion		E		U		+		U				CHD, distinctive face, figure 8 head shape, eyelid groove	AR	Unk	(168)
Senior-Loken			+							+		Nephronophthisis, tapeto retinal degeneration	AR	NPHP1,4,5	(169)
Setleis						+		U				Cutis aplasia with temporal scarring, abnormal eyelashes	AD,AR	Unk	(170)
Short rib, Beemer Langer			+		+	+		U				Hydrops, cleft lip, bowed long bones, atretic ear canal	AR	Unk	(171)
Short rib-polydactyly, type 1–3	1		+					Ua				Urethral fistula, CHD, cloacal/ urogenital sinus anomalies	AR	Unk	(171–172)
Silverman (dyssegmental dwarfism)						+		U				SS, flat face, cleft palate, generalized skeletal dysplasia	Uncertain	HSPG2	(173)
Simopoulos			+									Hydrocephalus, polydactyly	AR	Unk	(174)

Table 6-1 (Continued)

Syndromes	Urinary tract abnormalities											Other associated anomalies	Other associated anomalies	Gene(s)	Ref.
	Renal agenesis	Ectopia/ horseshoe	Cystic/dysplasia	Duplication	Hypoplasia	Hydronephrosis/ ureter	Diverticulae	Atresia/stenosis	Reflux	Nephritis/ sclerosis	Tumor/ nephromegaly				
Simpson-Golabi-Behmel			+			+					+	Overgrowth, polydactyly, typical face, arrhythmia	XR	GPC3	(175)
Sirenomelia sequence	1, 2	E	+	+	+	+	+	+	+			See text for details	Sporadic	Unk	
Smith-Lemli-Opitz	1		+		+							SS, ambiguous genitalia, 2–3 toe syndactyly, brain anomalies	AR	DHCR7	(176–177)
Sommer	1											Iris aplasia, corneal opacity, glaucoma, prominent forehead	AD	Unk	(178)
Sorsby (coloboma-brachydactyly)	1											Ocular coloboma, brachydactyly type B, bifid thumbs	AD	Unk	(179)
Sotos								Ua			+	Overgrowth, MR, embryonal tumors, advanced bone age	Sporadic	NSD1	(180)
Supernumerary nipples-renal anomalies		E	+	+	+	+						Familial polythelia	AD	Unk	(181)
Thalidomide, maternal use	1, 2	E, H	+		+	+	+		+			Limb reduction, phocomelia, neural tube defect	Sporadic	none	(182–183)
Thymic-renal-anal-lung	+		+					U				SS, absent thymus, parathyroid agenesis, urethral fistula	AR	Unk	(184)
Tolmie			+									Lethal multiple pterygia, long bone abnormalities	XR	CHRN	(185)
Townes-Brock	1		+		+			Ua	+			Triphalangeal thumb, imperforate anus, skin tag, deafness	AD	SALL1	(186)
Trimethadione, maternal use	1							U				SS, CHD, omphalocele, distinctive face	Sporadic	none	(187)
Tuberous sclerosis			+								+	MR, seizures, cortical tuber, facial angiofibroma	AD	TS1, TS2	(188)

Turner												See *Table 6-3*	Chromosomal		
Ulnar-mammary	1											Oligodactyly, ulnar ray defect, nipple aplasia, genital defects	AD	TBX3	(189)
Urogenital adysplasia	1, 2		+									Absent uterus, vaginal atresia, hydrometrocolpos	AD	RET, UnkPK3A	(190)
VATER (VACTERL) association	1	E, H	+	+	+	+	+	+	+			See text for details			
Velocardiofacial	1, 2	E, H		+	+	+		+	+			See *Table 6-3*			
von Hippel-Lindau			+								+	Cerebello-retinal angiomatosis, pheochromocytoma	AD	VHL	(191–192)
Weinberg-Zumwalt			+								+	Multiple lung cysts, ascites, accessory spleen	Uncertain	Unk	(193)
Wenstrup	1		+		+	+		U				Female pseudohermaphrodite, imperforate anus	Uncertain	Unk	(194)
Weyers		H	+	+		+		U				Oligodactyly, pterygia, sternal defect, cleft palate	AR	EVC	(195)
Williams		E			+	+	B, U	U				See *Table 6-3*	Chromosomal		
Wilms tumor-horseshoe kidney		H									+	Possible association	Sporadic	WT1	(196)
Wilms tumor-hemihypertrophy											+	Ipsilateral vascular malformation, cafe-au-lait spots	Sporadic	WT1	(106)
Wilms tumor-radial aplasia											+	Hypoplastic fibula/tibia, abnormal thumbs	Sporadic	Unk	(197)
Winter (oto-renal-genital)	1, 2		+									Middle ear anomalies, deafness, vaginal atresia	AR	Unk	(198)
Wolfram (DIDMOAD)						+			+			Diabetes mellitus/insipidus, optic atrophy, nerve deafness	AR, mitochondrial	WFS1-2	(199)
Zellweger	1		+			+						Hypotonia, seizures, hepatosplenomegaly, growth delay	AR	PEX	(148–150)

U ureter; *B* bladder; *Ua* urethra; *1* unilateral renal agenesis; *2* bilateral renal agenesis; *E* ectopia; *H* horseshoe; *ACC* agenesis of corpus callosum; *SS* short stature; *MR* mental retardation; *DD* developmental delay; *CHD* congenital heart disease; *FTT* failure to thrive; *IUGR* intrauterine growth retardation; *Unk* Gene unknown

Table 6-2

Well-known syndromes associated with occasional urinary tract anomalies

Syndromes	Renal agenesis	Ectopia/ horseshoe	Cystic/ dysplasia	Duplication	Hypoplasia	Hydro-nephrosis/ ureter	Diverticulae	Atresia/ stenosis	Reflux	Nephritis/ sclerosis	Tumor/ nephro-megaly	Other Associated Anomalies	Inheritance Pattern	Gene(s)	Reference.
Aase								Ua				Triphalangeal thumb, hypoplastic anemia	AD,AR	RPS19, 24	(200)
Achondrogenesis				+		+						Micromelic dwarfism, short trunk, fetal hydrops	AR	COL2A1	(201)
Acrocallosal	1		+									ACC, macrocephaly, polymicrogyria, polydactyly, CHD	AR	GLI3	(202)
Acro-facial dysostosis		H			+							Abnormal thumb/toe, facial bone defect,ear anomalies	AR	Unk	(203)
Acromelic frontonasal dysplasia		E		+								Polydactyly, ACC, encephalocele, Dandy-Walker anomaly	Sporadic	Unk	(204)
Adrenogenital	1			+		+						Ambiguous genitalia, vomiting,salt losing,UPJ obstruction	AR	CYP11,21	(205)
Adrenal hypoplasia-MR						+		U	+			Aminoaciduria, MR, muscular dystrophy, visual abnormality	X-linked	NROB1	(206)
Antley-Bixler		H		+								Craniosynostosis, radio-humeral synostosis, cardiac defects	AR	FGFR2	(207)
Apert (acrocephalo-syndactyly)			+			+						Acrocephaly, craniosynostosis, syndactyly	AD	FGFR2	(208)
Bloom											+	Short stature, telangiectasias, leukemia, lymphoma	AR	BLM	(209)
Bowen-Conradi		H		+								Micrognathia, arthrogryposis, cloudy cornea, brain anomaly	AR	Unk	(210)
Brachydactyly type E	1				+							Vertebral anomalies, narrow auditory canal	Uncertain	HOXD13	(211)
3C (Ritscher-Schinzel)						+						SS,Dandy-Walker anomaly,typical face, CHD	AR	Unk	(212)

C-trigonocephaly	1											Polysyndactyly, abnormal ear, hypospadias, dislocated joints	AR	CD96	(213)
Campomelic dysplasia	1		+			+						Tibial bowing, pretibial dimples, ambiguous genitalia	AD	SOX9	(214)
Carbohydrate deficient glycoprotein			+									FTT, abnormal fat pad, hepatosplenomegaly, neurodegeneration	AR	PMM2, MPI	(215)
Carpenter										+		Aminoaciduria, polysyndactyly, craniosynostosis	AD	RAB23	(216)
CHILD	1,2					+						Unilateral erythroderma, ipsilateral limb defect	X-linked	NSDHL	(217)
Chondrodysplasia punctata, non-rhizomelic			+									Flat face, microcephaly, cataract, short femora/humeri, stippled epiphyses	AR	Unk	(218)
Coffin-Siris	1					+						MR,sparse scalp hair, hirsutism,coarse face, thick lips	AR	Unk	(219)
Cutis la+a type I							B					GI tract diverticulae, emphysema, diaphragmatic defect	AR	FBLN5	(220)
Disorganization-like	+											Polydactyly, duplication of lower limbs, skin appendages	Sporadic	Unk	(221)
Duane anomaly-radial defects	1											Limited ocular abduction, radial defects, blepharophimosis	AD	SALL4	(222)
Ehlers-Danlos			+									Joint hypermobility, skin hyperextensibility, easy brusing	AD, AR, XR	COL5A, COL1A1	(223)
Epidermolysis bullosa						+		U				Skin blistering, pyloric stenosis, dystrophic nails, sparse hair	AR	LAMA3, LAMB3	(224–245)
Femoral hypoplasia-unusual facies	1											Various leg deformities, abnormal genitalia, typical face	Sporadic, AD	Unk	(226)
Floating-Harbor		E										SS, typical face, DD, delayed bone age	Uncertain	Unk	(227)

Table 6-2 (Continued)

Syndromes	Renal agenesis	Ectopia/ horseshoe	Cystic/ dysplasia	Duplication	Hypoplasia	Hydro-nephrosis/ ureter	Diverticulae	Atresia/ stenosis	Reflux	Nephritis/ sclerosis	Tumor/ nephro-megaly	Other Associated Anomalies	Inheritance Pattern	Gene(s)	Reference.
Focal dermal hypoplasia		H										Atrophy/linear skin pigmentation, hand/ vertebral anomalies	X-linked	PORCN	(228)
Freeman-Sheldon					+	+						Whistling face, ulnar deviation of hands, talipes equinovarus	AD	TNNT3, TNNI2	(229)
Fronto-metaphyseal dysplasia				+		+						Prominent supraorbital ridges, contractures, deafness	X-linked	FLNA	(230)
Frontonasal dysplasia	+	E										Hypertelorism, broad nasal tip, median cleft nose	Sporadic	Unk	(231)
Fryns			+									Digital hypoplasia, diaphragmatic defect, cleft palate	AR	Unk	(232)
G (Opitz/BBB)				+				+	+			See Opitz (G/BBB) syndrome			
Glutaric aciduria, type II			+									Cerebral anomalies, pancreatic dysplasia, biliary dysgenesis	AR	ETFA, B, DH	(233)
Grieg cephalopoly-syndactyly				+								Macrocephaly, polydactyly, hypertelorism	AD	GLI3	(234)
Hajdu-Cheney			+									SS, Wormian bones, acro-osteolysis, osteoporosis	AD	Unk	(235)
Hydrolethalus						+		Ua				Hydrocephalus, polydactyly, polyhydramnios,cleft lip	AR	HYLS1	(236)
Jarcho-Levin						+						Spondylothoracic dysplasia,fused ribs, hemivertebrae	AR	DLL3, MESP2	(237)
Johanson-Blizzard						+						Pancreatic insufficiency, spiky hair,small alae nasi	AR	UnkBR1	(238)
Killian-Pallister												See *Table 6-3*	Chromosomal		
Lacrimo-auriculo-dento-digital (LADD)	1											Nasolacrimal duct stenosis, malformed ears/enamel/digits	AD	FGFR2, 3	(239)
Larsen	1					+						Multiple joint dislocations, flat face	AD, AR	FLNB	(240, 241)

Lenz microphthalmia	1		+			+						Ocular coloboma, ear/facial anomalies, syndactyly/camptodactyly	X-linked	Unk	(242)
LEOPARD (multiple lentigines)	1											Hypertelorism, deafness, abnormal ECG, genital anomalies	AD	PTPN11, RAF1	(243)
Marfan			+							+		Tall thin habitus, aortic root dilatation, lens sublu+ation, arachnodactyly	AD	FBN1	(244, 245)
Marshall-Smith						+						MR, FTT, accelerated bone maturation, broad phalanges	Sporadic	Unk	(246)
Miller-Dieker (lissencephaly)	1		+									See *Table 6-3*	Microdeletion	LIS1	
Moebius-peripheral neuropathy				+								Peripheral neuropathy, anosmia, hypogonadism	Sporadic	Unk	(247)
Mohr-Majewski												See Oro-facio-digital syndrome type IV			
Multiple pterygium					+							Multiple soft tissue contractures, camptodactyly	AR	CHRNG	(248)
Myotonic dystrophy			+									Myotonia, muscle weakness, cataract, arrhythmia, diabetes	AD	DMPK	(249, 250)
Nijmegen breakage						+						MR, SS, microcephaly, immunodeficiency	AR	NBS1	(251)
Opitz (G/BBB)												Hypertelorism, hypospadias, cleft lip/palate, dysphagia	AD, X-linked	MID1	(252, 253)
Oro-facio-digital, type I			+			+						Midline cleft lip, multiple frenulae, polydactyly, tongue nodules	X-linked	CXORF5	(254)
Oro-facio-digital, type IV	1		+									Cleft palate, multiple frenulae, polysyndactyly, lobed tongue	AR	Unk	(254, 255)
Oro-facio-digital, type VI	1		+									Midline cleft lip, multiple frenulae, polydactyly, tongue nodules	AR	Unk	(254)
Pallister-Killian												See *Table 6-3*	Chromosomal		

Table 6-2 (Continued)

Syndromes	Renal agenesis	Ectopia/ horseshoe	Cystic/ dysplasia	Duplication	Hypoplasia	Hydro-nephrosis/ ureter	Diverticulae	Atresia/ stenosis	Reflux	Nephritis/ sclerosis	Tumor/ nephro-megaly	Other Associated Anomalies	Inheritance Pattern	Gene(s)	Reference.
Peutz-Jeghers			+									Hamartomatous intestinal polyposis, lip hyperpigmentation	AD	STK11	(257)
Poland anomaly	1			+	+							Hypoplastic pectoralis, ipsilateral upper limb reduction	Sporadic	Unk	(258, 259)
Restrictive dermopathy				U								Aplasia cutis, rigid skin, contractures, typical face	AR	LMNA, ZMPSTE24	(260)
Robinow				+		+						Mesomelic dwarfism, typical face, abnormal genitalia	AD, AR	ROR2	(261)
Rothmund-Thomson										+		Poikiloderma, alopecia, dysplastic nails, photosensitivity	AR	RECQL4	(262)
Serpentine fibula			+									Elongated curved fibulae, hirsutism, hypertelorism	Uncertain	Unk	(263)
Spondylocostal dysostosis	1		+									Sacral agenesis, anal atresia, bifid thumb, skin tags	Uncertain	Unk	(264)
Spondyloepimeta-physeal dysplasia						+		U				Joint laxity, kyphoscoliosis, talipes equinovarus, CHD	AR	COL2A1	(265)
Spondylometa-physeal dysplasia										+		SS, platyspondyly, co+a vara, vertebral/long bone anomalies	AD	Unk	(266)
Syndactyly, type V		E			+							Bladder exstrophy, fusion of 4th and 5th metacarpal bones	AD	HOXD13	(267)

U ureter, *B* bladder, *Ua* urethra, *1* unilateral renal agenesis, *2* bilateral renal agenesis, *E* ectopia, *H* horseshoe, *ACC* agenesis of corpus callosum, *SS* short stature, *MR* mental retardation, *DD* developmental delay, *CHD* congenital heart disease, *FTT* failure to thrive, *IUGR* intrauterine growth retardation, *Unk* Gene unknown

Table 6-3

Chromosomal disorders and their consistent associated urinary tract anomalies

Chromosomal disorders	Renal agenesis	Ectopia/ horseshoe	Cystic/ dysplasia	Duplication	Hypoplasia	Hydro-nephrosis/ ureter	Diverti-culae	Atresia/ stenosis	Reflux	Nephritis/ sclerosis	Tumor/ nephro-megaly	Other Associated Anomalies	Reported Familial Cases	Ref.
3p deletion		E,H				+						MR, growth delay, ptosis, postaxial polydactyly, micrognathia	No	(270)
3q duplication		H		+	+	+						MR, SS, seizures, hirsutism, typical face, cardiac defects	No	(271)
Williams syndrome (7q deletion)		E			+	+	B,U	U				SS, typical face, supravalvar aortic stenosis, hypercalcemia	Yes	(268)
Trisomy 9 mosaicism			+			+	B					MR, joint contractures, cardiac defects, brain anomalies	No	(269)
10q duplication		H	+									MR, ptosis, short palpebral fissures, camptodactyly	Yes	(270
Aniridia-Wilms tumor (WAGR) (11p13 deletion)											+	Ambiguous genitalia, hypospadias,short stature	AD	(271)
Pallister-Killian syndrome (tetrasomy 12p)			+									SS, MR, hypogonadism, seizures, diaphragmatic defect	No	(271)
Patau syndrome (trisomy 13)	1,2	H	+	+		+						Holoprosencephaly, midline anomalies, cleft lip/palate	No	(269)

Table 6-3 (Continued)

Chromosomal disorders	Renal agenesis	Ectopia/ horseshoe	Cystic/ dysplasia	Duplication	Hypoplasia	Hydro-nephrosis/ ureter	Diverti-culae	Atresia/ stenosis	Reflux	Nephritis/ sclerosis	Tumor/ nephro-megaly	Other Associated Anomalies	Reported Familial Cases	Ref.
Miller-Dieker syndrome (17p13 deletion)	1		+									MR, lissencephaly, microgyria, agyria, typical face, seizures	No	(272)
Edward syndrome (trisomy 18)	+	E,H	+	+		+						IUGR, CHD, clenched hands, rocker bottom feet	Yes	(269)
18q deletion		H										SS, MR, microcephaly, narrow external ear canals,long hands	Yes	(273)
Down syndrome (trisomy 21)	1	H	+	+								MR,hypotonia, CHD, typical face, clinodactyly	Yes	(274)
Cat eye syndrome (tetrasomy 22p)	1	H	+			+		U				MR, CHD, colobomas, anal/digital anomalies	Yes	(275)
Velocardiofacial syndrome (22q11 deletion)	1,2	E,H	+	+	+	+		+	+			Conotruncal CHD, thymic aplasia, typical face, cleft palate	Yes	(276, 277)
Turner syndrome (45,+ or 46,+,i (+q))	1	E,H	+	+	+	+			+			SS,amenorrhea, webbed neck, cubitus valgus, hypogonadism	No	(278)
Triploidy		H	+			+						Large molar placenta, IUGR, syndactyly of 3rd and 4th digit, others	No	(271)

U ureter; *B* bladder; *Ua* urethra, *1* unilateral renal agenesis, *2* bilateral renal agenesis, *E* ectopia, *H* horseshoe, *ACC* agenesis of corpus callosum, *SS* short stature, *MR* mental retardation, *DD* developmental delay, *CHD* congenital heart disease; *FTT* failure to thrive, *IUGR* intrauterine growth retardation

Prevalence of Urinary Tract Anomalies

The true incidence of urinary tract anomalies is difficult to ascertain because many anomalies are asymptomatic and therefore undetected. Many reported statistics have apparent bias of ascertainment because they are derived from symptomatic individuals. Furthermore, inconsistent terminology and clustering of data have decreased the power of much of the epidemiologic data. Long-term analysis of data collected through major national birth defect registries showed increasing prevalence of reported statistics for many congenital birth defects, not only from an actual increment but also from increased tendency to report several isolated and associated anomalies (1–4). For this reason, the practical use of the derived prevalence seems not to be meaningful. However, there currently are quite a number of reliable estimates for prevalence of specific isolated anomalies and of those associated with a specific syndrome. A large number of European birth cohorts during 1996–1998 (EUROSCAN) was prenatally studied and recently reported (4). *Table 6-4* shows a comparison of prevalence figures among various studies.

Approach to the Child with a Urinary Tract Anomaly

The approach to the child with a urinary tract anomaly is similar to that for other birth defects. The initial step is to make a specific diagnosis based on history taking, physical examination, and laboratory investigation. A thorough *family history* for both urinary tract anomalies and for any other type of congenital or developmental anomalies that may have occurred in the family must be obtained. Many genetic disorders have variable expression even within the same family. A careful *physical examination* looking specifically for major and minor anomalies should be performed. Sometimes, a pattern of multiple anomalies can be recognized immediately as a well-described syndrome. Patterns of anomalies that cannot be recognized may require a literature or database search, or referral to an expert in syndrome recognition, such as a clinical geneticist. The search for a specific diagnosis is optimally accomplished by identifying the least common and most distinctive anomalies, for which the list of differential diagnoses is limited. Many excellent textbooks, atlases, and databases are available (5–7). To aid

Table 6-4

Prevalence of urinary tract anomalies detected by various surveys

Anomalies	Rates per 1,000 births			
	EUROSCAN 1996–1998[a]	CBDMP 1983–1994[b]	WSBDR 1987–1989[c]	MACDP 1983–1988[d]
Total renal malformation	*~1.6*	*~2.31*	*~2.33*	~1.5
Unilateral renal agenesis	0.08			
Bilateral renal agenesis/dysplasia	0.13			
Unilateral multicystic dysplasia	0.14			
All renal agenesis /dysplasia	0.36	0.48	0.58	0.47
Horseshoe/ectopic kidney	0.04	0.04	0.16	No data
Cystic kidney	0.04	0.03	0.05	No data
Obstruction of kidney/ureter	0.43	1.27	1.27	0.8
Double ureter	no data	0.004	0.05	No data
Exstrophy of bladder	0.03	0.03	0.02	0.03
Obstruction of bladder/urethra	0.04	0.16	0.2	0.2
VATER, CHARGE, and MURCS associations	No data	0.21	No data	No data
Sirenomelia	No data	0.09	No data	No data

[a]European Renal Anomaly Detection Program (total 709,030 births)
[b]California Birth Defect Monitoring Program
[c]Washington State Birth Defect Registry
[d]Metropolitan Atlanta Congenital Defects Program

Table 6-5

Syndromes associated with unilateral renal agenesis

Acrocallosal syndrome
Acrorenal syndrome, Dieker type
Acro-renal-mandibular syndrome
Acro-renal-ocular syndrome
Adrenogenital syndrome
Aglossia-adactylia syndrome
Alagille syndrome (arterio-hepatic dysplasia)
Branchio-oto-renal syndrome
C-trigonocephaly syndrome
Campomelic dysplasia
Cat-eye syndrome
Chondroectodermal dysplasia
Coffin-Siris syndrome
Cornelia de Lange syndrome
Ectrodactyly-ectodermal dysplasia-clefting (ECC) syndrome
Femoral hypoplasia-unusual facies syndrome
Fetal alcohol syndrome
Goldenhar syndrome
Ivemark syndrome
Kallmann syndrome
Klippel-Feil anomaly
Lacrimo-auriculo-dento-digital syndrome
Larsen syndrome
Lenz microphthalmia syndrome
LEOPARD syndrome (multiple lentigenes)
Limb-body wall complex
Miller-Dieker syndrome
MURCS association
Neu-Laxova syndrome
Oro-facio-digital syndrome, types IV andVI
Pfeiffer syndrome
Poland anomaly
Renal dysplasia
Roberts syndrome
Rokitansky-Mayer-Kuster-Hauser syndrome
Rubella syndrome, congenital
Rubinstein-Taybi syndrome
Russell-Silver syndrome
Short rib polydactyly syndrome, types 1–3
Smith-Lemli-Opitz syndrome
Sorsby coloboma-brachydactyly syndrome
Spondylocostal dysostosis
Townes-Brocks syndrome
Trisomy 22
Turner syndrome
Ulnar-mammary syndrome
VATER (VACTERL) association
Zellweger syndrome

Table 6-6

Syndromes associated with unilateral or bilateral renal agenesis

Acrorenal, Johnson-Munson type
Alkylating agent, maternal use
Caudal duplication syndrome
Caudal regression syndrome
Cocaine, maternal use
CHARGE syndrome
Diabetic mother, infant of
DiGeorge syndrome
Fraser (cryptophthalmos) syndrome
Holzgreve syndrome
Pallister-Hall syndrome
Potter (oligohydramnios) sequence
Sirenomelia sequence
Thalidomide embryopathy
Urogenital abysplasia
Velocardiofacial syndrome
Winter syndrome

in this effort, refer to *Tables 6-1–6-3* in addition to a table listing the differential diagnosis that accompanies the description of each of the major urinary tract anomalies below (*Tables 6-5–6-15*). For example, it is preferable to search for syndromes with urethral agenesis (22 syndromes) rather than renal dysplasia (more than 80 syndromes) when the two anomalies coexist. A search based on the more common anomalies can be performed if the first search does not reveal a match. Even after careful evaluation, a substantial number of children with multiple congenital anomalies remain undiagnosed.

When a suspected syndrome is known to be caused by a gene mutation, confirmatory molecular genetic testing can be performed. DNA-based test is currently available on either a clinical service or research basis. Knowledge regarding a pathogenic mutation specific for each

Table 6-7
Syndromes associated with ectopic kidney

Acromelic frontonasal dysplasia
Acrorenal syndrome, Dieker type
Acrorenal syndrome, Siegler type
Acro-renal-ocular syndrome
Baller-Gerold syndrome
Beckwith-Wiedemann syndrome
Branchio-oto-renal syndrome
Caudal regression syndrome
CHARGE syndrome
Crossed ectopia-pelvic lipomatosis syndrome
DiGeorge syndrome
Drash (Denys-Drash) syndrome
Fanconi anemia syndrome
Fetal alchhol syndrome
Floating-Harbor syndrome
Frontonasal dysplasia
Goldenhar syndrome
Kaufman-McKusick syndrome
Klippel-Feil anomaly
Limb-body wall complex
MURCS association
Pallister-Hall syndrome
Penoscrotal transposition
Renal adysplasia
Rokitansky-Mayer-Kuster-Hauser syndrome
Rubinstein-Taybi syndrome
Schinzel-Giedion syndrome
Sirenomelia sequence
Turner syndrome
VATER (VACTERL) association
Velocardiofacial syndrome
Williams syndrome

Table 6-8
Syndromes associated with horseshoe kidney

Acro-facial dysostosis syndrome
Agnathia-holoprosencephaly syndrome
Antley-Bixler syndrome
Bowen-Conradi syndrome
Caudal regression syndrome
Diabetic mother, infant of
Fanconi anemia syndrome
Fetal alcohol syndrome
Focal dermal hypoplasia
Juberg-Hayward syndrome
Kabuki syndrome
Pallister-Hall syndrome
Pyloric stenosis
Roberts syndrome
Thalidomide embryopathy
Trisomy 13, 18, 21, and 22
Turner syndrome
VATER (VACTERL) association
Weyers syndrome
Wilms tumor

proband may potentially be useful for genetic counseling and future reproductive option in order to avoid intrafamilial recurrence. *Tables 6-1* and *6-2* list currently known causative gene(s) for each of the disorder.

A chromosome analysis is indicated in any child who has at least two major congenital anomalies or one isolated anomaly that is a pertinent feature of a chromosome abnormality, such as aniridia (microdeletion 11p). Growth or developmental delay and dysmorphic features or lack of familial resemblance should also prompt a chromosome analysis. Chromosome abnormalities are found in approximately 10–12% of all renal anomalies (3, 8). *Table 6-3* lists common and distinct chromosomal disorders with their reported urinary tract anomalies.

For a child with no known urinary tract anomaly, findings that should prompt an evaluation of the urinary tract are oligohydramnios, undefined abdominal mass, abnormal genitalia, aniridia, hypertension, preauricular pits or tags, branchial cleft cyst or sinus, imperforate anus, or symptoms indicative of renal dysfunction, urinary tract infection, or obstructive uropathy (3). For patients with known syndromes, the type of potentially associated urinary tract anomalies are listed in *Tables 6-1* and *6-2*.

The best initial evaluation to screen for urinary tract anomalies in general is an ultrasound examination because this study is noninvasive and gives good anatomic information about the urinary tract. It is also the only method routinely used for the prenatal diagnosis of urinary tract anomalies. Specific investigations such as intravenous pyelogram, voiding cystourethrogram, radionuclide renal and urinary system scan, and specialized genetic testing may then be used, depending on the working diagnosis. The type

Table 6-9
Syndromes associated with renal dysplasia/cystic kidney

Acro-renal-mandibular syndrome
Alagille syndrome (arterio-hepatic dysplasia)
Baller-Gerold syndrome
Bardet-Biedl syndrome
Beckwith-Wiedemann syndrome
Branchio-oto-renal syndrome
Campomelic dysplasia
Carbohydrate deficient glycoprotein syndrome
CHARGE syndrome
Chondrodysplasia punctata, non-rhizomelic
Cloacal exstrophy
Cornelia de Lange syndrome
Diabetic mother, infant of
Ectrodactyly-ectodermal dysplasia-clefting (EEC) syndrome
Fanconi anemia syndrome
Fetal alcohol syndrome
Fraser (cryptophthalmos) syndrome
Fryns syndrome
Glutaric aciduria, type II
Goldenhar syndrome
Hajdu-Cheney syndrome
Ivemark syndrome
Jeune syndrome
Joubert syndrome
Kaufman-McKusick syndrome
Lenz microphthalmia syndrome
Leprechaunism (Donohue) syndrome
Limb-body wall complex
Marden-Walker syndrome
Marfan syndrome
Marshall-Smith syndrome
Meckel-Gruber syndrome
MURCS association
Noonan syndrome
Omphalocele-Exstrophy of bladder-Imperforate anus-Spinal dysraphism (OEIS) complex
Oral-facial-digital syndrome, types I and VI
Pallister-Hall syndrome
Pallister-Killian syndrome
Potter (oligohydramnios) sequence
Prune belly syndrome
Renal adysplasia
Roberts syndrome
Rokitansky-Mayer-Kuster-Hauser syndrome
Rubella syndrome, congenital
Short rib-polydactyly syndrome
Smith-Lemli-Opitz syndrome
Thalidomide embryopathy
Trisomy 8, 9, 13, 18, 21, and 22
Tuberous sclerosis
VATER (VACTERL) association
Von Hippel-Lindau diesease
Zellweger and pseudo-Zellweger syndromes

of anomaly generally guides treatment. Corrective or reparative treatments are available for many anomalies (stenosis or atresia, bladder exstrophy, duplication. diverticula, and tumors). Symptomatic treatment for complications is often necessary.

Families who have a child with a urinary tract anomaly should be informed of the diagnosis when possible. A search for a related anomaly in first-degree relatives is automatically indicated only when the proband has renal agenesis by ultrasound examination (9). Otherwise, the decision to investigate family members should be based on a thorough family history and/or physical examination, and whether the child's disorder is a well described inherited syndrome. Genetic counseling should be provided to the family and should include a discussion of the manifestations of the disorder, the natural history, complications, available treatments, cause, and recurrence risk when these are known. Reproductive options should be discussed in a nondirective fashion. For an isolated anomaly without a family history of similar or related anomalies, an empiric risk can be provided. Accurate risk figures can be determined for Mendelian disorders, and estimated risks are available for associations.

All children with congenital anomalies need long-term, periodic follow-up to detect new abnormalities or complications of their birth defects. This is especially the case for children with undiagnosed multiple congenital anomalies, for whom follow-up examination may lead to a specific diagnosis. Additional relevant family information should be specifically sought for any newly affected member. Finally, for patients with a urinary tract anomaly who reach reproductive age, the recurrence risk for their offspring and reproductive options should be discussed.

The remainder of this chapter contains descriptions of the major types of urinary tract anomalies, including the etiology, pathogenesis, and associated disorders. First, a review of the embryology of the normal urinary tract

Table 6-10

Syndromes associated with hydronephrosis or hydroureter

Acro-cephalo-polysyndactylous dysplasia
Acrorenal syndrome, Dieker snd Johnson-Munson types
Barbet-Biedl syndrome
Branchio-oto-renal syndrome
Campomelic dysplasia
Caudal duplication and regression syndromes
CHARGE syndrome
Cloacal exstrophy
Coffin-Siris syndrome
Crossed ectopia-pelvic lipomatosis syndrome
Cornelia de Lange syndrome
Diabetic mother, infant of
Ectrodactyly-ectodermal dysplasia-clefting (EEC) syndrome
Fanconi anemia syndrome
Fetal alcohol syndrome
Goldenhar syndrome
Hydrolethalus syndrome
Kabuki syndrome
Kaufman-McKusick syndrome
Megacystis-microcolon syndrome
Noonan syndrome
Ochoa syndrome
Omphalocoele-Exstrophy of bladder-Imperforate anus-Spinal dysraphism (OEIS) complex
Pallister-Hall syndrome
Polydactyly-obstructive uropathy syndrome
Pyloric stenosis
Roberts syndrome
Sirenomelia sequence
Schinzel-Giedion syndrome
VATER (VACTERL) association

Table 6-11

Syndromes associated with duplication of ureters or collecting systems

Achondrogenesis
Acromelic frontonasal dysplasia
Adrenogenital syndrome
Antley-Bixler syndrome
Bardet-Biedl syndrome
Bowen-Conradi syndrome
Branchio-oto-ureteral syndrome
Braun-Bayer syndrome
Caudal duplication syndrome
Diabetic mother, infant of
Drash (Denys-Drash) syndrome
Ectrodactyly-ectodermal dysplasia-clefting (EEC) syndrome
Fanconi anemia syndrome
Fetal alcohol syndrome
Frontometaphyseal dysplasia
G (Opitz-Frias) syndrome
Goldenhar syndrome
Kabuki syndrome
Kaufman-McKusick syndrome
Mammo-renal syndrome
Noonan syndrome
Ochoa syndrome
Perlman syndrome
Poland anomaly
Prune belly syndrome
Robinow syndrome
Rubinstein-Taybi syndrome
Trisomy 8, 9, 13, 18, and 21
Turner syndrome
Weyers syndrome

will be useful in understanding structural urinary tract abnormalities.

Overview of Normal Embryogenesis of the Urinary System

Renal organogenesis is reviewed in chapter 1. Embryogenesis of the lower urinary tract includes development of the mesonephric duct and urogenital sinus. The mesonephric duct from which the ureteric bud arose inserts into the lower allantois, just above the terminal part of the hindgut, the cloaca. During the fourth to seventh weeks, mesoderm proliferates and forms the transverse mesodermal ridge, the urorectal septum that divides the cloaca into the anterior portion, the primitive urogenital sinus, and the posterior portion, the cloacal sinus or anorectal canal. The mesonephric ducts open into the urogenital sinus and later become the ureters. The urorectal septum develops caudally and fuses with the cloacal membrane, dividing it into the urogenital membrane (anterior) and the anorectal membrane (posterior) by the end of the

Table 6-12

Syndromes associated with bladder exstrophy

Axial mesodermal dysplasia
Caudal duplication syndrome
Caudal regression syndrome
Cloacal exstrophy
Frontonasal dysplasia
Omphalocoele-Exstrophy of bladder-Imperforate anus-Spinal dysraphism (OEIS) complex
Trisomy 18
Sirenomelia sequence
Syndactyly, type V

Table 6-13

Syndromes associated with urethral agenesis

Aase syndrome
Acrorenal syndrome, Dieker and Johnson-Munson types
Adrenogenital syndrome
Caudal regression syndrome
Cocaine, maternal use
Diabetic mother, infant of
Hydrolethalus syndrome
Kaufman-McKusick syndrome
Limb-body wall complex
Meckel-Gruber syndrome
Occipital horn syndrome
Ochoa syndrome
Omphalocoele-Exstrophy of bladder-Imperforate anus-Spinal dysraphism (OEIS) complex
Potter (oligohydramnios) Sequence
Prune belly syndrome
Renal adysplasia
Russell-Silver syndrome
Short rib-polydactyly syndrome, types 1–3
Sirenomelia sequence
Sotos syndrome
Townes-Brocks syndrome
Trisomy 21

Table 6-14

Syndromes associated with urethral duplication

Amniotic band disruption sequence
Limb-body wall complex
Omphalocoele-Exstrophy of bladder-Imperforate anus-Spinal dysraphism (OEIS) complex
Prune belly syndrome

Table 6-15

Syndromes associated with posterior urethral valves

Acrorenal syndrome, Johnson-Munson type
Caudal regression syndrome
Diabetic mother, infant of
Kaufman-McKusick syndrome
Limb-body wall complex
Neurofibromatosis, type I
Ochoa syndrome
Omphalocoele-Exstrophy of bladder-Imperforate anus-Spinal dysraphism (OEIS) complex
Polydactyly-obstructive uropathy syndrome
Potter (oligohydramnios) sequence
Prune belly syndrome
Renal adysplasia
Rubinstein-Taybi syndrome
Sirenomelia sequence
Townes-Brocks syndrome
VATER (VACTERL) association

seventh week. The primitive perineal body forms at the site of fusion.

The primitive urogenital sinus develops primarily into the urinary bladder. The superior portion, originally continuous with the allantois, later becomes a solid fibrous cord, the urachus or median umbilical ligament, which connects the bladder to the umbilicus. The inferior portion of the urogenital sinus in the male divides into a pelvic portion, containing the prostatic and membranous urethra, and the long phallic portion, containing the penile urethra. The inferior portion in the female forms a small portion of the urethra and the vestibule. At the same time, the distal portion of the mesonephric ducts is incorporated into the endodermal vesicoureteral primordium, forming the trigone of the bladder. A part of the distal end of both mesonephric ducts just proximal to the trigone develops into the seminal vesicles and ductus deferens in the male. Finally, at the end of the twelfth week, the epithelium of the superior portion of the prostatic urethra proliferates to form buds that penetrate the surrounding mesenchyme. In the male, these buds form

the prostate gland; in the female, they form the urethral and paraurethral glands.

Anomalies Involving the Urinary Tract

Kidney Defects

Renal Agenesis

By definition, renal agenesis refers to complete absence of one of both kidneys without identifiable rudimentary tissue. Renal agenesis is usually associated with agenesis of the ipsilateral ureter. The pathogenesis of renal agenesis is failure of formation of the metanephros. Causal heterogeneity has been shown, by both animal studies and human observations (10–12), including failure of ureteric bud formation, failure of the bud to reach the metanephric blastema, or failure of the bud and the metanephric blastema to create mutual inductive influence on one another. In addition, interruption in vascular supply and regression of a multicystic kidney can lead to renal agenesis in the fetal period (11).

Unilateral renal agenesis is usually asymptomatic and found incidentally, whereas bilateral renal agenesis results in severe oligohydramnios and fetal or perinatal loss. Renal agenesis can occur in either side without predilection. Birth prevalence in the United States for renal agenesis/hypoplasia ranges between 0.30 and 9.61 per 10,000 live births (3). Several studies have demonstrated that unilateral renal agenesis is associated with an increased frequency of anomalies in the remaining kidney (9, 13). Moreover, renal agenesis is often detected in conjunction with anomalies of other organ systems. These anomalies can occur both in contiguous structures (e, g., vertebrae, genital organs, intestines, and anus) and also in noncontiguous structures (e.g., limbs, heart, trachea, ear, and central nervous system). The diagnosis of renal agenesis is made by abdominal ultrasound. Care must be taken to exclude the possibility of ectopic kidney. Intravenous pyelography, computerized tomography scan, and radionuclide studies can be helpful in equivocal cases.

The recurrence risk for renal agenesis can be provided if the pattern of inheritance is known or if the proband has a recognizable syndrome. For nonsyndromic renal agenesis, an empiric risk of 3% can be used for families in which renal anomalies in first-degree relatives (siblings, parents) have been excluded (3). First-degree relatives of patients with nonsyndromic renal agenesis have an increased prevalence of related urogenital anomalies. In one study, 9% of first- degree relatives of infants with agenesis or dysgenesis of both kidneys had a related urogenital anomaly, and 4.4% had an asymptomatic renal malformation (13). In another recent retrospective review, empiric risks were 7% in offspring, 2.5% in siblings and 4.5% in parents (14). Moreover, offspring of an individual with unilateral agenesis is at a slightly increased risk for bilateral renal agenesis. Therefore renal ultrasound is recommended for the first-degree relatives of the proband unless renal agenesis in the proband is clearly sporadic or a specific cause without an increased recurrence risk is identified.

Tables 6-5 and *6-6* list the syndromes commonly associated with unilateral and bilateral renal agenesis, respectively. See also *Tables 6-1–6-3* for more information about these disorders and other less known conditions with renal agenesis.

Ectopic Kidney

The ectopic kidney derives from an error of ascent. Most are pelvic kidneys that fail to ascend out of the pelvic cavity. Rare case reports of thoracic kidney exist (15). Ectopic kidney can be unilateral or bilateral. In bilateral pelvic kidneys, the kidneys often fuse into a midline mass of renal tissue, with two pelves and a variable number of ureters, which is referred to as a pancake or discoid kidney. Fused pelvic kidney may in fact be due to fusion of ureteric buds or metanephric blastema. *Crossed renal ectopia* refers to an ectopic kidney whose ureter crosses the midline. It often fuses with the normal kidney. The embryogenesis of crossed renal ectopia is not well understood, but presumably involves abnormal migration of the ectopic kidney to the contralateral side. An ectopic kidney is usually hypoplastic, is rotated, and has numerous small blood vessels and associated ureteric anomalies. Ectopic kidneys may be asymptomatic and incidentally found, but complications from ureteral obstruction, infection, and calculi can occur. In a recent study, ectopic kidney without hypoplasia or hydronephrosis seems not to be associated with an appreciable increase frequency of associated anomaly and complication thus making further urologic investigation such as vesicourethrography unnecessary (16). *Table 6-7* provides a list of syndromes that include ectopic kidney. These are described in *Tables 6-1–6-3*.

Horseshoe Kidney

Horseshoe kidney refers to a condition in which both kidneys are fused at the lower poles with a renal parenchymal or, less commonly, fibrous isthmus.

The embryogenesis of horseshoe kidney with parenchymal isthmus is thought to be migration of nephrogenic cells across the primitive streak before the fifth gestational week. Horseshoe kidney with fibrous isthmus is believed to originate from mechanical fusion of the two developing kidneys at or after the fifth week before renal ascent (17). The concept of a narrow vascular fork leading to approximation and fusion of the two kidneys is no longer considered valid. Most horseshoe kidneys are located in the pelvis or at the lower lumbar vertebral level because ascent is further prevented when the fused kidney reaches the junction of the aorta and inferior mesenteric artery.

Complications of horseshoe kidneys include obstructive uropathy primarily related to ureteropelvic junction obstruction, calculi and urinary tract infection. Similar to other urinary tract anomalies, a horseshoe kidney is often associated with other genitourinary anomalies. In addition, there is an increased risk of various types of renal tumors developing in the horseshoe kidney compared with the normal kidney (18). Renal cell carcinoma is the most common, but Wilms' tumor, adenocarcinoma, transitional cell carcinoma, malignant teratoma, oncocytoma, angiomyolipoma, and carcinoid have all been reported. Horseshoe kidneys also carry an increased risk for renal pelvis carcinoma and higher proportion of squamous cell carcinoma than those in normal kidneys (19).

Table 6-8 lists syndromes associated with horseshoe kidney. See *Tables 6-1* and *6-2* for more details of these disorders.

Dysplasia and Polycystic Kidney

Renal dysplasia is the most common congenital urinary tract anomaly and the most common cause of an abdominal mass in children (3). Unilateral dysplasia is reported to occur in 1:1,000, whereas the prevalence of bilateral disease is estimated to be 1:5,000 (20). It may be unilateral or bilateral, and diffuse, segmental, or focal. Symptoms are variable from silent in unilateral or focal dysplasia to progressive renal dysfunction in diffuse or bilateral dysplasia. Dysplasia refers to abnormal differentiation or organization of cells in the tissue. Renal dysplasia is characterized histologically by the presence of primitive ducts and nests of metaplastic cartilage (20, 21). Although cysts are not always present in a dysplastic kidney, the dysplastic process often results in the formation of cysts that are variable in size and number. Several hypotheses are proposed for the embryogenesis of the dysplastic kidney. The most likely pathogenesis is an error of the mutual induction between the ureteric bud and the metanephric blastema. The molecular pathogenesis of cystic kidney, especially polycystic kidney, has been one of the most extensively studied aspects of nephrology and recent studies discovered few genes and pathways critical for renal cyst formation such as TCF2/hepatocyte nuclear factor 1ss (HNF1beta), PAX2 and uroplakins. Dysplastic kidneys are usually identified as enlarged bright kidneys on prenatal ultrasonography. If there is associated functional renal impairment, alteration in amniotic fluid volume could potentially be detected and signifies a poor prognosis. (22) Unilateral dysplasia carries an overall better postnatal prognosis than that of bilateral disease. However, up to 30–50% of those with unilateral dysplasia have associated contralateral urinary anomalies (22). Multicystic renal dysplasia is the most common among many causes of renal dysplasia and it is usually unilateral. Polycystic kidney diseases, both autosomal dominant (ADPKD) and autosomal recessive (ARPKD) forms, are in general far more common than other syndromic causes of renal dysplasia. *Table 6-9* summarizes well-known syndromes with renal dysplasia/cystic kidney. See also *Tables 6-1–6-3*.

Obstruction and Hydronephrosis

Urinary obstruction is a complication of a primary anomaly, which can be stenosis or atresia of the ureteropelvic junction, ureter, or urethra; a poorly functional bladder causing reflux; a malformed dilated ureteral end (ureterocele); or extrinsic compression by other structures, such as anomalous blood vessels or tumors. Hydronephrosis and pyelectasis (dilated renal pelvis) are the most common urinary tract abnormalities on prenatal ultrasound examination. Early diagnosis of collecting system dilatation can be achieved by ultrasound examination in the second trimester (23). Persistent dilatation almost always indicates an underlying anomaly. Isolated obstructive uropathies diagnosed prenatally may not require antenatal or immediate postnatal surgical intervention. Postnatally diagnosed obstructive uropathies are almost always symptomatic and require thorough investigation to delineate the anatomy of the urinary tract and to exclude associated anomalies.

Table 6-10 provides a list of syndromes commonly associated with obstruction and hydronephrosis. See also *Tables 6-1–6-3*.

Ureter Defects

Duplication

Double ureters or collecting systems are caused by duplication of the ureteric bud. Early duplication results in

duplicated kidney, which is usually smaller and fused with the ipsilateral kidney and has ureters that enter into the bladder separately. Duplication that occurs later results in double ureters that may have separate openings into the bladder or may join each other before the opening. On rare occasion, one of the ureters may have an ectopic opening into the vagina, vestibule, or urethra. In most double ureters, the two ureters cross each other, and that from the higher pelvis enters the bladder more caudally. Duplication anomalies are common but usually asymptomatic; therefore they often remain undetected. One autopsy study reported the prevalence of duplication anomalies to be as high as 1 in 25, with females about four times more likely to be affected than males (24). Unilateral duplication is five to six times more common than bilateral duplication (3). Double ureters are commonly associated with vesico-ureteral reflux due to their ectopic opening into the urinary bladder and/or the ureterocele (23). In addition, ureteric obstruction can occur at the level of vesico-ureteric junction or that of uretero-pelvic junction.

Table 6-11 summarizes syndromes associated with duplication, and *Tables 6-1–6-3* provide clinical information about these disorders.

Hydroureter

Hydroureter, or magaloureter, is caused by distal obstruction and is usually found with hydronephrosis, except in ureteropelvic junction obstruction. Hydroureter has the same etiology as hydronephrosis (see Obstruction and Hydronephrosis).

Bladder Defects

Anomalies of the bladder are rare. These include agenesis, hypoplasia, diverticulae, and dilatation or megacystis caused by distal obstruction or by non-obstructive causes. Agenesis of the bladder is usually associated with severe developmental anomalies of the urinary tract, such as in sirenomelia and caudal regression syndrome. Hypoplastic bladder can be found in conditions associated with bilateral renal agenesis because no urine is produced. Bladder diverticulae have heterogeneous causes. They result from an intrinsic defect in the bladder wall, such as in cutis laxa, or Ehlers-Danlos, Ochoa, occipital horn, and Williams syndromes. They can also be caused by increased intravesicular pressure from distal obstruction or by persistent urachus. See *Tables 6-1–6-3* for information about specific syndromes associated with bladder diverticulae.

Bladder Exstrophy

Bladder exstrophy refers to a urinary bladder that is open anteriorly because of the lack of an abdominal wall closure. It is usually associated with anomalies of the contiguous structures including epispadias and separation of the pubic rami. This anomaly is thought to result from an overdeveloped cloacal membrane that interferes with inferolateral abdominal mesenchymal closure. Therefore, when the cloacal membrane ruptures, the inferior abdominal wall has not completely closed and the bladder cavity is exposed. It has been suggested that bladder exstrophy belongs to the spectrum of omphalocele-cloacal exstrophy-imperforate anus-spinal dysraphism (OEIS) complex (25–27). The extent of anomalies is determined by the timing of the cloacal membrane rupture. Rupture that occurs after the separation of cloaca by the urorectal septum results in bladder exstrophy, whereas one that occurs before the separation results in the more severe cloacal exstrophy and OEIS complex. Bladder exstrophy is six times more common in males.

Table 6-12 lists syndromes associated with bladder exstrophy, and *Tables 6-1–6-3* provide information about these disorders.

Urethral Defects

Agenesis and Atresia

Urethral agenesis is rare, and its predominant occurrence in males probably reflects the greater complexity of embryogenesis of the male urethra. Urethral agenesis is often associated with bladder obstruction sequence. *Table 6-13* lists syndromes associated with urethral agenesis, and clinical information about these disorders is summarized in *Tables 6-1–6-3*.

Duplication

Duplication refers to complete or partial duplication of the urethra, which is a rare anomaly found only in a few syndromes. Those syndromes associated with urethral duplication are listed in *Table 6-14* and their findings are provided in *Tables 6-1–6-3*.

Posterior Urethral Valves

Posterior urethral valves refer to abnormal mucosal folds that function as a valve to obstruct urine flow. This is the

most common childhood cause of obstructive uropathy leading to renal failure. Posterior urethral valves can be suspected prenatally when a dilated bladder is seen in association with obstructive uropathy. A "keyhole" sign has been demonstrated in prenatal ultrasound of fetuses with subsequently confirmed posterior urethral valves (28). A voiding cystourethrogram or endoscopy is usually required for a definitive diagnosis. The embryogenesis of posterior urethral valves is unknown. Proposed hypotheses include an overdeveloped posterior urethral fold, a remnant of the mesonephric duct, and an anomalous opening of the ejaculatory duct. *Table 6-15* lists syndromes in which posterior urethral valves can be seen, and the other findings in these disorders are provided in *Tables 6-1*–*6-3*.

Associations and Sequences Involving the Urinary Tract

A number of associations and sequences involve anomalies of the urinary tract that may be important to both diagnosis and management. For this reason, such conditions are described in more detail in this section, in addition to the information presented in *Tables 6-1* and *6-2*.

VATER Association

VATER association is an acronym used to designate a non-random occurrence of Vertebral defects, imperforate Anus, Tracheo-Esophageal fistula, Radial and Renal anomalies (29, 30). An acronym VACTERL has been proposed to broaden the spectrum of VATER to include Cardiac defects and Limb anomalies. The term VATER is not a diagnosis per se, but the designation provides clues for potentially associated anomalies and for recurrence risk counseling when no specific syndromic diagnosis can be made. Patients with VATER association need a careful physical examination and investigation for potential multiorgan anomalies. A specific diagnosis should be sought. Causes of VATER association include: chromosomal disorders, such as trisomy 18; genetic syndromes, such as Goldenhar and Holt-Oram syndromes; and teratogenic exposures, such as infants of diabetic mothers and fetal alcohol syndrome. A family with a mitochondrial DNA mutation was identified in which the daughter was born with VACTERL association, and her mother and sister had classic mitochondrial cytopathy (31). Thus all patients suspected to have VATER association should have a chromosome analysis, a careful family and prenatal exposure history, and a thorough examination for dysmorphic features. The spectrum of anomalies seen in VATER is broad. Associated renal anomalies are usually agenesis, ectopy, or obstruction (29, 30).

Because there is apparent causal heterogeneity for VATER association, the inheritance pattern and recurrence risk vary with the cause. VATER association is usually sporadic with an empirical recurrence risk of 1 to 3% when a specific cause cannot be identified (32). Autosomal recessive and X-linked inheritance have been reported for subsets of patients, such as for VATER with hydrocephalus, and recurrence risk in these families can be as high as 25% (32).

CHARGE Syndrome (CHARGE Association)

CHARGE syndrome – previously designated as an association but now recognized to have a major causative gene- is an acronym used to designate an association of Coloboma of iris, choroid or retina, Heart defects, Atresia choanae, Retarded growth and development, Genital anomalies or hypogonadism, and Ear anomalies or deafness (33–36). In addition, unilateral facial palsy is a common finding. Renal anomalies occasionally found in CHARGE syndrome include ectopy, dysplasia, renal agenesis, and ureteric anomalies. The presence of two or more anomalies associated with CHARGE syndrome should prompt a search for the others. To prevent overuse of the term, it was suggested that at least three anomalies are required for the term CHARGE to be applied, and one of the anomalies should be either coloboma or choanal atresia (34). To date, consistent features in CHARGE syndrome have been ocular coloboma, choanal atresia and semicircular canal hypoplasia (37). Conditions with anomalies in the spectrum of CHARGE include trisomy 13, trisomy 18, and Wolf-Hirschhorn (deletion 4p), cat-eye, Treacher-Collins, velocardiofacial, Apert, Crouzon, and Saethre-Chotzen syndromes. Therefore, a careful physical examination for malformations and dysmorphic features should be conducted. Recently, *CHD7* mutation has been found by an array CGH study to be the cause of this syndrome in about 60 percent of typical patients thus making a molecular confirmation possible. In those without *CHD7* mutation, chromosome analysis including specific fluorescence in situ hybridization (FISH) probes for velocardiofacial syndrome (deletion 22q) and 4p deletion should be performed. Because most cases of CHARGE association are sporadic, the empirical recurrence risk in sibling is low (33, 36).

MURCS Association

MURCS association refers to a rare occurrence of Mullerian duct aplasia, Renal aplasia and Cervicothoracic Somite dysplasia (38). Anomalies include absence of the proximal two thirds of the vagina; uterine hypoplasia or aplasia; unilateral renal agenesis; ectopic kidney; renal dysplasia; C5-T1 vertebral anomalies (hypoplasia of vertebrae, fusion, hemivertebrae, and butterfly vertebrae); and short stature. Additional anomalies are common, including rib defects, facial asymmetry, limb anomalies, hearing loss, and brain anomalies, such as encephalocoele and cerebellar cyst (39).

The pathogenesis of MURCS association is unknown, but is thought to be related to defects in the paraxial mesoderm, which gives rise to the cervicothoracic somites and the adjoining intermediate mesoderm. Most patients are diagnosed because of primary amenorrhea or infertility associated with normal secondary sexual characteristics, followed by recognition of reproductive organ atresia. MURCS association is usually sporadic. A report of vertebral and renal anomalies associated with azoospermia was proposed to represent the male version of MURCS association (40).

Oligohydramnios Sequence

Oligohydramnios of whatever cause leads to a recurrent pattern of abnormalities that has been called the oligohydramnios sequence (3, 6). Oligohydramnios may be caused by decreased production of fatal urine from bilateral renal agenesis or dysplasia or by urinary obstruction, or it can result from amniotic fluid leakage. When the oligohydramnios is prolonged and severe, the condition is lethal because of pulmonary hypoplasia. Moderate oligohydramnios from amniotic fluid leakage may result in a liveborn child with multiple congenital anomalies. These anomalies are both malformations and deformations. Intrauterine constraint leads to mechanical compression that leads to the characteristic flat facial profile (Potter's facies), limb deformities (e.g., talipes equinovarus), and intrauterine growth retardation (IUGR). Decreased fetal movement as a result of intrauterine constraint causes multiple joint contractures (arthrogryposis). Breech presentation is common. Pulmonary hypoplasia can be the consequence of compression of the chest cavity coupled with decreased inspiration of amniotic fluid. Liveborns have respiratory distress caused by pulmonary hypoplasia, and the lungs may have insufficient volume to support life.

Because the initial defect has many causes, recurrence risk is based on the underlying defect. When oligohydramnios is due to nonsyndromic bilateral renal agenesis or dysgenesis, related renal malformations occur at an increased frequency in first-degree relatives (13), and recurrence risk can be as high as 4–9%. The recurrence risk can be as high as 25% for an autosomal recessive disorder causing bilateral renal agenesis or dysplasia.

Urethral Obstruction Sequence

The initial defect in this sequence is obstruction of the urethra leading to dilation of the proximal urinary tract, bladder distension, and hydroureter (3, 6, 41). Obstruction of urine flow interferes with normal nephrogenesis, resulting in renal dysplasia. Other potential anomalies related to bladder distension include cryptorchidism, malrotation of colon, persistent urachus, and limb deficiency caused by iliac vessel compression. In addition, oligohydramnios results from lack of urine and leads to the oligohydramnios sequence.

Prune-belly syndrome (41, 42) is a rare entity referring to a constellation of anomalies that includes megacystis, abdominal wall muscle deficiency, hydroureter, renal dysplasia, and characteristic wrinkled abdominal skin. This condition, previously thought to be a form of urethral obstruction sequence, is in fact a non-obstructive cause of bladder distension that results from a malformation, thus now being properly designated a syndrome (28).

The most common cause of urethral obstruction is posterior urethral valves, but urethral agenesis/atresia or bladder neck obstruction can also be the cause. This anomaly occurs mostly in males, with a male: female ratio of 20:1. Survival is rare in fetuses with complete obstruction, and severe urinary tract dysfunctions are always present in those that are liveborn. Prenatal diagnosis by ultrasound examination can detect the abnormally dilated bladder at the beginning of the second trimester (43), and intrauterine urinary decompression procedures, such as vesicoamniotic shunts, are options for treatment in order to decrease the occurrence of pulmonary hypoplasia, although their benefits have not been unequivocally shown (44, 45).

Sirenomelia Sequence

Sirenomelia is a malformation characterized by the presence of a single lower extremity with posterior alignment of the knees and feet, sacral agenesis and other lower vertebral defects, imperforate anus and rectal agenesis,

and absence of external and internal genitalia (46). The current view of the embryogenesis is that sirenomelia results from a vascular steal phenomenon (47). This is supported by the presence of abnormal vasculature in the caudal part of affected embryos. A single large vessel originating from the aorta, a derivative of the vitelline artery complex, connects the iliac arteries to the placenta rather than the two normal umbilical arteries. The area caudal to the origin of this vessel has minimal blood supply because of the lack of aortic branches. Therefore, a "vascular steal phenomenon" is generated, leading to a vascular disruption sequence. Alternatively, since sirenomelia shares a number of anomalies with caudal regression syndrome, it is thought to potentially be causally similar and represent different patterns in the same spectrum.

Sirenomelia is a rare condition and has a broad spectrum of anomalies. Virtually any urinary tract anomaly can occur in sirenomelia sequence. Renal agenesis occurs in two-thirds of cases and a variable degree of renal dysplasia is present in one-third of cases (3). Absence of the ureter and bladder are common. All cases of sirenomelia are sporadic and almost uniformly fatal because of pulmonary hypoplasia. Sirenomelia has been noted with an increased frequency among monozygotic twins in which only one of the twins in usually affected.

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Homeostasis

7 Sodium and Water

Howard Trachtman

Introduction

> "Wasted away again in Margaritaville, searching for my lost shaker of salt. . ."
>
> Jimmy Buffet

There must be something about the topic of sodium and water homeostasis that reaches deep within the human psyche and prompts authors to wax poetic in search of literary aphorisms (1, 2). In the past, the author looked to the ancients to demonstrate that human beings probably have an intuitive sense of the critical role played by salt balance and the integrity of the plasma compartment for the maintenance of life in terrestrial species. For the second time, the author turns to a contemporary voice for inspiration in this chapter that reviews the physiological mechanisms involved in the control of sodium and water homeostasis. Using this knowledge as a basis, there will be an analysis of the common diseases that arise when these systems malfunction and a discussion of the optimal therapy for these conditions.

Body Fluid Compartments and their Composition

Total Body Water and its Compartments

Water is vital for the maintenance of life and has several key physiological functions including providing an aqueous environment for cytosolic chemical reactions, a solvent for elimination of waste products, a medium for transport of nutrients, key molecules and gases, and thermoregulation via sweat production (1). On average, water comprises 60% of total body weight in adults. This proportion is higher in infants and even greater in babies born prematurely and very low birth weight neonates. It declines during early infancy and reaches the adult value by the end of the first year (3). The percentage of body weight, i.e., water, is lower in postpubertal girls because they have a higher percentage of body mass, i.e., fat. In addition, it may be altered in disease states that are associated with altered salt handling such as cystic fibrosis and endocrinopathies.

Total body water is divided into two principle components – the intracellular (ICW) and the extracellular water (ECW) spaces (4). These spaces are apportioned in a 2:1 ratio. When there is an increase in the total body water, this is clinically manifested by an increase in the ECW space, because the ICW compartment is not accessible to direct assessment. The ECW compartment is further divided into the interstitial and intravascular spaces, which are separated in a 3:1 ratio. Thus, the intravascular space constitutes 1/12 of the total body water, i.e., ⅓ × ¼ (*Fig. 7-1*). A component of the ECW, namely the interstitial fluid in skin and connective tissue, may serve as a reservoir that can mobilize water into the plasma volume to sustain circulation during conditions of hypovolemia (5). Finally, there are transcellular water compartments, such as the gastrointestinal lumen or cerebrospinal fluid, which need to be considered as a distinct category. They are not in direct contact with the rest of the fluid spaces and are separated by an epithelial membrane. Water and electrolytes enter these spaces via tightly regulated active transport processes.

Composition of Body Water Compartments

All of the major fluid compartments in the body are separated by semipermeable membranes (4, 5). This type of barrier permits free passage of the aqueous solvent but limits movement of selective solutes across the membrane. Water always moves down its concentration gradient to ensure that the osmolality of the solution is the same on both sides of the membrane. Water channels called aquaporins are a group of proteins that are selectively expressed in specific cell membranes such as the erythrocytes and distinct nephron segments, which facilitate water movement in response to an osmotic gradient (6).

Because of the presence of active transporters and selective channels for various solutes within the cell membrane, there is an uneven distribution of solutes in the ICW and ECW compartments. The presence of the Na-K ATPase pump in localized cell membrane domains ensures that potassium and sodium are the principle

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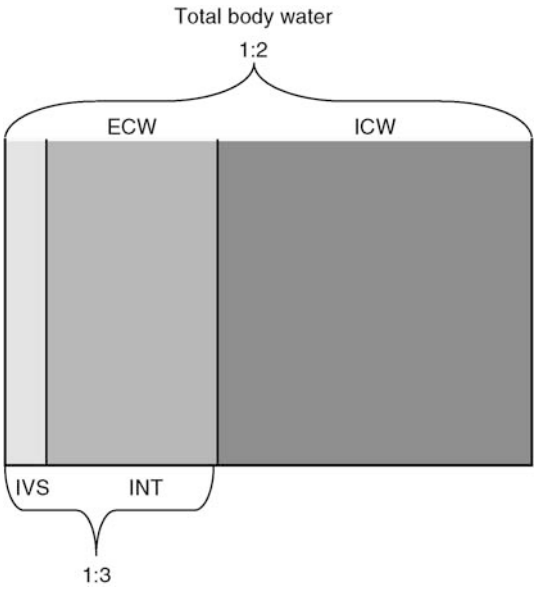
Figure 7-1

Graphic illustration of the total body water compartments and the relative size of the intracellular water (ICW) to the extracellular water (ECW) spaces, and intravascular (IVS) to interstitial water space.



cations in the ICW and ECW spaces, respectively (7). The secondary movement of Na^+ and K^+ through other pathways such as the amiloride sensitive epithelial sodium channel (ENaC) or ROMK channel is driven by the primary operation of the Na-K-ATPase (8). Limitations in membrane permeability to chloride and bicarbonate confine these anions almost exclusively to the ECW space, while proteins and phosphate comprise the major intracellular anions. The differences in cell permeability and binding characteristics of specific ions are reflected in the coefficients that are used to determine the volume of distribution for individual solutes. For example, because of the permeability of cell membranes to water, the volume of distribution for sodium is equal to the entire body water compartment even though sodium is confined to the ECW space. In contrast, the volume of distribution of bicarbonate is 0.3 × total body water. These considerations are important in formulating therapeutic regimens to treat specific disorders of sodium and water homeostasis (9).

Besides distinctive membrane permeability characteristics for specific solutes, the unequal distribution of ions across the membrane is in part due to the Gibbs–Donnan effect, which arises because of the presence of impermeant, negatively charged proteins, primarily albumin, in the intravascular space (3). Although there are significant differences in the composition of cationic and anionic solutes in various body water compartments, under equilibrium conditions, there is electroneutrality and tonicity or osmolality, i.e., the sum of all osmotically active particles is equal in all body water compartments.

Under normal conditions, the serum osmolality is 286 ± 4 mosm/kg water. Because sodium is the major cation in the ECW, osmolality can be closely estimated by the formula:

$$\text{Serum osmolality} \approx 2 \times [\text{serum sodium concentration}] \quad (1)$$

A reflection coefficient of 1.0 indicates a totally nonpermeant solute, while freely permeable molecules have a reflection coefficient of zero. The reflection coefficient for urea is approximately 0.4. Similarly, in the absence of insulin, the reflection coefficient for glucose is 0.5. Thus, when there is a pathological elevation in the serum urea nitrogen, e.g., acute renal failure, or glucose concentration, e.g., diabetic ketoacidosis, these solutes will also contribute to osmolality, albeit less than sodium. Therefore, the following formula should be used to calculate serum osmolality:

$$\begin{aligned}\text{Serum osmolality} &= 2 \times [\text{serum sodium concentration}] \\ &\quad + [\text{serum urea nitrogen}]/2.8 \\ &\quad + [\text{serum glucose concentration}]/18\end{aligned} \quad (2)$$

This formula is based on the molecular weights of urea nitrogen (28 Da) and glucose (180 Da) and the standard practice of reporting the serum concentrations as mg/100 mL. The calculated serum osmolality is normally within 1–2% of the value obtained by direct osmometry in clinical chemistry laboratories. If the calculated serum osmolality is significantly lower than the value obtained by measurement with an osmometer, this indicates the presence of an "osmolal gap" and reflects the accumulation of unmeasured osmoles. Clinically relevant examples are the organic solutes that are produced after an ingestion of ethanol or ethylene glycol (antifreeze) (10, 11).

Maintenance Sodium and Water Requirements

Sodium

Sodium is an essential dietary component that is required for normal growth. Wassner et al. (12) demonstrated

that somatic growth of experimental animals is impaired if they are fed a sodium-deficient diet. This effect is independent of the protein or calorie content of the diet. Interestingly, in contrast to chronic potassium deficiency induced by diuretics, compromised sodium intake does not cause structural damage to the kidney (13).

Balance studies indicate that the daily sodium requirement is 2–3 mmol/kg body weight. This quantity is nearly two- to threefold higher in term and very low birth weight premature infants (14). This reflects the immaturity in renal tubular function coupled with the increased need for sodium to achieve the high rate of growth during the first few years of life. It will be exaggerated by intrinsic (diarrhea, increased losses via chronic peritoneal dialysis, genetic defect in tubular sodium transport) or exogenous (administration of diuretics) factors that promote sodium loss. In most developed countries, the daily sodium intake is in excess of the amount needed to promote growth or maintain body function. Under normal circumstances, the principal anion that accompanies sodium is chloride. The identity of the anion that is present with sodium and a variety of other dietary constituents impacts on the adverse consequences of excessive sodium intake, such as hypertension (15). In certain disease states such as renal tubular acidosis, metabolic acidosis associated with chronic renal insufficiency, or urolithiasis, it may be advisable to provide a portion of the daily sodium requirement as the bicarbonate or the citrate salt.

Water

The daily requirement for water is traditionally expressed as mL per metabolic kg (16). However, in clinical practice, this is a very cumbersome and impractical method and all calculations are based on body weight and size. There are three methods that are currently used to estimate the daily fluid requirement. The first is a direct extension of the use of metabolic kg and utilizes the following formula:

(1) Daily water requirement
= 100 ml/kg for a child weighing less than 10 kg
+50 ml/kg for each additional kg up to 20 kg
+20 ml/kg for each kg in excess of 20 kg

The second method is based on body surface area and utilizes the following formula:

(2) Daily water requirement
= 1500 mL/m^2 body surface area (BSA)

The last method is a refinement of the second and utilizes the following formula:

(3) Daily water requirement = Urine output
+ insensible water losses

Based on clinical experience, under normal circumstances, urine output is approximately 1,000 mL/m^2/day and insensible losses amount to 500 mL/m^2/day.
Thus, for a child weighing 30 kg and 123 cm in height with a BSA of 1.0 m^2, according to the first method the daily water requirement is 1700 mL while the second method yields 1500 mL/day. The first method is easier to apply, but it tends to overestimate the water requirement as body weight increases. The third method is the most precise and should be applied in more complicated circumstances such as the patient in the intensive care unit with oliguria, secondary to acute kidney injury or the child with increased insensible losses, e.g., diarrhea, increased ambient temperature, tachypnea, burns, or cystic fibrosis (17). In addition to the daily energy requirement and insensible losses that are represented in the formulas, the amount of water excreted by the kidney on a daily basis is dependent upon the solute load. Because urine has a minimum osmolality, approximately 50 mosm/kg H_2O, even in the absence of arginine vasopressin (AVP), increased dietary intake of solute will result in a larger obligatory urine volume to accommodate the larger solute load (18, 19).

The daily sodium and water requirement are generally provided enterally. Intravenous administration of fluids and electrolytes should be resorted to only under clinical circumstances that interfere with normal feeding such a persistent vomiting, gastrointestinal tract surgery, or states of altered consciousness.

In recent years, there has been an ongoing controversy about which fluid is most appropriate for the administration of daily maintenance of water and sodium requirements. The standard recommendation is to use hypotonic fluids containing approximately 50–75 mmol NaCl/L (0.33–0.5% normal saline), based on the original studies done by Holliday that linked fluid requirements to metabolic needs. This guideline has been repeated in several recent reports (20, 21, 22a). However, there are nephrologists who have questioned the risk:benefit ratio of this prevailing practice, based on the occurrence of hyponatremia and neurological complications in

hospitalized children who receive hypotonic fluids parenterally (23, 24). To prevent cerebral edema and neurological consequences of hyponatremia, they advocate routine administration of isotonic saline to all pediatric patients who must receive maintenance fluids intravenously (25b). There are a number of gaps that need to be filled in to clarify this important issue, including the incidence of hyponatremia in large unselected patient populations studied prospectively, the frequency of neurological complications arising from hyponatremia, and the results of a randomized trial comparing safety and efficacy of the two fluid regimens, i.e., hypotonic and isotonic solutions. In the interim, it is important to emphasize that in the face of clinically significant acute ECW contraction, there is universal agreement that isotonic fluids are necessary to replete the intravascular compartment. Careful clinical and laboratory monitoring is a key to ensure good outcomes in all children who are given maintenance fluids and electrolytes parenterally (25a).

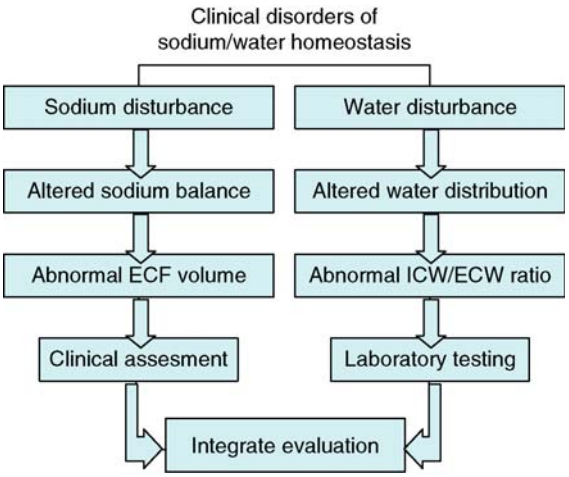
Distinct Roles of Sodium and Water in Body Fluid Homeostasis

Sodium and water are inextricably linked in the determination of the serum sodium concentration. However, it is critical to recognize that sodium and water serve two distinct functions within the body. Sodium is instrumental in the maintenance of the size of the extracellular fluid (ECF) space and the vascular perfusion compartment, while water is critical to the maintenance of the size of individual cells. The regulation of sodium and water homeostasis represents two distinct processes with discrete sensing and effector mechanisms. Although these systems overlap from a physiological and clinical perspective, a complete understanding of body fluids and electrolytes mandates separate evaluation of sodium and water (*Fig. 7-2*).

As mentioned earlier, sodium is confined primarily to the ECW compartment as a consequence of active transport mechanisms in the cell membrane. Because sodium is the principal cation in this space, disturbances in total body sodium content are reflected by expansion or contraction in the ECW compartment. Adequacy of the ECW compartment is essential to maintain the intravascular space and sustain perfusion of vital organs. In terrestrial mammals living in an environment where ECW volume depletion is a constant threat, the kidney is designed for maximal sodium reabsorption as the default mode unless physiological signals instruct it to respond otherwise. This contrasts with potassium, in which the threat is an elevated serum concentration and for which the default mode is tubular secretion of the cation.

Figure 7-2

This scheme illustrates the importance of independent assessment of sodium and water handling in managing patients with clinical disorders of sodium-water homeostasis.



The primary step in the pathogenesis of disturbances in ECW compartment size is a perturbation in sodium balance. When total body water and sodium content are within the normal range, net sodium balance is zero and the daily intake of sodium is matched by losses in the urine, stool, and insensible losses. Provided kidney function is normal, the dietary sodium intake can be as low as 0.1 mmol/kg or in excess of 10 mmol/kg without any derangement in ECF compartment size. If the alterations in diet are not abrupt, then sodium balance is maintained even when kidney function is markedly impaired (26). In contrast, if the daily input of sodium exceeds losses, there is expansion of the ECF space that manifests as edema while if the input does not match the daily losses, there will be symptoms and signs related to ECW space contraction. These disturbances are not associated with any obligatory parallel changes in the serum sodium concentration.

Water homeostasis is a prerequisite for the normal distribution of fluid between the ICW and ECW

compartments. Cell function is dependent on stabilization of cell volume in order to keep the cytosolic concentration of enzymes, co-factors, and ions at the appropriate level. Perturbations in water balance result in fluctuations in serum osmolality. Because cell membranes are semipermeable and permit free movement of water down its osmolal gradient, this causes obligatory shifts in water between the cell and the ECW space. Any disorder that alters the 2:1 ratio of water volume in the ICW:ECW spaces will be reflected by changes in cell size and subsequent cellular dysfunction. Under hypoosmolal conditions, water will move from the intravascular compartment into the cell, causing relative or absolute cell volume expansion. Conversely, if the serum osmolality is elevated, water will exit from the cell to the ECW space resulting in absolute or relative cellular contraction (27).

Disturbances in cell function related to abnormalities in cell size are most prominent in cerebral cells. There are two reasons for this phenomenon. First, the blood–brain barrier, which is constituted by tight junctions between adjacent endothelial cells, limits the movement of solute between the ICW and ECW compartments, while permitting unrestricted flow of water down an osmolal gradient (28). Second, the brain is contained within the skull, which is a closed, noncompliant space, and is tethered to the cranial vault by bridging blood vessels, which limits its tolerance of cell swelling or contraction. Thus, alterations in water balance and serum osmolality are dominated by clinical findings of central nervous system dysfunction, including, lethargy, seizures, and coma (27).

In the same way that disturbances in sodium balance do not necessarily predict specific abnormalities in serum sodium concentration, the presence of a disturbance in water balance and serum osmolality is not linked to a specific abnormality in the ECW compartment size. The independent nature of disturbances in sodium and water balance is illustrated in *Table 7-1*. Alterations in ECW size can occur in patients with hypotonicity, isotonicity, or hypertonicity. Similarly, each alteration in serum osmolality can develop in patients with contraction or expansion of the ECW compartment.

The presence of disturbances in sodium and water homeostasis must be addressed separately in the clinical evaluation of patients with derangements in ECF volume or tonicity. This must then be integrated to obtain a comprehensive view of what is abnormal, and determine how to restore sodium and water homeostasis effectively and with minimal side effects (*Fig. 7-2*). The clinical approach to these problems will be outlined later.

Sensor Mechanisms: Sodium and Water

For both sodium and water homeostasis, the sensor mechanisms that maintain the equilibrium state are primarily designed to be responsive to the consequences of abnormalities in sodium or water balance, i.e., changes in ECW and cell size, respectively, rather than measuring the primary variable. In this regard, they differ from a recently described acid-base sensor that is directly responsive to changes in pH (29). They operate using negative feedback loops in which deviations from normal are detected, counter-regulatory mechanisms are activated that antagonize the initiating event, and the system is restored to its original state.

Sodium

The detection of abnormalities in sodium balance is based on systems that sense the consequences of these changes. Thus, net sodium deficit is detected as a decrease in ECW space size, while net sodium excess is perceived as an obligatory expansion of the ECW space. These receptors, which are influenced by the filling pressure within the circulation, are called baroreceptors or mechanoreceptors. These signals are supplemented in certain instances by chemoreceptors that respond directly to changes in the serum sodium concentration and trigger adaptive modifications in renal sodium handling. These receptors may effect change by altering nervous system activity or by activating upstream promoter elements and stimulating the expression of relevant genes (30).

Atrial Receptors

There are ECW volume receptors on the venous (low pressure) and arterial (high pressure) sides of the circulation. Within the right atrium, sensors possess the distensibility and compliance needed to detect alterations in intrathoracic blood volume provoked by increasing negative intrathoracic pressure or head-out water immersion. Both of these maneuvers, which increase the central blood volume and raise central venous and right atrial pressures, are followed by a brisk natriuresis and diuresis (31). These relative changes are triggered even in the absence of a concomitant change in the total ECW space size. Neural receptors that respond to mechanical stretch or changes in right or left atrial pressure convey the signal via the vagus nerve (31, 32).

Table 7-1

Clinical diseases of sodium and water homeostasis: relationship between ECW size and tonicity

Tonicity ECW volume	Low	Normal	High
Low	Addison's disease	Isotonic diarrheal Dehydration	Hypertonic diarrheal Dehydration
	Salmonella diarrhea		Diabetes insipidus
	Mannitol infusion		
Normal	SIADH	No disease	Acute sodium bicarbonate infusion
High	Acute renal failure	Nephrotic syndrome	Salt intoxication
	Nephrotic syndrome		Salt water drowning
	Cirrhosis		
	Congestive heart Failure		

Hepatic Receptors

The enhanced renal sodium excretion, triggered by saline infusions, directly into the hepatic vein versus the systemic circulation suggests that there are low-pressure sensors within the portal vein or hepatic vasculature. The hepatic responses to changes in sodium balance have been divided into two categories (33). The "hepatorenal reflex" involves direct activation of sodium chemoreceptors and mechanoreceptors in the hepatoportal region, via the hepatic nerve, and causes a reflex decrease in renal nerve activity. The "hepatointestinal reflex" utilizes chemoreceptors to respond to changes in sodium concentration and modulate intestinal absorption of sodium via signals conveyed along the vagus nerve. Activation of these hepatic volume sensors may contribute to the sodium retention and edema states that develop secondary to chronic liver disease and cirrhosis with the associated intrahepatic hypertension.

Pulmonary Receptors

There may also be pressure sensors within the pulmonary circulation that are activated by changes in pulmonary perfusion or mean airway pressure (34). The receptors in the lung may be located in the interstitial spaces and influence the physical forces that modulate paracellular absorption of sodium and water. They resemble receptors in the renal interstitium that also influence paracellular absorption of fluid and solutes along the nephron, especially in the proximal tubule segment (35).

Carotid Arch Receptors

There are also volume-dependent sensors on the high-pressure side of the circulation including the carotid arch, the brain, and the renal circulation. Thus, occlusion of the carotid leads to increased sympathetic nervous system activity and alterations in renal sodium handling (32). The responsiveness of the carotid arch receptors may be modulated by chronic changes in ECF volume. For example, a head-down bed position and a high salt diet blunt carotid baroreceptor activity (36).

Cerebral Receptors

Increases in the sodium concentration of the CSF or brain arterial plasma promote renal sodium excretion (37). Lesions in discrete anatomic areas of the brain such as the anteroventral third ventricle alter renal sodium reabsorption, confirming that there are central mechanisms of sensing changes in sodium balance and ECF volume. Derangements in the sensing system within the brain in patients with long-standing central nervous system diseases may contribute to the cerebral syndrome. Intracerebral expression of angiotensin converting enzyme (ACE) isoforms contribute to peripheral sodium and water handling (38).

If the arterial sensors perceive underfilling of the vascular space, this activates counter-regulatory mechanisms to restore the ECW compartment size even if the receptors in the venous system detect adequate or even overfilling of the venous tree. This implies that despite normal or even excess total body sodium and net positive sodium balance, there are conditions in which the body perceives

an inadequate circulating plasma volume. This has given rise to the notion of the "effective" intravascular volume, a concept that is applicable in the edema states such as congestive heart failure, cirrhosis, and nephrotic syndrome (39). For example, in patients with cardiac pump failure, perceived underfilling of the arterial tree may occur despite significant venous distention (25b). Similarly, women who develop edema during pregnancy may have primary peripheral vasodilatation and excess total body sodium (39).

In summarizing the sensor mechanisms that are involved in the regulation of sodium balance, the primary ones are those that are directly linked via mechanoreceptors to the status of the ECF volume. These sensor systems are activated by a decreased size of the ECW compartment and respond to "underfilling" of the vasculature tree. However, there are secondary mechanisms that are activated by chemoreceptors or localized intra-organ disturbances in perfusion that are dissociated from the ECF volume. These sensors can cause overfilling of the vascular compartment by stimulating renal sodium reabsorption. Correct interpretation of the balance between these two processes involved in sodium balance is critical to the proper diagnosis and management of the edema states.

Water

The receptors that are responsible for regulating water homeostasis are primarily osmoreceptors and are sensitive to alterations in cell size (40). These osmoresponsive cells are located in the circumventricular organs and anterolateral regions of the hypothalamus, adjacent to but distinct from the supraoptic nuclei. They shrink or swell in response to increases or decreases in plasma tonicity and this change in cell size triggers the release of AVP and/or the sensation of thirst. The anatomic configuration of these cells enables them to be exposed to circulating peptides that are involved in water homeostasis.

AVP

AVP is a peptide containing nine amino acids and has a molecular weight of 1,099 Da. It is synthesized by the cells in the hyopothalamus, transported down the axon, and stored in the posterior pituitary in conjunction with larger proteins, called neurophysins (41). The gene for AVP is located on chromosome 20 and has a cAMP response element in the promoter region. Prolonged stimulation of AVP release leads to upregulation of the AVP gene; however, synthesis does not keep up with the need for the peptide because pituitary levels of AVP are usually depleted in states such as chronic salt loading and hypernatremia (42).

The principle solute that provokes the release of AVP is sodium. Infusion of sodium chloride to increase plasma osmolality results in increased secretion of AVP in the absence of parallel changes in ECW volume. This underscores the primary role of plasma osmolality per se in stimulating AVP release (40). Mannitol, an exogenous solute that is used in clinical practice to treat increased intracranial pressure, is nearly as effective as sodium in stimulating AVP release. Urea and glucose are <50% as effective as sodium in provoking AVP secretion because they are more permeable than sodium and cause less pronounced changes in osmoreceptor cell volume. However, in disease states such as acute renal failure or diabetic ketoacidosis, in which urea or glucose, respectively, act as osmotically active molecules or following the exogenous administration of mannitol, these solutes also stimulate increases in AVP release. There is coupling between mechanical changes in membrane structure and hormone release. However, the exact mechanism and the neurotransmitters that mediate the actions of the osmoreceptors on the cells of the posterior pituitary have not been identified.

A variety of nonosmotic stimuli to AVP release may contribute to water handling in various disease states (40). Vomiting and acute hypoglycemia promote AVP release by neural–hormonal pathways that are not well defined. Stress associated with pain or emotional anxiety, physical exertion, high body temperature, acute hypoxia, and acute hypercapnia are other conditions that lead to increased secretion of AVP in the absence of a primary disturbance in water balance. Numerous drugs directly influence the hypothalamic release of AVP including carbamazepine, cyclophosphamide, and vincristine. Finally, hemodynamic changes arising from primary alterations in sodium balance and the ECW space can trigger AVP release. If the ECW volume disturbance is mild, then the stimulation of AVP release is modest. However, in the face of severe ECW volume contraction, there is marked secretion of AVP. Under these circumstances, the imperative to protect the effective circulating blood volume takes precedence over the need to maintain plasma osmolality and ECW volume is restored at the expense of hypoosmolality. This clinical observation indicates that despite rigorous intellectual attempts to separate sodium and water homeostatic mechanisms, these two factors are closely linked in vivo and there can be significant overlap in sensor and effector mechanisms in the regulation of ECW and ICW compartment size. *Table 7-2* summarizes the factors that modulate AVP release.

Table 7-2
Factors that increase AVP release

↑Plasma osmolality
Hemodynamic
↓Blood volume
↓Blood pressure
Emesis
Hypoglycemia
Stress
Elevated body temperature
Angiotensin II
Hypoxia
Hypercapnia
Drugs

In addition to AVP release, the osmoreceptor cells also respond to the changes in serum osmolality in an independent manner to stimulate thirst and increase drinking (43). The stimuli for thirst are generally the same as those for AVP release, with hypernatremia being the most potent trigger. The osmotic threshold for thirst in humans appears to be higher than for AVP secretion, i.e., 295 mosm/kg. The sensing mechanism that leads to this increase in water intake is even more obscure than that for AVP release. It is likely that changes in ECW volume are also involved in this process because angiotensin II, which rises in states of ECW volume contraction, is a potent dipsogen (44). Recent studies suggest that the day-to-day regulation of thirst by osmoreceptors is under the control of dopamine-mu opioid neurotransmitters in the brain while angiotensin II may be activated under more stressful conditions (1).

Efferent Mechanisms: Sodium and Water

The efferent mechanisms involved in maintaining sodium and water balance include the neural and endocrine-humoral systems. There often is an overlap in the action of these effectors, with an individual effector having distinctive effects on both sodium and water balance.

Sodium

Renin–Angiotensin–Aldosterone Axis

The major components of this system – renin, angiotensinogen, and ACE – are found within the kidney and the vasculature of most organs. These elements are linked in a large feedback loop involving the liver, kidney, and lung as well as smaller loops within individual organs. This accounts for the often-disparate data about plasma renin activity (PRA) and the expression of individual components within the kidney during disturbances in ECW compartment size.

Angiotensin II is the major signal generated by this axis (45). There are two distinct forms of ACE and the ACE2 isoform may metabolize angiotensin II to nonpressor breakdown products that react with specific receptors and that are less likely to promote the development of hypertension (46). This introduces another layer of complexity in the regulation of sodium balance by the renin–angiotensin axis. Angiotensin II interacts with two different receptors, and most of its biological activity is mediated by the angiotensin type 1 (AT1) receptor. The AT2 receptor is more prominently expressed in the fetal kidney; however, interaction of angiotensin II with the AT2 receptor postnatally stimulates the release of molecules such as nitric oxide (NO) that counteract the primary action of the peptide (47). In addition, angiotensin I can be processed to the heptapeptide angiotensin 1–7, which interacts with a separate mas receptor and modulates the biological effects of angiotensin II (46). More research is needed to elucidate the role of alternate forms of angiotensin such as angiotensin 1–7 on sodium and water balance in children.

The best-known effects of angiotensin II include peripheral vasoconstriction to preserve organ perfusion and stimulation of adrenal synthesis of aldosterone to enhance renal sodium reabsorption. These two actions restore the ECW space to normal. However, angiotensin II also has direct actions on tubular function and stimulates both proximal and distal sodium reabsorption. The proximal tubule cells contain all of the elements needed to synthesize angiotensin II locally and the peptide increases the activity of the sodium–hydrogen exchanger (48). In the distal tubule, angiotensin II modulates this exchanger as well as the amiloride-sensitive sodium channel (45). The effects of aldosterone on the renal tubule include an immediate effect to increase apical membrane permeability to sodium and more extended effects that involve enhanced gene transcription and de novo synthesis of Na-K ATPase. Aldosterone stimulates the synthesis of other enzymes involved in renal cell bioenergetics such a citrate synthase that are needed to sustain maximal tubular sodium transport (49). Finally, aldosterone induces a state of glucose-6-phosphate dehydrogenase deficiency in endothelial cells which may contribute to oxidant stress and altered reactivity of blood vessels in response to disturbances in sodium balance (50).

Endothelin

This vasoactive molecule is part of a family of three peptides of which endothelin-1 (ET-1) is the most important in humans (51). It is converted in two steps from an inactive precursor to a biologically active 21-amino acid peptide. Endothelins react with two receptors, ETA and ETB, and cause vasoconstriction, resulting in a decrease in renal blood flow and glomerular filtration rate (GFR). With regard to sodium balance, the primary effect of endothelin is sodium retention mediated by the reduction in GFR. This suggests that endothelin acts in concert with angiotensin II to protect ECW compartment size under conditions of sodium deficit. However, the situation may be more complicated because direct exposure of proximal tubule and medullary collecting duct cells to endothelin in vitro inhibits sodium absorption.

Renal Nerves

There is abundant sympathetic nervous innervation of the renal vasculature and all tubular segments of the nephron (52). The efferent autonomic fibers are postganglionic and originate in splanchnic nerves. The renal innervation is primarily adrenergic and involves α1 adrenoreceptors on blood vessels and both α1 and α2 receptors along the basolateral membrane of the proximal tubule. Renal sympathetic nervous system activity contributes to preservation of ECF volume by (1) promoting renal vasoconstriction and lowering GFR and (2) increasing sodium reabsorption. Among the catecholamines involved in adrenergic transmission, norepinephrine exerts an antinatriuretic effect. Dopamine, another sympathetic nervous system neurotransmitter, promotes a natriuresis, suggesting that there is internal regulation of the effect of nerve activation on renal sodium handling (53).

Renal sympathetic nervous activity is inversely proportional to dietary salt intake (52). Drug-induced sodium retention and volume-dependent hypertension, e.g., with the use of cyclosporine, is mediated in part by activation of the sympathetic nervous system (54). Increased adrenergic nervous signaling within the kidney is instrumental in the initiation of hypertension in experimental animals by causing a right-shift in the pressure–natriuresis curve (52). However, sodium balance is normal and ECF volume is maintained in the denervated transplanted kidney, implying that the role of the sympathetic nervous system in maintaining sodium homeostasis is redundant and can be taken over by other regulatory mechanisms (52).

Atrial Natriuretic Peptide

Atrial natriuretic peptide (ANP) is a 28-amino acid peptide that is a member of a group of proteins that includes C-type natriuretic peptide (55). It is synthesized as a prohormone that is stored in granules in the cardiac atria. There are other molecular isoforms of the hormone including brain natriuretic peptide (BNP) whose circulating levels are altered and which can be monitored at diagnosis and in response to treatment in conditions such as congestive heart failure (56).

Increases in right atrial pressure provoke cleavage and release of the mature peptide. For each 1 mm Hg rise in central venous pressure, there is a corresponding 10–15 pmol/L increase in circulating ANP levels. Conversely, declines in atrial pressure secondary to sodium depletion or hemorrhage inhibit ANP release. There are two receptors for ANP and both are coupled to guanylate cyclase. The activation of this enzyme results in cytosolic accumulation of cGMP, which in turn diminishes agonist-stimulated increases in intracellular calcium concentration. The principle effects of ANP are to promote an increase in GFR, diuresis, and most importantly, natriuresis. The augmented renal sodium excretion is, in part, mediated by an increased filtered load secondary to the rise in GFR. However, ANP also exerts direct actions on renal tubular cells to diminish sodium reabsorption including inhibition the Na-K-Cl co-transporter in the loop of Henle and the amiloride-sensitive sodium uptake in the medullary collecting duct. Finally, ANP antagonizes the action of several antinatriuretic effectors, including sympathetic nervous system activity, angiotensin II, and endothelin. The overall effects of ANP to counteract increases in ECW compartment have been demonstrated by short-term studies in which acute infusions of ANP improved cardiac status in patients with congestive heart failure and promoted a diuresis in patients with acute renal failure (57). However, despite the potent actions of ANP on fluid balance, clinical trials assessing its efficacy in chronic congestive heart failure have been disappointing and may be associated with an increased risk of kidney failure (58).

Prostaglandins

The kidney contains the enzymes required for constitutive (COX-1) and inducible (COX-2) cyclooxygenase activity that convert arachidonic acid to prostaglandins (59). The major products of these pathways are PGE_2, $PGF_{2\alpha}$, PGD_2, prostacyclin (PGI_2), and thromboxane (TXA_2).

In the cortical regions, PGE_2 and PGI_2 predominate, while PGE_2 is the major prostaglandin metabolite in the medulla. These two compounds increase GFR and promote increased urinary sodium excretion. In addition, they antagonize the action of AVP. These actions may mediate the adverse effects of hypercalcemia and hypokalemia on renal tubular function (59). The natriuretic effects of prostaglandins, in response to normal alterations in dietary sodium intake are unclear. The role of prostaglandins as efferent signals is more apparent in conditions associated with increased vasoconstrictor tone such as congestive heart failure or reduced renal perfusion, where prostaglandins counteract the vasoconstrictor and sodium-retaining effects of angiotensin II and norepinephrine. Inhibition of prostaglandins with cyclooxygenase inhibitors is associated with dramatic declines in GFR and profound sodium retention and edema (60).

Kinins

Kinins are produced within the kidney and act via B_1 and B_2 receptors. Because the half life of kinins in the plasma is very short, in the range of 20–40 s, it is likely that their actions in the kidney are regulated locally through production and proteolytic processing in the tissue (61). Their principal action is to promote renal vasodilatation and natriuresis. The kinins act primarily in the distal tubule to reduce sodium reabsorption (61, 62).

Nitric Oxide

The kidney contains all three isoforms of nitric oxide synthase (NOS) – neuronal NOS in the macula densa, inducible NOS in renal tubules and mesangial cells, and endothelial NOS in the renal vasculature – involved in NO synthesis. The neuronal and endothelial isoforms are calcium-dependent enzymes and produce small, transient increases in NO synthesis. The inducible isoform is upregulated by various cytokines and inflammatory mediators, resulting in large sustained elevations in NO release.

Activation of eNOS within the kidney increases the activity of soluble guanylate cyclase and causes vasodilatation and an increase in GFR. In addition to its effect on renal blood flow and GFR, NO directly inhibits Na-K ATPase in cultured proximal tubule and collecting duct cells (63, 64). The specific isoform of NOS that is responsible for modulating urinary sodium excretion is not well defined. Studies with inducible NOS, neuronal NOS and endothelial NOS knockout mice suggest that only the first two isoforms are involved in the regulation of sodium and water reabsorption in the proximal tubule (63). A role of NO in maintaining sodium balance under normal conditions is suggested by the observation that alterations in dietary salt intake are associated with parallel changes in urinary excretion of nitrite, the metabolic byproduct of NO (65). In normotensive Wistar-Kyoto rats and spontaneously hypertensive rats, increased dietary sodium intake is associated with a modest increase in urinary nitrite excretion (66). This effect is not well-documented in pediatric patients. Along with ANP and bradykinin, NO is part of the defense system against sodium excess and expansion of the ECW compartment. Derangements in renal NO synthesis and responsiveness to cGMP may be instrumental in the pathogenesis of salt-dependent hypertension in experimental animals (67).

Adrenomedullin

Adrenomedullin is a 52-amino acid peptide that was isolated from human pheochromocytoma cells (68). It reacts with a G-protein cell receptor and causes vasodilatation, an effect that may be mediated by increased synthesis of NO. The resultant natriuresis secondary to the increase in GFR is accompanied by direct inhibition of tubular sodium reabsorption. Its role in sodium balance is under investigation.

Water

AVP

The primary efferent mechanism in the maintenance of water homeostasis is AVP. This peptide fosters water retention by the kidney and stimulates thirst. The plasma AVP concentration is approximately 1–2 pg/mL under basal conditions (40). It is not known whether there is tonic release of AVP or whether there is pulsatile secretion in response to minute fluctuations in plasma osmolality. The set point, or osmotic threshold for AVP release, ranges from 275–290 mosm/kg H_2O. The circulating hormone concentration rises approximately 1 pg/mL for each 1% increase in plasma osmolality. The sensitivity of the osmoreceptors in promoting AVP release varies from person-to-person with some individuals capable of responding to as small as a 0.5 mosm/kg H_2O increase in osmolality and others requiring greater than a 5 mosm/kg H_2O increment to stimulate AVP release. Patients with essential hypernatremia possess osmoreceptors that have

normal sensitivity, but the osmotic threshold for AVP release is shifted to the right. Because the relative distribution of water between the ECW and ICW compartments is undisturbed, these patients are unaffected by their abnormally high serum sodium concentration. Although there may be sex-related differences in AVP secretion in response to abnormal water homeostasis with increased sensitivity in women, this is not a relevant clinical concern in prepubertal children.

Angiotensin

Angiotensin II serves as an efferent system in water homeostasis primarily by acting as a potent dipsogen and stimulating drinking (44). Its role in water handling within the kidney is minor and may be related to modulation of the renal response to AVP.

Thirst

Thirst, or the consciously perceived desire to drink, is a major efferent system in water homeostasis (43). It is estimated that for each 1 pg/mL increase in the circulating plasma AVP level, there is parallel rise of 100 mosm/kg H_2O in urinary concentration. If the basal plasma osmolality and AVP concentration are approximately 280 mosm/kg H_2O and 2 pg/mL, respectively, and the steady state urine osmolality is 200 mosm/kg H_2O, then as soon as the plasma osmolality and AVP concentration reach 290 mosm/kg H_2O and 12 pg/mL, respectively, the urine is maximally concentrated. Beyond this point, the only operational defense against a further rise in plasma osmolality is increased free water intake, underscoring the essential role of thirst as an efferent mechanism in water homeostasis. It highlights the increased risk of hyperosmolality in patients who do not have free access to water such as infants, the physically or mentally incapacitated, or the elderly (69).

Thirst is a difficult biological function to quantitate because it is an expression of a drive rather than an actual behavior. At present, visual analog scales using colors or faces are the most useful tools for quantitating thirst under controlled condition. There can be dissociation between water intake and the sensation of thirst as in patients with psychogenic polydipsia (e.g., schizophrenia, neurosis). It is not known whether specific drugs directly stimulate the dipsogenic response. The role of diet, e.g., high salt intake, in the regulation of thirst is also unknown. The osmotic control of thirst may be suboptimal in newborn infants and in the elderly (69).

Thirst and drinking behavior are stimulated by significant contraction of the ECF space or by hypotension. In addition, thirst and drinking behavior are modulated by signals that originate in the oropharnyx and upper gastrointestinal tract. Animals with hypernatremia who are given access to water as the sole means of correcting the hyperosmolal state stop drinking sooner than animals corrected in part with supplemental intravenous fluid. This is most likely due to oropharyngeal stimuli that curtail drinking prior to complete normalization of plasma osmolality (70).

Effector Mechanisms: Sodium and Water

The kidney is the principal organ that acts in response to sensory input, delivered via neural or humoral signals, to restore ECW volume size to normal following the full range of clinical problems. Although absorption of sodium and water across the intestinal epithelium may be modulated by chemoreceptors in the hepatic vasculature, the role of the gastrointestinal tract in the control of sodium balance is clearly secondary to the function of the kidney.

Sodium

GFR

In children with normal kidney function, changes in GFR are generally associated with parallel alterations in sodium balance. This is accomplished by glomerular-tubular balance in which proximal tubule sodium absorption and delivery of filtrate to the distal tubule is modulated in response to GFR (71). Tubular sodium reabsorption increases in parallel with an increase in GFR, which reflects the load-dependent nature of sodium reabsorption in the proximal tubule. In addition, changes in GFR lead to changes in the oncotic pressure in the peritubular capillaries that influence sodium reabsorption (72). Thus, an increased GFR is associated with higher hydrostatic pressures in the peritubular capillary network that retard fluid and solute reabsorption in the proximal tubule. Finally, tubuloglomerular feedback is activated by alterations in solute delivery to the distal nephron to bring GFR in line with alterations in tubular function. Many of the efferent signals including renin, angiotensin, NO, adenosine, and prostaglandins participate in this particular pathway. The release of these effector molecules is activated via myogenic stretch receptors

and chemoreceptors located in the macula densa region of the distal nephron. Even in children with compromised renal function (GFR < 20–30 mL/min/1.73 m^2), in whom there are adaptive changes in tubular function, e.g., increased fractional excretion of sodium (FENa), glomerulotubular balance is maintained in the face of gradual changes in GFR. However, patients with chronic kidney disease are unable to respond to abrupt changes in sodium balance and ECF volume changes as rapidly as in healthy children and are susceptible to volume contraction or hypervolemia if sodium intake is substantially reduced or increased over a short period of time (26).

Most of the neural and humoral factors described previously can modulate GFR. Agents that lower GFR act predominantly on the vascular tone of the afferent arteriole and reduce renal blood flow and the filtration fraction. Agents in this category include adrenergic nerve stimulation and endothelin. In contrast, angiotensin II acts primarily on efferent arteriolar tone. This tends to preserve GFR more than renal blood flow and the filtration fraction (RBF/GFR) is increased. This pattern is most evident in states of compromised effective perfusion such as congestive heart failure, cirrhosis, and nephrotic syndrome (39, 73, 74). The critical role of angiotensin II in maintaining GFR and sodium excretion in these conditions is manifested during the reversible functional decline in GFR that occurs after the administration of ACE inhibitors (75). This phenomenon also explains the reduction in kidney function and sodium retention that are observed in patients with a critical renal artery stenosis in a kidney transplant following initiation of ACE inhibitor therapy (75) and which are corrected by discontinuation of the medication.

Proximal Tubule

Nearly 60–70% of the filtered sodium and water load are reabsorbed in the proximal tubule. Sodium and fluid reabsorption are isosmotic in this nephron segment. These processes are driven by Na-K ATPase activity along the basolateral membrane surface with secondary active transport of solute across the apical membrane. The bulk of sodium reabsorption is driven by the sodium–hydrogen exchanger, with a lesser contribution by other co-transport systems for glucose, phosphate, organic anions and amino acids. The linkage between disturbances in ECF volume and sodium reabsorption in the proximal tubule is created, in part, by changes in the physical forces that govern fluid and solute movement. These include changes in peritubular capillary hydrostatic pressure, peritubular capillary protein concentration and oncotic pressure, and changes in renal interstitial pressure that modulate water and solute movement across cells (transcellular) and along the paracellular pathway.

Sympathetic nervous stimulation, norepinephrine release, and both filtered and locally synthesized angiotensin II stimulate the activity of the sodium–hydrogen antiporter and promote sodium reabsorption in conditions associated with decreased ECF volume. Conversely, ANP and the kinins act on proximal tubular cells to inhibit sodium reabsorption and limit expansion of the ECW space.

Distal Nephron Including Collecting Duct

This portion of the nephron is responsible for the reabsorption of approximately 10–25% of the filtered sodium and water load. Under most circumstances, it adapts to changes in delivery arising from alterations in proximal tubule function. This segment of the nephron is responsive to virtually all of the humoral efferent signals and accomplishes the final renal homeostatic response to fluctuations in sodium balance. Sodium reabsorption in the distal tubule and connecting segment is responsive to circulating levels of aldosterone (49). In the collecting tubule, mineralocorticoid-responsive sodium reabsorptive pathways achieve the final modulation of sodium excretion in response to alterations in sodium intake. Aldosterone enhances sodium reabsorption by inducing a number of transport proteins whose synthesis is triggered by activation of SGK1, serum and glucocorticoid-inducible kinase (76). The most prominent of these is the ENaC. This transepithelial protein is composed of three distinct chains – α, β, and γ – each of which is encoded by a separate gene (54). The complete protein has two membrane-spanning domains with an amino and carboxyl terminus within the cell. The α-chain appears to constitute the actual sodium conducting pathway while the β- and γ-chains may represent regulatory components that control the open/closed status of the channel. Genetic defects in each individual component have been described and linked to human disease. Thus, pseudohypoaldosteronism has been mapped to mutations in the α, β, and γ chains, and Liddle's syndrome has been attributed to truncation in the β-chain with increased ubiquitinylation and proteasomal degradation of the abnormal protein (76, 77, 78).

Water

AVP

AVP acts along several segments of the nephron. However, its primary site of action for maintenance of water homeostasis is the collecting tubule (66). In that segment of the nephron, AVP reacts with the V2 receptor, a 371-amino acid protein that is coupled to a heterotrimeric G-protein, along the basolateral membrane of the distal tubule and collecting duct cells. The V2 receptor gene has been localized to region 28 of the X chromosome. This epithelial cell receptor is distinct from the V1 receptor in the vasculature that is linked to Ca-activation of the inositol triphosphate cascade and which mediates vasoconstrictor response to the hormone (66).

Binding of AVP to the V2 receptor activates basolateral adenylate cyclase and stimulates the formation of cAMP within the cytosol. This intracellular second messenger then interacts with the cytoskeleton, specifically microtubules and actin filaments, and promotes fusion of intramembrane particles that contain preformed water channels with the apical membrane of principal cells in the collecting duct. The AVP-induced entry of preformed water channels involves clathrin-coated pits. Withdrawal of AVP leads to endocytosis of the membrane segment containing the water channels into vesicles that are localized to the submembrane domain of the cell, which terminates the hormone signal. Recycling of water channels from vesicles to the apical membrane and then back into vesicles has been demonstrated in freeze-fracture studies of cells exposed to AVP (66). The importance of the V2 receptor in water homeostasis is confirmed by genetic mutations and corresponding abnormalities in protein structure in children with X-linked congenital nephrogenic diabetes insipidus (79).

The water channels that mediate transmembrane movement of water across the collecting tubule in response to AVP are called aquaporins (80). There are nine known members of this group of proteins, all of which contain six membrane-spanning domains. The first member to be identified was aquaporin-1 (AQP-1) (originally called channel-forming integral membrane protein of 28 kDa or CHIP-28), which mediates water movement across the erythrocyte membrane and along the proximal tubule. Mice that do not express AQP-1 have a normal phenotype and concentrate their urine normally. AQP-2 is the major AVP-sensitive water channel in the collecting tubule (81). Immunogold electron microscopy studies have confirmed that AQP-2 represents the water channel in the cytosolic vesicles which fuse with the apical membrane following exposure of principal cells to AVP. The contribution of AQP-3 and AQP-4 to the normal urinary concentrating mechanism has been confirmed in mice that have been genetically manipulated and which do not express these two proteins (82).

The importance of AQP-2 in mediating the normal response to AVP has been verified by the discovery of mutations in the AQP-2 gene in children with non-X-linked, autosomal recessive forms of nephrogenic diabetes insipidus (83). Moreover, alterations in AQP-2 protein expression have been documented in other states associated with a urinary concentrating defect such as lithium exposure, urinary tract obstruction, hypokalemia, and hypercalcemia (80).

Water reabsorption in the collecting duct is not completely dependent upon the presence of AVP. In animals that are genetically deficient in AVP (Brattleboro rats) or in patients with central diabetes insipidus, urinary osmolality increases slightly above basal levels in the face of severe ECF volume contraction. This may be the consequence of reduction in urinary flow rate along the collecting duct that enables some passive equilibration between the luminal fluid and the hypertonic medullary interstitium.

Although the collecting duct is the primary site of regulation of net water reabsorption, the proximal tubule contributes to water balance under circumstances of decreased ECW compartment size. Whereas the proximal tubule normally reabsorbs approximately 60% of the filtered water load, this proportion may exceed 70% when the ECF volume is diminished. Furthermore, by decreasing fluid delivery to the distal nephron, this enhances the AVP-independent reabsorption of water along the collecting tubule. These combined effects may explain the clinical benefit achieved by the administration of thiazide diuretics to patients with nephrogenic diabetes insipidus (84).

Countercurrent Mechanism

The primary locus of the urinary concentrating mechanism is the medulla and involves the thin descending limb of Henle, medullary thick ascending limb of Henle, cortical thick ascending limb of Henle, and collecting duct (85). Sodium and water reabsorption are isosmotic in all segments of the nephron proximal to the loop of Henle. In order to concentrate or dilute the urine, water and solute must be separated to enable excretion of free water or

urine that is hyperosmolal relative to plasma. This process begins in the medullary and cortical thick ascending limb of Henle where NaCl is reabsorbed independently of water, generating a hypotonic luminal fluid. This action is linked in series to the low water permeability of the distal tubule and the connecting segment, which together with continued sodium reabsorption, enhances the hypotonicity of the urine in this segment. In a secondary step, the permeability to water along this segment of the nephron is much lower than in the descending limb of the loop of Henle. This enables water to move down its osmolal gradient from the tubule lumen into the interstitium as it enters the medulla in the descending limb. Finally, the third critical component of the countercurrent mechanism is the presence of vasa recta, which perfuse the inner medulla via vascular bundles that contain hairpin loop-shaped blood vessels. This facilitates efficient removal of water that exits the descending limb of Henle from the medullary interstitium without washing out the solute gradient that passively drives water reabsorption in the collecting tubule. The final effector mechanism is the alteration in the water permeability of the collecting tubule in response to AVP and the generation of a concentrated urine or the excretion of solute free water in the absence of AVP (*Table 7-3*).

Osmoprotective Molecule (Compatible Osmolytes)

Besides the presence of effector mechanisms to maintain water balance, cells possess a wide range of adaptive mechanisms to counteract the undesirable movement of water between the cell and the ECW during hypotonic and hypertonic states and to prevent neurological dysfunction. These include early response genes that mediate the prompt accumulation of chaperone molecules to counteract the adverse effects of altered cell size on protein function (27). This is followed temporally by the uptake or extrusion of electrolytes as an acute response to altered size cell. Because there are inherent limits on the ability to regulate cell volume exclusively with inorganic electrolytes, the more extended response involves membrane transport and/or synthesis/degradation of a variety of compatible solutes, called osmolytes, whose cytosolic concentration can be safely altered without perturbing enzymatic activity and cell function. These osmoprotective molecules include carbohydrates (sorbitol, myo-inositol), amino acids (taurine, glutamate), and methylamines (betaine, glycerophosphorylcholine) (27). They accumulate in the cytosol to preserve cell function during chronic osmolal disturbances. The cell volume regulatory response can be activated by electrolytes such as sodium or neutral molecules, e.g., urea and glucose (27a). The adequacy of the cell volume regulatory response and the accumulation of osmoprotective molecules in cerebral and renal cells depend on the rate of rise in osmolality as well as the magnitude of the absolute change (86).

Experimental data in animals and clinical experience in premenopausal women suggest that estrogens may impair the cell volume regulatory response to disturbances in plasma osmolality. This increases the risks associated with both the untreated abnormalities and therapy (87). The cell volume regulatory adaptation is fully operational during maturation. The accumulation of osmoprotective molecules in the face of chronic hypernatremia is normal in preweanling rats with a higher set-point to preserve the increased brain cell water content (88).

Failure to adequately account for the cell volume regulatory response to osmolal disorders contributes to some of the adverse effects associated with inappropriate correction of abnormalities in plasma osmolality. These include neurological dysfunction, specifically seizures, during the treatment of hypernatremia, osmotic demyelinating syndrome following rapid reversal of hyponatremia, dialysis dysequilibrium syndrome after the initiation of dialysis in patients with acute or chronic renal failure, and cerebral edema and brain herniation in patients with a first episode of acute diabetic ketoacidosis (89, 90).

Table 7-3

Factors that contribute to the countercurrent mechanism

Na-Cl-K-mediated solute absorption in the medullary thick ascending limb of Henle
Low water permeability of the distal tubule and connecting segment
High water permeability in the descending limb of Henle
Vasa recta and elimination of interstitial water volume
AVP-responsiveness of the collecting tubule

Laboratory Assessment of Sodium and Water Balance

There are no normal values for sodium and water intake or excretion, a reflection of the wide range of normal daily dietary intake for both sodium and water. Healthy individuals are in balance and the excretion of sodium and water matches the daily intake. Therefore, laboratory

assessment of sodium and water homeostasis is confined to disease states in which the clinician must determine whether renal sodium and water handling are appropriate for the clinical circumstances, will maintain external balance, and prevent disturbances in ECF volume or water distribution between the ICW and ECW compartments.

Sodium

The urine sodium concentration is not a valid index of sodium balance because the value may vary depending upon the volume and concentration of the sample. Therefore, the renal handling of sodium is best evaluated using the FENa. After obtaining a random urine sample and a simultaneous blood sample and measuring the sodium and creatinine concentrations in both specimens, the FENa is calculated using the following formula:

$$\begin{aligned}\text{FENa} &= \text{Excreted sodium}/\text{Filtered sodium}\\ &= \text{Urinary sodium concentration}\\ &\quad \times \text{urine flow rate}\\ &\quad / \text{ Plasma creatinine concentration}\\ &\quad \times \text{GFR}\\ &= \frac{\text{Urine sodium concentration}\,/\,\text{Plasma sodium concentration}}{\text{Urine creatinine concentration}\,/\,\text{Plasma creatinine concentration}}\\ &= \frac{\text{Urine sodium concentration}\times\text{Plasma creatinine concentration}}{\text{Plasma sodium concentration}\times\text{Urine creatinine concentration}}\end{aligned} \tag{3}$$

This formula is based on the insertion of the creatinine clearance as a measurement of GFR in the second equation and the cancellation of the urine flow rate term in the numerator and denominator. Therefore, the determination of the FENa is an especially useful test in clinical practice because it can be done using spot samples and does not require a timed urine collection. In healthy individuals, the FENa varies depending upon the daily sodium intake. However, in patients with ECF volume contraction who are responding appropriately to retain sodium, the FENa is $<1\%$ ($<3\%$ in neonates). Conversely, patients with expansion of the ECW compartment, the FENa will exceed 3% unless there is concomitant renal disease.

Water

Determination of the urine specific gravity or osmolality in a random sample varies depending upon the water intake in past 2–4 h. Therefore, the assessment of water handling is best judged by determining these values under more controlled conditions such as in the water-deprived state or following administration of a water load (10–20 mL/kg body weight) to evaluate the urinary concentrating or diluting capacity, respectively.

The functional aspects of renal water handling are best assessed by determining the free water clearance. This represents the amount of solute-free water excreted by the kidney. It is calculated using the following formula:

$$\begin{aligned}\text{Free water clearance} &= \text{Urine volume} - \text{osmolal clearance}\\ &= \text{Urine volume} - [\text{Urine osmolality}\\ &\quad \times \text{urine flow rate}]/\text{Plasma osmolality}\end{aligned} \tag{4}$$

If the free water clearance is a positive number, then the urine/plasma osmolality ratio is <1, the urine is dilute, and the kidney is in a diuretic mode. When a water diuresis is maximal, the free water clearance measures the capacity of the kidney to excrete free water. Under these circumstances, urine volume is directly related to the dietary solute intake (19). In contrast, patients who are in an antidiuretic mode and can concentrate the urine have a urine/plasma osmolality ratio >1 and a negative free water clearance. As the solute excretion rate increases, both the maximum values for free water clearance and free water reabsorption increase. At any given solute excretion rate, the free water clearance greatly exceeds the free water reabsorption. This indicates that the renal water homeostatic mechanisms designed to protect against overhydration and dilution of the ECW are more robust than those used to defend against water deficit and dehydration.

Overview of the Evaluation of Fluid and Water Abnormalities

In practice, the clinical information and laboratory data used to evaluate patients overlap with one another. However, in view of the different physiological roles of sodium and water balance in body fluid homeostasis, the distinct regulatory mechanisms activated to control these factors, and the varied therapeutic strategies that must be employed to restore sodium and water balance in disease

states, it is essential that disturbances in sodium and water balance be evaluated separately. In real-time, these separate assessments are done in parallel, a reflection of the body's own method of operation.

When confronted with a child with a sodium and water disturbance, the first question that must be addressed is whether the size of the ECF volume is altered to the extent that perfusion of vital organs or pulmonary gas exchange is jeopardized. Thus, it is necessary to clarify whether the ECF volume is decreased or expanded, which represents a clinical assessment of sodium balance. Depending on the severity of the problem, such patients have a life-threatening condition and may require emergency therapy such as volume resuscitation or acute dialysis.

After making this acute determination and instituting appropriate emergency therapy, it is important to grade the magnitude of the disturbance in ECF volume. There is no single or combination of laboratory tests that can substitute for clinical judgment. Because acute changes in body weight always reflect alterations in sodium balance and ECW compartment size, serial measurements in body weight are the most reliable indicator of the presence and severity of disturbances in ECF volume. However, these measurements are often unavailable or will have been done in different locations using different equipment. Therefore, the evaluation of sodium balance is based upon a wide range of clinical findings including changes in mental status, level of alertness, irritability, presence of thirst, pulse rate, blood pressure, orthostatic changes, fullness of the anterior fontanelle in infants, the presence of tears, dryness of the mucus membranes, skin color, elasticity of the skin or tenting, capillary refill or turgor, peripheral edema, shortness of breath, and the presence of rales on auscultation of the chest. Capillary refill may be the most useful test to rapidly and accurate assess ECF volume and the response to treatment (91). Other findings on clinical examination include urinary specific gravity, and central venous pressure. Laboratory investigations include BUN, serum creatinine and bicarbonate concentrations.

It is important to emphasize that assessment of ECF volume is a *clinical determination*. There is no single or combination of laboratory tests that is a valid surrogate marker. Moreover, despite the frequency of clinical disturbances in sodium balance, especially ECF volume contraction, no suitable scoring has been devised to accurately and reliably distinguish different degrees of ECF volume contraction or expansion. This contrasts to the Glasgow coma score or the APACHE score that have been successfully applied to the initial assessment of patients with acute neurological or multisystem organ failure.

The third step in the evaluation of a patient with a sodium and water disturbance is to measure the plasma osmolality, which indicates an abnormal distribution of water between the ECW and ICW compartments. The most likely symptoms in affected patients arise secondary to central nervous dysfunction and include confusion, irritability, lethargy, obtundation, and seizures. These manifestations overlap significantly in patients with hyperosmolality or hypoosmolality. Moreover, there is no obligatory change in ECF volume. In contrast to disorders of ECF volume, disturbances in water balance and distribution require a laboratory determination for confirmation and grading. The steps involved in the initial evaluation of a child with a disturbance in sodium and water balance are summarized in *Table 7-4*. The following sections will describe the individual clinical entities responsible for derangements in sodium and water balance and outline the general treatment of these conditions.

Sodium Balance Disturbances: Deficit and Excess

Sodium Deficit

Diagnosis and Evaluation

In children with normal kidney function, steady consumption of a diet that is low or high in sodium does not cause a net negative or positive sodium balance because the kidney can adaptively modify sodium reabsorption in parallel with salt intake if the changes are not too abrupt or massive in nature.

Sodium deficits and ECF volume contraction are dangerous because decreased size of the intravascular space leads to reduced perfusion and ischemia of vital organs

Table 7-4

Steps in the initial evaluation and treatment of a child with a disturbance in sodium and/or water balance

Step 1: Determine if there is a life-threatening alteration in ECF volume
• Volume resuscitation if there is ECF volume contraction
• Consider dialysis if there is ECF volume expansion
Step 2: Grade severity of defect in sodium balance
• Clinical determination of ECF volume
Step 3: Determine if there is a defect in water balance
• Laboratory measurement of plasma osmolality

such as the brain, heart, and kidneys. In children, the absence of concomitant atherosclerosis disease or endothelial dysfunction secondary to essential hypertension, smoking, hyperlipidemia and diabetes, decreases this risk. However, there are groups of pediatric patients who may be more susceptible to the adverse consequences of hypovolemia. These include newborn babies in whom high circulating levels of vasoconstrictor hormones and impaired autoregulation render the glomerular microcirculation sensitive to reduced perfusion (92). In addition, underlying diseases or medications may hinder the counterregulatory responses to ECF volume contraction and heighten the risks of hypovolemia.

Diseases that cause sodium deficiency can originate outside the kidney or within the kidney. It is unfortunate that the word dehydration is routinely used to describe these states because it deflects attention from the primary defect, namely a net negative sodium balance and suggests that water deficit is the major pathophysiological problem in these conditions (93, 94). The critical role of the ECF space and sodium balance in the pathogenesis of states of volume contraction is highlighted by comparing the situation that occurs in patients with diabetes insipidus. When sodium balance is perturbed, >30% of the fluid loss is derived from the ECW compartment provoking the rapid onset of symptoms. In contrast, only 1/12 or 8% of the pure water loss that occurs in diabetes insipidus is derived from the ECF (⅓ × ¼), accounting for the rare evidence of ECF volume contraction in children with central or nephrogenic diabetes insipidus (*Fig. 7-1*). Use of the term "denatration" may provide a more accurate depiction of what is occurring in patients with primary deficits in sodium balance and contraction of the ECF volume (93).

Extrarenal causes can occur from losses of sodium in any body fluid or across any epithelial surface including the CSF, pleural fluid, biliary tree, gastrointestinal losses, or skin. They can be the result of a disease process or they may be iatrogenic. Chronic kidney disease can cause sodium deficit because the lower GFR compromises the homeostatic capacity of the renal tubules. Alternatively, there may be primary renal sodium loss that is not the consequence of a decrease in kidney function. Finally, renal sodium reabsorption may be diminished because of reduced circulating levels of aldosterone or unresponsiveness to the hormone. The major causes of sodium deficiency are summarized in *Table 7-5*.

The diagnosis of the cause of a disturbance in sodium balance is made based on a thorough history and physical examination. In most cases, this information is adequate to identify the source of the sodium losses. Previously, the degree of ECF volume contraction was categorized as mild, moderate, or severe if the changes in body weight were estimated to be <5, 5–10, or >10%, respectively. Life-threatening ECF volume contraction was thought to represent >15% decrease in weight. Recent data, based upon systematic body weights at the time of hospitalization and immediately after correction of the sodium deficit, suggest that these numbers overestimate the degree of sodium deficit and that the ECF volume contraction is better estimated to be <3, 3–6, and >6% with >9% change in body weight representing an emergency (95).

If the losses are primarily extra-renal, then renal sodium reabsorptive mechanisms will be activated and the urinary specific gravity will be >1.015 and the FENa will

Table 7-5

Causes of net sodium deficit

Renal causes
Compromised GFR
Acute decrease in sodium intake or increased losses
Tubular disorders
Osmotic diuresis
Diabetic ketoacidosis
Renal tubular acidosis
Pseudohypoaldosteronism
Obstructive uropathy
Bartter's syndrome
Renal dysplasia/hypoplasia
Central nervous system
Cerebral salt wasting
CSF drainage procedures
Hepato-biliary system
Biliary tract drainage
Gastrointestinal tract
Infectious diarrhea
Chloride diarrhea
Laxative abuse
Malignancy (carcinoid, tumor-related)
Adrenal diseases
Salt-losing congenital adrenal hyperplasia
Addison's disease
Skin losses
Cystic fibrosis
Neuroectodermal diseases
Burns

be low, generally <1% except in infants. Failure to increase the urine concentration and lower FENa in the face of clinical signs of ECF volume contraction points towards a renal or adrenal cause for the disorder. A renal ultrasound documenting the presence of small, misshaped kidneys or hydronephrosis may be indicative of congenital abnormalities of the kidney such as dysplasia or obstructive uropathy. Adrenal insufficiency is suggested by concomitant hyponatremia and hyperkalemia in a child with ECF volume contraction.

Treatment

If the ECW compartment size is so severely contracted that vital organ perfusion is compromised, based upon an altered mental status, orthostatic changes, and azotemia, then fluid resuscitation must be initiated on an emergent basis. This is necessary to prevent the development of acute kidney injury, which may occur if there is sustained hypotension and renal ischemia secondary to ECF volume contraction. This term has been introduced to replace acute tubular necrosis which implies the presence of pathologic changes that are usually absent in kidney biopsy samples (9). The risk of acute kidney injury is higher in children with preexisting renal disease, who are receiving nephrotoxic medications or who have hemoglobinuria or myoglobinuria, e.g., crush injury or compartment syndrome. If there is no evidence of cardiac or pulmonary disease, then the optimal therapy under these conditions is infusion of isotonic crystalloid (0.9% NaCl, Ringer's lactate), 20 mL/kg body weight. While transfusion of whole blood is optimal for treatment of hemorrhagic shock, infusion of crystalloid solutions avoids difficulties caused by extravasation of the colloid into the interstitial compartment. Moreover, a systematic review of the literature does not support the use of colloid solutions for volume replacement in critically ill patients (96). This fluid is appropriate regardless of what the initial serum sodium or osmolality is and concerns about infusing Ringer's lactate are misplaced in view of the low potassium concentration (4 mmol/L) in this solution. A catheter should be placed in the bladder to facilitate monitoring of urine output. The infusion should be as rapid as possible and the fluid dose should be repeated as often as necessary to achieve some evidence of clinical improvement such as improved mental status, decrease in pulse, rise in blood pressure, or improved capillary refill.

After correcting life-threatening hypoperfusion, a fluid repletion plan should be initiated as soon as possible. Preference should be given to correcting sodium and electrolyte deficits with oral rehydration solutions (ORS). In general, patients can be repleted with a rapid (1–2 h) intravenous infusion to restore ECF volume followed immediately by initiation of ORS (97). The only conditions that represent contraindications to the use of ORS are impaired neurological status, persistent vomiting, or diseases associated with mucosal damage in the gastrointestinal lumen.

ORS fluids introduced by the World Health Organization contain sodium 90, potassium 20, bicarbonate 30, chloride, and glucose 111 mmol/L (20 g/L). The sodium and glucose are present in a molar ratio that maximizes the secondary active uptake of these solutes via the sodium–glucose co-transporter across the gastrointestinal epithelium. Water is absorbed passively down its osmolal gradient. The presence of other solutes such as potassium and bicarbonate are not critical to the successful utilization of ORS. These fluids have been utilized for over 30 years. They can be administered ad libitum in response to the child's own thirst and they are effective and safe with minimal occurrence of hypernatremia or hyperkalemia. Various alternatives to glucose such as rice-syrup or amylase-resistant starch may facilitate sodium and water reabsorption from ORS, decrease fecal fluid loss, and shorten recovery time after an episode of cholera (98, 99). Clinical studies are needed to confirm the utility of these additives because they may increase the cost and decrease the shelf life of ORS. These are important considerations in developing countries, where there is a high incidence of infectious diarrhea in infants and children.

When parenteral therapy is required to correct sodium and water deficits, the following guidelines can be applied when devising a therapeutic plan. First, in the absence of reliable data regarding the acute weight loss, it is easiest to calculate maintenance and deficit therapy based on the clinical estimate of the percentage decrease in body weight. Second, it is advisable to discount any emergency fluid therapy, such as bolus infusions of isotonic saline, in computing the fluid prescription. Third, if the clinical problem has developed in <48 h then it should be considered an acute process and the sodium and fluid losses are derived from the ICW and ECW in the ratio of 80:20%. If the patient has been ill for more than 48 h and the process is chronic, then the sodium and fluid losses are derived from the ECW and ICW in the ratio of 60:40%. Under most conditions in which the sodium and water losses are isotonic, the ECW portion of the loss, in liters, can be multiplied by 140 mmol/L to determine the sodium loss. Similarly, the ICW portion of the loss, in

liters, can be multiplied by 140 mmol/L to determine the potassium deficit. If the ECW and ICW fluid losses together with the respective sodium and potassium deficits are added to the maintenance requirements, then a total fluid volume can be determined. After selecting a fluid that most closely approximates the total sodium and water losses, the total fluid volume is divided by the time frame of the correction to determine the intravenous infusion rate.

As an example, if a child weighing 10 kg is judged to have a 5% decrease in body weight over 36 h, then the total fluid deficit of 500 mL can be divided into two components – 400 mL ECW with 56 mmol sodium and 100 mL ICW containing 14 mmol potassium. The daily maintenance water and sodium requirements are 1,000 mL and 30 mmol sodium. Adding these two quantities together results in a fluid that closely resembles 0.33–0.45% saline (61 mmol NaCl/L) containing 30 mmol KCl/L, and the infusion rate is approximately 60 mL/h if the correction is designed to occur over 24 h. In all cases, it is important to monitor and replace ongoing losses to insure that there is full resolution of the underlying clinical problem.

Sodium Excess

Diagnosis and Evaluation

These conditions, which generally are less common than sodium deficit, are evidenced by clinical signs of ECF volume expansion. They can arise secondary to exogenous addition of sodium or abnormal retention of endogenous sodium. Because the normal kidney has the adaptive capacity to rapidly excrete a sodium load, in order for the excess sodium to cause clinical symptoms and signs, there must be a concomitant factor that limits natriuresis.

Common causes of excess exogenous sodium are salt-water drowning, ingestion of a diet abnormally high in sodium, or therapeutic infusion of sodium-containing intravenous solutions, such as sodium bicarbonate during the resuscitation after a cardiopulmonary arrest. Examples of dietary excess of sodium include errors in the preparation of infant formulas. The widespread use of premixed baby formulas may decrease the incidence of these accidents.

Conditions associated with excessive endogenous sodium retention include acute renal failure and the edema states, namely nephrotic syndrome, cirrhosis, and congestive heart failure. In the first condition, which may occur due to glomerulonephritis or tubular necrosis, the sodium retention is directly related to the decrease in GFR and diminished filtration of sodium. In the latter three states, renal sodium and water reabsorption are activated by a combination of mechanisms that are termed, underfill and overfill. A critical review of the evidence in patients with nephrotic syndrome suggests that those diseases that are associated with an inflammatory infiltrate in the kidney develop primary sodium retention and overfilling of the ECF volume. This histopathological feature is absent in minimal change nephrotic syndrome and the underfill mechanism predominates (100). Thus, in nephrotic syndrome, total body sodium excess may be coupled with a diminished, normal, or expanded effective ECF volume. This explains the normal distribution of PRA values and wide range of measured plasma volume in these patients. The causes of net total body sodium excess are summarized in *Table 7-6*.

The major clinical problems that accompany these conditions are related to pulmonary venous congestion, impaired gas exchange, and dyspnea. In idiopathic nephrotic syndrome, the intra-alveolar pressure is often sufficient to prevent frank pulmonary edema. However, in other circumstances, intrinsic capillary leak together with lowered plasma oncotic pressure promotes the development of pulmonary interstitial fluid. Peripheral edema may be associated with skin infection, peritonitis, or thromboembolic events.

Conditions associated with sodium excess should be evident on physical examination. The FENa will be high if there is sodium loading and renal function is normal. In contrast, in the states characterized by retention of endogenous sodium, the FENa will be very low. The FENa is more useful than the urinary specific gravity because it is likely to be elevated in all cases of sodium excess.

Table 7-6
Causes of net sodium excess

Exogenous sodium
Salt-water drowning
Errors in formula preparation
Infusion of hypertonic sodium solutions
e.g., after cardiopulmonary arrest
Endogenous sodium
Acute renal failure (glomerulonephritis, ATN)
Nephrotic syndrome
Cirrhosis
Congestive heart failure

Therapy

The optimal therapy is targeted at correcting the underlying disease. This is most important in patients with cirrhosis or congestive heart failure. Ancillary therapies include administration of diuretics to facilitate urinary sodium excretion. Although thiazide diuretics may be adequate, more potent loop diuretics may be required if the GFR is diminished. Supplemental oxygen may be necessary to alleviate shortness of breath and hypoxemia. Patients with nephrotic syndrome may require combinations of diuretic agents that act in the proximal (e.g., metolazone) and distal (e.g., furosemide) segments of the nephron to promote an adequate natriuresis and diuresis. Infusions of albumin (1 g/kg bodyweight) can augment the medication-induced diuresis, especially in those with severe ECF volume contraction, reduced GFR, and azotemia (101). In the most severe cases, acute dialysis using peritoneal or hemodialysis techniques may be necessary to foster rapid clearance of the excess sodium. Finally, hemofiltration may be a safe and effective means to rapidly remove sodium and water in severely ECF-overloaded children with nephrotic syndrome (102).

Water Balance Disturbances: Deficit and Excess

Hyponatremia

Diagnosis and Evaluation

Patients with hyponatremia have relative or absolute expansion of the ICW compartment. If renal function is normal, then excess free water is eliminated within 2–4 h. Therefore, for this problem to occur two conditions must be satisfied – there must be continued AVP release that is inappropriate to the serum sodium concentration and the patient must have continued access to free water. The symptoms and signs of hyponatremia, which generally involve central nervous system dysfunction, are vague and nonspecific. Laboratory confirmation is required to diagnose this abnormality. The presence of hyponatremia has no predictive value about the status of the ECF volume and this later issue must be assessed clinically to determine whether the child has hypovolemic hypotonicity, isovolemic hypotonicity, or hypervolemic hypotonicity.

The laboratory measurement of serum sodium concentration is no longer susceptible to technical artifacts. In the past, laboratory analyzers measured sodium concentration in the total supernatant obtained after centrifugation of blood samples, including lipids and proteins. Because these molecules are not water soluble, they displace sodium into a smaller aqueous phase leading to a spurious reduction in the serum sodium concentration. All biochemical analyzers currently in use at large medical centers assay sodium concentration solely in the aqueous phase and yield an accurate determination of the serum sodium level. Therefore, the entity called pseudohyponatremia is no longer clinically relevant. In contrast, the reduced serum sodium concentration noted in patients with increased circulating levels of an impermeant solute such as mannitol, urea, contrast media, or glucose in patients with diabetic ketoacidosis is valid and reflects osmotic redistribution of water from the ICW to the ECW space. The phenomenon is reflected in the following formula which enables adjustment of the serum sodium in patients with severe hyperglycemia:

$$\begin{aligned}&\text{“Physiological” sodium concentration}\\&\quad = \text{measured serum sodium concentration}\\&\qquad + 1.6 \times [\text{Each 100 mg/dl increment in}\\&\qquad \text{serum glucose above 100 mg/dl}]\end{aligned} \tag{5}$$

The most common clinical causes of hyponatremia are classified by the concomitant ECF volume status in *Table 7-7*. In some diseases, such as congestive heart failure, the degree of hyponatremia may be a reflection of circulating AVP levels and sympathetic nervous system activation and provide a marker of disease severity. This relationship has not been demonstrated in patients with nephrotic syndrome or cirrhosis.

The syndrome of inappropriate AVP or ADH (SIADH) release causes hyponatremia with mild to modest ECF volume expansion. It can occur as a consequence of central neurological lesions, pulmonary disease, or tumors. In addition, numerous drugs can result in abnormal secretion or action of AVP and lead to chronic hyponatremia. A list of these agents is provided in *Table 7-8*. The diagnosis of SIADH requires confirmation that the urine is excessively concentrated relative to the plasma osmolality without any evidence of ECF volume contraction, or adrenal or thyroid insufficiency. These two hormones are required to maintain the low water permeability of the collecting duct in the absence of AVP. Deficiencies of either hormone impair free water clearance leading to euvolemic hyponatremia. In practice, diagnosing SIADH requires comparison of the urine specific gravity or osmolality with the concurrent serum

Table 7-7
Causes of hyponatremia

Hypovolemic: ECF volume contraction
Renal
Mineralocorticoid deficiency
Mineralocorticoid resistance
Diuretics
Polyuric acute renal failure
Salt-wasting renal disease
Renal tubular acidosis
Metabolic alkalosis
Bartter's syndrome/Gitelman's syndrome
Gastrointestinal
Diarrheal dehydration
Gastrointestinal suction
Intestinal fistula
Laxative abuse
Transcutaneous
Cystic fibrosis
Heat exhaustion
"Third space" loss with inadequate fluid replacement
Burns
Major surgery, trauma
Septic shock
Euvolemic: Normal ECF volume
Glucocorticoid deficiency
Hypothyroidism
Mild hypervolemia: ECF volume expansion
Reduced renal water excretion
Antidiuretic drugs
Inappropriate secretion of ADH
Hypervolemic: ECF volume expansion
Acute renal failure (glomerulonephritis, ATN)
Chronic renal failure
Nephrotic syndrome
Cirrhosis
Congestive heart failure
Psychogenic polydipsia/compulsive drinking

osmolality. The urine should normally be maximally dilute if the serum sodium concentration is <130 mmol/L or the plasma osmolality <270 mosm/kg H_2O. A urinary sodium concentration >40 mmol/L is adequate evidence against ECF volume contraction.

Table 7-8
Drugs that cause water retention and the syndrome of inappropriate AVP release (according to mode of action)

Increasing water permeability of the nephron
AVP (arginine or lysine vasopressin)
Vasopressin analogs, e.g., 1-deamino, 8-D-arginine vasopressin (DDAVP)
Oxytocin
Promoting AVP release
Barbiturates
Carbemazepine
Clofibrate
Colchicine
Isoproterenol
Nicotine
Vincristine
Inhibition of prostaglandin synthesis
Salicylates
Indomethacin
Acetaminophen (paracetamol)
Other nonsteroidal anti-inflammatory drugs
Potentiation of the action of AVP
Chlorpropamide
Cyclophosphamide

Treatment

In all cases, the first line of therapy should be directed at the underlying cause of the low serum sodium concentration. However, hyponatremia often warrants specific corrective treatment. Much ink has been spilled in detailing the appropriate therapy of this electrolyte abnormality. At times, this issue has been quite contentious and the world has been divided into two supposedly distinct camps – those who advocate "rapid" versus "slow" correction of hyponatremia. The former group asserts that hyponatremia has direct adverse effects on central nervous system function including impaired oxygenation that can lead to seizures or cardiopulmonary collapse prior to initiation of therapy (103). Such a sequence of events has been documented in experimental animals with acute hyponatremia (104). This risk may be especially prominent in premenopausal women. In contrast, there are others who emphasize the cerebral cell volume regulatory response to hyponatremia and highlight the risk of brain cell dehydration and osmotic demyelinating syndrome in patients who are corrected too quickly (105).

Taking into account the entire literature on the subject, current evidence suggests that the risk of hyponatremia is more closely related to the acuity of the change rather than the absolute size of the drop in serum sodium concentration (106). Thus, therapy should be guided by the time frame in which hyponatremia has developed. If the hyponatremia is acute, i.e., <12 h in duration, then the brain will behave as a perfect osmometer leading to potentially life-threatening cerebral cell swelling. Under these circumstances, there is an urgent need to rapidly reverse the hyponatremia to counteract cell swelling. Clinical experience indicates that infusion of a 3% NaCl solution (513 mmol/L) in a volume designed to raise the sodium concentration by 3–5 mmol/L is sufficient to halt central nervous system dysfunction (107). The benefits and lack of adverse effects of acute correction have been confirmed in a series of 34 infants and children with acute water intoxication caused by the ingestion of dilute infant formula (108). After this is achieved, the hyponatremia can be corrected more slowly. For example, if a 6-year old child weighing 20 kg develops a seizure after a tonsillectomy and is noted to have a serum sodium concentration of 115 mmol/L, then 36–60 mmol of sodium are needed to raise the sodium concentration by 3–5 mmol/L. This is accomplished by infusing 72–120 mL of the hypertonic saline infusion.

If hyponatremia has developed over more than 12 h or the duration of the problem is unclear, especially if the patient has no signs of neurological dysfunction, then slow correction is the prudent course of action (105, 106). The current definition of slow correction includes two features: (1) the rate of rise in serum sodium concentration should be <0.6 mmol/L throughout the correction phase; and (2) the total increment and/or the final serum sodium concentration after 48 h of treatment should not exceed 25 or 130 mmol/L, respectively. The more cautious criterion should be applied depending on the initial serum sodium level. If a child develops acute changes in mental status or new neurological findings, during or shortly after the fluid treatment, then a serum sodium concentration should be checked. Imaging studies, specifically an MRI of the brain, may reveal the changes of osmotic demyelinating syndrome.

There are several therapeutic options for patients with SIADH whose underlying cause cannot be corrected. Restriction of free water intake to match insensible losses and urine output may be adequate to stabilize the serum sodium concentration. If this is not well-tolerated, then administration of furosemide 1–2 mg/kg/day to promote a hypotonic diuresis together with oral administration of NaCl 1–2 g/day may correct the hyponatremia. If these measures fail, consideration can be given to treatment with lithium or demeclocycline, two drugs that interfere with AVP-action in the collecting tubule to foster excretion of free water and raise the serum sodium concentration (109). Finally, nonpeptide vasopressin receptor antagonists have been developed that may be introduced into clinical practice for the treatment of chronic hyponatremia (110, 111). Conivaptan is the only FDA-approved agent in this class and is useful for short-term intravenous use. Tolvaptan is an oral agent that has been demonstrated to safely correct hyponatremia in several dose response studies involving adults with euvolemic hyponatremia or hypervolemic hyponatremia (112). Safety and efficacy of these drugs have not been assessed in pediatric patients.

Hypernatremia

Diagnosis and Evaluation

Hypernatremia may arise due to excessive intake of sodium and ECF volume expansion. However, excessive water loss relative to the sodium deficit with hypovolemia is far more common. In a recent survey of hypernatremia in hospitalized children, the vast majority had significant underlying medical problems and 76% of the cases were secondary to inadequate water intake (113). The prevalence of this electrolyte abnormality is much lower than hyponatremia. One of the common causes is diarrheal illness in infants; however, the reduction in the sodium concentration of most baby formulas to match the level in human breast milk has resulted in a dramatic decrease in the incidence of hypernatremic dehydration. Nonetheless, changes in medical practice with early discharge of newborn infants after delivery have resulted in the steady occurrence of hypernatremic dehydration in breast-fed babies (114). Patients with hypernatremia have relative or absolute contraction of the ICW compartment. Similar to the situation with hyponatremia, the clinical clues to the presence of hypernatremia are nonspecific and laboratory confirmation is mandatory to diagnose this abnormality. Moreover, the hypernatremia must be evaluated in light of the clinically determined ECF volume status.

Patients with hypernatremia and ECF volume expansion are easy to diagnose. The children who represent a serious problem are those with hypovolemia. They may have some distinct features including marked irritability, a high-pitched cry, and a doughy skin texture. Because the hyperosmolality of the ECW compartment provokes movement of water from the ICW down its osmolal gradient, these patients preserve ECF volume until

Table 7-9
Causes of hypernatremia

Hypovolemic: ECF volume contraction
Gastrointestinal (diarrhea and vomiting)
Evaporative (high fever, high ambient temperature)
Hypothalamic diabetes insipidus (ADH deficiency)
Head trauma
Infarction (Sheehan's syndrome)
Tumors (e.g., craniopharyngioma)
Histiocytosis
Degenerative brain diseases
Infections
Hereditary central diabetes insipidus (usually dominant)
Idiopathic
Nephrogenic diabetes insipidus (ADH resistance)
Chronic renal failure
Hypokalemia
Hypercalcemia
Damage to renal medulla
Sickle cell disease
Nephronophthisis
Renal papillary necrosis
Chronic pyelonephritis (reflux nephropathy)
Euvolemic: Normal ECF volume
Unconscious patients
Infants
Lack of access to water (lost in the desert)
Primary adipsia
Essential hypernatremia (osmoreceptor destruction or malfunction)
Hypervolemic: ECF volume expansion
Inappropriate IV fluid therapy
Salt poisoning
Mineralocorticoid excess

late in the disease course. Thus, their illness is usually chronic and there is a greater contribution of the ICW to the water and electrolyte deficits. Assessment of the FENa is useful in assessing the ECF volume in these patients. The causes of hypernatremia are listed in *Table 7-9*.

Treatment

Because children with hypovolemic hypernatremia are usually very ill, they often require bolus infusions of isotonic saline to restore organ perfusion. Once this is accomplished, then the fluid regimen should include the maintenance fluids, the estimated deficit with the assumption that 60% is derived from the ECW and 40% from the ICW. In addition, there is a free water deficit. This can be calculated from the following formula:

$$\text{Water deficit (in ml)} = 0.6 \times \text{Body weight} \times [\text{Actual serum sodium concentration}/140 - 1] \quad (6)$$

This formula may overestimate the water deficit and it has been recommended that the following alternative equation be used to estimate the increase in serum sodium concentration that will be achieved following the infusion of 1 L of a given solution (115):

$$\text{Change in sodium concentration} = [\text{Infusate } Na^+ - \text{serum } Na^+]/\text{total body water} + 1 \quad (7)$$

Finally, with regard to the rate of correction, the standard practice is to correct hypovolemic hypernatremia gradually over at least 48 h. After the groundbreaking work of Finberg et al. (116), the risk of cerebral edema following rapid correction of chronic hypernatremia is now attributed to the inability of brain cells to extrude the osmoprotective solutes that accumulate during sustained hyperosmolal conditions in parallel with the decline in plasma osmolality during fluid therapy (117, 118). Therefore, the osmolal gradient will be reversed with plasma osmolality lower than cerebral cell osmolality leading to ICW expansion and clinical signs of cerebral edema, findings that have been confirmed in NMR spectroscopy studies of the brain (119). The experimental observations were confirmed in a randomized trial, which demonstrated that the safest and most effective fluid therapy for hypovolemic hypernatremia is 0.18% NaCl given slowly over 48 h compared to 0.45% saline given slowly or rapidly over the same time period (120). Because hyperosmolality impairs insulin and PTH release, patients should be monitored for hyperglycemia and hypocalcemia during the correction period.

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8 Potassium

Lisa M. Satlin

Potassium is the most abundant intracellular cation. Approximately 98% of the total body potassium content is located within cells, primarily muscle, where its concentration ranges from 100–150 mEq/L; the remaining 2% resides in the extracellular fluid, where the potassium concentration is tightly regulated within a narrow range (3.5–5.0 mEq/L in the adult). The ratio of the intra- to extracellular potassium concentration determines, in large part, the resting membrane potential, and is thus critical for normal function of electrically excitable cells, including nerve and muscle. Maintenance of a high intracellular potassium concentration is essential for many cellular processes, including DNA and protein synthesis, cell growth and apoptosis, mitochondrial enzyme function, and conservation of cell volume and pH (1–7). Because of the many vital processes dependent on potassium homeostasis, multiple complex and efficient mechanisms have developed to regulate total potassium balance and distribution.

Potassium Homeostasis

Total body potassium content reflects the balance between intake and output, the latter regulated primarily by renal and, to a lesser extent, fecal excretion; the amount of potassium lost through sweat is negligible. The homeostatic goal of the healthy adult is to remain in zero potassium balance. Thus, ~90% of the daily potassium intake, which typically averages 1 mEq/kg body weight, is eliminated by the kidneys and the residual ~10% lost through the stool (8).

Total body potassium content increases from approximately 8 mEq/cm body height at birth to >14 mEq/cm body height by 18 years of age (9, 10) (*Fig. 8-1*), with the rate of accumulation of body potassium per kilogram body weight in the infant exceeding that in the older child and adolescent. The increase in total body potassium content correlates with an increase in cell number and potassium concentration (at least in skeletal muscle) with advancing age (10–12). This robust somatic growth early in life requires the maintenance of a state of positive potassium balance (13, 14), as has been demonstrated in growing infants greater than approximately 30 weeks gestational age (GA) (15, 16). The tendency to retain potassium early in postnatal life is reflected, in part, in the higher plasma potassium values in infants, and particularly in preterm neonates (16, 17).

Thirty to fifty percent of very low birth weight and premature infants < 28 weeks GA exhibit nonoliguric hyperkalemia, defined as a serum potassium concentration of >6.5 mEq/L, during the first few days of life, despite the intake of negligible amounts of potassium (18–22). This phenomenon, not observed in mature infants or VLBW infants after 72 h (20, 21), has been proposed to reflect principally an intra- to extracellular shift of potassium (20, 21, 23). Prenatal steroid treatment may prevent this nonoliguric hyperkalemia via induction of sodium-potassium-adenosine triphosphatase (Na-K-ATPase) activity (see below) in the fetus (24, 25).

Studies in rats suggest that the accumulation of potassium in the growing fetus is facilitated by the active transport of potassium across the placenta from mother to fetus (26). This notion is further supported by studies in dog (27) and rat (28) that show that fetal plasma potassium concentrations are maintained during maternal hypokalemia, an adaptation proposed to be due to an increase in the ratio of maternofetal-to-fetomaternal unidirectional potassium transport.

Regulation of Internal Potassium Balance

The task of maintaining potassium homeostasis is challenging because the daily dietary intake of potassium in the adult (~50–100 mEq) approaches or exceeds the total potassium normally present within the extracellular fluid space (~70 mEq in 17 L of extracellular fluid with a potassium concentration averaging ~4 mEq/L). To maintain zero balance in the adult, all the dietary intake of potassium must be ultimately eliminated, a task performed primarily by the kidney. However, excretion of potassium by the kidney is sluggish. Only 50% of an oral or intravenous load of potassium is excreted during the first 4–6 h after it is administered (29, 30). Life-threatening

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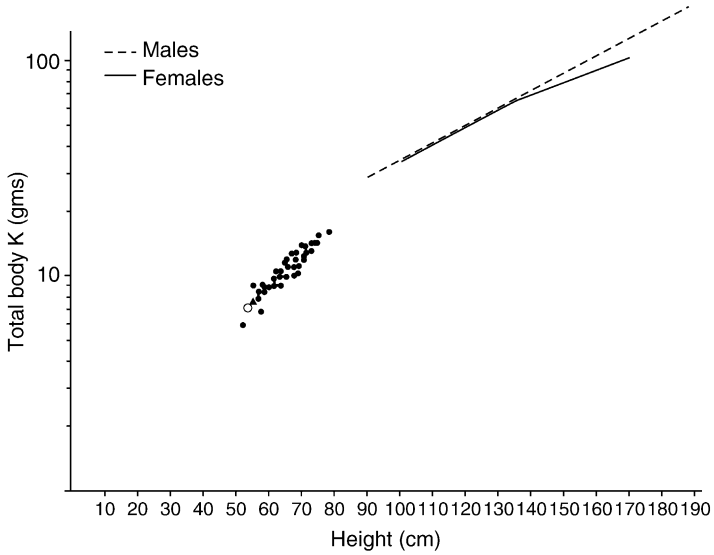
Figure 8-1

Relationship between total body potassium (gm) and height (cm) for infants and children. The rate of accretion of body potassium in the neonate is faster than in later childhood, likely reflecting both an increase in cell number and potassium concentration, at least in skeletal muscle, with advancing age (10).



hyperkalemia is not generally observed during this period because of the rapid (within minutes) hormonally mediated translocation of ~80% of the retained potassium load from the extracellular space into cells (31). The buffering capacity of the combined cellular storage reservoirs, which includes muscle, bone, liver, and red blood cells (RBCs), is capable of sequestering up to approximately 3,500 mEq of potassium and is vast compared with the extracellular pool (8).

Cells must expend a significant amount of energy to maintain the steep potassium (and sodium) concentration gradients across their cell membranes. This is accomplished by the ubiquitous Na-K-ATPase which transports 3 sodium ions out of and 2 potassium ions into the cell at the expense of the hydrolysis of cytosolic ATP. The unequal cation exchange ratio produces a charge imbalance across the cell membrane, and thus the Na-K-ATPase is defined as an electrogenic pump. Positively charged potassium ions, present in high concentration within the cell, passively leak out of cells down a concentration gradient through ubiquitously expressed potassium-selective channels. A steady state is reached at which the outward movement of positively charged potassium is opposed by the negative cell potential. At this cell equilibrium potential, the net transmembrane flux of potassium is zero.

The basic functional unit of the Na-K-ATPase is comprised of a catalytic α and a β subunit; the β subunit acts a molecular chaperone that directs the correct membrane insertion of the α subunit (32). The α/β-heterodimer complexes with phospholemman (PLM, FXYD1) in heart and skeletal muscle (33); the latter interaction modulates pump activity (33). The cardiac glycoside digoxin binds to the catalytic α subunit of the enzyme, inhibiting its activity. Thus, digoxin overdose may thus be associated with hyperkalemia, especially in the presence of a concomitant perturbation of potassium homeostasis.

Na-K-ATPase is regulated by changes in its intrinsic activity, subcellular distribution, and cellular abundance. Long-term stimulation of pump activity is generally mediated by changes in gene and protein expression, whereas short-term regulation typically results from changes in the intracellular sodium concentration, alterations in the phosphorylation status of the pump and/or interaction with regulatory proteins, or changes in membrane trafficking of preexisting pumps (33–35). Regulation of internal potassium balance in the neonate may be influenced by developmental stage-specific expression of potassium transporters, such as Na-K-ATPase, as well as channels, receptors, and signal transduction pathways (36, 37).

The chemical, physical, and hormonal factors that acutely influence the *internal* balance of potassium are listed in *Table 8-1*, and are discussed below. Potassium uptake into cells is acutely stimulated by insulin, β_2-adrenergic agonists, and alkalosis and is impaired by α-adrenergic agonists, acidosis, and hyperosmolality. Generally, deviations in extracellular potassium concentration

Table 8-1

Factors that regulate internal potassium balance and their effects on cell uptake of potassium

Physiologic factors	
Plasma K concentration	
Increase	Increases uptake
Decrease	Decreases uptake
Insulin	Increases uptake
Catecholamines	
α-Agonists	Decreases uptake
β-Agonists	Increases uptake
Pathologic factors	
Acid-base balance	
Acidosis	Decreases uptake
Alkalosis	Increases uptake
Hyperosmolality	Enhances cell efflux
Cell breakdown	Enhances cell efflux

arising from fluctuations in internal distribution are self-limited as long as the endocrine regulation of internal balance and mechanisms responsible for regulation of *external* balance are intact.

Plasma Potassium Concentration

Active basolateral cellular potassium uptake by the ubiquitous Na-K-ATPase in large part determines the intracellular pool of potassium. An increase in potassium input into the extracellular fluid space, which may arise from exogenous or endogenous sources or result from a chronic progressive loss of functional renal mass, decreases the concentration gradient against which the Na-K-ATPase must function and thus favors an increase in cellular potassium uptake. Sources of exogenous potassium input may not be readily apparent and include not only diet, but also potassium-containing drugs (potassium penicillin G), salt substitutes, protein-calorie supplements, herbal medications and packed RBCs (38). The extracellular fluid potassium concentration can also increase in response to endogenous release of potassium as accompanies tissue breakdown (rhabdomyolosis, tumor lysis syndrome) and exercise, the latter mediated by adenosine triphosphate (ATP) depletion and opening of ATP-dependent potassium channels. In those epithelial cells of the kidney and colon specifically responsible for potassium secretion, the resulting increase in intracellular potassium maximizes the concentration gradient between cell and lumen, thereby promoting potassium diffusion into the tubular lumen and thus potassium excretion.

Hormones

Insulin, the most important hormonal regulator of internal potassium balance, stimulates Na-K-ATPase-mediated cellular potassium uptake and thus the rapid transfer of potassium from the extracellular to the intracellular fluid space of insulin-responsive cells in liver, skeletal muscle, adipocytes, and brain, a response that is independent of the hormonal effects on glucose metabolism (39). The mechanism of insulin action in these tissues differs, in part, because of differences in the isoform composition of the catalytic α-subunit of the pump. Insulin stimulates Na-K-ATPase activity by promoting the translocation of preformed pumps from intracellular stores to the cell surface (40–44), and/or increasing cytoplasmic sodium content (45–47) or the apparent affinity of the enzyme for sodium (48).

Basal insulin secretion is necessary to maintain fasting plasma potassium concentration within the normal range (29). An increase in plasma potassium in excess of 1.0 mEq/L in the adult induces a significant increase in peripheral insulin levels to aid in the rapid disposal of the potassium load, yet a more modest elevation of approximately 0.5 mEq/L is without effect (29, 49, 50). In the setting of insulin deficiency, i.e., diabetes, there is a reduction in uptake of potassium by muscle and liver (31, 51).

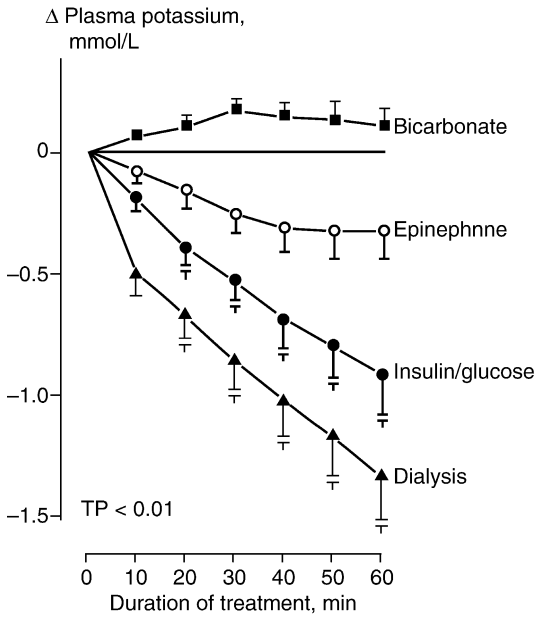
Catecholamines enhance the cell uptake of potassium via stimulation of Na-K-ATPase activity in skeletal muscle and hepatocytes through β_2-adrenergic receptors (52, 53). The effect of epinephrine on potassium balance in the adult is biphasic and is characterized by an initial increase, followed by a prolonged fall in plasma potassium concentration to a final value below baseline. The initial transient rise in plasma potassium results from α-adrenergic receptor stimulation which causes release of potassium from hepatocytes and impairs cell uptake of potassium (51, 54–56). β_2-Receptor stimulation, via stimulation of adenylate cyclase leading to generation of the second messenger cyclic adenosine monophosphate (cAMP) and activation of downstream protein kinases, stimulates the sodium pump and thus promotes enhanced uptake of potassium by skeletal and cardiac muscle, effects that are inhibited by nonselective β-blockers including propranolol and labetalol (51, 55, 57–60). The observation that the potassium-lowering effects of insulin and epinephrine are additive suggests that their responses are mediated by different signaling pathways.

The effects of these hormones on the distribution of potassium between the intracellular and extracellular compartments have been exploited to effectively treat disorders of homeostasis (*Fig. 8-2*). Administration of β_2-adrenoreceptor agonists (albuterol or salbutamol via nebulizer), which promote potassium uptake by cells, has been to treat hyperkalemia in neonates, children and adults (62–64). A single dose of nebulized albuterol can lower serum potassium by as much as 0.5 mEq/L (65, 66). Transient side effects associated with this class of drugs include mild tachycardia, tremor and mild vasomotor flushing (67). Administration of glucose alone (to induce endogenous insulin release) or glucose plus insulin is efficacious, although patients must be monitored for complications of hyperglycemia (without insulin) or hypoglycemia (with insulin), especially in neonates (68–70). It should be kept in mind that the treatment options discussed thus far are only temporizing. To remove potassium from the body, renal potassium excretion must be enhanced, either by stimulating potassium secretion in the distal nephron (see below) or, in the presence of renal insufficiency, by dialysis.

Aldosterone is best known for its effect on transporting tissue, increasing potassium secretion in distal segments of the nephron and colon (see below). Triiodothyronine (T3) also promotes Na-K-ATPase-mediated potassium cellular uptake in skeletal muscle (34). Whereas T3 had been thought to act as a direct transcriptional activator of target genes, recent studies emphasize the importance of nongenomic effects, including the stimulation of translocation of Na-K-ATPase to the plasma membrane by a pathway that requires activation of MAPK and phosphatidylinositol 3-kinase (PI3K) (71, 72). The postnatal increases in Na-K-ATPase expression in kidney, brain, and lung depend on normal thyroid hormone status (73).

Figure 8-2

Changes in plasma potassium concentration (mmol/L) during intravenous infusion of bicarbonate (8.4%), epinephrine, or insulin and glucose, and during hemodialysis in adult patients on maintenance hemodialysis (61).



Acid–Base Balance

It is well known that the transcellular distribution of potassium and acid-base balance are interrelated (74–77). Whereas acidemia (increase in extracellular hydrogen ion concentration) is associated with a variable increase in plasma potassium secondary to potassium release from the intracellular compartment, alkalemia (decrease in extracellular hydrogen ion concentration) results in a shift of potassium into cells and a consequent decrease in plasma potassium. However, the reciprocal changes in plasma potassium that accompany acute changes in blood pH differ widely among the four major acid-base disorders; metabolic disorders cause greater disturbances in plasma potassium than do those of respiratory origin, and acute changes in pH result in larger changes in plasma potassium than do chronic conditions (74).

Acute metabolic acidosis after administration of a mineral acid that includes an anion that does not readily penetrate the cell membrane, such as the chloride of hydrochloric acid or ammonium chloride, consistently results in an increase in plasma potassium. As excess extracellular protons, unaccompanied by their nonpermeant anions, enter the cell where neutralization by intracellular buffers occurs, potassium is displaced from the cells, thus maintaining electroneutrality and leading to hyperkalemia. However, comparable acidemia induced by acute organic anion acidosis (lactic acid in lactic acidosis, acetoacetic and β-hydroxybutyric acids in uncontrolled diabetes mellitus) may not elicit a detectable change in plasma potassium (74, 78, 79). In organic acidemia, the associated anion diffuses more freely into the cell and thus does not require a shift of potassium from the intracellular to the extracellular fluid.

In respiratory acid-base disturbances, in which carbon dioxide and carbonic acid readily permeate cell membranes, little transcellular shift of potassium occurs because

protons are not transported in or out in association with potassium moving in the opposite direction (74).

Changes in plasma bicarbonate concentration, independent of the effect on extracellular pH, can reciprocally affect plasma potassium concentration (80). Movement of bicarbonate (outward at a low extracellular bicarbonate concentration and inward at a high extracellular bicarbonate concentration) between the intracellular and extracellular compartments may be causally related to a concomitant transfer of potassium. This relationship may account for the less marked increase in plasma potassium observed during acute respiratory acidosis, a condition characterized by an acid plasma pH with an elevated serum bicarbonate (leading to inward net bicarbonate and potassium movement), as compared with acute metabolic acidosis with a low serum bicarbonate concentration (leading to outward net bicarbonate and potassium movement). Though recommended as a mainstay of therapy, alkalinization of the extracellular fluid with sodium bicarbonate to promote the rapid cellular uptake of potassium may not be useful in patients on maintenance hemodialysis for end stage renal disease (61) (*Fig. 8-2*). However, this maneuver remains valuable if metabolic acidosis is at all responsible for the hyperkalemia. The major toxicities of bicarbonate therapy include sodium overload and precipitation of tetany in the face of preexisting hypocalcemia.

Other Factors

A number of other pathologic perturbations alter the internal potassium balance. An increase in plasma osmolality secondary to severe dehydration or administration of osmotically active agents causes water to shift out of cells. The consequent increase in intracellular potassium concentration exaggerates the transcellular concentration gradient and favors movement of this cation out of cells. The effect of hyperosmolality on potassium balance becomes especially problematic in diabetic patients with hyperglycemia, in whom the absence of insulin exacerbates the hyperkalemia.

Succinylcholine, a depolarizing paralytic agent and an agonist of nicotinic acetylcholine receptors, which are found predominantly in skeletal myocyte membranes, may lead to efflux of potassium from myocytes into the extracellular fluid under certain pathologic states associated with upregulation and redistribution of the receptors, including states characterized by physical or chemical upper or lower motor neuron denervation, immobilization, infection, muscle trauma or inflammation and burn injury (81, 82).

Finally, parenteral administration of cationic amino acids such as lysine, arginine or epsilon-amino caproic acid (used to improve hemostasis in patients undergoing cardiac surgery) may lead to electroneutral exchange of cell potassium for the cationic amino acid in skeletal myocytes (83–85).

Regulation of External Potassium Balance

Renal Contribution

The kidney is the major excretory organ for potassium. In the adult, urinary potassium excretion generally parallels dietary intake. However, the renal adaptation to variations in dietary intake is rather sluggish. Extreme adjustments in the rate of renal potassium conservation cannot be achieved as rapidly as for sodium, nor are the adjustments as complete; whereas urinary sodium can be virtually eliminated within 3–4 days of sodium restriction, there is a minimum urinary potassium loss of about 10 mEq/day in the adult, even after several weeks of severe potassium restriction (86). An increase in dietary potassium intake is matched by a parallel increase in renal potassium excretion within hours, yet maximal rates of potassium excretion are not attained for several days after increasing potassium intake. In adults, renal potassium excretion follows a circadian rhythm, presumably determined by hypothalamic oscillators, and it is characterized by maximum output during times of peak activity (87). It is unknown whether a circadian cycle of urinary potassium excretion prevails in infancy.

Children and adults ingesting an average American diet that contains sodium in excess of potassium excrete urine with a sodium-to-potassium ratio greater than one (16, 88). Although breast milk and commercially available infant formulas generally provide a sodium-to-potassium ratio of approximately 0.5–0.6, the urinary sodium-to-potassium ratio in the newborn up to 4 months of age generally exceeds 1. This high ratio may reflect the greater requirement of potassium over sodium for growth. In fact, some premature (<34 weeks GA) newborns may excrete urine with a sodium-to-potassium ratio greater than 2, a finding suggesting significant salt wasting and a relative hyporesponsiveness of the neonatal kidney to mineralocorticoid activity (16).

Net urinary potassium excretion in the fully differentiated kidney reflects the sum of three processes:

glomerular filtration, reabsorption along the proximal tubule and the loop of Henle, and bidirectional transport (secretion and reabsorption) in the distal nephron (8, 89). Most of the factors known to modulate potassium excretion do so by altering the rate of potassium secretion.

Renal potassium clearance is low in newborns, even when it is corrected for their low glomerular filtration rate (16, 17). Infants, like adults, can excrete potassium at a rate that exceeds its glomerular filtration, reflecting the capacity for net tubular secretion. However, they are unable to excrete an exogenously administered potassium load as efficiently as the adult (90); specifically, the rate of potassium excretion normalized to body weight or kidney weight is less in newborn than older animals (91, 92). Comparison of the fractional delivery of potassium to the early distal tubule with that present in the final urine reveals that the distal nephron of the young (2-week-old) rat secretes approximately fivefold less potassium than the older (5-week-old) rat (93). Similarly, clearance studies in saline-expanded dogs also provide indirect evidence of a diminished secretory and enhanced reabsorptive capacity of the distal nephron to potassium early in life (94). Furthermore, premature neonates studied weekly after birth exhibited a 50% reduction in the fractional excretion of potassium between 26 and 30 weeks GA, in the absence of significant change in absolute urinary potassium excretion (15). To the extent that the filtered load of potassium increased almost threefold during this same time interval, the constancy of renal potassium excretion could be best explained by a developmental increase in the capacity of the kidney for potassium reabsorption (15). Please note that, in general, the limited potassium secretory capacity of the immature kidney becomes clinically relevant only under conditions of potassium excess.

Sites of Potassium Transport along the Nephron

Proximal Tubule

Potassium is freely filtered at the glomerulus. Thus, the concentration of potassium entering the proximal convoluted tubule (PCT) is similar to that of plasma. Approximately 65% of the filtered load of potassium is reabsorbed along the initial two-thirds of the proximal tubule, a fractional rate of reabsorption similar to that of sodium and water (95–97) (*Fig. 8-3*). A similar fraction (50–60%) of the filtered load of potassium is reabsorbed along the proximal tubules of suckling (13–15 days old) rats (93, 99).

Reabsorption of potassium along the early proximal tubule is passive, closely following that of sodium and water (100), and has been proposed to occur via solvent drag via the paracellular pathway and diffusion (101–103). Solvent drag depends on active sodium reabsorption, which generates local hypertonicity in the paracellular compartment, providing an osmotic force driving water reabsorption that entrains potassium in the reabsorbate. Potassium diffusion is driven by the lumen-positive transepithelial voltage in the second half of the proximal tubule, and the slightly elevated concentrations of potassium in the lumen (104).

In the proximal tubule, as in all other nephron segments discussed below, transepithelial sodium reabsorption requires the coordinated function of apical sodium transport proteins and the basolateral Na-K-ATPase which actively extrudes intracellular sodium into the interstitium, and thereby maintains the low intracellular sodium concentration and steep sodium concentration gradient critical to the driving force for apical sodium entry (*Fig. 8-3*). There is no evidence for specific regulation of potassium reabsorption along the proximal tubule, and most observed modulation of proximal reabsorption of this cation can be accounted for by alterations in sodium transport.

Electrogenic sodium-coupled entry of substrates such as amino acids and glucose across the luminal cell membrane of the proximal tubule as well as bicarbonate exit across the basolateral cell membrane are driven by the potential differences across the respective cell membranes, which are maintained by potassium flux through potassium channels (105). Electrophysiological studies in isolated perfused proximal tubules suggest that potassium movement from the cell to lumen maintains the electrical driving force for sodium-coupled cotransport in the proximal tubule. Immunohistochemical studies reveal that KCNE1 and KCNQ1, which together constitute the slowly activated component of the delayed rectifying potassium current in heart, also colocalize in the luminal membrane of the proximal tubule in mouse kidney, as does the cyclic nucleotide-gated, voltage-activated potassium channel KCNA10 (106, 107). The observation that KCNE1 knock-out mice exhibit an increased fractional excretion of fluid (with accompanying volume depletion), sodium, chloride, and glucose compared to their wild type littermates supports the critical role of KCNE1 in repolarizing the membrane potential in proximal tubule in response to sodium-coupled transport (107).

Notably, mutations in KCNQ1 give rise to the long QT syndrome (108).

Thick Ascending Limb of the Loop of Henle (TALH)

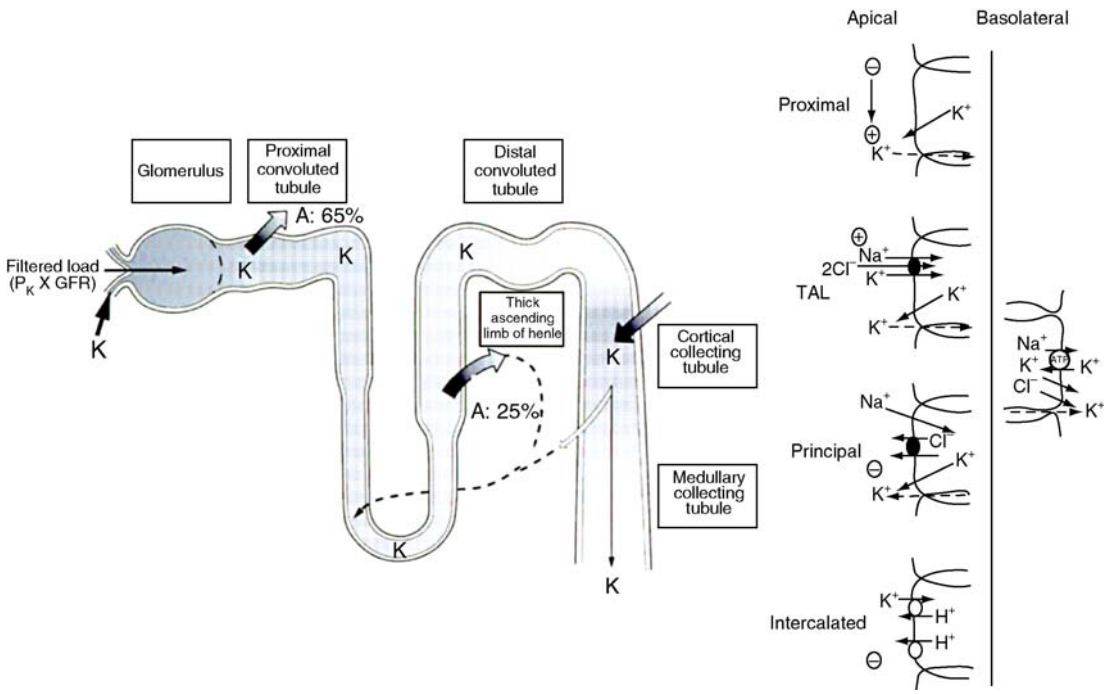
Approximately 10% of the filtered load of potassium reaches the early distal tubule of the adult rat (96), an observation that implies that significant reabsorption of this cation occurs beyond the proximal tubules. The site responsible for this additional avid potassium reabsorption is the TALH where potassium reabsorption is mediated, at least in part, by the apical Na-K-2Cl cotransporter (NKCC2) that translocates a single potassium ion into the cell accompanied by one sodium and two chloride ions (*Fig. 8-3*). This secondary active transport is ultimately driven by the low intracellular sodium concentration, established by the basolateral Na-K-ATPase, which drives sodium entry from the lumen into the cell. The diuretics furosemide and bumetanide specifically inhibit NKCC2 and thus block sodium, potassium and chloride reabsorption at this site.

Critical to the function of NKCC2 is the presence of a secretory potassium channel in the urinary membrane which provides a pathway for potassium, taken up into the cell via the cotransporter, to recycle back into the lumen. This "recycling" of potassium ensures that a continuous supply of substrate is available for the apical cotransporter. Potassium secretion into the urinary space creates a lumen positive transepithelial potential difference, which in turn provides a favorable electrical driving force that facilitates paracellular reabsorption of sodium, potassium, calcium and magnesium.

The luminal potassium secretory channel in the TALH has been identified as ROMK, a channel originally cloned from the TALH in the rat outer medulla (109–111); this low-conductance ATP-sensitive potassium channel is encoded by the KCNJ1 gene. Loss-of-function mutations in ROMK lead to antenatal Bartter syndrome (type 2),

Figure 8-3

Potassium transport along the nephron. (left panel) The percentages of filtered potassium reabsorbed along the proximal tubule and thick ascending limb of the loop of Henle (TALH) are indicated for the adult (A). *Arrows* show direction of net potassium transport (reabsorption or secretion). GFR = glomerular filtration rate. (right panel) Simplified cell models of potassium transport along the nephron, showing apical transporters unique to discrete nephron segments and cells therein, and basolateral transporters which are similar in all nephron segments (adapted from 98).



also known as the hyperprostaglandin E syndrome, which is characterized by severe renal salt and fluid wasting, electrolyte abnormalities (hypokalemia, hypomagnesemia, and hypercalciuria), and elevated renin and aldosterone levels (112). The clinical picture observed is similar to that of chronic administration of loop diuretics. The typical presentation of antenatal Bartter syndrome includes polyhydramnios, premature delivery, life threatening episodes of dehydration during the first week of life, and profound growth failure (113). It should be noted that mutations in NKCC2 (SLC12A1) or the basolateral chloride channel CLC-Kb (CLCNKB) can also give rise to Bartter syndromes type 1 and 3, respectively (114, 115).

In contrast to the situation in the adult, up to 35% of the filtered load of potassium reaches the early distal tubule of the young (2-week-old) rat (93), suggesting that the TALH undergoes a significant postnatal increase in its capacity for reabsorption. Consistent with this premise are the observations from studies in rats of significant increases in the (i) fractional reabsorption of potassium along the TALH, expressed as a percentage of delivered load, between the second and sixth weeks of postnatal life (93), and (ii) osmolarity of early distal fluid between the second and fourth weeks of life (116). While these findings provide compelling evidence for a developmental maturation of potassium absorptive pathways and diluting capacity of the TALH, respectively, direct functional analysis of the transport capacity of this segment in the developing nephron has not been performed.

Molecular studies in rat kidney indicate that mRNA encoding NKCC2, absent at birth, is first expressed on postnatal day 8 in rat, coincident with completion of nephronogenesis (37), and increases, at least in medulla, between postnatal days 10 and 40 (117). Na-K-ATPase activity in the TALH of the neonatal rabbit is only 20% of that measured in the adult when expressed per unit of dry weight (118). The postnatal increase in pump activity is associated with a parallel increase in expression of medullary Na-K-ATPase mRNA (117). Although the balance of the studies summarized above identifies a functional immaturity of the TALH early in life and would thus predict limited effects of inhibitors of NKCC2 on transepithelial transport, administration of furosemide (2 mg/kg) to newborn lambs leads to a natriuretic response equivalent to that observed in adult animals (119).

Distal Nephron

Within the distal nephron of the fully differentiated kidney, the late DCT, CNT and the CCD are considered to be the primary sites of potassium secretion, and thus urinary potassium excretion, which can approach 20% of the filtered load (96, 97, 120, 121) (*Fig. 8-3*). The DCT secretes a constant small amount of potassium into the urinary fluid (122). Regulated bidirectional potassium transport occurs in the CNT and CCD, comprised of two major populations of cells, each with distinct functional and morphologic characteristics. CNT/principal cells reabsorb sodium and secrete potassium, whereas intercalated cells regulate acid-base balance but can reabsorb potassium in response to dietary potassium restriction or metabolic acidosis (123, 124) (*Fig. 8-3*). Thus, the direction and magnitude of net potassium transport in these segments depend on the metabolic needs of the organism and reflect balance of potassium secretion and absorption, processes mediated by CNT/principal and intercalated cells, respectively.

Potassium Secretion

Potassium secretion in the CNT and CCD is critically dependent on the reabsorption of filtered sodium delivered to these segments. Sodium passively diffuses into the CNT/principal cell from the urinary fluid down a favorable concentration gradient through the luminal amiloride-sensitive epithelial Na channel (ENaC) and is then transported out of the cell at the basolateral membrane in exchange for the uptake of potassium via the basolateral Na-K-ATPase (*Fig. 8-3*). The accumulation of potassium within the cell and the lumen-negative voltage, created by movement of sodium from the tubular lumen into the cell and its electrogenic extrusion, creates a favorable electrochemical gradient for intracellular potassium to diffuse into the urinary space through apical potassium-selective channels. The magnitude of potassium secretion is determined by its electrochemical gradient and the apical permeability to this cation. Basolateral potassium channels in these same cells provide a route for intracellular potassium ions to recycle back into the interstitium, thereby maintaining the efficiency of the Na-K pump. Any factor that increases the electrochemical gradient across the apical membrane or increases the apical potassium permeability will promote potassium secretion. An apical electroneutral potassium-chloride cotransporter has also been functionally identified in the CCD (125, 126).

Two apical potassium-selective channels have been functionally identified in the distal nephron: the small-conductance secretory potassium (SK) channel and the high-conductance maxi-K channel. The density of these channels appears to be greater in the CNT than in the CCD (127).

The SK channel, restricted to the CNT/principal cell, mediates baseline potassium secretion (128, 129). ROMK, originally cloned from the TALH (described in the section above on the TALH), is considered to be a major functional subunit of the SK channel. A complex interplay of hormones, second messengers and kinases/phosphatases regulate the SK/ROMK channel in the distal nephron, thereby allowing the kidney to respond appropriately to the metabolic needs of the organism (130, 131). Protein kinase A (PKA)-induced phosphorylation of the channel is essential for its activity (129, 132), and may account for the well-documented stimulatory effect of vasopressin on renal potassium secretion (133). Protein tyrosine kinase (PTK) mediates the endocytosis of ROMK channels in the rat CCD in the face of dietary potassium restriction (134, 135). Tyrosine phosphorylation of ROMK enhances channel internalization and thus the removal of channels from the plasma membrane (136), leading to a reduction in number of apical channels and net potassium secretion. Tyrosine phosphorylation of ROMK channels decreases in response to dietary potassium loading (137).

The "with-no-lysine-kinases," or WNKs, comprise a recently discovered family of serine/threonine kinases that act as molecular switches that direct differential effects on downstream ion channels, transporters, and the paracellular pathway to allow either maximal sodium chloride reabsorption or maximal potassium secretion in response to hypovolemia or hyperkalemia, respectively (138). WNK4 inhibits sodium and chloride absorption in the DCT by reducing the surface expression of the apical thiazide-sensitive NaCl cotransporter NCCT (139), an effect that would be expected to increase sodium delivery to and reabsorption by the CCD, in turn augmenting the driving force for potassium secretion. However, WNK4 decreases surface expression of ROMK by enhancing endocytosis of this channel (140), thereby negating the effect of the augmented electrochemical gradient on stimulation of net potassium secretion. Mutations in WNK1 or 4 lead to pseudohypoaldosteronism type II (PHA II; Gordon's Syndrome), an autosomal dominant disorder characterized by hypertension sensitive to thiazide diuretics, hyperkalemia, and metabolic acidosis (141). Loss-of-function mutations in WNK4 lead to increased apical expression of the NaCl cotransporter and stimulation of sodium absorption in the DCT (139, 142). The consequent reduction in sodium delivery to the CNT and CCD would be expected to reduce potassium secretion. However, the same mutations in WNK4 that relieve the inhibition of NCCT further decrease surface expression of ROMK, reduce potassium secretion in the CCD, and likely are the cause of hyperkalemia in patients with Gordon's Syndrome. WNK1 suppresses the activity of WNK4. Therefore, a gain-in-function mutation in WNK1 will also produce the clinical signs and symptoms of PHA II (141).

The maxi-K channel, present in CNT/principal and intercalated cells, is considered to mediate flow-stimulated potassium secretion (143, 144). In the CNT and CCD, the density of conducting maxi-K channels is greater in intercalated than CNT/principal cells (145, 146). The maxi-K channel is comprised of two subunits: a channel pore-forming α subunit and a regulatory β subunit. This channel is rarely open at the physiologic resting membrane potential, but can be activated by cell depolarization, membrane stretch, and increases in intracellular Ca^{2+} concentration, as accompany increases in urinary flow rate (146–149). The proposed role of the maxi-K channel in flow-stimulated urinary potassium secretion has been confirmed in a mouse model with targeted deletion of the β1 subunit; the fractional excretion of potassium in maxi-K $\beta1^{-/-}$ mice subjected to acute volume expansion was significantly lower than that in wild type mice (150).

The maxi-K channel appears to assume great importance in regulating potassium homeostasis under conditions where SK/ROMK channel-mediated potassium secretion is limited. Thus, adult animals with targeted deletion of ROMK (i.e., Bartter phenotype) are not hyperkalemic, as would be expected in the absence of a primary potassium secretory channel, but instead lose urinary potassium (151). The sensitivity of distal potassium secretion in this rodent model of Bartter syndrome to iberiotoxin, a specific inhibitor of maxi-K channels, presumably reflects recruitment of the latter channels to secrete potassium in response to high distal flow rates as accompany loss-of-function of the TALH NKCC2 cotransporter (151). Similarly, although infants with antenatal Bartter syndrome due to loss-of-function mutations in ROMK may exhibit severe hyperkalemia during the first few days of life (152), the hyperkalemia is not sustained. In fact, these patients typically exhibit modest hypokalemia beyond the neonatal period (153, 154).

Potassium Absorption

In response to dietary potassium restriction or metabolic acidosis, the distal nephron may reverse the direction of net potassium transport from secretion to absorption. Potassium reabsorption is mediated by a H-K-ATPase, localized to the apical membrane of acid-base transporting intercalated cells, that couples potassium reabsorption to proton secretion (*Fig. 8-3*) (123, 155–157). Two isoforms of the H-K-ATPase are found in the kidney: the gastric isoform, HKAg, is normally found in gastric parietal cells and is responsible for acid secretion into the

lumen whereas the colonic HKAc isoform is a structurally related H-K-ATPase found in distal colon that mediates active potassium reabsorption (156). Expression of the apical gastric-like H-K-ATPase in the rat and rabbit intercalated cell is increased in response to dietary potassium restriction and metabolic acidosis (123, 155, 158, 159).

A reduction in potassium intake leads to a fall in potassium secretion by the distal nephron within 5–7 days in rat (160). This adaptation is associated with a decrease in the number of apical SK/ROMK (161) and maxi-K (162) channels and stimulation of H-K-ATPase-mediated potassium reabsorption in intercalated cells in the distal nephron (163). The reduction in number of SK/ROMK channels in potassium-restricted animals is mediated by the effect of dietary potassium on circulating levels of aldosterone and other effectors, such as PTK, as described above. Stimulation of luminal H-K-ATPase activity in intercalated cells results not only in potassium retention, but also in urinary acidification and metabolic alkalosis.

Developmental Regulation of Distal Potassium Transport

Potassium secretion in the distal nephron, and specifically in the cortical collecting duct (CCD) studied in vitro, is low early in life and cannot be stimulated by high urinary flow rates (121). Indeed, basal potassium secretion can not be detected in the rabbit CCD until after the third week of postnatal life, with potassium secretory rates increasing thereafter to reach adult levels by 6 weeks of age (121). Consistent with the relatively undifferentiated state of the newborn CCD are the ultrastructural and morphometric findings in neonatal principal cells of few organelles, mitochondria and basolateral infoldings, the site of Na-K-ATPase (164, 165).

The limited capacity of the CCD for potassium secretion early in life could be explained by either an unfavorable electrochemical gradient across the apical membrane and/or a limited apical permeability to this ion. Cumulative evidence suggests that the electrochemical gradient is not limiting for potassium secretion in the neonate. Activity of the Na-K-ATPase, present along the basolateral membrane of corticomedullary collecting ducts in the neonatal rabbit (166), is 50% of that measured in the mature nephron; the observation that the intracellular potassium concentration in this segment is similar in the neonate and adult presumably reflects a relative paucity of membrane potassium channels in the distal nephron early in life (118, 165, 167). Concordant with the measurements of sodium pump activity, the rate of sodium absorption in the CCD at 2 weeks of age is approximately 60% of that measured in the adult (121). However, electrophysiologic analysis has confirmed the absence of functional SK/ROMK channels in the luminal membrane of the neonatal rabbit CCD with the number of open channels per patch increasing progressively after the second week of life (168). Thus, the postnatal increase in the basal potassium secretory capacity of the distal nephron appears to be due primarily to a developmental increase in number of SK/ROMK channels, reflecting an increase in transcription and translation of functional channel proteins (168–170).

The appearance of flow-stimulated net potassium secretion is a relatively late developmental event. Flow-stimulated potassium secretion can not be elicited in rabbit CCDs until the fifth week of postnatal life, which is approximately 2 weeks after basal net potassium secretion is first detected (121, 171). The absence of flow-stimulated potassium secretion early in life is not due to a limited flow-induced rise in net sodium absorption and/or intracellular calcium concentration, each of which is required for flow stimulation of potassium secretion and is equivalent to that detected in the adult by the second week of postnatal life (171). The observation that mRNA encoding the maxi-K channel α-subunit and immunodetectable channel protein can not be demonstrated until the fourth and fifth weeks of postnatal life, respectively (171) suggests that flow-dependent potassium secretion is determined by the transcriptional/translational regulation of expression of maxi-K channels.

While the neonatal distal nephron is limited in its capacity for potassium secretion, indirect evidences suggests that this nephron segment absorbs potassium. As indicated above, saline-expanded newborn dogs absorb 25% more of the distal potassium load than do their adult counterparts (91). Functional analysis of the rabbit collecting duct has shown that the activity of apical H-K-ATPase in neonatal intercalated cells is equivalent to that in mature cells (123). The latter data alone do not predict transepithelial potassium absorption under physiologic conditions, as the balance of transport will depend on the presence and activity of apical and basolateral potassium conductances and the potassium concentration of the tubular fluid delivered to this site. The high distal tubular fluid potassium concentrations, as measured In vivo in the young rat, may facilitate lumen-to-cell potassium absorption mediated by the H-K-ATPase (93).

Luminal and Peritubular Factors Affecting Potassium Transport

The major factors that influence the external balance of potassium are listed in *Table 8-2* and are discussed in the following sections.

Table 8-2

Factors that regulate external potassium balance

Renal factors
Distal sodium delivery and transepithelial voltage
Tubular (urinary) flow rate
Potassium intake/plasma potassium concentration
Hormones (mineralocorticoids, vasopressin)
Acid-base balance
Gastrointestinal tract factors
Stool volume
Hormones (mineralocorticoids)

Sodium Delivery and Absorption

The magnitude of sodium reabsorption in the distal nephron determines the electrochemical driving force favoring potassium secretion into the luminal fluid, as described above. Processes that enhance distal sodium delivery and increase tubular fluid flow rate, such as extracellular volume expansion or administration of a variety of diuretics (osmotic diuretics, carbonic anhydrase inhibitors, loop and thiazide diuretics), lead to an increase in urinary excretion of both sodium and potassium. The kaliuresis is due not only to the increased delivery of sodium to the distal nephron, but also to the increase in tubular fluid flow rate, which maximizes the chemical driving forces, as described below, favoring potassium secretion.

Processes that decrease sodium delivery to less than 30 mM in the distal tubular fluid (172, 173) and/or sodium reabsorption sharply reduce potassium secretion in the CCD and can lead to hyperkalemia. In vivo measurements of the sodium concentration in distal tubular fluid generally exceed 35 mEq/L both in healthy adult and suckling rats and thus should not restrict distal sodium secretion (93, 116, 172, 174). However, in edema-forming states, including congestive heart failure, cirrhosis and nephrotic syndrome, the urinary sodium concentration typically falls to less than 10 mEq/L; a reduction in potassium excretion in these patients may be ascribed to the low rates of distal sodium delivery as well as urinary flow. The potassium-sparing diuretics, amiloride and triamterene, inhibit ENaC and thus block sodium absorption, thereby diminishing the electrochemical gradient favoring potassium secretion (175). Trimethoprim and pentamidine can also limit urinary potassium secretion via the same mechanism (177, 178).

Sodium delivered to the distal nephron is generally accompanied by chloride. Chloride reabsorption occurs predominantly via the paracellular pathway. The movement of the negative charged chloride out of the lumen dissipates the lumen negative potential, creating a less favorable driving force for luminal potassium secretion (179). When sodium delivered to the distal nephron is accompanied by an anion less reabsorbable than chloride, such as bicarbonate (in proximal renal tubular acidosis), β-hydroxybutyrate (in diabetic ketoacidosis), or carbenicillin (during antibiotic therapy), luminal electronegativity is maintained, thereby eliciting more potassium secretion than occurs with a comparable sodium load delivered with chloride (180).

Tubular Flow Rate

High rates of urinary flow in the mature, but not the neonatal or weanling, distal nephron, as elicited by extracellular fluid volume expansion or administration of diuretics or osmotic agents, stimulate potassium secretion (121). There are a number of factors responsible for the flow-stimulation of potassium secretion. First, increases in tubular fluid flow rate in the distal nephron enhance sodium reabsorption due to an increase in the open probability of ENaC (time the channel spends in the open state), which augments the electrochemical gradient favoring potassium secretion (176, 181). Second, the higher the urinary flow rate in the distal nephron, the slower the rate of rise of tubular fluid potassium concentration because secreted potassium is rapidly diluted in urine of low potassium concentration (182). Maintenance of a low tubular fluid potassium concentration maximizes the potassium concentration gradient (and thus the chemical driving force) favoring net potassium secretion. Finally, increases in luminal flow rate transduce mechanical signals (circumferential stretch, shear stress, hydrodynamic bending moments on the cilium decorating the apical surface of virtually all renal epithelial cells) into increases in intracellular calcium concentration, which in turn activate apical maxi-K channels to secrete potassium, thereby enhancing urinary potassium excretion (143, 144, 171).

Potassium Intake and Cellular Potassium Content

The kidney adjusts potassium excretion to match input, in large part by regulating the magnitude of potassium secretion and reabsorption in the distal nephron. Thus, for example, an increase in dietary potassium intake

stimulates whereas a decrease in intake reduces net potassium secretion (97). An increase in potassium concentration in the extracellular fluid space increases potassium entry into principal cells via the basolateral Na-K-ATPase, which in turn maximizes the concentration gradient favoring apical potassium secretion into the urinary fluid. Simultaneously, the increase in circulating levels of plasma aldosterone that accompanies potassium loading enhances the electrochemical driving force favoring potassium secretion in the distal nephron by stimulation of ENaC-mediated sodium absorption and its electrogenic absorption via the Na-K-ATPase. Within 6 h of an increase in dietary potassium intake, the density of apical ROMK channels increases in principal cells in rats due to activation of a previously "silent" pool of channels or closely associated proteins (183). Chronic potassium loading also increases maxi-K channel message, apical protein, and function in the distal nephron (162). Finally, the inhibition of proximal tubule and loop of Henle salt and water reabsorption in response to a potassium load (184, 185) increases tubule fluid flow rate which in turn stimulates potassium secretion via activation of maxi-K channels and increased distal of delivery of sodium to the distal nephron.

The trigger for the renal adaptation to dietary potassium loading remains uncertain. It is now believed that after a potassium-rich meal, a reflex increase in potassium excretion is initiated by sensors in the splanchnic bed (gut, portal circulation, and/or liver) that respond to local increases in potassium concentration that occur in the absence of or before changes in plasma potassium concentration are detected (87, 186). In support of the notion of potassium sensing, intraportal delivery of potassium chloride to rats leads to an increase in hepatic afferent nerve activity and urinary potassium excretion, responses that are unaccompanied by increases in plasma potassium concentration (187). Increases in the intraportal concentration of glucagon, as follows ingestion of a protein- and potassium-rich meal, also increases renal excretion of potassium (188, 189), a response proposed to be due to the release of cAMP, the second messenger of glucagon in the liver, into the circulation and its uptake by kidney (186).

Chronic potassium loading leads to *potassium adaptation,* an acquired tolerance to an otherwise lethal acute potassium load (8). Potassium adaptation, which begins after a single potassium-rich meal, includes increases in the rate of skeletal muscle uptake of potassium from the extracellular fluid (53) due to stimulation of Na-K-ATPase activity (190) and secretion of potassium by the distal nephron and colon. The process is facilitated by the increase in circulating levels of aldosterone elicited by the increase in serum potassium concentration (191). A similar adaptive response is seen in renal insufficiency such that potassium balance is maintained during the course of many forms of progressive renal disease, as long as potassium intake is not excessive (192). The molecular mechanisms underlying this adaptation in the distal nephron (and colon) include not only an increase in the density of apical membrane potassium channels, but also an increase in the number of conducting ENaC channels and activity of the basolateral Na-K pump. The latter two processes result in increases in transepithelial voltage and the intracellular potassium concentration, events that enhance the driving force favoring potassium diffusion from the cell into the urinary fluid.

Hormones

Mineralocorticoids are key regulators of renal sodium absorption and potassium excretion, and thus of circulating volume, blood pressure and sodium and potassium homeostasis. The major stimuli for aldosterone release from the zona glomerulosa in the adrenal gland are angiotensin II and elevations in serum potassium concentrations (193). ACE inhibitors, by reducing the conversion of angiotensin I to angiotensin II and thus aldosterone secretion by the adrenal gland, may lead to hyperkalemia as the ability of the distal nephron to secrete potassium is impaired. Angiotensin receptor blockers (ARBs), by competitively binding to angiotensin II type 1 (AT1) receptors and thus antagonizing the action of angiotensin II on aldosterone release, may have similar effects.

Aldosterone stimulates sodium reabsorption and potassium secretion in principal cells of the fully differentiated aldosterone-sensitive distal nephron (ASDN) (194, 195). Circulating mineralocorticoids bind to their cytosolic receptors in the ASDN; the aldosterone antagonist spironolactone competitively inhibits this binding. The hormone-receptor complex translocates to the nucleus where it promotes the transcription of aldosterone-induced physiologically active proteins (e.g., Na-K-ATPase). Among the cellular and molecular effects of an increase in circulating levels of aldosterone are increases in density of ENaC channels, achieved by the recruitment to and retention of intracellular channels at the apical membrane, de novo synthesis of new ENaC subunits, and activation of preexistent channels, as well as and stimulation of Na-K-ATPase activity by translocation of preformed transporters to the membrane and translation of new sodium pump subunits (196–201). The sum effect of the stimulation of apical

sodium entry and Na-pump-mediated reabsorption is an increase in lumen negative transepithelial voltage and thus electrochemical driving force favoring potassium exit across the apical membrane (199, 200, 202).

The effects of aldosterone on ENaC and, to some extent, the Na-K pump appear to be indirect, mediated by aldosterone-induced proteins, including serum and glucocorticoid-inducible kinase (sgk). Aldosterone rapidly induces Sgk1 in the distal nephron (203). Phosphorylated Sgk stimulates sodium reabsorption, in large part by inhibiting ubiquitin-ligase Nedd4-2-mediated endocytic retrieval of ENaCs from the luminal membrane (204). Renal water and electrolyte excretion is indistinguishable in sgk1-knockout ($sgk1^{-/-}$) and wild-type ($sgk1^{+/+}$) mice fed a normal diet (205), indicating that the kinase is not necessary for basal sodium absorption. However, dietary sodium restriction reveals an impaired ability of $sgk1^{-/-}$ mice to reduce sodium excretion despite increases in plasma aldosterone levels and proximal tubular sodium and fluid reabsorption (206). $Sgk1^{-/-}$mice exhibit an impaired upregulation of renal potassium excretion in response to potassium loading, presumably due to the impact of the mutation on ENaC and/or Na-K-ATPase activity and thus the electrochemical gradient favoring potassium secretion (207). Corticosteroid hormone induced factor (CHIF) is another aldosterone-induced protein that is expressed in the basolateral membrane of the collecting duct where it increases the affinity of Na-K-ATPase for sodium (208–211).

Plasma aldosterone concentrations in premature infants and newborns are higher than those measured in the adult (16, 212). Yet, clearance studies in fetal and newborn animals demonstrate a relative insensitivity of the immature kidney to the hormone (16, 213–215). The density of aldosterone binding sites, receptor affinity, and degree of nuclear binding of hormone-receptor are believed to be similar in mature and immature rats (215).

The transtubular potassium gradient (TTKG) provides an indirect, semiquantitative measure of the renal response to mineralocorticoid activity in the aldosterone-sensitive cortical distal nephron and is calculated using the equation:

$$\text{TTKG} = \frac{[K^+]\text{urine}}{[K^+]\text{plasma}} \times \frac{\text{plasma osmolality}}{\text{urine osmolality}}$$

where $[K^+]$ equals the potassium concentration in either urine (U) or plasma (P), as indicated (216–218). Measurements of TTKG have been reported to be lower in 27 than 30-week GA preterm infants followed over the first 5 weeks of postnatal life (219). The low TTKG has been attributed to a state of relative hypoaldosteronism (219), but may also reflect the absence of potassium secretory transport pathways (i.e., channel proteins).

Acid-Base Balance

Disorders of acid-base homeostasis induce changes in tubular potassium secretion in the distal nephron (179). In general, acute metabolic acidosis causes the urine pH and potassium excretion to decrease, whereas both acute respiratory alkalosis and metabolic alkalosis increase urine pH and potassium excretion (179, 220). Chronic metabolic acidosis has variable effects on urinary potassium excretion.

Acute metabolic acidosis reduces cell potassium concentration and leads to a reduction in urine pH, which in turns inhibits activity of the SK/ROMK channel and thereby limits potassium secretion in the distal nephron (220–223). The effect of chronic metabolic acidosis on potassium secretion is more complex and may be influenced by modifications of the glomerular filtrate (e.g., chloride and bicarbonate concentrations), tubular fluid flow rate, and circulating aldosterone levels (8, 179). The latter two factors may lead to an increase rather than a decrease in potassium secretion and excretion.

The alkalosis-induced stimulation of potassium secretion reflects two direct effects on principal cells: an increase in net sodium absorption (224), which enhances the electrochemical gradient for net potassium secretion, and (74) an increase in the permeability of the apical membrane to potassium resulting from an increase in duration of time the potassium-selective channels remain open (8). Alkalosis also decreases acid secretion in intercalated cells, thereby reducing H-K-ATPase-mediated countertransport of potassium.

Potassium deficiency stimulates proton secretion in the distal nephron, increases the production of the urinary buffer ammonia (225), and may stimulate bicarbonate generation by increasing expression H-K-ATPase in the distal nephron (226).

Contribution of the Gastrointestinal Tract

Under normal conditions in the adult, 5–10% of daily potassium intake is excreted in the stool. The colon is considered to be the main target for regulation of intestinal potassium excretion (227). Potassium transport in the colon represents the balance of secretion and absorption (228). Under baseline conditions, net potassium secretion predominates over absorption in the adult, whereas the neonatal gut is poised for net potassium absorption (227).

Potassium secretion requires potassium uptake by the Na-K-ATPase and furosemide-sensitive Na-K-2Cl cotransporter located in the basolateral membrane of colonocytes; potassium is then secreted across the apical membrane through potassium channels, including a calcium-activated maxi-K channel similar to that found in the distal nephron (229–233). Potassium absorption is mediated by two H-K-ATPases localized to the apical membrane of the distal colon (234).

Stool potassium content can be enhanced by any factor that increases colonic secretion, including aldosterone, epinephrine, and prostaglandins (31, 235, 236). Indomethacin and dietary potassium restriction reduce potassium secretion by inhibiting the basolateral transporters and apical potassium channels. Diarrheal illnesses are typically associated with hypokalemia, presumably due to the presence of nonreabsorbed anions (which obligate potassium), an enhanced electrochemical gradient established by active chloride secretion, and secondary hyperaldosteronism due to volume contraction (237).

Potassium adaptation in the colon is demonstrated by increased fecal potassium secretion after potassium loading and in the face of renal insufficiency. Fecal potassium excretion may increase substantially to account for 30–50% of potassium excretion in patients with severe chronic renal insufficiency (31, 238–240). The enhanced colonic potassium secretion characteristic of renal insufficiency requires induction and/or activation of apical maxi-K channels in surface colonic epithelial cells (241).

Net colonic potassium absorption is significantly higher in young compared to adult rats (227). The higher rate of potassium absorption during infancy is due to robust activity of apical K-ATPases and limited activity of the basolateral transporters that mediate secretion (227, 231, 242).

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9 Acid-Base Homeostasis

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Introduction

Acid-base homeostasis operates to maintain extracellular arterial pH between 7.35 and 7.45, and intracellular pH between 7.0 and 7.3 in order to provide an optimal milieu for enzymatic and metabolic processes. Since pH is equal to –log [H^+], with the concentration of hydrogen ions in Eq/L (or mol/L), this normal extracellular pH correlates with a hydrogen ion concentration of 35–45 nEq/L, or 35×10^{-6} to 45×10^{-6} mEq/L. Thus, hydrogen ion concentration is maintained within extremely narrow limits, and is just a tiny fraction, roughly one millionth, of the concentration of sodium, potassium and chloride. Deviations in either direction outside the physiological range could impair cardiopulmonary or neurologic function or even lead to death. Several regulatory processes enable the body to efficiently dispose of physiologic daily load of carbonic acid (as volatile CO_2) and nonvolatile acids; and defend against the occasional addition of pathologic quantities of acid and alkali. Chemical buffers within the extracellular and intracellular compartments serve to blunt changes in pH that could occur with retention of either acid or bases. In addition, the control of CO_2 tension (PCO_2) by the central nervous system and the respiratory system and the control of HCO_3^- by the kidneys constitute the complex regulatory processes that act in concert to maintain the arterial pH within a very narrow range.

Buffering Systems

An acid is a proton donor and a base is a proton acceptor. An acid dissociates reversibly into a conjugate base (A^-) and a proton, H^+.

$$HA \rightleftarrows A^- + H^+$$

The acidity of the solution is calculated from the Henderson-Hasselbach equation:

$$pH = pKa + \log[(A^-)/(HA)]$$

pKa indicates the pH at which HA = A^- and $\log[(A^-)/(HA)] = 0$. A strong acid has a low pKa and dissociates freely in solution because its conjugate base has a weak affinity for protons; whereas a weak acid has a high pKa and dissociates poorly because its conjugate base has a strong affinity for protons. A weak acid only partially dissociates and forms a solution which contains both the acid and base forms, enabling it to partially resist changes in pH when a strong acid or base is introduced. This dampening of pH changes is called buffering and is maximal when the pKa is close to the physiological pH.

The major extracellular buffer is the CO_2-HCO_3 system:

$$H_2O + CO_2 \rightleftarrows H_2CO_2 \rightleftarrows H^+ + HCO_3^-$$

Although the pKa of the CO_2-HCO_3 buffer system (6.1) is well below 7.4, this buffer is very effective because of its abundance and because $PaCO_2$ is rapidly regulated by the lungs. Pulmonary participation in acid-base homeostasis relies on the excretion of CO_2 by the lungs. The reaction is catalyzed by the enzyme carbonic anhydrase. Large amounts of CO_2 (10–12 mol/day in adults) accumulate as metabolic end products of tissue metabolism. This CO_2 load is transported in the blood to the lungs as hemoglobin-generated HCO_3^- and hemoglobin-bound carbamino groups.

Introduction of a strong acid HA is rapidly (within minutes) distributed within the extracellular fluid and results in the titration of plasma HCO_3^-:

$$HA + NaHCO_3 \rightleftarrows NaA + H_2O + CO_2$$

The resulting changes in HCO_3 are ultimately corrected by the kidneys. Other buffers include plasma proteins and hemoglobin and intracellular proteins and phosphates. Intracellular buffering follows extracellular buffering and takes up to hours. Approximately 60% of a nonvolatile acid load is buffered outside the extracellular fluid by bone and soft tissues (1) *Table 9-1*.

Renal Acidification

The kidneys play major roles in acid-base homeostasis. Two renal processes are essential: reabsorption of filtered bicarbonate and secretion of acid generated from the metabolism of dietary protein as well as from bone formation, the latter especially significant in the growing child. About 4,000–4,500 mmoles of bicarbonate are filtered per day in

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the adult depending on glomerular filtration rate. Normally almost all of the filtered bicarbonate is reabsorbed. The proximal tubule accounts for the majority of this reabsorption (about 80%). The thick ascending limb of the nephron reabsorbs ~10% and the distal nephron reabsorbs the remaining 10% such that very little filtered HCO_3^- is excreted in the urine. The second function of the kidney is to secrete acid by generating new bicarbonate, to cope with dietary or endogenous metabolic acids. Dietary and endogenous acids amount to about 1 mEq/kg body weight per day in adults on a typical western diet (2). This is accomplished by excretion of titratable acid (TA) and excretion of NH_4^+. The production of new bicarbonate, which is not filtered, by the kidney is quantitated by urinary net acid excretion (NAE), calculated as:

$$NAE = NH_4^+ + TA - HCO_3^-$$

TA refers to acid excreted that has titrated urinary buffers. It equals the amount of acid (H^+) that is added to the tubular fluid by the nephron and is a function of both urine pH and buffering capacity (3). Urinary NH_4^+ excretion produces new bicarbonate from the metabolism of glutamine to bicarbonate and NH_4^+ (4). Addition of bicarbonate to plasma accomplishes acid secretion whereas loss of bicarbonate in the urine is equivalent to gain of acid.

Table 9-1

Summary of response of an acute nonvolatile acid load, including buffering, increased ventillatory rate, and increased renal acid excretion, resulting in ultimate correction of the acidemia

	Response	Time course
	Extracellular buffering by HCO_3	Immediate
Acid load	Respiratory buffering by pCO_2	Minutes to hours
Response	Intracellular and bone buffering	2–4 h
	Renal acid excretion	Hours to days

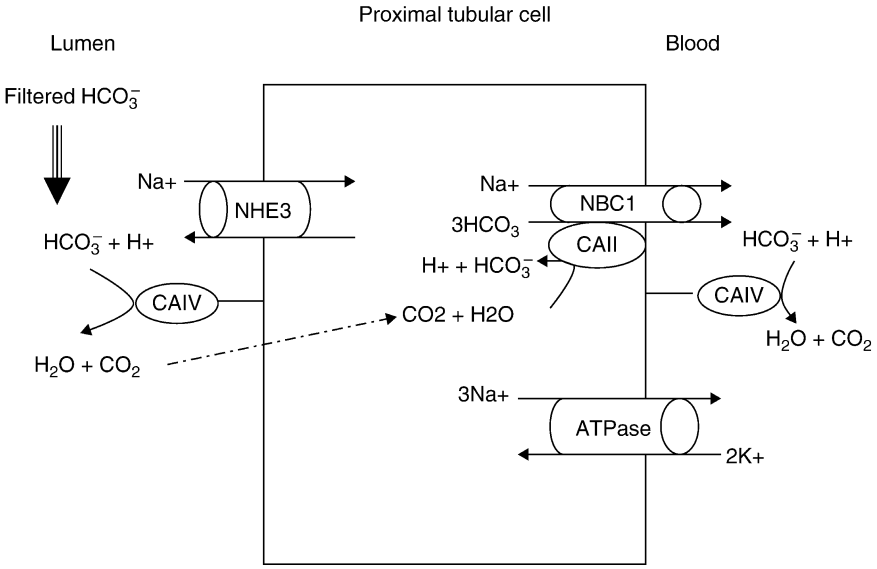
Modified with permission from (133)

Proximal Mechanisms

The proximal tubule of the nephron is responsible for reabsorbing approximately 80% of the bicarbonate that is filtered by the glomerulus. The ability of the proximal tubular cell to reclaim filtered bicarbonate is achieved indirectly by the secretion of hydrogen ions into the tubular lumen. There is no evidence for direct bicarbonate

Figure 9-1

Model for acid base homeostasis in the proximal tubular cell. Filtered bicarbonate is reabsorbed via secreting protons into the tubular lumen. H^+ secretion is achieved via the sodium hydrogen exchanger (NHE3) on the brush border and by electrogenic ($3HCO_3$:1Na) sodium bicarbonate cotransport (NBC1) on the basolateral surface. Carbonic anhydrase (CAIV and CAII) facilitates bicarbonate entry and efflux.



reabsorption (5) (Fig. 9-1). The rate of bicarbonate reabsorption can vary along the proximal segment, with a lower rate in the terminal S3 segment compared with the proximal S1 segment (6, 7). The major mechanism used to secrete H^+ into the lumen is through the Na^+/H^+ exchanger (NHE) located on the luminal surface of the cell (8–11).

Na^+/H^+ Exchanger (NHE)

To date, nine NHE isoforms have been described varying in their tissue expression and cellular location (12). NHE1 is the "housekeeping" isoform that is ubiquitously expressed in virtually all tissues within the plasma membrane. The isoforms NHE2-NHE5 are also located within the plasma membrane but are more restricted in their tissue distribution, while the NHE6-NHE9 isoforms are also ubiquitously expressed but are usually located intracellularly (12). NHE3, an integral membrane protein, is the predominant isoform in the proximal tubular cell and is located at the apical surface (13–19). Like other isoforms, NHE3 facilitates movement of one sodium molecule into the cell in exchange for secreting one hydrogen molecule into the tubular lumen. In addition, NHE3 regulates intracellular pH and cell volume and is responsive to growth factors (20–23). This exchanger is sensitive to amiloride and its analogs (10, 24, 25). Recent studies have identified a second transporter, NHE8, within the proximal tubule (24, 26, 27) that may play a role in acid base homeostasis (28).

Na^+/K^+ ATPase

Hydrogen ion secretion within the proximal tubule is sodium dependent (29). Sodium moves passively into the proximal tubular cell by the concentration gradient generated within the cell. The luminal concentration of sodium is maintained throughout the length of the proximal tubule and resembles that of isotonic sodium chloride. The sodium concentration gradient within the cell is generated by the basolateral Na^+/K^+ ATPase located on the basolateral surface of the tubular cell. This is an active process, which extrudes three sodium ions in exchange for two potassium ions. Within the proximal tubule, the Na^+/K^+ ATPase pump indirectly provides the energy that allows most of the transport proteins to translocate filtered solutes. Murine studies have suggested that two-thirds of the bicarbonate reabsorption in the proximal tubule is achieved through the NHE (15–19). The remaining one third is achieved through a ATPase similar to those in the distal nephron (30). The H^+-ATPase (15–19) is an apically located, multi-subunit vacuolar-type (31–33) ATPase that is blocked by DCCD (N,N'-dicyclohexylcarbondiimide), NEM (N-ethylmaleimide) and bafilomycin (34, 35). Studies have shown that there is a maturational increase in both NHE activity (36–40) and basolateral Na^+/K^+-ATPase activity (41, 42) resulting in an increase in proximal tubule acidification during development. Thus, most of the H^+ secreted by the proximal tubule is used to reclaim bicarbonate. The remaining secreted H^+ will be combined with phosphate as titratable acid.

Carbonic Anhydrase (CA)

The filtered bicarbonate combines with the secreted hydrogen ions within the lumen to form carbonic acid (H_2CO_3). The dehydration of H_2CO_3 to CO_2 and H_2O is catalyzed by the zinc metalloenzyme, carbonic anhydrase isoenzyme type IV (CA IV), which is tethered to the luminal membrane of the proximal tubular cells. The CO_2 that is produced by this reaction rapidly diffuse into the tubular cells. Within the cells it combines with intracellular H_2O to produce intracellular H_2CO_3. Soluble cytoplasmic carbonic anhydrase isoenzyme type II (CA II) catalyzes the conversion of intracellular H_2CO_3 to H+ and HCO_3^- (43). Currently, there are 15 identified CA isoenzymes that enhance H^+/HCO_3^- transport (44). CA II accounts for 95% of the CA activity in the kidney and resides primarily within the cytosol. CA IV and CA XII account for the remaining 5% and are membrane-associated. CA II interacts with a number of transporters that facilitate movement of HCO_3^- (45).

$Na^+/HCO3^-$Cotransporter (NBC)

In general, there are three main groups of bicarbonate transporters: chloride-bicarbonate exchangers, sodium bicarbonate cotransporter (NBC), and sodium dependent chloride bicarbonate exchanger (NDCBE). These transporters are part of the solute-linked carriers (SLCs) and are members of the SLC4 family (46). The sodium bicarbonate cotransporter 1 (NBC1), otherwise known as SLC4A4, is an electrogenic sodium bicarbonate transporter located on the basolateral cell membrane of the proximal tubule (47). In the proximal tubule, NBC1 is responsible for transporting three bicarbonate molecules and one sodium molecule into the blood (48). Although dependent on Na^+, NBC1 appears to be independent of chloride. The electrogenic Na^+/HCO_3^- cotransporter is blocked by disulfonic stilbenes SITS and DIDS (21).

NBC1 appears to be the dominant pathway for HCO_3^- reabsorption in the S1 and S2 segments of the proximal tubule but a basolateral Cl-HCO_3 exchanger plays a role in the S3 segment (49–51). Thus, both the secretion of H^+ within the tubular lumen and the movement of HCO_3^- into the blood are sodium-dependent.

Distal Mechanisms

The distal nephron is responsible for net acid excretion. As in the proximal tubule, processes are at work to reabsorb the remaining filtered bicarbonate. In addition, protons are actively transported to the tubular lumen where they combine with NH_3 and HPO_4^{2-} to form $H_2PO_4^-$ and NH_4^+ resulting in urinary acidification.

Loop of Henle and Thick Ascending Loop (TAL)

The loop of Henle is responsible for reabsorbing the HCO_3^- that escapes proximal tubular reabsorption. This can be as much as 15% of the total filtered HCO_3^- and mostly occurs in the TAL (52–54). HCO_3^- reabsorption in the thick ascending loop is dependent on the concentration of HCO_3^- in the lumen and, like the proximal tubule, on luminal H^+ secretion (55). H^+ secretion in the TAL is mediated by Na^+/H^+ exchange at the luminal surface (56). The NHE3 isoform is predominantly involved (57–59) although, NHE2 has also been demonstrated to be present (60). The driving force mediating the exchange is the Na^+/K^+-ATPase (3Na:2K) on the basolateral surface (55). Efflux of HCO_3^- across the basolateral membrane into the peritubular fluid occurs via the electroneutral (1:1) basolateral Na^+/HCO_3^- isoform (NBCn1) (61–63), but other mechanisms such as Cl^-/HCO_3^- exchange (AE2) and K^+/HCO_3^- transport have been suggested to play a role (64–66).

Renal synthesis and excretion of ammonia is an important function of the kidney in maintaining acid base homeostasis. Ammonia is synthesized in the proximal tubule from the metabolism of glutamine (67, 68), which enters the proximal cell on the basolateral surface via the glutamine amino acid transporter, SNAT3 (69). NH_3 produced from the metabolism of glutamine enters the tubular lumen by diffusion where it combines with H^+ to produce NH_4^+. The TAL reabsorbs NH_4^+ allowing its secretion in the thin descending loop facilitating acid secretion distally. The TAL participates in generating the high NH_4^+ concentration in the medulla and the establishment of countercurrent multiplication system (70). NH_4^+ absorption in the TAL occurs by both active transport and passive diffusion and a number of transporters have been described. NH_4^+ is carried across the apical membrane by a $Na^+/K^+/2Cl^-$ cotransporter (NKCC2) that is sensitive to furosemide and bumetanide (71). Other cellular transport pathways on the luminal surface of the TAL include a K^+/H^+ (NH_4^+) antiport (NHE3 and/or NHE2), which exchanges NH_4^+ for H^+ and nonselective cation conductance (72). Basolateral carriers that transport NH_4^+ into the interstitium are $Na^+/K^+(NH_4^+)$-ATPase, NH_4^+ (K+)/Cl^- cotransport (KCC4), NH_4^+ conductance and Na^+/K^+ (NH_4^+) antiport (NHE1) (73–75). The final excretion of NH_3/NH_4^+ into the lumen of the collecting duct may be mediated by carrier proteins. RhBG and RhCG have been suggested as candidates (76, 77).

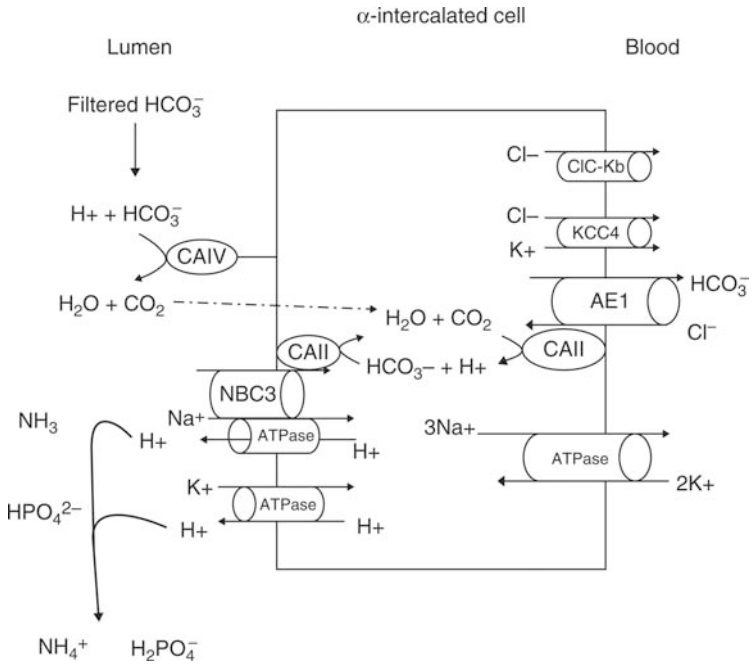
Collecting Duct

Urine acidification occurs mainly in the collecting duct (78). Two major cell types are found interspersed in the collecting duct: the principal cell and the intercalated cell. The principal cell is primarily involved with Na^+ reabsorption and K^+ secretion and plays little role in acid base balance. The intercalated cell is primarily involved in acid base transport and regulation. There are two types of intercalated cells that display different functions. The α-intercalated cells secrete protons while the β-intercalated cells secrete bicarbonate. The cortical collecting duct contains both α- and β-intercalated cells while the outer medullary collecting duct contains mostly α- intercalated cells.

The α-intercalated cell (*Fig. 9-2*) actively transports H^+ into the urine via a vacuolar H^+ -ATPase and to a lesser extent a H^+/K^+-ATPase on the luminal surface (79). Mutations in either the β1 or α4 subunits of the human H^+-ATPase can cause clinical acidosis (80–82). The distal tubule H^+-ATPase isoform differs from the proximal tubule isoform (83). Intracellular CAII is required to generate intracellular H^+ for active transport. An apical Na^+/H^+ exchanger (NHE2) also secretes H^+ into the tubule (59, 84). Proton secretion in the collecting duct serves to reabsorb the remaining HCO_3^- that has evaded earlier transport mechanisms of the nephron. In addition, secreted H^+ combines with HPO_4^{2-} to form $H_2PO_4^-$ and NH_3 to trap NH_4^+. HCO_3^- is transported from the cell to the interstitium via a basolateral Cl^-/HCO_3^- anion exchanger (AE1). AE1 exchanges one bicarbonate ion for one chloride ion. Mutations in AE1 can also cause a metabolic acidosis (85). Chloride returns to the peritubular space via a conductive pathway thereby continuing to drive

Figure 9-2

Model for acid base homeostasis in the α-intercalated cell of the collecting duct. α-intercalated cells acidify the urine by (1) secreting protons via the H^+ ATPase and less so via H^+/K^+-ATPase, (2) reabsorbing residual filtered HCO_3^- via electroneutral sodium bicarbonate cotransport (NBC3) and chloride bicarbonate exchange (AE1). Carbonic anhydrase (CAII) facilitates bicarbonate efflux.



HCO_3^- extrusion. Other exchangers such as AE2, AE4 and SLC26A7 (pendrin) have been identified on the basolateral membrane of selective cells within the inner or outer medullary collecting cells contributing to HCO_3^- exchange (65, 86–89). Mutations in the K^+/Cl^- cotransporter, KCC4, in mice have also been shown to result in acidosis (90).

The β-intercalated cell is opposite in polarity to the α-intercalated cell. The function is to actively secrete HCO_3^- into the urine coupled to Cl^- reabsorption but independent of Na^+. $HCO_3^-$is moved from the lumen into the cell through a Cl^-/HCO_3^- exchanger on the apical membrane. Pendrin (SLC26A4) is thought to be this exchanger. Mice deficient in pendrin are unable to secrete HCO_3^- by their collecting ducts (91–93). On the basolateral surface, acetazolamide sensitive H^+-ATPase pumps H^+ into the peritubular space assisting in HCO_3^- secretion (94).

Genetics of Renal Tubular Acidosis (RTA)

Renal tubular acidosis (RTA) is classified according to the location of the defect in acid-base balance. Molecular and genetic advances have identified various mutations in the transporters of the nephron that can result in metabolic acidosis and alkalosis. Inherited disorders of acidosis and alkalosis are listed in *Tables 9-2* and *9-3*.

Inherited Distal Renal Tubular Acidosis

Distal RTA is diagnosed when the kidney fails to acidify the urine in the setting of systemic metabolic acidosis or after an induced acid load. The inability to acidify the urine is usually associated with hypocitraturia, hypercalciuria, and nephrocalcinosis. The nephrocalcinosis can result in chronic interstitial disease, electrolyte abnormalities, and urinary concentration defects. Severe hypokalemia can result in muscle weakness, periodic paralysis, or cardiac arrhythmias. Affected children usually present with failure to thrive and growth retardation.

Both dominant and recessive forms of inherited distal RTA have been described. Mutations in either the basolateral AE1 transporter or the apical H^+-ATPase of the α-intercalated cell results in an inability of

Table 9-2

The inherited renal tubular acidosis

Type of RTA	Subtype and inheritance	Age at presentation	Clinical features	Protein	Gene(s)	OMIM
Distal (type 1)	Dominant	Older/adult	Mild/compensated metabolic acidosis	AE1	*SCL4A1*	179800
			Hypokalemia (variable)			
			Hypercalciuria			
			Hypocitraturia			
			Nephrolithiasis			
			Nephrocalcinosis			
			Sometimes rickets/ osteomalacia			
			Secondary erythrocytosis			
	Recessive	Childhood	Metabolic acidosis with hemolytic anemia	AE1	*SCL4A1*	602722
			Only reported in Southeast Asian populations			
	Recessive with early onset hearing loss	Infancy/ childhood	Metabolic acidosis	B1 subunit of H^+-ATPase	*ATP6V1B1*	267300
			Early nephrocalcinosis			
			Vomiting/dehydration			
			Growth retardation			
			Rickets			
			Bilateral sensorineural hearing loss, from childhood			
	Recessive with later onset hearing loss	Infancy/ childhood	As above, but later onset hearing loss in some (a few with normal hearing)	A4 subunit of H^+-ATPase	*ATP6V0A4*	602722
Proximal (type 2)	Recessive with ocular abnormalities	Infancy	Metabolic acidosis	NBC1	*SLC4A4*	604278
			Hypokalemia			
			Ocular abnormalities (band keratopathy, cataracts, glaucoma)			
			Growth retardation			
			Defective dental enamel			
			Intellectual impairment			
			Basal ganglia calcification			
Combined proximal and distal (type 3)	Recessive with osteopetrosis	Infancy/ childhood	Metabolic acidosis	CA II	*CA2*	259730
			Hypokalemia			
			Osteopetrosis			
			Blindness			
			Deafness			
			Early nephrocalcinosis			

Adapted with permission from (95)

Table 9-3

Different subtypes of Bartter's and Gitelman's syndrome, with the responsible genes, the resulting transport proteins, their localization and function

Type	Gene locus	Gene	Gene product	Localization	Function
Type I Bartter's syndrome	15q15-21	SLC12A1	Na^+-K^+-$2Cl^-$ Cotransporter NKCC2	TAL	NaCl reabsorption
Type II Bartter's syndrome	11q24-25	KCNJ1	K^+ channel ROMK	TAL	K^+ supply for NKCC2
				CCD	Renal excretion of diet K^+
Type III Bartter's syndrome	1p36	CLCNKB	Cl^- channel C1C-Kb	TAL	Cl^- reabsorption
				Distal tubule and CCD	Cl^- reabsorption
Type IV Bartter's syndrome	1p31	BSND	Barttin	TAL	β-Subunit of ClC-Kb
				Thin ascending limb	β-Subunit of ClC-Ka
				Stria vascularis	β-Subunit of ClC-Kb/ ClC-Ka
Type V Bartter's syndrome	3q13.3-q21	CASR	Ca^{2+} sensing receptor CaSR	TAL	Inhibition of NKCC2
Gitelman's syndrome	16q13	SLC12A3	Na^+-C^1-cotransporter NCCT	Distal tubule	NaCl reabsorption

TAL thick ascending limb of the loop of Henle, *CCD* cortical collecting duct
Adapted with permission from (119)

the distal nephron to acidify the urine (95). E1 is the Cl^-/HCO_3^- exchanger on the basolateral surface of the α-intercalated cell. The gene that encodes for the AE1 protein is SLC4A1 is located on human chromosome 17q21–22. AE1 is expressed in the kidney and in red blood cells. Mutations in the red blood cells isoform result in deformities such as hereditary spherocytosis and Southeast Asian ovalocytosis. Eight different mutations in the kidney isoform of AE1 have been reported to cause distal RTA (96–101). Dominant and recessive mutations of AE1 that result in distal RTA have been found (102). The defect does not appear to be abnormal anion exchange but rather the mutations appear to alter the polarity of the cell causing intracellular retention of the AE1 transporter or misplacement of the transporter to the apical surface (101, 103–107).

The renal proton pump is a member of the vacuolar multisubunit ATP-dependent proton pumps (108, 109). The pump has a soluble cytoplasmic domain, that displays the ATPase activity, and a membrane-associated domain that displays the proton translocation pathway. Two genes responsible for recessive distal RTA have been identified: ATP6V1B1 (on chromosome 2p13) and ATP6V0A4 (on chromosome 7q33–34) (110–112). Their expression is restricted to the kidney (111, 112). Bilateral sensorineural hearing loss is associated with mutations in the β1 subunit and not the α4 subunit. Hearing loss is thought to be due to impaired proton secretion which thought to be required for normal cochlear development and hair cell survival (111).

Inherited Proximal Renal Tubular Acidosis

Inherited mutations in the proximal tubule that result in isolated RTA are rare and characterized by impaired proximal tubular bicarbonate reabsorption with preservation of other proximal reabsorption functions, such as those for glucose, amino acids, phosphate, and citrate. Inherited isolated autosomal recessive proximal RTA can present with growth retardation alone, or together with mental retardation and ocular abnormalities such as

band keratopathy, cataracts, and glaucoma. Calcification in the basal ganglia has been described (113).

Sodium bicarbonate cotransport across the basolateral membrane of proximal tubular cells is required for proximal bicarbonate reabsorption. Two missense mutations were initially found in human kidney NBC1 isoform (SLC4A4) gene to be associated with reduced NBC1 activity (114). Other NBC1 mutations have since been found (113, 115, 116) and have been associated with intracytoplasmic retention and aberrant localization (117). The NHE3 deficient mouse exhibits reduced proximal tubular HCO_3 reabsorption with distal tubular compensation and a mild metabolic acidosis (118). Human NHE3, encoded by SLC9A3, is a candidate gene for proximal RTA. However, mutations have not been reported.

Genetics of Metabolic Alkalosis

Mutations in six genes have been found to cause Gitelman's and Bartter's syndromes (131) (*Table 9-3*). In the clinic, Gitelman's and Barter's syndromes are characterized by hypochloremic metabolic alkalosis, hypokalemia with increased fractional excretion of potassium and normal to low normal blood pressure. In the newborn period Bartter's syndrome presents with polyuria and polydipsia, and hypercalciuria with associated nephrocalcinosis. In childhood, classic Bartter's syndrome presents with polyuria and polydipsia along with vomiting and failure to thrive but nephrocalcinosis is not usually seen. Both forms demonstrate increased urinary prostaglandin E2 concentration. Gitelman's syndrome is usually diagnosed later in life where only mild laboratory abnormalities (mild hyokalemia and alkalosis) are observed. Patients may develop muscle weakness, cramps and tetany.

Classification of the syndromes as described below is based on the genetic mutations that have been reported (131). *Type I Bartter's syndrome* is caused by mutations of the sodium potassium chloride cotransporter (NKCC2) expressed on the apical surface of the TAL (120). The cotransporter is encoded by the SLC12A1 gene located on chromosome 15q15-q21 and is responsible for Na^+ reabsorption (121). *Type II Bartter's syndrome* is caused by mutations of the voltage-dependent potassium channel ROMK expressed on the apical surface of the TAL and the cortical collecting duct (122). The channel is encoded by the KCNJ1 gene on chromosome 11q24–25 and facilitates NKCC2 activity by providing the intraluminal potassium. *Type III Bartter's syndrome* is caused by mutations of a chloride channel (ClC-Kb) expressed in the basolateral surface of the TAL, the distal convoluted tubule, the connecting tubule and the α-intercalated cell of the collecting duct (123). The channel protein is encoded by the CLCNKB gene located on chromosome 1p36 and is responsible for transporting chloride across basolateral membrane (123–125). *Type IV Bartter's syndrome* is caused by mutations of barttin, a β-subunit of the ClC-Kb channel and is expressed in the basolateral surface of the TAL and in the cochlea (126, 127). The protein is encoded by the Bartter syndrome and sensorineural deafness (BSND) gene located on chromosome 1p31 (126, 128, 129). *Gitelman's syndrome* is caused by mutations of the renal thiazide-sensitive sodium chloride cotransporter (NCCT) in the apical membrane of the distal convoluted tubule. The cotransporter is encoded by the SLC12A3 gene located on chromosome 16q13 and is responsible sodium reabsorption in the distal tubule (130, 131).

Physiologic Response to Nonvolatile Acid Loads

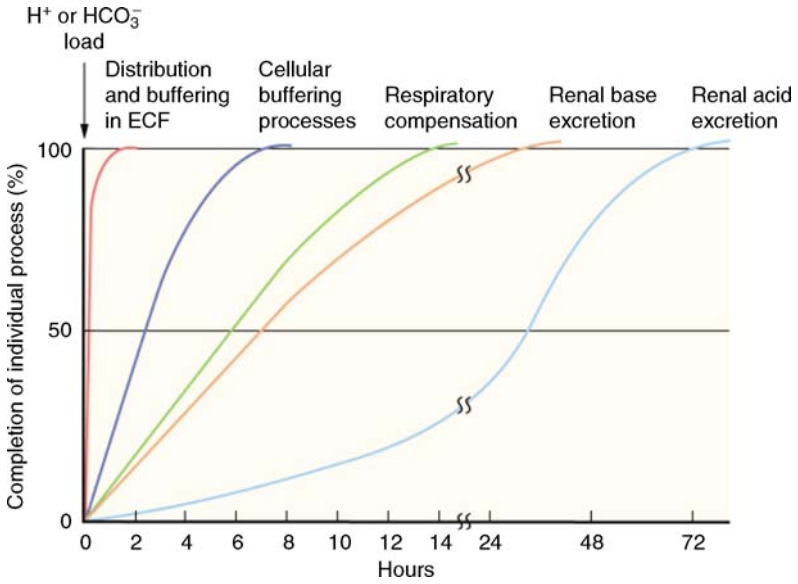
Acute non-volatile acid loads are distributed rapidly and attenuated by extracellular buffers within 30 min. A second phase of buffering by intracellular processes then occurs. Approximately two-thirds of this intracellular buffering is through Na^+/H^+ exchange and one-third through either K^+/H^+ or Cl^-/ HCO_3^- exchanges (132). These intracellular processes function to restore arterial pH within 6–8 h. This is followed by respiratory compensation and renal acid excretion. The time course of these physiologic compensatory mechanisms is summarized in *Fig. 9-3*.

An important response to an acid load is the neurorespiratory control of ventilation. A fall in the systemic arterial pH is sensed by chemo-receptors which stimulate ventilation and reduce $PaCO_2$. The fall in arterial pH is therefore blunted. Approximately 12–24 h is required for full respiratory compensation for metabolic acidosis (*Fig. 9-3*).

In response to a HCO_3^- load, the kidneys efficiently retain all filtered base and attempt to generate enough new bases to normalize the arterial pH. Acidosis enhances proximal HCO_3^- absorption, decreasing delivery of HCO_3^- out of the proximal tubule and enhances distal acidification. Net acid excretion is increased by stimulation of $NH4^+$ production and excretion. In addition, hyperaldosteronism and the effect of nonreabsorbable anions can act synergistically to strengthen the renal defense to an acid challenge (133).

Figure 9-3

Time course of acid-base compensatory mechanisms. In response to a metabolic acid or alkaline load, component approaches to completion of the distribution and extracellular buffering mechanisms, of cellular buffering events and of respiratory and renal regulatory processes are presented as a function of time. *ECF* extracellular fluid. (Reproduced with permission from reference 133.)



Physiologic Response to Alkaline Loads

The physiologic response to an alkaline load is dependent on the same three responses for defense of an acid challenge: cellular buffering, distribution within the ECF, respiratory and renal excretion. The cellular defense against a base load is somewhat less effective than the defense against an acid load. There is also poorer stabilization of intracellular pH in the alkaline than in the acid range (133).

An acute alkaline load in the form of a HCO_3^- load is rapidly distributed in the extracellular fluid within 25 min. This is followed by cellular buffering, which has a half life of about 3 h. The volume of distribution of the HCO_3^- load is inversely proportional to the preexisting serum HCO_3^- concentration. Two thirds of the HCO_3^- load is retained in the ECF. One third of the HCO_3^- load is buffered by cellular processes, principally by Na^+/H^+ exchange. A small amount is buffered by Cl^-/HCO_3^- exchange and increased lactate production (134). Modest hypokalemia also results. Neutralization of the HCO_3^- load by buffers results in an increase in PCO_2, which stimulates ventilation acutely. However, if the respiratory system is compromised, dangerous hypercapnia may ensue.

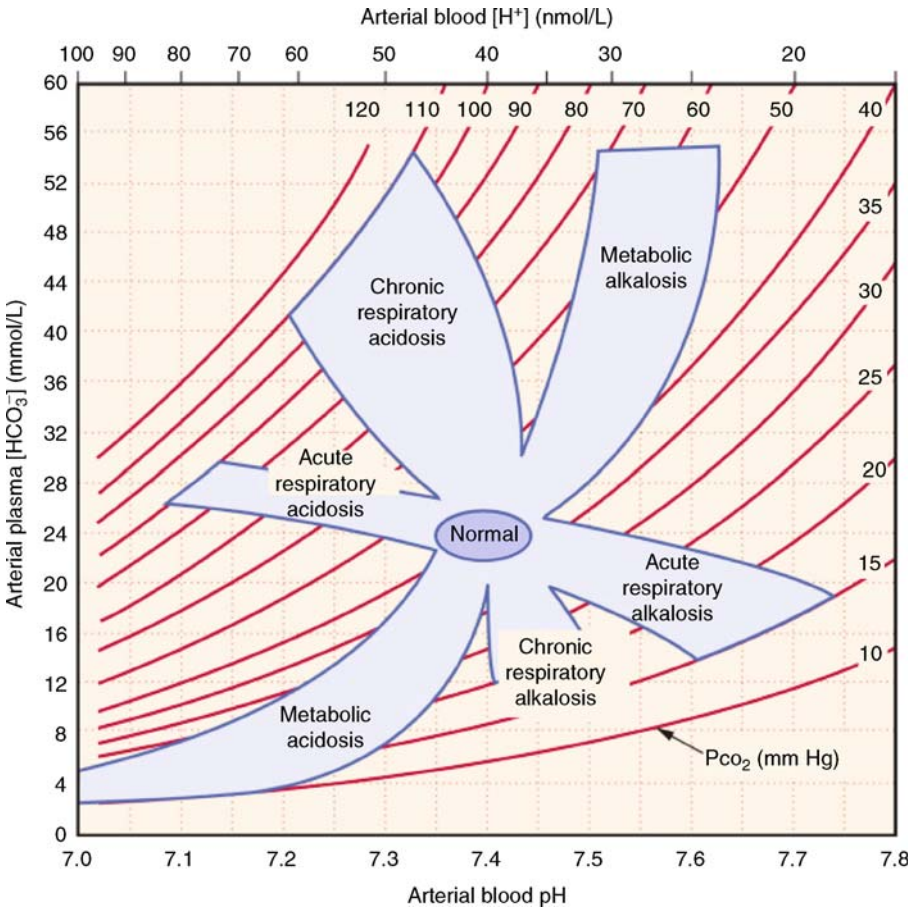
The kidney excretes HCO_3^- more rapidly than acid. A base load is excreted almost entirely within 24 h. The proximal tubule is responsible principally for HCO_3^- excretion when blood HCO_3^- increases. Glomerular ultrafiltrate HCO_3^- rises in conjunction with higher serum HCO_3^- but absolute proximal HCO_3^- reabsorption does not increase because of suppression of proximal acidification processes by alkali. The most sensitive renal response to an alkaline load is a decline in the excretion of NH_4^+. In addition, there is a rise in the excretion of unmeasured anions. Excretion of citrate and 2-oxoglutarate is increased significantly with modest base loads. Bicarbonaturia is quantitatively important only with the large alkaline loads. This process of limiting changes in urine pH without sacrificing acid-base balance lessens the risk of kidney stone formation (135).

Acid-Base Map

A convenient approach to acid-base disorders is an acid-base map. Although not always reliable, this defines the 95% confidence limits in acid-base disorders (*Fig. 9-4*).

Figure 9-4

Acid-base normogram (or map). Shaded areas represent the 95% confidence limits of normal respiratory and metabolic compensations for primary acid-base disturbances. Data falling outside the shaded areas denote a mixed disorder if a laboratory error is not present. (Reproduced with permission from reference 133.)



If the arterial HCO_3^- and H^+ values fall within one of the shaded areas, a simple acid-base disturbance is present. Two broad types of acid-base disorders are metabolic and respiratory. Metabolic acidosis and alkalosis are characterized by primary disturbances of plasma HCO_3^-. Common examples of metabolic acidosis and alkalosis are listed in *Tables 9-2*, *9-3* and *9-4*. On the other hand, respiratory disorders of acid-base balance are characterized by primary disturbance of $PaCO_2$. Common examples of respiratory disorders of acid-base balance are listed in *Tables 9-5*–*9-7*.

Primary metabolic acid-base disorders evoke secondary respiratory responses and primary respiratory acid-base disorders evoke secondary metabolic responses. Compensation is a predictable physiologic consequence of the primary disturbance and does not present a secondary acidosis or alkalosis. Mixed acid-base disorders are situations which exceed the physiologic limits of compensation. Values that fall outside the shaded areas in *Fig. 9-4* indicate mixed disorders. Common examples of mixed disorders of acid-base balance are listed in *Tables 9-8* and *9-9* (136).

Physiologic Response to Changes in Carbon Dioxide Tension

Respiratory acidosis (*Tables 9-6* and *9-7*) which follows hypercapnia, is initiated by an increase in $PaCO_2$ and elicits acidification of body fluids. An acute increase in plasma HCO_3^- occurs and is complete within 5–10 min. This results in acidic titration of non-bicarbonate buffers,

Table 9-4

Causes of metabolic acidosis

Mechanism	Class of agents	Clinical conditions
↑ Production of acid	β-Hydroxybutyric acid andacetoacetic acid	Fasting or starvation
		Insulin deficiency
		Ethanol intoxication
		Ketotic hypoglycemia with hypoalaninemia
	Lactic acid	Hypoxia
		Muscular exercise
		Ethanol ingestion
	Type 1 glycogen storage disease	Fructose-6-diphosphate deficiency
		Leukemia
		Diabetes mellitus
		Pancreatitis
		Cirrhosis
	Incompletely identified organic acids	Ethylene glycol ingestion
		Paraldehyde intoxication
		Salicylate intoxication
		Methanol intoxication
		Methylmalonic aciduria
		Propionyl coenzyme A carboxylase deficiency
	Acidifying salts	Arginine hydrochloride
		Ammonium chloride
		Lysine chloride
		Hyperalimentation
	Sulfuric acid	Methionine
		Neutramigen
		High-protein milk formula
↑ Extra renal losses of base	Bicarbonate (or combustible base)	Diarrhea
		Ureterosigmoidostomy
		Drainage of pancreatic, biliary, or small bowel secretion
		Ingestion of calcium chloride, cholestyramine, and magnesium sulfate
Dilutional acidosis	Infusion of bicarbonate-free isotonic or hypertonic solutions	Impaired renal acidification
		Oliguria or salt-retaining states
		Renal tubular acidosis
Impaired renal acidification	Accumulation of fixed, nonmetabolizable acids	Polycystic kidney disease
		Hyperparathyroidism
		Adrenal insufficiency
		Pseudohypoaldosteronism
		Leigh's syndrome

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Table 9-5

Causes of metabolic alkalosis

Mechanism	Class of agents	Clinical conditions
Excessive loss of acid with volume contraction	Chloride deficiency syndromes	Normal blood pressure, high rennin and aldosterone, low potassium: vomiting of gastric juices, gastric drainage fistula; diuretic and laxative abuse; Bartter's syndrome; chloride-deficient infant formula
		Cystic fibrosis; villous adenoma of the colon; Congenital alkalosis with chloride diarrhea (Darrow)
Excessive gain of base	Base overload	Iatrogenic, especially in the context of renal
		insufficiency; milk alkali syndrome
	Conversion of lactate, acetate to base	Iatrogenic; dialysis excess
	Nonmetabolizable acid into cells	Glucose-induced alkalosis in fasting
	Excess proximal tubular bicarbonate reabsorption	Posthypercapnic state
	Hypoparathyroidism	Phosphate excess
Increased (distal) bicarbonate reabsorption	Volume expansion, mineralo-corticoid excess	Hypertension, high renin and aldosterone; secondary nonedematous aldosteronism (e.g., renal artery stenosis, intrarenal vascular disease, accelerated hypertension)
		Renin-secreting tumors
		Hypertension, low rennin, high aldosterone: primary hyperaldosteronism; dexamethasone-suppressible hyperaldosteronism; adrenal carcinoma
		Hypertension, low rennin, low aldosterone: adrenocorticosteroid excess; deficiency of 11-hydroxylation/17-hydroxylation; adrenal carcinoma; Liddle syndrome; licorice (glycyrrhizic acid) excess

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such as phosphates, hemoglobin and intracellular protein buffers. When respiratory acidosis is chronic, renal adjustments exacerbate the acidemia by a further increase in plasma HCO_3^-. This chronic adjustment phase takes 3–5 days to complete and involves upregulation of the renal acidification mechanisms.

Respiratory alkalosis is initiated by a decrease in $PaCO_2$ from different causes (*Table 9-6*). Respiratory alkalosis causes alkalinization of body fluids. The acute response consists of a decrease in plasma HCO_3^- and is complete within 5–10 min from the onset of hypocapnia. It occurs by alkaline titration of nonbicarbonate buffers of the body as well as increased production of organic acids. When respiratory alkalosis is chronic, renal adjustments worsen the alkalemia by an additional decrease in plasma HCO_3^-. This adaptation takes 2–3 days to complete and involves downregulation of renal acidification mechanisms. Chronic but not acute respiratory acidosis stimulates activity of H^+-ATPase and H^+ K^+-ATPase in the proximal tubule, medullary thick ascending limb and collecting duct. In contrast, both acute and chronic respiratory alkalosis decrease both H^+-ATPase and H^+K^+-ATPase proton pumps. The stimulatory effect of respiratory acidosis and the inhibitory effect of respiratory alkalosis appear to be potassium and aldosterone independent. Although the precise mechanisms are not known, direct of PCO_2, pH or HCO_3^- delivery may be involved (137).

Molecular and Cellular Renal Regulation in Acid-Base Disorders

The proximal tubule responds to systemic changes in acid base balance to restore homeostasis. An acute acid load (decrease in peritubular pH) will result in increased H+ secretion and HCO_3^- reabsorption while an acute base load (increase in peritubular pH) will result in decreased H^+ secretion and HCO_3^- reabsorption by the proximal tubule (138–140). Metabolic acidosis results in increased insertion of NHE into the apical membrane as well as an

Table 9-6

Causes of acute respiratory acidosis

Mechanism	Conditions
Airway obstruction	Aspiration of vomitus or foreign body
	Laryngospasm and edema
	Broncospasm
	Obstructive sleep apnea
Neuromuscular impairment	Injury of brain stem and high cord
	Botulism
	Tetanus
	Guillain-Barre' syndrome
	Myasthenia gravis crisis
	Overdose or narcotic, sedatives
	Toxic agents (curare, succinylcholine)
	Aminoglycoids, organophosphate
	Hypokalemic myopathy
	Familial hypokalemic periodic paralysis
Thorax or pulmonary disorders	Respiratory distress system
	Pneumothorax
	Hemothorax
	Smoke inhalation
	Severe pneumonitis
Inadequate ventilation	Large dead space mechanical ventilation
	Erroneous settings for tidal volume
Vascular accidents	Massive pulmonary embolism and edema
	Cardiac arrests
Central nervous system depression	General anesthesia
	Tranquilizer overdose
	Cerebral trauma or infection
	Central sleep apnea

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Table 9-7

Causes of chronic respiratory acidosis

Mechanism	Conditions
Airway obstruction	Chronic obstructive airway disease; bronchitis, emphysema
	End-stage interstitial lung disease
Respiratory center depression	Chronic narcotic or tranquilizer overdose
	Primary hypoventilation (Ondine's curse)
	Brain tumor
Restrictive lesions	Kyphoscoliosis, spinal arthritis
	Diaphragmatic paralysis
	Hydrothorax
	Fibrothorax
	Interstitial fibrosis
	Prolonged pneumonitis
	Obesity hypoventilation syndrome (Pick-wickian syndrome)
Neuromuscular defects	Poliomyelitis
	Multiple sclerosis
	Muscular dystrophy
	Amyotrophic lateral sclerosis
	Myxedema
	Myopathic polymyositis
	Acid maltase deficiency

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increase in its activation. On the basolateral surface there is an increase in posttranslational modifications of NBC resulting in increased HCO_3^- reabsorption (141). The amount of HCO_3^- reabsorbed depends upon the luminal concentration of HCO_3^- and the luminal flow rate (142, 143).

The distal tubule responds to acidosis by increasing H^+ secretion and HCO_3^- reabsorption in the superficial distal and inner medullary tubules. A decrease in peritubular HCO_3^- stimulates basolateral Cl^-/HCO_3^- exchange and stimulates insertion of vesicles containing H^+-ATPase in the apical membrane to enhance H^+ secretion. A decrease in luminal pH will inhibit H^+-ATPase activity. In the collecting duct, acidosis has been shown to result in loss of apical Cl^-/HCO_3^-exchange but acquired basolateral function. It is well understood that Na^+ delivery to the distal nephron with or without volume depletion results in increased H^+ secretion (144–146), while potassium depletion increases HCO_3^-reabsorption in the superficial distal tubule and collecting duct (147, 148).

Chronic acid loading (7 days) is associated with an increase in apical NHE-3 in the renal proximal tubule. Because NHE-3 mediates both proton secretion and sodium reabsorption, compensatory changes in sodium handling develop, involving decreases in the abundance of the thiazide-sensitive Na^+/Cl^- transporters of the distal convoluted tubule and both the β and γ subunits of the amiloride-sensitive epithelial sodium channel of the

Table 9-8
Causes of respiratory alkalosis

Mechanism	Conditions
Reflex excitation of respiratory center via pulmonary stretch receptors	Pulmonary edema, cardiopulmonary disease
	Embolus
	Interstitial pulmonary disease
Primary excitation of central respiratory center	Anxiety
	Hyperventilation (voluntary or mechanical)
	Encephalitis, meningitis
	Cerebrovascular incidents, head trauma, brain tumor, or vascular accidents
	Medications; salicylate, nicotine, xanthine, pressor agents, progesterone
	Heat exposure, fever, pain
	Pregnancy
Reflex excitation of respiratory center via peripheral chemoreceptor	Low inspirational oxygen (e.g., high altitude)
	Hypotension
	Tissue hypoxia (e.g., anemia, congestive heart failure, asthma)
	Arterial hypoxemia
Multiple mechanisms	Hepatic failure
	Gram negative sepsis
	Shock

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collecting duct. In addition, the renal cortical abundance of the proximal type 2 Na-dependent phosphate transporter is markedly decreased (147). The adaptation of renal $NH4^+$ synthesis and transport is mediated by key enzymes of ammoniagenesis (mitochondrial glutaminase and glutamine dehydrogenase) and gluconeogenesis (phosphoenolpyruvate carboxykinase) in the proximal tubule and the apical $Na^+/K^+(NH4^+)$-2 Cl^- cotransporter of the medullary collecting ducts. An acid pH and glucocorticoids are the two major factors which control the expression of these transporters and act in concert to coordinate the adaptation during metabolic acidosis (148).

A variety of hormones (e.g., endothelin-1, cortisol, and angiotensin II) can influence proximal tubular function by increasing NHE3 and/or NBC1 activity. Endothelin-1 (ET-1), produced by proximal tubular cells in response to acidosis, binds to its receptor and increases levels of NHE3 in the apical membrane of proximal tubular cells (151–156). The increased ET-1 levels have also been suggested to stimulate distal tubule acid secretion by increasing Na^+/H^+ exchange and decreasing HCO_3^- secretion (157, 158). Acidosis also increases cortisol levels, which increase NHE3 insertion into the apical membrane (159–162) and increases both NBC1 levels and activity (163, 164). Mineralocorticoids have also been shown to enhance H^+ secretion by directly stimulating H^+-ATPase in the distal nephron (165, 166). Angiotensin II is produced by the proximal tubule and increases insertion of NHE3 into the apical membrane as well as enhances its activity (167–169). Basolateral NBC activity is also increased (170, 171) by decreasing cAMP, activation of PKC and activation of src/MAPK pathways (172–175). This results in net H^+ secretion and HCO_3^- reabsorption. In the distal nephron angiotensin II has opposing effects by increasing HCO_3^- reabsorption in the superficial tubules but decreasing it in the outer medullary duct (176–178).

Concept of Serum Anion Gap

All solutions of dissolved salts contain equal number of dissociated positive and negative charges. This simple principle was used by Gamble as a means of analyzing clinical acid-base disorders (179). A practical approach takes advantage of the fact that most plasma ions normally exist at relatively low concentrations. The three ions with the highest plasma concentrations and the largest variances are Na^+, Cl^- and HCO_3^-. The plasma concentration of Na^+ normally exceeds the sum of Cl^- and HCO_3^- and such comparisons therefore yield what is called the anion gap:

$$Na^+ - (Cl^- + HCO_3^-)$$

The anion gap is a virtual and entirely arbitrary measurement and is a function of the specific ions incorporated in, or excluded from the equation. Anion gap calculations can also include potassium:

$$(Na^+ + K^+) - (Cl^- + HCO_3^-)$$

Since the potential absolute changes in plasma K^+ is small compared to the other 3 ions, most clinicians do not include this ion.

Usually when metabolic acidosis (or chronic respiratory alkalosis) reduces the plasma HCO_3^-, the concentration of another anion increases, and the magnitude of that increase is similar to that of the HCO_3^- reduction.

Table 9-9

Causes of mixed acid-base disorders

Mechanism	Disorders	Adaptation	Blood pH
Inadequate response	Mixed metabolic acidosis and respiratory acidosis	$PaCO_2$ ↑↑ HCO_3 ↓↓	Depressed
	Mixed metabolic alkalosis and respiratory alkalosis	$PaCO_2$ ↓↓ HCO_3 ↑↑	Elevated
Excessive response	Mixed metabolic acidosis and respiratory alkalosis	$PaCO_2$ ↓↓ HCO_3 ↓↓	Normal or decreased or increased
	Mixed metabolic alkalosis and respiratory acidosis	$PaCO_2$ ↑↑ HCO_3 ↑↑	Normal or increased or decreased
Triple acid-base disorders	Mixed metabolic alkalosis (diuretics or Cl-deficient intake) metabolic acidosis (lactic acids of sepsis to hypoxemia, hypotension), and respiratory acidosis or alkalosis	$PaCO_2$ inappropriate, HCO_3 inappropriate, anion gap exceeds 20 mEq/L	Variable
Chronic respiratory acidosis obstructive lung disease, superimposed acute respiratory acidosis from pneumonitis or congestive heart failure, acute respiratory alkalosis (intubation) mechanical ventilation	HCO_3 inappropriate	$PaCO_2$ inappropriate	Variable

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This simple calculation separates the acid-base disorder into two groups, thereby restricting the diagnostic possibilities. When the anion gap remains normal and increased Cl^- is the dominant change, the clinical situation is designated a normal-anion gap disorder or hyperchloremic metabolic acidosis (or compensated chronic respiratory alkalosis). Alternatively, if the HCO_3^- reduction is associated with an increased concentration any other anion (such as lactate or ketoacid anion) an elevated anion gap metabolic acidosis is diagnosed. Furthermore, when anion gap acidosis exists, the increase in anion gap should quantitatively mirror the fall in HCO_3^-. Disruption of this expected relationship is indicative of certain mixed acid-base disorders. For example, if the anion gap increases from normal to a far greater degree than the HCO_3^- decrease, this may indicate that the initial HCO_3^- was supranormal (pre-existing metabolic alkalosis) or that additional HCO_3^- was generated or administered during or after the metabolic acidosis. Both these possibilities define mixed anion-gap metabolic acidosis and metabolic alkalosis. Occasionally, the anion gap is abnormally small or even has a negative value. If the possibility of laboratory error is eliminated, the small or negative anion gap is likely to result from reduced concentration of a normal unmeasured anion such as albumin, pseudohyperchloremia (from hyperlipidemia) or increased concentration of unmeasured cations such as charged globulins (multiple myeloma) or lithium (as in lithium poisoning). Recognition of an unexpected abnormal anion gap is helpful in consideration of common as well as obscure disorders (179).

Normal anion gap in children is less than 12 mmol/L. Hypoalbuminemia reduces the anion gap by about 2–3 mmol/L (180). An anion gap of 12 mmol/L could represent clinically important lactic acidosis in a hypoalbuminemic patient. The normal range is higher for children younger than 2 years, 16 ± 4 mEq/L.

In recent years, another method of acid-base interpretation was introduced by Stewart, called "strong ion difference analysis" (181). This approach is similar to the anion-gap concept but requires many more quantitative measurements and calculations which makes it complicated and cumbersome for routine clinical utility. However, research in this area may help elucidate several unusual acid-base and electrolyte disorders.

Common examples of high and normal plasma anion gap acidosis are listed in *Table 9-9*.

Concept of Urine Anion Gap

In chronic metabolic acidosis, urine ammonium excretion should be elevated if renal tubular function is intact. Because ammonium is a cation, it should balance part of the negative charge of chloride. Therefore, the urine anion gap (UAG) should become progressively negative as the rate of ammonium excretion increases in response to acidosis or acid loading. UAG could therefore be used as a surrogate estimation of urinary ammonium. UAG is calculated as:

$$\mathrm{UAG} = [\mathrm{Na}^+ + \mathrm{K}^+]_{\mathrm{urine}} - [\mathrm{Cl}^-]_{\mathrm{urine}}$$

In the assessment of patients with normal serum anion-gap or hyperchloremic metabolic acidosis, a negative urine anion gap suggests gastrointestinal loss of bicarbonate whereas a positive urine anion gap suggests the presence of altered distal urinary acidification (182). The detailed approach to the clinical assessment and diagnosis of renal tubular acidosis will be discussed in a subsequent chapter (*Tables 9-10* and *9-11*).

Nutrition and Acid-Base Balance

Dietary intake, daily generation of organic acids (lactic acid, pyruvic acid and acetic acid) and excretion of bicarbonate in the stool results in net daily acid production of approximately 1 mEq hydrogen ions per kilogram body weight in adults (183). Dietary intake contributes to the majority of this daily acid production, with the latter two processes making a minimal contribution. During human evolution, with the development of agriculture and animal husbandry, our diets changed from net base or alkali-producing to net acid-producing. This occurred as net acid-producing animal foods and cereal grains replaced alkali-rich fruits and vegetables (184).

The typical modern western diet produces a net acid load or hydrogen ion in an adult of approximately 50–100 mEq/day (185, 186). Vegetarian diets that consist of vegetables, fruits and nuts generate net base, whereas non-vegetarian diets including meat, seafood and dairy products generate net acid (187). This load is markedly increased in infants who are solely fed cow milk based infant formula, which produces a net acid load of approximately 2 mEq/kg/day compared to about 0.8 mEq/kg/day when fed human milk (188). The dietary net acid load in children in the west is roughly 15–80 mEq/day, or roughly 1–3 mEq/kg/day, data obtained from population based studies, being higher in adolescents than younger children, and in males compared to females (189–192).

Metabolism of sulfur-containing amino acids cysteine and methionine, cationic amino acids arginine and lysine, and phosphorus produce acid. These substances are found in high concentrations in animal proteins, cereals,

Table 9-10
Causes of decreased and increased anion gap

Decreased anion gap	Increased anion gap
Increased cations (not Na^+)	Increased anions (not Cl^- or HCO_3^-)
↑ Ca^{2+}, Mg^{2+}	↑ Albumin concentration
↑ Li^+	Alkalosis
↑ IgG	↑ Inorganic anions
Decreased anions (not Cl^- or HCO_3^-)	Phosphate
↓ Albumin concentration (hypoalbuminemia)[a]	Sulfate
Acidosis	↑ Organic anions
Laboratory error	L-Lactate
Hyperviscosity	D-Lactate
Bromism	Ketones
Uremic	
↑ Exogenously supplied anions	
Toxins	
Salicylate	
Paraldehyde	
Ethylene glycol	
Propylene glycol	
Methanol	
Toluene	
Pyroglutamic acidosis	
↑ Unidentified anions	
Toxins	
Uremic	
Hyperosmolar, nonketotic states	
Myoglobinuric acute renal failure	
Decreased cations (not Na^+)	
↓ Ca^{2+}, Mg^{2+}	

[a]For each decline in albumin by 1 g/dL from normal (4.5 g/dL), anion gap decreases by 2.5 mEq/L
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Table 9-11

Clinical causes of high anion gap and normal anion gap acidosis

High anion gap acidosis
Ketoacidosis
Diabetic ketoacidosis (acetoacetate)
Alcoholic ketoacidosis (β-hydroxybutyrate)
Starvation ketoacidosis
Lactic acid acidosis
L-Lactic acid acidosis (types A and B)
D-Lactic acid acidosis
Toxins
Ethylene glycol
Methyl alcohol
Salicylate
Propylene glycol
Pyroglutamic acidosis
Normal anion gap acidosis
Gastrointestinal loss of HCO_3^- (negative urine anion gap)
Diarrhea
Fistulae external
Renal loss of HCO_3^- or failure to excrete NH_4^+ (positive urine anion gap = low net acid excretion)
Proximal renal tubular acidosis (RTA)
Acetazolamide (or other carbonic anlydrase inhibitor)
Classic distal renal tubular acidosis (low serum K^+)
Generalized distal renal tubular defect (high serum K^+)
Miscellaneous
NH_4Cl ingestion
Sulfur ingestion
Dilutional acidosis

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nuts and dairy products. Conversely, metabolism of anionic amino acids aspartic acid and glutamic acid, found in wheat, soy protein, cow's milk and vegetables; and potassium and magnesium containing foods like fruits, vegetables, nuts and dairy products, consume hydrogen ion (191).

Physiologically, two types of acid are important. Carbonic acid, which is a volatile acid, and produced by the metabolism of dietary carbohydrates and fats, and non-carbonic acids, produced from the metabolism of proteins. Metabolism of dietary carbohydrates and fats results in generation of carbon dioxide that can be excreted by alveolar ventilation. Approximately 15,000 mmol of volatile acid is produced per day (193). On the other hand, renal urinary excretion eliminates excess non-carbonic or non-volatile acids, mainly sulfuric acid (H_2SO_4) that is generated endogenously or from dietary intake of sulfur-containing amino acids.

Intracellular and extracellular acids and alkali are initially buffered. This process serves to neutralize the acid or base in order to minimize change in pH. Buffering does not remove acid or alkali from the body.

Two subsequent processes play an important role in maintaining normal acid-base balance: (1) the respiratory excretion of volatile acid as carbon dioxide, and (2) the ability of the kidney to excrete net acid. The non-volatile acid produced from metabolism and dietary intake is balanced with renal net acid excretion, thus maintaining acid-base equilibrium. The normal healthy kidney easily handles dietary net acid, but in the setting of reduced renal function, excess dietary load provides a challenge for excretion. In fact, there is evidence that even with normal aging, gradual reduction in glomerular filtration rate results in metabolic acidosis on a normal diet (193).

Goodman et al. demonstrated that despite minor daily fluctuations, the cumulative acid balance in normal individuals is approximately zero (194). Typical acid production in adults, estimated from extrapolation of urinary sulfate and organic anion, is equal to urinary acid output. With chronic renal insufficiency, serum CO_2 content reaches the acidotic level over a period of 6 days after withdrawal of bicarbonate therapy. Net acid production exceeds compromised or reduced net acid excretion, resulting in a positive balance of 21–30 mEq of acid per day (195). Withdrawing the alkaline therapy of a patient with chronic kidney disease may lead to metabolic acidosis resulting primarily from hydrogen ion retention.

Acid-Base Balance in Neonates, Infants and Children

Newborns and infants have a lower serum bicarbonate of about 20 mEq/L compared to older children and adults, whose serum bicarbonate is 24 mEq/L. The imbalance between increased acid load and decreased ability for net acid excretion, results in increased susceptibility of neonates and infants to development of metabolic acidosis.

The acid load from diet and metabolism in infants is approximately one hundred percent higher than that of adults, adjusted for body weight. The increased acid load is exaggerated in infants fed cow's milk formula. In small preterm babies, renal acid excretion capacity is low compared to term newborns. In both preterm and term infants, maximum renal net acid excretion improves rapidly during the first few weeks of life (189) (*Fig. 9-5*).

Insipient late metabolic acidosis is common in premature infants in the first year of life and its pathophysiology is multifactorial (189) (*Fig. 9-6*).

The growing bones of neonates and children results in production of additional acid as a result of hydroxyapatite production for bone mineralization. Approximately 0.92 mmol of protons are released into the extracellular circulation for every 1 mmol of calcium incorporated into the skeleton (196).

Broadly speaking, renal regulation of hydrogen ion balance involves (1) glomerular filtration, (2) reclamation of filtered bicarbonate, thus repleting body stores, and (3) excretion of hydrogen ion as titratable acid or ammonium, which excretes the non-volatile acid from metabolism, dietary intake, and in children, bone growth. Net urinary acid excretion consists of the sum of titratable acid (hydrogen ion buffered by phosphate and sulfate) plus ammonium.

Since the beginning of the twentieth century, it was observed that urine pH is higher in preterm and term neonates and gradually decreases after several weeks. When preterm and term infants fed human milk or formula were challenged with acute or chronic acid loading using ammonium chloride or calcium chloride, to determine maximal net renal acid excretion, the results revealed lower net acid excretion in preterm compared to term infants (188). Furthermore, there was an increase in maximum net renal acid excretion during the first few weeks in term and preterm infants (188) (*Fig. 9-3*).

Animal studies point to decreased activity and immaturity of almost every transporter involved in bicarbonate reabsorption and acid secretion as a cause for the reduced ability of the neonatal kidney to excrete acid. While it may not be possible to study these mechanisms in humans, animal studies have provided substantial insights into potential mechanisms. Less bicarbonate reclamation occurs in the proximal convoluted tubule of infants compared to adults. Whereas in adults, approximately 85% of filtered bicarbonate is reabsorbed proximally, only 65% of the bicarbonate is reabsorbed proximally in infants (189).

Studies in rabbit proximal convoluted tubules show that the lower neonatal bicarbonate transport is due to lower activities of all the transporters involved in proximal bicarbonate reabsorption, that is, the apical Na^+/H^+ antiporter, apical H^+-ATPase, the basolateral $Na^+/3HCO_3^-$ transporter, and the basolateral Na^+-K^+-ATPase (197–200). The activity of all these transporters reached adult levels by the age of 6–7 weeks in these studies. One of the major hormones that stimulate the development of bicarbonate transport is glucocorticoids. Neonates

Figure 9-5

Maximum renal excretion of net acid and ammonium in preterm and term infants on nutrition with human milk or formulas. (Reproduced with permission from reference 189.)

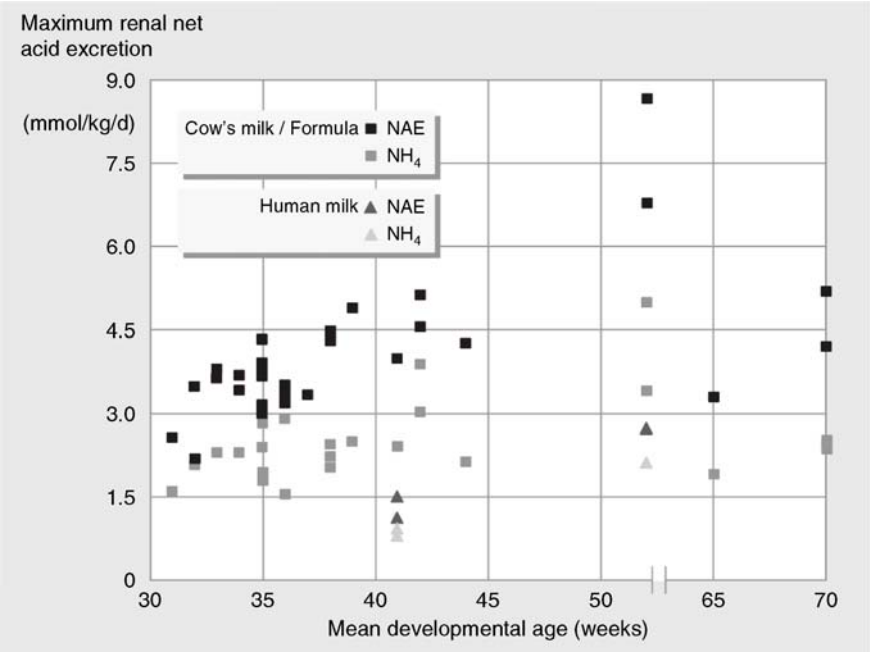
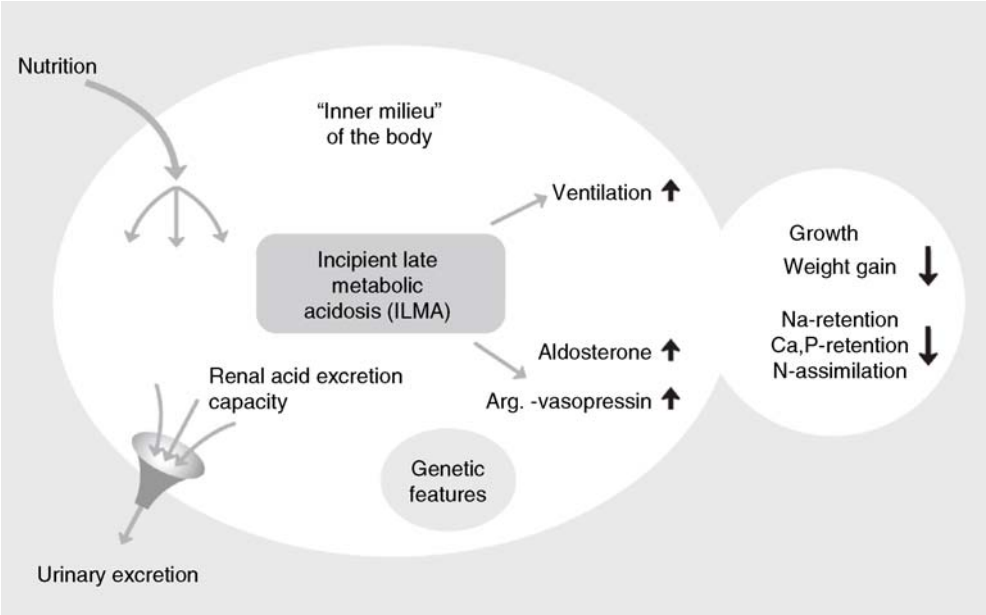


Figure 9-6

Pathophysiological mechanisms in premature infants with incipient late metabolic acidosis (ILMA). Reproduced with permission from reference 189.



are relatively glucocorticoid-deficient during the first weeks of life. There is a developmental increase in glucocorticoids which precedes the increase in bicarbonate transport. Indeed, if glucocorticoids are administered to pregnant animals prior to giving birth, the neonates have proximal tubular bicarbonate transport rates which are comparable to those in adults (197). Thyroid hormone also affects the development of bicarbonate transport but plays a much less important role.

The limited ability for urinary acidification may in part be due to carbonic anhydrase II deficiency in immature renal tubules. This isoform of carbonic anhydrase is found in the cytoplasm of proximal and distal tubular cells. Karashima et al. found that carbonic anhydrase II levels increased from 14% of adult levels in 1 week old rats to 40% at age 3 weeks and 97% at age 7 weeks (200). Carbonic anhydrase IV is the isoform that is membrane bound on the luminal surface of tubular cells. There is decreased activity of carbonic anhydrase IV in neonatal rabbit kidneys compared with adults (201, 202).

However, human studies provided contradictory data. Studies of kidney tissue from human embryos showed positive staining for carbonic anhydrase in proximal and distal tubules from 12 to 15 weeks gestation. The catalytic activity and the amount of carbonic anhydrase increased as gestational age increased from 19 to 26 weeks, being comparable to those of adult renal cortex at 26 weeks (203). Thus, in humans, carbonic anhydrase may not play a major role in the maturational changes associated with renal acid-base homeostasis.

Intercalated α and β cells in the collecting duct are responsible for distal urinary acidification. There are fewer intercalated cells in the rabbit neonatal kidney and the activity of the apical H^+-ATPase is reduced (204). Additionally, the capacity for ammonium excretion is reduced in newborn animals (205, 206). Thus, the ability for distal urinary acidification by excreting hydrogen ions is limited in newborns.

Therefore, maturational deficiencies in almost every transporter involved in acid-base homeostasis throughout the renal tubule puts the neonate at higher risk for developing metabolic acidosis. This limited capacity for acid excretion, in conjunction with increased acid load from the diet and bone formation in growing children are important factors in acid-base homeostasis.

Mechanisms of Growth Retardation in Chronic Acidosis

Poor growth occurs in many chronic disorders such as chronic kidney disease, cystic fibrosis, rheumatologic

conditions and inflammatory bowel disease. Growth retardation is a frequent, long-recognized complication of chronic metabolic acidosis (207).

Studies in acidotic children with classic renal tubular acidosis have shown a blunted release of growth hormone in response to provocative stimuli. The growth retardation associated with acidosis can be reversed by systemic bicarbonate therapy (208). The release of growth hormone under acidotic conditions has been studied (209). Exploration in rats with normal anion gap acidosis from ingestion of ammonium chloride demonstrated aberrant GH secretion. Significant inhibition of pulsatile growth hormone secretion was present in the acidotic rats such that both the amplitude of the GH secretory pulse and the area under the curve were significantly smaller than in controls. These reductions in pulse amplitude and area were correlated to decreased growth (weight) in the acidotic rats (209). Using in situ hybridization histochemistry in combination with immunocytochemistry, the expression of GH/insulin-like growth factor (IGF)-I in the tibial epiphyseal growth plate has been examined. Evaluation of tibial epiphyseal growth plate (IGF-I) gene expression in acidotic and control rats revealed that IGF-I messenger RNA abundance was lower in the acidotic growth plates. IGF-I peptide was predominantly localized to the hypertrophic zone of chondrocytes and was weakly detectable in the proliferative zone in both the acidotic and control rats' growth plates. Anthropomorphic measurements demonstrated that acidotic rats grew less than did control rats in both length and weight, and these physical measurements were reflected in the size of the tibial epiphyseal growth plates being significantly smaller in the acidotic rats compared with the control group (210). Taken together, these observations suggest that metabolic acidosis reduces IGF-I message abundance and induces resistance to IGF-I peptide action within the tibial epiphyseal growth plate. The use of growth hormone to stimulate growth in normal anion gap acidosis has been ineffective experimentally, despite enhancement of IGF-I and IGF binding protein immunoreactivity within the stem cell chondrocyte zone of the tibial epiphyseal growth plate (210).

Effect of Acidosis on Bone and Calcium Homeostasis

The renal response to acidosis, which consists of hydrogen ion excretion, is relatively slow and takes days. The initial response to acidosis is buffering by extracellular bicarbonate and by cellular and bone buffers. This role of the skeleton as buffer occurs at the expense of bone mineral content resulting in loss of calcium and phosphate (211). Bone sodium and potassium is lost in exchange for hydrogen ion, and carbonate consumed as a buffer. Positive acid and negative calcium balance (due to hypercalciuria) occurs in healthy individuals made chronically acidotic by ammonium chloride loading (212, 213). A similar profile is found in patients with renal tubular acidosis. Correction of the metabolic acidosis with bicarbonate therapy results in return of acid balance to zero and less negative calcium balance (214). Furthermore, bicarbonate administration corrects bone mineral loss in patients with acidosis (215, 216).

Acutely, bone resorption is primarily due to physicochemical mineral dissolution, while cell-mediated mechanisms predominate after 24 h (211). Chronic metabolic acidosis stimulates calcium efflux from bone due to increased osteoclastic bone resorption and decreased osteoblastic activity. The negative calcium balance observed in acidotic patients is due to calcium mobilization from bone, resultant hypercalciuria (217, 218), and lack of concomitant increase in intestinal calcium absorption (215, 219). There is net loss of body calcium. The hypercalciuria predisposes to osteoporosis, nephrocalcinosis and nephrolithiasis. The latter renal effects result in renal impairment as evidenced in the study by Goodman et al., in which few adult patients with RTA had normal glomerular filtration rate (194).

Patients with chronic kidney disease with concomitant metabolic acidosis and hyperparathyroidism are at increased risk for skeletal demineralization. Krieger et al. studied the effect of acidosis with and without parathyroid hormone on calvariae (211). They found that acidosis and PTH independently stimulated calcium efflux from bone, inhibited osteoblastic collagen synthesis and stimulated osteoclastic beta-glucuronidase secretion. Furthermore, the effects of acidosis and PTH on bone resorption were additive.

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10 Calcium and Phosphorus

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Calcium

Calcium Homeostasis

Calcium Distribution in the Body

Calcium is the most abundant electrolyte in the human body, and in healthy adults, accounts for about 2%, or 1,300 g, of body weight. Approximately 99% of body calcium is in the skeleton mainly in the form of hydroxyapatite crystals [$Ca_{10}(PO_4)_6(OH)_2$]; the remainder is in teeth, soft tissue, and extracellular fluid. By contrast, at birth calcium accounts for only about 0.9% of body weight (1). From birth to approximately 20 years of age, when the skeleton reaches its full size and density, calcium content increases by some 40-fold (2). During this period, the increase in skeletal weight and calcium content requires the net retention of about 150–200 mg of calcium per day. Thus, in growing individuals, calcium balance must be positive to meet the needs of skeletal growth and consolidation. In adults, calcium balance is zero after peak bone mass is attained and becomes slightly negative as bone is slowly lost with aging.

Calcium Chemistry

Calcium in plasma exists in three fractions: protein-bound calcium (40%), which is not filtered by the renal glomerulus, and ionized calcium (48%) and complexed calcium (12%), which are filtered (3). Complexed calcium is that bound to various anions such as phosphate, citrate, and bicarbonate. Albumin accounts for 90% of the protein binding of calcium in plasma; globulins the remainder. Conditions that affect the concentration of albumin in plasma, such as nephrotic syndrome or hepatic cirrhosis, will affect the measurement of total serum calcium concentration. A decrease in albumin concentration of 1 g/dl results in a decrease in protein-bound and hence total calcium concentration of about 0.8 mg/dl. Binding of calcium to albumin is strongly pH-dependent between pH 7 and pH 8; an acute increase or decrease in pH of 0.1 pH units will increase or decrease, respectively, protein-bound calcium by about 0.12 mg/dl. Thus, in hypocalcemic patients with metabolic acidosis, rapid correction of acidemia with sodium bicarbonate can precipitate tetany, due to increased binding of calcium to albumin and a consequent decrease in the ionized calcium concentration.

Extracellular Calcium Homeostasis

Calcium homeostasis is maintained by the interaction between three major organ systems, bone, intestine, and kidney, and is regulated principally by parathyroid hormone (PTH) and 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), and to a lesser extent, by calcitonin. In healthy adults, net intestinal absorption of calcium is approximately 20–25% of dietary intake. To meet the demands of rapid skeletal growth, fractional calcium absorption in infants is higher, 40–45%, reaching values as high as 80% in low-birth-weight, breast-fed infants (4, 5). The efficiency of calcium absorption also is increased during adolescence, during pregnancy, and with administration of vitamin D metabolites, and is decreased with vitamin D deficiency, in the elderly, and with estrogen deficiency. Calcium is absorbed principally in the duodenum and proximal jejunum, both by a saturable, active transport mechanism that requires stimulation by $1,25(OH)_2D$, and by a nonsaturable, passive diffusion mechanism. A small amount of calcium is secreted into the intestinal lumen, presumably by paracellular diffusion. An overall schema of calcium metabolism is depicted in *Fig. 10-1*.

Absorbed calcium enters the extracellular calcium pool, which is in equilibrium with the bone calcium pool; the latter includes a rapidly exchangeable pool which plays an important role in maintaining extracellular calcium concentration, and a more stable bone mineral pool. Calcium is filtered by the renal glomerulus and is nearly completely reabsorbed by the renal tubule. In subjects in zero calcium balance, the amount of calcium excreted by the kidney is equal to the net amount absorbed by the intestine, and in growing children is less than the net amount absorbed due to deposition of calcium in bone.

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Figure 10-1

Calcium fluxes between body pools in the normal adult human in zero calcium balance.

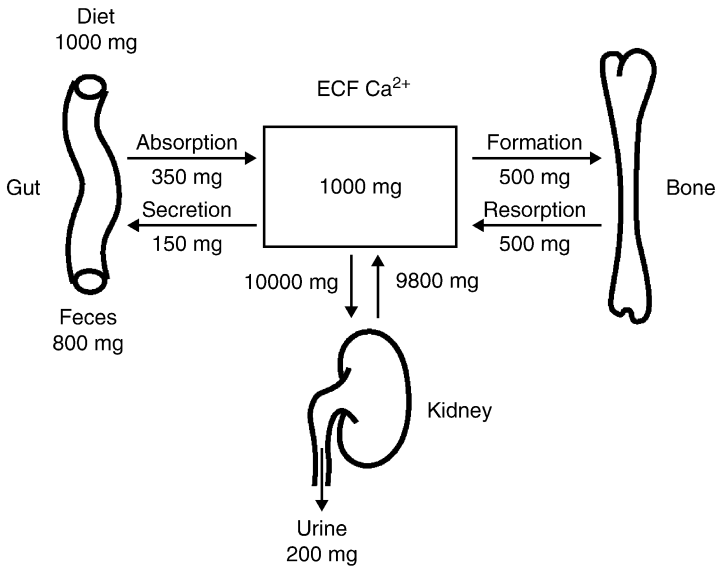
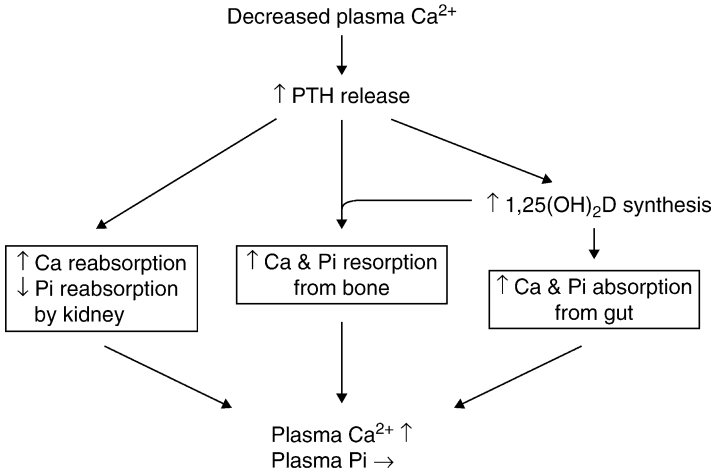


Figure 10-2

The homeostatic response to hypocalcemia.



In response to a decrease in extracellular concentration of ionized calcium, secretion of PTH from the parathyroid gland is increased (*Fig. 10-2*). PTH acts on the kidney to decrease excretion of calcium, to increase excretion of phosphate (Pi), and to stimulate the production of $1,25(OH)_2D$. $1,25(OH)_2D$ acts on the intestine to stimulate active absorption of calcium and Pi, and together with PTH, acts on bone to stimulate release of calcium and Pi into the extracellular fluid. PTH action on bone is thought to occur in two phases, an initial rapid mobilization of bone mineral that occurs within hours, is associated with increased metabolic activity of osteoclasts, and does not require protein synthesis, and a later phase that occurs after 12–24 h of exposure to PTH, is associated with an increase in both activity and numbers of osteoclasts, and does require protein synthesis (6). The combined

effects of PTH and $1,25(OH)_2D$ on their target tissues result in an increase in extracellular calcium concentration toward normal values, with the serum Pi concentration being little changed. Conversely, in response to an increase in blood ionized calcium concentration, secretion of PTH and production of $1,25(OH)_2D$ are decreased, and release of calcitonin is stimulated. The exact physiologic role of calcitonin in reducing hypercalcemia is unknown (7). The combined effects of these hormonal changes on bone, kidney, and intestine are opposite to those occurring with hypocalcemia, resulting in a decrease in calcium concentration toward normal values.

The total serum calcium concentration exhibits a circadian rhythm characterized by a nadir at 1–3 am and a peak at 12–1 pm, with amplitude (nadir to peak) of about 0.5 mg/dl (*Table 10-1*) (8–10). This rhythm is thought to reflect hemodynamic changes in serum albumin concentration that result from changes in body posture (11). Prolonged upright posture or venostasis can cause hemoconcentration and thus increases of about 0.5 mg/dl in serum calcium concentration. There is little difference between values taken in fasting and non-fasting states.

Normal values for serum total calcium concentration differ among clinical laboratories, and in general range from 9.0 to 10.6 mg/dl. In children, the calcium concentration is higher than in adult subjects, being highest at 6–24 months of age, mean ~10.2 mg/dl, decreasing to a plateau of ~9.8 mg/dl at 6–8 years and decreasing further to adult values at 16–20 years (*Table 10-2*) (12). In men, the calcium concentration decreases from a mean of ~9.6 mg/dl at age 20 to ~9.2 mg/dl at age 80 years; the decrease can be accounted for by a decrease in serum albumin concentration (13). In women, no change is observed with age. Ionized calcium is the fraction of plasma calcium that is important for physiologic processes such as muscle contraction, blood coagulation, nerve conduction, hormone (PTH and $1,25(OH)_2D$) secretion and action, ion transport, and bone mineralization. Measurement of the blood ionized calcium concentration is most useful in critically ill patients, particularly those in whom serum protein levels are decreased, acid–base disturbances are present, or to whom large amounts of citrated blood products are given, such as with cardiac surgery or hepatic transplantation. A decrease in the blood ionized calcium concentration can occur due to increased binding of calcium to albumin, such as with metabolic alkalosis, or due to increased complexing with other anions. For example, in severe uremia, the ionized fraction of calcium can decrease due to increased complexing with Pi, sulfate, and citrate (14). Based on in vitro studies of human serum (15), an increase in serum Pi concentration of 3.7 mg/dl was required to induce a decrease in ionized calcium of 0.1 mg/dl, the smallest decrease thought necessary to stimulate release of PTH (16, 17).

In normal individuals, values of ionized calcium will vary somewhat among laboratories depending on which technique is employed and whether the measurement is made in serum, plasma, or heparinized whole blood. In healthy infants, ionized calcium levels decrease from ~5.8 mg/dl (1.4 mmol/l) at birth to a nadir of 4.9 mg/dl (1.2 mmol/l) at 24 h of life (18), and increase slightly during the first week of life (*Table 10-2*) (19). Values in young children are slightly higher (~0.2 mg/dl) than those in adults until after puberty. In adult men and women, normal serum ionized calcium concentrations range from 4.6 to 5.3 mg/dl (1.0–1.3 mmol/l), without significant sex differences (20, 21). The blood ionized calcium concentration exhibits a circadian rhythm characterized by a peak at 10 am and a nadir at 6–8 pm, with an amplitude of 0.3 mg/dl (*Table 10-1*) (8). Specimens must be obtained anaerobically to avoid spurious results due to ex vivo changes in pH.

Calcium-Sensing Receptor

The calcium-sensing receptor (CaR) plays a central role in regulating calcium homeostasis. Extracellular ionized calcium concentration is maintained within a tight range (1.1–1.3 mM) by activation and inhibition of the CaR

Table 10-1

Characteristics of the circadian rhythms in blood mineral concentration in humans

	Concentration (mg/dl)		Amplitude (mg/dl)	Phase (hour)	
	Fasting	24-h mean	(Nadir to peak)	Nadir	Peak
Total serum calcium	9.6	9.4	0.5	03:00	13:00
Blood ionized calcium	4.67	4.52	0.3	19:00	10:00
Serum phosphorus	3.6	4.0	1.2	11:00	02:00

Data are from references (8, 10)

Table 10-2

Representative normal values for concentrations of blood ionized calcium, serum total calcium and phosphorus at various ages

	Age	Blood ionized		Serum total	
		Calcium		Calcium	Phosphorus
	(yr)	(mg/dl)	(mM)	(mg/dl)	(mg/dl)
Infants	0–0.25	4.9–5.6	(1.22–1.40)	8.8–11.3	4.8–7.4
	1–5	4.9–5.3	(1.22–1.32)	9.4–10.8	4.5–6.2
Children	6–12	4.6–5.3	(1.15–1.32)	9.4–10.3	3.6–5.8
Men	20	4.5–5.2	(1.12–1.30)	9.1–10.2	2.5–4.5
	50	4.5–5.2	(1.12–1.30)	8.9–10.0	2.3–4.1
	70	4.5–5.2	(1.12–1.30)	8.8–9.9	2.2–4.0
Women	20	4.5–5.2	(1.12–1.30)	8.8–10.0	2.5–4.5
	50	4.5–5.2	(1.12–1.30)	8.8–10.0	2.7–4.4
	70	4.5–5.2	(1.12–1.30)	8.8–10.0	2.9–4.8

Data are from references (12, 13, 18, 19, 21, 239, 240, 403)

located on the plasma membrane of parathyroid cells. When serum ionized calcium concentrations are increased, the CaR is activated, leading to a decrease in PTH synthesis and secretion and suppression of parathyroid cell proliferation; the opposite occurs when serum ionized calcium concentrations are decreased (22). The CaR has been cloned from bovine, human, and rat parathyroid tissue (23–25), from rat kidney (26), and from several other mammalian and non mammalian organisms (27–29). The nucleic acid and amino acid sequence of CaR is highly conserved throughout evolution, with retention of functionally important structural features and limited divergence (22).

The CaR has a predicted molecular weight of ~120 kD and is a member of family C II of the superfamily of guanine-nucleotide-regulatory (G) protein-coupled receptors (GPCRs). CaR activates many intracellular signaling pathways including phospholipase A2, C and D, and mitogen activated protein kinase (MAPK) pathways (22), with phospholipase C being the major downstream mediator of the biological response. Activation of the CaR results in increased activity of phospholipase-C, which catalyzes the hydrolysis of the membrane-bound phospholipid, inositol 4,5-bisphosphate, to two second messengers, inositol 1,4,5-trisphosphate (IP3) and diacylglycerol. Intracellular accumulation of IP3 induces release of calcium from storage pools and thereby a rapid increase in cytosolic calcium concentration, and possibly an increase in movement of calcium from the extracellular to the cellular compartment. In parathyroid cells, the increase in cytosolic calcium concentration is associated with a decrease in PTH secretion. Thus, CaR enables the parathyroid gland to tightly regulate PTH secretion in response to changes in serum calcium levels. CaR is also widely expressed along the entire nephron in the kidney, in bone, cartilage and intestine. The physiology and pathophysiology of CaR and the role of calcimimetic drugs in human disorders of calcium homeostasis are detailed in several comprehensive reviews (22, 30, 31).

Vitamin D

Vitamin D exists as either ergocalciferol (vitamin D_2) produced by plants, or cholecalciferol (vitamin D_3) produced by animal tissues and by the action of near ultraviolet radiation (290–320 nm) on 7-dehydrocholesterol in human skin. Both forms of vitamin D are biologically inactive pro-hormones that must undergo successive hydroxylations at carbons #25 and #1 before they can bind to and activate the vitamin D receptor. The 25-hydroxylation of vitamin D occurs in the liver, catalyzed by one or more enzymes including the microsomal cytochrome P450 enzyme, CYP2R1 (32). The activity of hepatic 25-hydroxylation is not under tight physiologic regulation, and thus circulating concentrations of 25-hydroxyvitamin D (25OHD) are determined primarily by dietary intake of vitamin D and exposure to sunlight. Although 25OHD is the most abundant form of vitamin D in the blood, it has minimal capacity to bind to the vitamin D receptor and elicit a biologic response. Circulating 25OHD is almost entirely bound to vitamin D binding

protein (DBP). In DBP knockout mice, serum 25OHD concentrations are low due to increased catabolism of 25OHD in the liver and its increased excretion in the urine (33). 25OHD bound to DBP is filtered by the glomerulus and reabsorbed by the proximal tubule where its uptake at the apical membrane is mediated by two endocytic receptors, megalin and cubulin. Mice with megalin or cubulin deficiency develop vitamin D deficiency due to increased urinary losses of 25OHD (34–36), as occurs in DBP-null mice. Therefore DBP, megalin and cubulin are responsible for targeted delivery of 25OHD to the renal proximal tubule cells for further bioactivation.

The active form of vitamin D, 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), is produced by the 1α-hydroxylation of 25OHD by the mitochondrial enzyme, 25-hydroxyvitamin D-1α-hydroxylase (1α-hydroxylase or P450c1α) in the renal proximal tubule. The circulating concentration of $1,25(OH)_2D$ primarily reflects its synthesis in the kidney; however, 1α-hydroxylase activity also is found in keratinocytes, macrophages, and osteoblasts (37–39). The 1α-hydroxylation is the rate-limiting step in the bioactivation of vitamin D, and enzyme activity in the kidney is tightly regulated. $1,25(OH)_2D$ is one of the principal hormonal regulators of calcium and Pi metabolism and thus is critically important for normal growth and mineralization of bone. The classical actions of $1,25(OH)_2D$ are to stimulate calcium and Pi absorption from the intestine, thereby maintaining plasma concentrations of these ions at levels sufficient for normal growth and mineralization of bone. $1,25(OH)_2D$ also has direct actions on bone, kidney, parathyroid gland, and on many other tissues unrelated to mineral metabolism (reviewed in (40)).

The other important vitamin D-metabolizing enzyme, the 25-hydroxyvitamin D-24-hydroxylase (24-hydroxylase), is found in kidney, intestine, lymphocytes, fibroblasts, bone, skin, macrophages, and possibly other tissues (41). The enzyme can catalyze the 24-hydroxylation of 25OHD to $24,25(OH)_2D$ and of $1,25(OH)_2D$ to $1,24,25(OH)_3D$; both reactions are thought to initiate the metabolic inactivation of vitamin D via the C24-oxidation pathway. The kidney and intestine are major sites of hormonal inactivation of vitamin D by virtue of their abundant 24-hydroxylase activity.

The synthesis of $1,25(OH)_2D$ in the kidney is subject to complex regulation by PTH, calcium, Pi, fibroblast growth factor 23 (FGF-23), and $1,25(OH)_2D$ (40, 42, 43, 346). Synthesis of $1,25(OH)_2D$ can be stimulated by PTH, insulin-like growth factor 1, and phosphorus deficiency, and suppressed by plasma ionized calcium, FGF-23, and $1,25(OH)_2D$ itself. The renal 1α-hydroxylase enzyme is a mitochondrial cytochrome P450 mixed-function oxidase that requires the presence of two electron transport intermediates for catalytic activity, a flavoprotein termed ferredoxin reductase and an iron/sulfur protein termed ferredoxin (44); these two proteins mediate the transfer of electrons from NADPH to the 1α-hydroxylase. The complementary DNA (cDNA) for the 1α-hydroxylase has been cloned from human, rat, mouse, and pig (45–50). The human 1α-hydroxylase cDNA is 2.4 kb in length and encodes a protein of 508 amino acids with a predicted molecular mass of 56 kDa (45). The human gene for 1α-hydroxylase, designated CYP27B1, is single copy, comprises nine exons and eight introns, and is located on chromosome 12 (46, 51). Although it is a substantially smaller gene, 5 kb, than those for other mitochondrial P450 enzymes (51), its intron/exon organization is very similar, especially to that of P450scc (51, 52). This strongly suggests that although the mitochondrial P450 enzymes retain only 30–40% amino acid sequence identity with each other, they all belong to a single evolutionary lineage. The mouse P450c1α gene also has been cloned (53, 54).

Loss-of-function mutations in the human CYP27B1 gene result in the autosomal recessive disease, vitamin D 1α-hydroxylase deficiency (45, 55–62), also known as hereditary pseudo-vitamin D deficiency rickets (PDDR) (63), vitamin D dependency (64), or vitamin D-dependent rickets type I. As of this writing a total of 36 different mutations have been found in 54 patients since the first description of gene mutations in 1997 (45, 60).

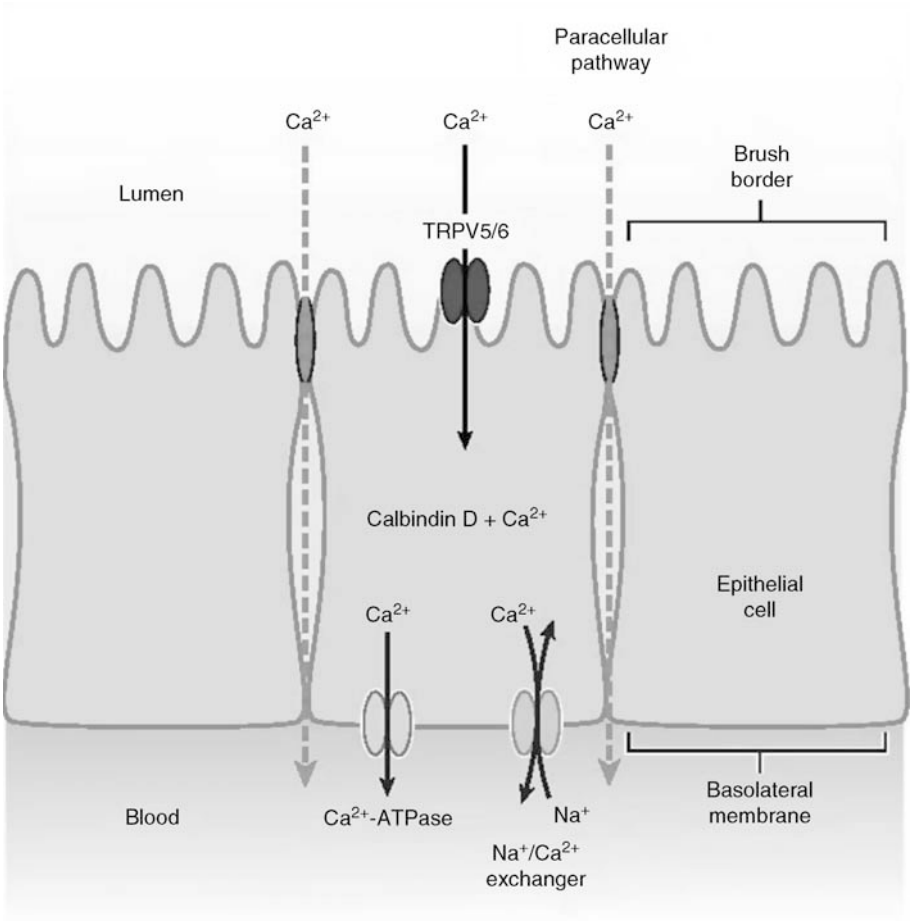
Transepithelial Calcium Transport

Transport of calcium across the plasma membrane of calcium-absorbing epithelia such as the intestine and renal tubule occurs via either the paracellular (between cells) or the transcellular (across cells) pathway, or via both pathways (*Fig. 10-3*). Paracellular transport is passive, linked to the net paracellular absorption (lumen-to-interstitium) of water, a process termed solvent drag or convection. Paracellular transport of calcium also can occur by passive diffusion driven by a chemical gradient, as occurs in the proximal convoluted tubule (PCT), or a lumen-positive transepithelial potential difference that results from sodium chloride reabsorption, as occurs in the thick ascending limb of Henle's loop.

Transcellular transport of calcium in the intestine and kidney is a three-step process (*Fig. 10-3*). Calcium enters the cell across the apical membrane via the epithelial calcium channels, TRPV5 in the kidney and TRPV6 in the intestine (65). It is then ferried across the cytosol by

Figure 10-3

Mechanism of epithelial Ca^{2+} absorption in the intestine and kidney. In the intestine, the TRPV6 epithelial Ca^{2+} channel, which is expressed in the brush border membrane, mediates the first step in transepithelial Ca^{2+} absorption. Within the epithelial cell, Ca^{2+} binds to calbindin-D9k. Ca^{2+} exits the cell at the basolateral membrane via the Ca^{2+}-ATPase, PMCA1b, and possibly the Na^+/Ca^{2+} exchanger, NCX1 (SLC8A1). Ca^{2+} reabsorption in the renal distal convoluted tubule proceeds in a similar fashion but with the following variations: (a) Ca^{2+} entry at the luminal membrane is mediated predominantly by the epithelial Ca^{2+} channel, TRPV5. (b) Ca^{2+} is shuttled to the basolateral membrane via both calbindin-D9k and calbindin-D28k. (c) Ca^{2+} exit proceeds via PMCA1b and NCX1 (SLC8A1). Under high luminal Ca^{2+} conditions, Ca^{2+} is absorbed, via the paracellular route, through the tight junctions down the transepithelial Ca^{2+} gradient. (Data from (65)).



calcium binding proteins – calbindin-D9k (in the intestine) and calbindin-D28K (in the kidney). Active extrusion across the basolateral membrane is mediated by a high affinity Ca^{2+}-ATPase (PMCA1b) and Na^+–Ca^{2+} exchanger (NCX1) (66).

Calcium Entry

Calcium channels. Although calcium entry across the luminal membrane is favored by both electrical and chemical gradients (67, 68), the physical chemical properties of lipid bilayer membranes prevent passive diffusion of the positively charged calcium ion (Ca^{2+}) across cell membranes. Thus, calcium entry across the luminal membranes of the kidney and intestine is thought to occur through Ca^{2+} channels. Studies using a variety of approaches including Ca^{2+} channel agonists and antagonists, patch-clamp analysis, and cell-attached electrophysiological techniques, have shown the presence of Ca^{2+} channels in proximal and distal nephron segments and in cultured distal renal tubule cells (69). Using

an expression cloning strategy, Hoenderop et al. (70) cloned and characterized the rabbit cDNA encoding a new epithelial Ca^{2+} influx channel, which was named ECaC/ECaC1 by analogy with the amiloride-sensitive, aldosterone-dependent epithelial sodium channel, ENaC (71, 72). The rabbit ECaC cDNA encodes a protein of 730 amino acids with a predicted molecular mass of 83 kDa (70). ECaC has been identified from several species including rabbit, rat, mouse, and human (69, 73). The amino acid sequence and predicted structure of ECaC closely resemble those of the superfamily of transient receptor potential (TRP) proteins, and hence ECaC was renamed TRPV5. Shortly after identification of the renal epithelial calcium channel, an expression cloning strategy was used to clone and characterize the cDNA for calcium transport protein (CaT1/ECaC2) from rat duodenum (74). CaT1 showed 75% homology to ECaC and was later renamed TRPV6. Studies in puffer fish show the presence of a single gene encoding a calcium channel (FrECaC) that closely resembles TRPV6, suggesting that early in the evolution of vertebrates, TRPV5 and TRPV6 evolved from a single ancestral gene (75, 76). Thus, the epithelial Ca^{2+} channels TRPV5 in the kidney and TRPV6 in the intestine are the gatekeepers of Ca^{2+} entry into the cell.

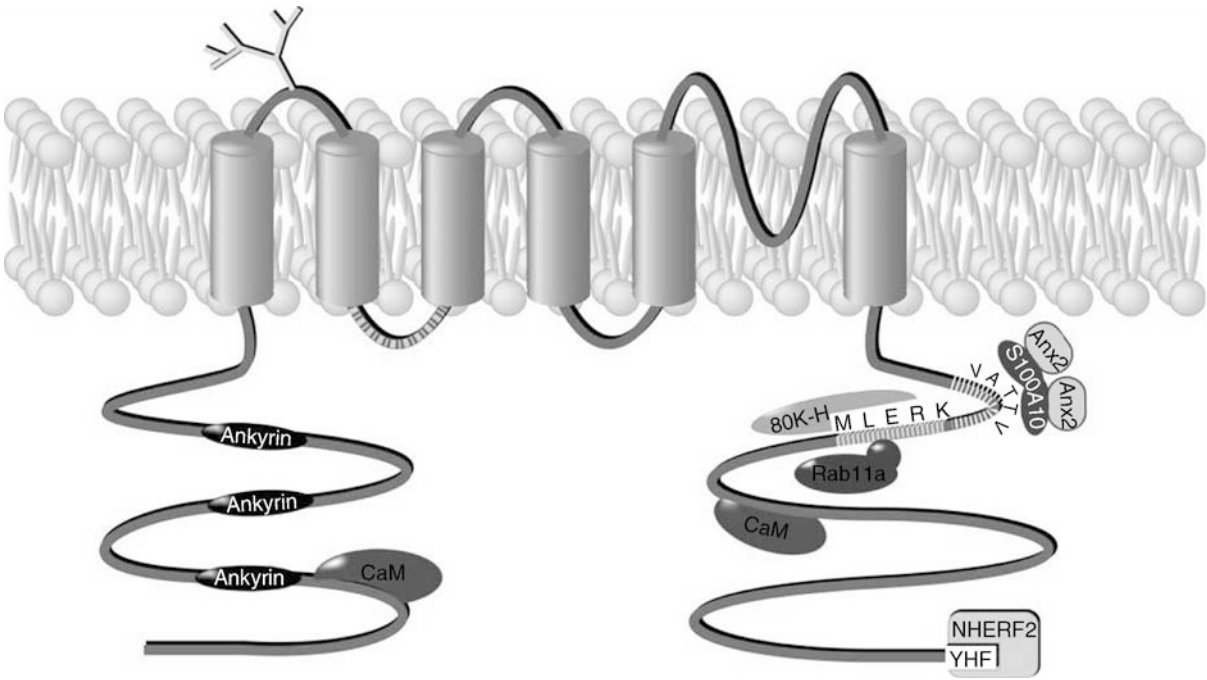
The TRP family of proteins is a diverse group of voltage-independent, cation-permeable channels that are organized into six protein subfamilies. TRPV5 and TRPV6 belong to the subgroup, TRPV (named after the founding member, vanilloid receptor) and are highly selective for Ca^{2+}. TRP channels possess six transmembrane domains with N- and C-termini located in the cytoplasm (*Fig. 10-4*) (77). TRPV5 and TRPV6 share typical topological features with other members of the TRP family. A hydrophobic loop region between transmembrane regions five and six is predicted to form the cation pore. The large intracellular N- and C-terminal domains contain putative regulatory regions that regulate channel activity and trafficking (69). Some of the regulatory sites identified are phosphorylation sites, postsynaptic density protein (zona occludens) motifs, and ankyrin repeat domains. Electrophysiological studies in HEK 293 cells show that both TRPV5 and TRPV6 are permeable to monovalent and divalent cations with a high selectivity for Ca^{2+}. A single aspartic residue in the pore region at position number 542 (D542) is critical for Ca^{2+} permeation (78, 79). TRPV5 and TRPV6 can form homo- and hetero-tetrameric ion channels, suggesting that the four aspartic residues form a negatively charged ring that selectively filters Ca^{2+} (80). The current–voltage relationship of TRPV5 and TRPV6 show inward rectification (unlike other TRPV channels which show outward rectification) and exhibit a Ca^{2+}-dependent feedback mechanism of regulating channel activity (81, 82). However, TRPV5 and TRPV6 exhibit different channel kinetics with respect to Ca^{2+}-dependent inactivation and recovery (82, 83). They also are differentially expressed in mammalian tissues as described below.

TRPV5 and TRPV6 are coexpressed in several tissues such as duodenum, jejunum, colon, kidney, pancreas, prostate, mammary, salivary, and sweat glands (84). However, the relative mRNA expression of TRPV5 and TRPV6 differ in various tissues; in the kidney, TRPV5 is 100 times more abundant than TRPV6, whereas in the intestine, TRPV6 is the major Ca^{2+} channel expressed. In rabbit kidney, TRPV5 is expressed exclusively in the distal nephron, specifically in the apical membranes of the distal convoluted tubule (DCT), connecting tubule (CNT), and cortical collecting duct, where it colocalizes with calbindin-D28k (70, 85). The distal nephron is the major site of regulation of Ca^{2+} reabsorption by PTH and $1,25(OH)_2D$, and TRPV5 is thought to play a major role in such regulation. In the intestine, TRPV6 is the predominant calcium channel that facilitates Ca^{2+} absorption, and it also is regulated by PTH and $1,25(OH)_2D$.

Mouse models of targeted disruption of the TRPV5 and TRPV6 genes have provided insights into the important physiological role of these Ca^{2+} channels in maintaining calcium homeostasis. Ablation of the TRPV5 gene induces severe hypercalciuria, increased serum $1,25(OH)_2D$ levels, and decreased bone mineral density (86). The increase in $1,25(OH)_2D$ induced a compensatory increase in duodenal TRPV6 and calbindin-D9k expression to maintain normocalcemia (87). However, despite high serum $1,25(OH)_2D$, which stimulates distal nephron calcium reabsorption, no compensatory increase in Ca^{2+} reabsorption was observed due to lack of TRPV5 expression. Therefore, TRPV5 channel activity was established as the rate-limiting step in renal tubular Ca^{2+} reabsorption. Surprisingly, TRPV6 knockout mice displayed a more severe phenotype than did the TRPV5 knock out mice, in that TRPV6-null mice developed growth retardation, reduced fertility, alopecia, and dermatitis in addition to decreased bone mineral density. These mice also exhibit impaired intestinal calcium absorption, hypercalciuria on a normal (1%) calcium diet, and hypocalcemia on a restricted (0.25%) calcium diet. The lack of TRPV6 expression resulted in failure to appropriately increase intestinal and renal Ca^{2+} reabsorption despite high PTH and $1,25(OH)_2D$ levels (88). Thus, TRPV6 was established as the rate-limiting step in intestinal Ca^{2+} absorption, but it also plays a significant role in other tissues such as skin and gonads.

Figure 10-4

Predicted topology of TRPV5/6 including the various binding sites identified for each associated protein. The extracellular loop between transmembrane domain (TM) 1 and TM2 is glycosylated. At the COOH terminus, Rab11, 80K-H, and S100A10 bind within a 30-amino acid-containing helical stretch juxtaposed to TM6. The S100A10-annexin 2 (Anx2) complex interacts with a conserved region within this stretch containing the VATTV amino acid sequence, whereas Rab11a and 80K-H share a COOH terminal binding site that flanks this sequence. Na^+/H^+exchanger regulatory factor 2 (NHERF2) specifically interacts with TRPV5 at the extreme COOH terminus of this channel. Calmodulin (CaM) has multiple binding sites present in the NH2 terminus, COOH terminus, and transmembrane region. The region between TM2 and TM3 of TRPV6 (indicated by vertical lines) is functionally linked to the fast Ca^{2+}–dependent inactivation of this channel, suggesting the binding of an unidentified protein to this region. (Data from (401)).



Transcytoplasmic Calcium Movement

Calbindins. Calcium diffusion through the cytoplasm is currently thought to be facilitated by the calcium binding proteins, calbindins-D, whose synthesis is dependent on $1,25(OH)_2D$. Calbindins also are proposed to act as an intracellular Ca^{2+} buffer to keep the otherwise tightly regulated cytosolic calcium concentration within physiologic levels during periods of stimulated transcellular Ca^{2+} transport (89–93). Two forms of calbindin-D have been described; a 28 kDa protein (calbindin-D28k) found in highest concentration in avian intestine and avian and mammalian kidney, brain, and pancreas, and a 9 kDa protein (calbindin-D9k) found in highest concentration in mammalian intestine, placenta, and uterus but also present in kidney, lung, and bone (94, 95). Much is now known about the amino acid sequence, X-ray crystal structure, and biophysical and calcium-binding properties of the calbindins. Calbindin-D28k is highly conserved in evolution, with a high degree of sequence homology observed among the various mammalian and avian D28k-calbindins (96). By contrast, calbindin-D9k is not highly conserved, and there is no amino acid sequence similarity between calbindin-D28k and calbindin-D9k. The genes for both rat calbindin species and for chicken calbindin-D28k have been cloned and sequenced and their transcriptional regulation by $1,25(OH)_2D$, glucocorticoids, and other factors has been investigated (reviewed in (94, 95, 97, 98)).

The calbindins belong to the superfamily of EF-hand helix-loop-helix, high affinity calcium binding proteins (K_d of 10^{-8} to 10^{-6} M) which contains more than 250 proteins. Calbindin-D28k binds four moles of calcium per mole of protein and calbindin-D9k, two moles of calcium

per mole protein. In the intestine, $1,25(OH)_2D$ stimulates both the synthesis of calbindin and the transfer of calcium across the luminal brush-border membrane. The rate and time course of active calcium absorption correlate well with the amount of calbindin D over a wide variety of physiological conditions (99, 100), providing strong support for the role of calbindin-D28k and calbindin-D9k in vitamin D-dependent active calcium transport. Calbindin D may play a similar role in mediating active renal tubular reabsorption of calcium (101, 102). In several mammalian species, both calbindin-D28k and TRPV5 have been localized in the DCT and CNT (70, 85, 103, 104), which are the major sites of active calcium reabsorption. Calbindin-D9k also has been localized to the distal nephron in the rat and mouse (105, 106). The creation of knockout mouse models has elucidated the relative importance of the calcium transport proteins in maintenance of calcium homeostasis. Calbindin-D28k knockout mice showed no change in serum concentrations of calcium, Pi, PTH and $1,25(OH)_2D$ when compared to wild type mice (107, 108). No compensatory increase in gene expression of other calcium transport proteins was observed in kidney or intestine (108). Similarly, calbindin-D9k knockout mice showed no significant difference in phenotype when compared to wild-type mice (109). In summary, the phenotypic findings in calbindin-D28k and calbindin-D9k knockout mice were not different from those in wild type mice, unlike the marked disturbances in calcium homeostasis observed in TRPV5 and TRPV6 knockout mice, which suggests that the role of calbindins in cellular transport of calcium is not rate-limiting.

Calcium Exit

At the basolateral membrane, calcium is actively extruded from the cell against its electrochemical gradient, mediated via a high affinity, magnesium-dependent Ca^{2+}-ATPase or an electrogenic $3Na^+/1Ca^{2+}$ exchanger.

Ca^{2+}-ATPase. The plasma membrane Ca^{2+}-ATPase (Ca^{2+} pump/PMCA) is an obligatory component of eukaryotic plasma membranes that mediates efflux of calcium from the cell. It is thought to play the most important role in maintaining the cytosolic calcium concentration within the normal range. PMCA belongs to the family of P-type ATPases in that it forms a phosphorylated intermediate (an aspartylphosphate) during the reaction cycle. PMCA has a high affinity for calcium, with an estimated K_m of 0.2 mM, and an apparent molecular weight of 120,000–140,000 daltons (reviewed in (110–116)). The pump is activated by direct interaction with calmodulin, a specific calcium receptor protein present in the cytosol, resulting in an increase in both the pump's affinity for calcium and its maximum transport velocity (Vmax). The pump also is activated by cAMP-dependent and protein kinase C-dependent phosphorylation of the pump protein, by limited proteolysis, and by exposure to acidic phospholipids. Transport of calcium from the cell is balanced by countertransport of hydrogen ion (H^+), and thus the activity of the Ca^{2+} pump can be either electroneutral ($Ca^{2+}/2H^+$) or electrogenic (Ca^{2+}/H^+).

The Ca^{2+} pump is located exclusively in the basolateral portion of the plasma membrane of renal tubule cells (117, 118). In earlier studies, activity of the Ca^{2+} pump was found along the entire length of the rabbit nephron, with the activity highest in the distal tubule where the majority of active calcium reabsorption occurs (119). In later studies using monoclonal antibodies against the erythrocyte plasma membrane Ca^{2+} pump, an epitope of this enzyme was identified in human and rat kidneys in the basolateral portion of only the distal convoluted tubule (117, 120). The purified Ca^{2+} pump protein colocalizes with calbindin-D28k in this nephron segment (117, 120). Analysis of different nephron segments of rat kidney using reverse transcription-polymerase chain reaction (RT-PCR) revealed that the Ca^{2+} pump is expressed in both the distal and proximal nephron (121). In the intestine, the Ca^{2+} pump is stimulated by calmodulin and by $1,25(OH)_2D$, which acts to increase pump activity by increasing its Vmax (122).

Four isoforms (PMCA1–4) of the plasma membrane Ca^{2+} pump have been identified and their cDNAs cloned (123–127). In humans and rats, the isoforms are encoded by a family of four genes (ATP2B1–4) that have been mapped to chromosomes 12, 1, 3, and X; additional isoforms of the enzyme (denoted by letters a, b, etc.) are created by alternative RNA splicing of the primary gene transcript (115). The isoforms exhibit 81–85% amino acid homology among themselves, and a single isoform exhibits about 99% homology among different species (128, 129). The deduced amino acid sequences of rat and human isoforms of the plasma membrane Ca^{2+} pump predict a secondary structure that contains ten transmembrane domains, with four main units containing most of the pump mass protruding into the cytoplasm (110, 111, 129). PMCA1b is the predominant isoform found in abundance in the small intestine and in the CNT and CCD of rabbit kidney (130). The expression and activity of PMCA1b is higher in enterocytes from the villus tip as compared to those from the villus crypt, which supports the idea that mature enterocytes have the greatest capacity for transcellular Ca^{2+} movement (131).

Na^+/Ca^{2+} exchanger. The Na^+/Ca^{2+} exchanger is an integral membrane protein that normally exports calcium from the cell, although under some circumstances it mediates calcium influx. The exchanger is a low-affinity, high-capacity transport system for calcium, which is driven by the inwardly directed transcellular electrochemical Na^+ gradient that is normally maintained by the basolateral Na^+/K^+-dependent ATPase. Activity of the exchanger is regulated by intracellular calcium, by changes in the transmembrane voltage being inhibited by sodium ionophores and ouabain which reduce the transmembrane sodium gradient, and by ATP (reviewed in (132–137)).

Complementary cDNAs encoding functional Na^+/Ca^{2+} exchangers have been isolated from heart, kidney, and brain from a variety of species (138–144), suggesting that the protein plays an important role in different physiologic processes in various cell types. Three mammalian isoforms of the Na^+/Ca^{2+} exchanger (NCX), designated NCX1, NCX2, and NCX3 have been cloned and are the products of separate genes (145–148); NCX1 is expressed most abundantly in heart but is found in most tissues including kidney, whereas expression of NCX2 and NCX3 is restricted to brain and skeletal muscle (148, 149). A number of alternative splicing variants of NCX1 are expressed in a tissue-specific fashion (149). The three NCX proteins share 68–75% amino acid sequence identity, and all are predicted to share the same topology of 11 membrane-spanning segments with a large hydrophilic cytoplasmic loop located between membrane-spanning segments 5 and 6. In NCX1, the cytoplasmic loop is thought to be a regulatory region and contains the binding site for Ca^{2+} and the location of the exchanger inhibitory peptide (XIP) sequence.

In the kidney activity of the Na^+/Ca^{2+} exchanger is found only in basolateral membrane preparations of renal tubules (150) and is localized exclusively to the distal tubule in the rabbit and rat (151). In rabbit kidney, immunolocalization was detected predominately along the basolateral plasma membrane of cortical connecting tubules, with weak staining of principal cells of the collecting duct; no staining was detected in other cell types in either the cortex or medulla (152). Using PCR to localize the exchanger in microdissected segments of rat nephron, NCX1 expression was observed in the DCT with little or no expression in other segments (153). Thus, the distal nephron exhibits Ca^{2+} pump and Na^+/Ca^{2+} exchanger activity, mRNA expression, and protein expression, consistent with the important role of this nephron segment in hormone-regulated calcium reabsorption. In the intestine, the Na^+/Ca^{2+} exchanger has been detected in rats (154), mice (155) and chicks (131), but not in rabbits (85). This transporter can operate in either a forward mode (Ca^{2+} exit) or in a reversed mode (Ca^{2+} entry), which depends on the Na^+ and Ca^{2+} gradients and the potential across the plasma membrane (156). The relative contribution of PMCA vs NCX1 in the extrusion of Ca^{2+} from the cytoplasm is unknown as there are no known inhibitors for NCX1, and its gene deletion results in fetal death (157, 158).

Renal Calcium Transport

Physiology and Tubular Localization

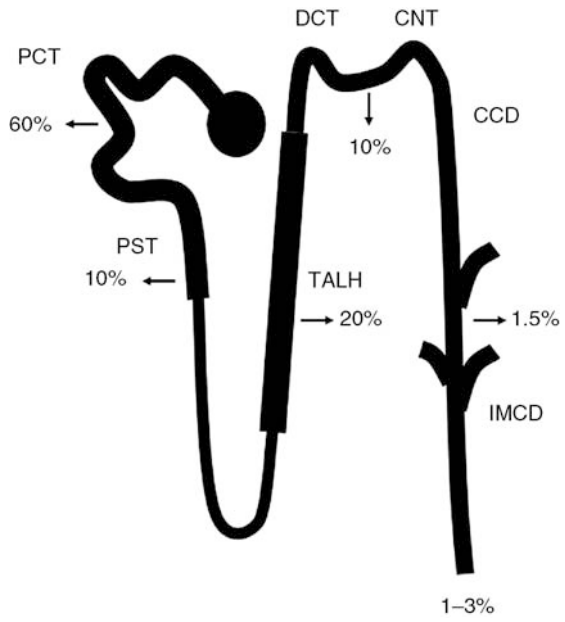
Approximately 60% of plasma calcium is freely filtered by the glomerulus, as shown by a glomerular filtrate to plasma (GF:P) ratio for calcium that ranges between 0.63 and 0.70 (159, 160). The fraction of plasma calcium that is filterable represents ionized calcium and complexed calcium. To maintain zero calcium balance, 98–99% of the filtered load of calcium, estimated at about 8 gm/day in the adult, must be reabsorbed by the renal tubules. Clearance studies in humans and experimental animals show that an increase in the filtered load of calcium, as occurs with calcium infusion, results in an increase in both urine excretion and absolute tubular reabsorption of calcium (161–165). Thus, there is no apparent maximum tubular reabsorptive rate (T_m) for calcium within the normal physiologic range (166).

Approximately 70% of filtered calcium is reabsorbed in the proximal tubule, about 20% is reabsorbed between the late proximal and early distal tubule, primarily in the thick ascending limb of Henle's loop (TALH), 5–10% is reabsorbed in the distal tubule, and less than 5% is reabsorbed in the collecting duct (*Fig. 10-5*) (159, 167–169). Thus, 1–3% of filtered calcium is excreted in the urine. As discussed below, the site of physiologic regulation of renal calcium reabsorption is the distal nephron.

Proximal tubule. The majority of filtered calcium is reabsorbed in the proximal tubule, with approximately 60% being reabsorbed by the end of the accessible portion of the superficial proximal tubule and an additional 10% reabsorbed in the proximal straight tubule. In the early proximal convoluted tubule (PCT) (S1 and S2 segments), calcium is reabsorbed passively principally via the paracellular pathway, in parallel with the reabsorption of sodium and water, mediated by convection (solvent drag) across the tight junctions. Evidence for passive reabsorption of calcium in the early PCT is the finding that in several species studied by micropuncture, the ratio of

Figure 10-5

Profile of calcium reabsorption along the nephron, as derived from micropuncture data. PCT, proximal convoluted tubule; PST, proximal straight tubule; TALH, thick ascending limb of Henle's loop; DCT, distal convoluted tubule; CNT, connecting tubule; CCD, cortical collecting duct; IMCD, inner medullary collecting duct. (Modified from (168)).



calcium concentration in tubular fluid to that in glomerular ultrafiltrate is approximately 1.0 (169). In the late S1 segment, reabsorption of calcium lags slightly behind that of sodium, thus creating a favorable chemical gradient for reabsorption downstream. In the S2 segment of the PCT, the transepithelial voltage is the lumen positive, thus the electrical gradient is positive for passive calcium reabsorption. In rabbit early S2 segments, net flux of calcium was zero in the absence of both water transport and an electrochemical gradient (170), providing further evidence for passive calcium reabsorption. There also is evidence that calcium reabsorption is active in the proximal nephron, particularly in the earliest segments of the PCT where the transepithelial voltage is lumen-negative (169). Calcium reabsorption also appears to be active in the S3 segment of the proximal tubule, as it is not dependent on sodium, occurs against an electrochemical gradient, and is not inhibited by ouabain (171).

Henle's Loop. In the thin descending and ascending limbs of Henle's loop, calcium transport is negligible (171, 172). However, in the TALH approximately 20% of the filtered calcium load is reabsorbed (169). In isolated, perfused segments of TALH, calcium reabsorption is passive, driven by the large lumen-positive transepithelial voltage in this segment (173–175) that results from the secondary-active transport of NaCl out of tubular fluid. An increase in NaCl reabsorption is attended by an increase in luminal positivity, which leads to stimulation of calcium reabsorption. Inhibition of NaCl transport with furosemide reduces the transepithelial voltage and thus increases calcium excretion. In some studies, however, calcium transport in the TALH is found to be active (172, 176, 177). It has been proposed that axial heterogeneity may account for some of the differences observed in the studies reported (178). Calcium transport in the cortical TALH can be increased by addition of PTH to the bath (179) and in medullary segments, by addition of calcitonin or cyclic adenosine monophosphate (cAMP) (180).

Distal Convoluted Tubule and Connecting Tubule. Physiologic regulation of calcium excretion occurs in the distal convoluted tubule (DCT), which reabsorbs up to 10% of the filtered calcium load. The capacity for calcium transport in this segment appears to be high and to be limited mainly by the availability of transportable ions. Although calcium transport in the DCT normally occurs in parallel with that of sodium, it is not dependent on either sodium or the transepithelial voltage and occurs against an electrochemical gradient; thus, it is active and presumably transcellular. Reabsorption of calcium in the DCT can be dissociated from that of sodium by administration of thiazide diuretics, which increase reabsorption of calcium and decrease that of sodium. As discussed above, luminal calcium entry in the DCT is thought to be mediated via the apical Ca^{2+} channel TRPV5, which is the rate-limiting step for Ca^{2+} entry from the tubular lumen. Both vitamin D-dependent calbindin-D28k, and the Ca^{2+} pump PMCA1b, co-localize in the DCT and are thought to facilitate transcellular movement and basolateral extrusion of calcium, respectively. The Na^{+}/Ca^{2+} exchanger NCX1 also is localized, perhaps exclusively, to the basolateral membrane of the DCT and connecting tubule (106, 151–153), although its physiologic role in these segments remains to be defined.

Collecting Tubule. Net reabsorption in the collecting tubule accounts for less than 5% of filtered calcium (169). In the cortical collecting tubule, calcium transport probably is active, as TRPV6 and calbindin-D28k in mice, and NCX1, calbindin-D28k, and the plasma membrane Ca-ATPase in humans, are found in this nephron segment (169). In the medullary collecting tubule, about 1% of the filtered load may be reabsorbed (181).

Regulation of Renal Calcium Transport

A number of factors can influence renal tubular reabsorption and urine excretion of calcium (*Table 10-3*) (166, 169).

Parathyroid hormone. PTH stimulates renal calcium reabsorption and is thought to be the principal hormonal determinant of urine calcium excretion. PTH acts to decrease calcium excretion in part by decreasing GFR via a reduction in glomerular capillary ultrafiltration coefficient, K_f (182), thus decreasing the filtered load of calcium. PTH receptors are found throughout the nephron (183, 184), and PTH increases tubular calcium reabsorption in the cortical TALH, DCT, and connecting tubule of the rabbit, with the principal effect being on the DCT (173, 179, 180, 185–188). PTH action is attributed to activation of both the cAMP-protein kinase A (PKA) and phospholipase A-protein kinase C (PKC)-dependent signaling pathways (168). The PTH-induced increase in calcium reabsorption is associated with an increase in the cytosolic calcium concentrations in cortical TALH, DCT, and connecting tubule (188–190). Recently, the molecular regulation of renal calcium transport proteins by PTH was studied in vivo and in vitro. In parathyroidectomized rats, the mRNA abundance of TRPV5, Calbindin-D28k, and NCX1 were decreased, and continuous infusion of PTH restored the expression to near normal levels (191). In addition, in primary cultures of rabbit connecting tubule/cortical collecting duct cells, PTH induced an

Table 10-3

Factors affecting renal calcium excretion

Factor	Ca excretion	Mechanism/nephron site
Dietary		
Volume expansion	↑	↓ Distal reabsorption
Sodium chloride	↑	Undefined
Protein	↑	↑ Net acid and sulfate excretion
Phosphorus	↓	↓ Production of $1,25(OH)_2D$ ↓ Intestinal absorption of Ca ↑ Distal reabsorption
Metabolic		
Acidosis	↑	↓ Proximal and distal reabsorption
Hypercalcemia	↑	↑ Filtered load of Ca ↓ Proximal and distal reabsorption (PTH)
Glucose	↑	↓ Proximal and distal reabsorption
Alkalosis	↓	↑ Proximal and distal reabsorption
Hormones		
Insulin	↑	↓ Proximal and distal reabsorption
Glucagon	↑	↑ RBF and GFR
Growth hormone	↑	Undefined
Thyroid hormone	↑	↑ Filtered load of Ca, ↓PTH
Glucocorticoids	↑	? ↓ Bone resorption, volume expansion
PTH	↓	↑ Reabsorption TALH, DCT and CNT
Vitamin D	↓	↑ Distal reabsorption; other sites
Calcitonin	↓	↑ Reabsorption TALH?
Estrogens	↓	↑ Distal reabsorption
Diuretics		
Mannitol	↑	↓ Proximal reabsorption
Furosemide	↑	↓ Reabsorption TALH
Thiazides, amiloride	↓	↑ Proximal reabsorption

increase in apical Ca^{2+} entry that was associated with increased mRNA expression of TRPV5, Calbindin-D28k, and NCX1 (191). These data support the critical role of PTH in regulating renal calcium reabsorption independent of vitamin D.

Vitamin D. The effect of vitamin D on renal calcium reabsorption is variable, depending on vitamin D status, activity of PTH, and species studied (66). Although vitamin D has no detectable effect on calcium transport by the proximal tubule (192), $1,25(OH)_2D$ stimulates Ca^{2+} transport in distal nephron segments, including DCT of the dog and CNT and cortical collecting duct of the rabbit (66, 193). The effect of $1,25(OH)_2D$ on expression of TRPV5 was studied in vitamin D-deficient rats. Administration of $1,25(OH)_2D$ induced an increase in the abundance of TRPV5 mRNA and protein in the distal part of the DCT and in CNT (194). The human TRPV5 promoter contains several putative vitamin D responsive elements, suggesting that $1,25(OH)_2D$ stimulates TRPV5 expression at least in part at the transcriptional level (194). Administration of $1,25(OH)_2D$ to vitamin D-deficient mice also stimulates the mRNA expression of Calbindin-D28k and NCX1 in the kidney (195). Thus, it is thought that $1,25(OH)_2D$ acts on the distal nephron to increase calcium reabsorption by increasing the expression of the calcium transport proteins in this nephron segment.

Other hormonal factors. Urine calcium excretion is increased by exposure to the following: insulin, glucose, glucagon, growth hormone, thyroid hormone, and corticosteroids; urine calcium is decreased by calcitonin and estrogens (169). Estrogen plays an important role in calcium homeostasis, and estrogen deficiency results in negative calcium balance as occurs in post-menopausal osteoporosis (196, 197). The negative calcium balance is attributed to increased renal excretion and intestinal malabsorption of calcium due to estrogen deficiency, which is corrected by estrogen therapy (198–201). Estrogen regulates intestinal and renal calcium absorption via TRPV6 and TRPV5 channels respectively (202–204), and this regulation is transcriptionally mediated and independent of vitamin D.

Dietary Factors. Changes in dietary calcium within the normal range have only a modest effect on urine calcium excretion. A high calcium diet decreases intestinal and renal calcium absorption, and a restricted calcium diet has the opposite effect. These changes are mediated by changes in expression of TRPV5, TRPV6, Calbindin-D28k, PMCA1b, and NCX1 by unknown mechanisms that are independent of vitamin D (205). A linear relationship exists between dietary protein intake and urine calcium excretion (206), an effect that is exaggerated in patients with recurrent nephrolithiasis. An increase in either oral or parenteral intake of phosphorus is associated with a decrease in urine calcium excretion, an effect mediated in part by increased calcium reabsorption in the distal nephron (207). Phosphorus loading can reduce intestinal calcium absorption and stimulate PTH secretion, both in part by decreasing the renal production and serum concentration of $1,25(OH)_2D$ (10, 208). Conversely, phosphorus restriction increases urine calcium excretion (166, 169). This effect is independent of vitamin D and PTH and is attributed to a reduction in tubular calcium reabsorption, principally in distal nephron segments.

Volume Status. Expansion and contraction of the ECF volume induces an increase and decrease, respectively, in excretion of both calcium and sodium. Acute infusion of sodium chloride increases urine calcium excretion, an effect attributed to inhibition of calcium reabsorption in both the proximal and late distal tubule (209). An increase in dietary sodium chloride induces an increase in urine calcium, although the intrarenal mechanisms responsible have not been defined.

Acid–base Status. Both acute and chronic metabolic acidosis, as induced by ammonium chloride loading in humans and experimental animals, is attended by an increase in urine calcium excretion (210–212). This increase is irrespective of a change in filtered load of calcium or in circulating PTH and is attributed to a decrease in calcium reabsorption in the distal nephron (210). Hypercalciuria is reversed when the acidosis is corrected with administration of alkali (210, 213, 214). Recently, the molecular mechanism of this regulation was studied in wild type mice administered ammonium chloride to induce metabolic acidosis. Hypercalciuria was associated with a concomitant decrease in TRPV5, Calbindin-D28k, and NCX1 mRNA and protein abundance in the kidney (215). Conversely, metabolic alkalosis is associated with a decrease in calcium excretion (216, 217). The effect of respiratory acid–base changes are similar to those of metabolically-induced changes (218, 219). Hypercalcemia, by increasing the filtered load of calcium, is associated with an increase in its excretion. This effect is mitigated to some extent by hypercalcemia-induced reduction in the ultrafilterability of calcium and Pi (220) and in GFR (221); the reduction in GFR is attributed to a PTH-dependent decrease in K_f. Hypercalcemia can decrease calcium reabsorption in the PCT, TALH, and distal nephron (166); the effect on this latter segment requires the presence of parathyroid glands (222).

Diuretic Agents. Loop diuretics (furosemide and ethacrynic acid) which act on the TALH, induce an increase in sodium excretion by inhibiting the apical Na^+–K^+–$2Cl^-$ cotransporter, NKCC2. Such inhibition reduces the

lumen-positive transepithelial voltage, leading to a decrease in paracellular reabsorption of calcium. Therefore, administration of loop diuretics induces both natriuresis and hypercalciuria (169). By contrast, thiazide diuretics, which inhibit the apical sodium chloride cotransporter, NCC, in the DCT, decrease the excretion of calcium (223). The molecular mechanisms for the hypocalciuric effect of thiazide diuretics are reviewed in (224). Recent studies (225–227) support the hypothesis that chronic administration of thiazide diuretics induces an increase in calcium reabsorption in the proximal tubule. In TRPV5 knockout mice, chronic administration of thiazide diuretics induces hypocalciuria, even though active calcium transport in the distal nephron is absent (225). Furthermore, in normal rats the hypocalciuric effect of thiazides can be reversed when extracellular volume (ECV) contraction is prevented by sodium repletion (226). Thus, the thiazide-induced ECV contraction leads to a compensatory increase in proximal tubular reabsorption of sodium. The electrochemical gradient thus generated drives paracellular calcium reabsorption in the proximal tubule.

Phosphorus

Inorganic phosphate (Pi) is fundamental to cellular metabolism and, in vertebrates, to skeletal mineralization. To accomplish these functions, transport systems have evolved to permit the efficient transfer of negatively charged Pi ions across hydrophobic membranes. Ingested Pi is absorbed by the small intestine, deposited in bone, and filtered by the kidney where it is reabsorbed and excreted in amounts that are determined by the specific requirements of the organism. The kidney is a major determinant of phosphorus homeostasis due to its ability to increase or decrease its Pi reabsorptive capacity to accommodate Pi need. Accordingly, significant advances have been made in our understanding of the molecular mechanisms involved in renal tubular Pi reabsorption and its hormonal regulation and modulation by dietary Pi intake. This section will focus on Pi homeostasis and the cellular and molecular aspects of intestinal and renal Pi transport and their regulation. For a more detailed discussion of renal Pi wasting disorders in humans, see chapter 11.

Phosphorus Homeostasis

Phosphorus Distribution in the Body

Phosphorus accounts for about 0.6% of body weight at birth and about 1% of body weight, or 600–700 gm, in the adult (1). Approximately 85% of body phosphorus is in the skeleton and teeth, approximately 15% is in soft tissue, and the remainder (~0.3%) is in extracellular fluid. Pi is an important constituent of bone mineral, and in growing individuals, the balance of Pi must be positive to meet the needs of skeletal growth and consolidation; in the adult, Pi balance is zero. Pi deficiency results in osteomalacia in both children and adults.

Phosphate Chemistry

Phosphorus exists in plasma in two forms, an organic form consisting principally of phospholipids and phosphate esters, and an inorganic form (228). Of the total plasma phosphorus concentration of approximately 14 mg/dl (4.52 mM), about 4 mg/dl (1.29 mM) is in the inorganic form. Of this, about 10–15% is protein bound and the remainder, which is filtered by the renal glomerulus, exits principally either as the undissociated or "free" Pi ions or as Pi complexed with sodium, calcium, or magnesium. At physiological pH, only HPO_4^{2-} and $H_2PO_4^{-}$ are present at significant concentrations in plasma. The ratio of the divalent to monovalent forms can be determined by the Henderson-Hasselbalch equation, pH = pKa+ log (HPO_4^{2-} /$H_2PO_4^{-}$). The dissociation constant, pKa, for Pi is 6.8. Thus, at a pH of 7.4, the ratio of divalent (HPO_4^{2}) to monovalent ($H_2PO_4^{-}$) Pi anions is essentially 4:1, and the composite valence of Pi in serum (or intravenous solutions) is 1.8. At this pH, 1 mmol Pi is equal to 1.8 meq. In clinical settings, only the inorganic orthophosphate form of Pi is routinely measured.

The terms "phosphorus concentration" and "phosphate concentration" are often used interchangeably, and for clinical purposes the choice matters little. Phosphorus in the form of the phosphate ion circulates in blood, is filtered by the renal glomerulus, and is transported across plasma membranes. However, the content of "phosphate" in plasma, urine, tissue, or foodstuffs is measured and expressed in terms of the amount of elemental phosphorus contained in the specimen, hence use of the term "phosphorus concentration."

Extracellular Phosphate Homeostasis

In the adult in zero Pi balance, net intestinal Pi absorption (dietary Pi minus fecal Pi) is approximately 60–65% of dietary intake. To satisfy the demands of rapid growth of bone and soft tissue, intestinal Pi absorption in infants is higher than in the adult and can exceed 90% of dietary intake (229, 230). Metabolic balance studies in normal adult humans reveal that over the customary range of

dietary Pi, net absorption is a linear function of intake (231), with no indication of saturation. Thus, inadequate Pi absorption results primarily from decreased Pi availability rather than from changes in the intrinsic capacity of intestinal Pi transport. A small amount of Pi is secreted into the intestinal lumen in digestive fluids. Absorbed Pi enters the extracellular Pi pool, which is in equilibrium with the bone and soft tissue Pi pools. Pi is filtered at the glomerulus and is reabsorbed to a large extent by the renal tubule. In subjects in zero Pi balance, the amount of Pi excreted by the kidney is equal to the net amount absorbed by the intestine, and in growing children is less than the net amount absorbed due to deposition of Pi in bone. An overall schema of Pi metabolism is depicted in *Fig. 10-6*.

Renal tubular reabsorption of Pi plays a central role in the regulation of plasma Pi concentration and Pi homeostasis. In response to a decrease in the extracellular Pi concentration, urine Pi excretion decreases promptly due to an increase in Pi reabsorption by the proximal tubule (*Fig. 10-7*). This acute response reflects a decrease in the filtered load of Pi, an adaptive increase in proximal tubule Pi reabsorption induced by hypophosphatemia or decreased dietary Pi intake, and a decrease

Figure 10-6

Phosphorus fluxes between body pools in the normal human adult in zero phosphorus balance.

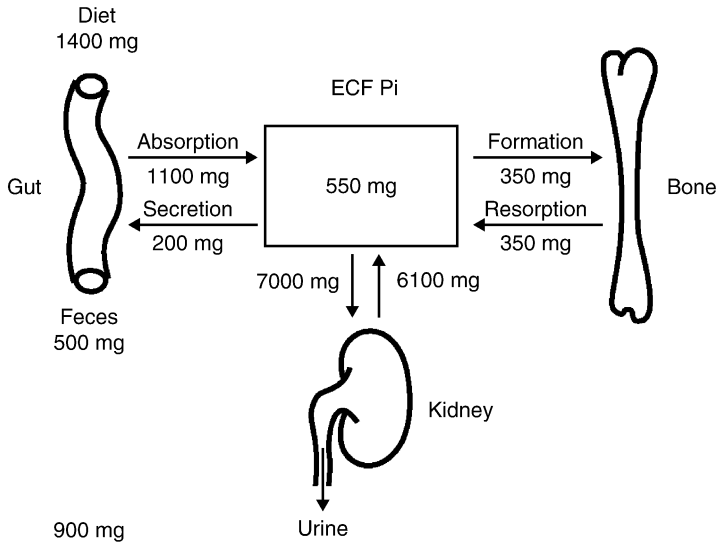
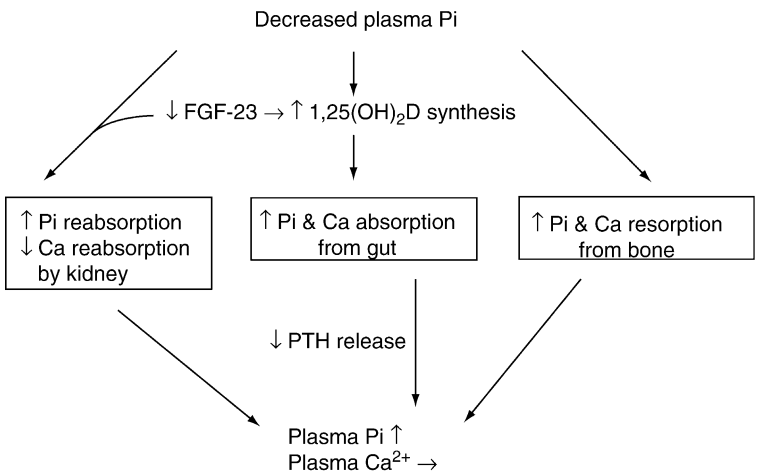


Figure 10-7

The homeostatic response to hypophosphatemia.



in circulating FGF-23 (discussed below). Hypophosphatemia also is a potent stimulus for the renal synthesis of $1,25(OH)_2D$ (208, 232–236). The resulting increase in serum $1,25(OH)_2D$ acts to stimulate intestinal absorption of Pi and calcium and their mobilization from bone. Hypophosphatemia itself also can directly promote mobilization of Pi and calcium from bone. With an increase in plasma calcium concentration, PTH is suppressed which leads to a further decrease in urine Pi excretion but an increase in calcium excretion. These homeostatic adjustments result in an increase in extracellular Pi concentration toward normal values, with little change in serum calcium concentration. Conversely, in response to an increase in plasma Pi concentration, production of $1,25(OH)_2D$ is decreased and release of PTH and FGF-23 are increased. The effect of hyperphosphatemia on bone, kidney, and intestine are opposite to those occurring with hypophosphatemia, the net result being a decrease in Pi concentration toward normal values.

In healthy subjects ingesting typical diets, the serum Pi concentration exhibits a circadian rhythm, characterized by a rapid decrease in early morning to a nadir shortly before noon, a subsequent increase to a plateau in late afternoon, and a small further increase to a peak shortly after midnight (8, 10) (*Table 10-1*). The amplitude of the rhythm (nadir to peak) is approximately 1.2 mg/dl, or 30% of the 24-h mean level. Restriction or supplementation of dietary Pi induces a substantial decrease or increase, respectively, in serum Pi concentrations during the late morning, afternoon, and evening, but induces less or no change in the morning fasting Pi concentration (10). To minimize the impact of changes in dietary Pi on the serum Pi concentration, one should obtain specimens for analysis in the morning fasting state. Specimens obtained in the afternoon are more likely to be affected by diet and thus may be more useful to monitor the effect of dietary Pi on serum Pi concentration, as in patients with renal insufficiency receiving Pi-binding agents to treat hyperphosphatemia.

Other factors can affect the serum Pi concentration. Presumably because of movement of Pi into cells, the serum Pi concentration can be decreased acutely by intravenous infusion of glucose or insulin, ingestion of carbohydrate rich meals, acute respiratory alkalosis, or by infusion or endogenous release of epinephrine. The decrease in Pi concentration induced by acute respiratory alkalosis can be as great as a 2.0 mg/dl (237). Serum Pi concentration can be increased acutely by metabolic acidosis and by intravenous infusion of calcium (238).

There are substantial effects of age on the fasting serum Pi concentration (*Table 10-2*). In infants in the first 3 months of life, Pi levels are highest (4.8–7.4 mg/dl, mean 6.2 mg/dl [2 mM]) and decrease at age 1–2 years to 4.5–5.8 mg/dl (mean 5.0 mg/dl [1.6 mM]) (239). In mid-childhood, values range from 3.5 to 5.5 mg/dl (mean 4.4 mg/dl [1.42 mM]) and decrease to adult values by late adolescence (12, 240). In adult males, serum Pi is ~3.5 mg/dl at age 20 years and decreases to ~3.0 mg/dl at age 70 (13, 240). In women, the values are similar to those of men until after the menopause, when they increase slightly from ~3.4 mg/dl at age 50 years to 3.7 mg/dl at age 70.

Intestinal Phosphate Absorption

Cellular Aspects

Dietary Pi is absorbed in the small intestine, primarily in the duodenum and jejunum with minimal absorption in the ileum. Pi absorption occurs via two mechanisms, nonsaturable, passive diffusion through the paracellular pathway and an active transcellular process that has been localized to the mucosal surface. Under usual conditions of excess dietary intake, Pi absorption occurs primarily via paracellular diffusion which is largely unregulated, whereas active transport plays an important role when luminal Pi concentration is low, as when dietary Pi is restricted (241, 242). The active absorption of Pi across the mucosal membrane is saturable, sodium-dependent, and driven by a Na^+-gradient (outside > inside) that is maintained by the basolateral membrane-associated Na^+, K^+-dependent ATPase. The exit of Pi at the serosal (basolateral) surface occurs down an electrochemical gradient and has not been well characterized. However, evidence suggests that the process is carrier-mediated, Na^+-independent, and electrogenic.

Regulation

Active transport of Pi across the mucosal membrane is the rate limiting step in intestinal Pi absorption and is regulated by $1,25(OH)_2D$ and dietary Pi intake (243). Administration of $1,25(OH)_2D$ to either vitamin D deficient or replete animals induces a significant increase in net Pi absorption which is associated with a corresponding increase in sodium-dependent Pi (Na/Pi) cotransport Vmax across the mucosal brush border membrane (BBM). The increase in intestinal Pi transport induced by $1,25(OH)_2D$ is dependent on protein synthesis, occurs several hours after its administration, and apparently

occurs after weaning. In a pig model in which mutant animals exhibit defective renal synthesis of $1,25(OH)_2D$, intestinal BBM Na/Pi cotransport Vmax in mutants is similar at birth but significantly reduced after weaning when compared to age-matched wild-type animals (244). Administration of $1,25(OH)_2D$ had no effect on mucosal Pi transport in newborn mutants but it corrected the Pi transport defect in weanling mutants (244). Low dietary Pi intake also induces an increase in mucosal Na/Pi cotransport. However, the response to dietary Pi restriction is likely mediated by $1,25(OH)_2D$. Renal synthesis of $1,25(OH)_2D$ is stimulated by hypophosphatemic states (232–234), and the adaptive intestinal response to Pi restriction is blunted in vitamin D deficient animals (245).

Molecular Mechanisms

In an effort to identify intestinal Na/Pi cotransporters, a full-length cDNA was generated from an EST clone and found to exhibit sequence homology with the renal type II Na/Pi cotransporter (discussed below) (246). When expressed in Xenopus oocytes, the cDNA induced Na/Pi cotransport that was electrogenic, with a pH-dependence that resembled that of intestinal BBM Na/Pi cotransport, i.e., higher transport at pH 6 than at pH 7.4 (246). Based on its high homology to the renal type II transporter, it was designated type IIb (NPT2b) (solute carrier series SLC34A2) and the renal isoform was renamed type IIa (NPT2a). The NPT2b/Npt2b genes map to human and mouse chromosome regions 4p15.2 and 5C1, respectively (247–249). NPT2b mRNA is expressed in a variety of tissues including small intestine, but not in kidney (246). NPT2b protein is localized to the apical membrane of enterocytes (246), and western blotting and immunohistochemical analysis revealed that its expression in the small intestine is regulated by dietary Pi intake (250). In mice, chronic Pi restriction induces an increase in small intestinal BBM Na/Pi cotransport and a corresponding increase in apical membrane NPT2b protein abundance. In contrast, with chronic feeding of a high Pi diet, Na/Pi cotransport was decreased, and NPT2b protein was no longer detectable in the intestinal apical membrane (250). The correlation between BBM Na/Pi cotransport and NPT2b protein expression in these studies is consistent with the notion that NPT2b plays an important role in intestinal Pi absorption and its regulation by dietary Pi intake.

Small intestinal NPT2b expression also is regulated by vitamin D. In mice, injection of cholecalciferol elicits comparable increases in small intestinal BBM Na/Pi cotransport and apical abundance of NPT2b protein (250). However, corresponding increases in intestinal NPT2b mRNA were not evident, suggesting that the effect of vitamin D cannot be explained by increased NPT2b gene transcription. In the rat, however, administration of $1,25(OH)_2D$ stimulated Na/Pi cotransport and increased both NPT2b mRNA and protein (251). In addition, in cultured intestinal epithelial cells, $1,25(OH)_2D$ induced an increase in NPT2b mRNA abundance and in NPT2b promoter activity (251). Both intestinal Na/Pi cotransport and apical NPT2b mRNA expression decrease with age in rats, being approximately fourfold higher in immature (2-week old) than in adult (14 weeks) rats, in the absence or presence of vitamin D (251).

Methylprednisolone induces a significant decrease in small intestinal BBM Na/Pi cotransport and a concomitant decrease in NPT2b mRNA and protein expression (252). The inhibition by methylprednisolone was evident in mice ranging from 14 days to 9 months of age, although apical transport activity and intestinal NPT2b expression were significantly higher in the suckling mice than in adult animals (252).

Renal Phosphate Transport

Physiology and Tubular Localization

Much of the material discussed here is covered in greater detail in review articles (253–260).

The proximal tubule is the major site of Pi reabsorption, with approximately 70% of the filtered load reclaimed in the proximal convoluted and approximately 10% in the proximal straight tubule. In addition, a small but variable portion (<10%) of filtered Pi is reabsorbed in the distal segments of the nephron (238) (*Fig. 10-8*). Clearance studies in humans and experimental animals show that when the filtered load of Pi is progressively increased, Pi reabsorption increases until a maximum tubular reabsorptive rate for Pi, or TmP, is reached, after which Pi excretion increases in proportion to its filtered load. The measurement of TmP varies among individuals and within the same individual, due in part to variation in GFR. Thus the ratio, TmP/GFR, or the maximum tubular reabsorption of Pi per unit volume of GFR, is the most reliable quantitative estimate of the overall tubular Pi reabsorptive capacity and is considered to reflect the quantity of Na/Pi cotransporters available per unit of kidney mass (241). The serum Pi concentration at which Pi reabsorption is maximal is called the "theoretical renal Pi threshold";

Figure 10-8

Profile of phosphate reabsorption along the mammalian nephron, as derived from micropuncture data. PCT, proximal convoluted tubule; PST, proximal straight tubule; TALH, thick ascending limb of Henle's loop; DCT, distal convoluted tubule; CCD, cortical collecting duct; IMCD, inner medullary collecting duct. (Data from (238)).

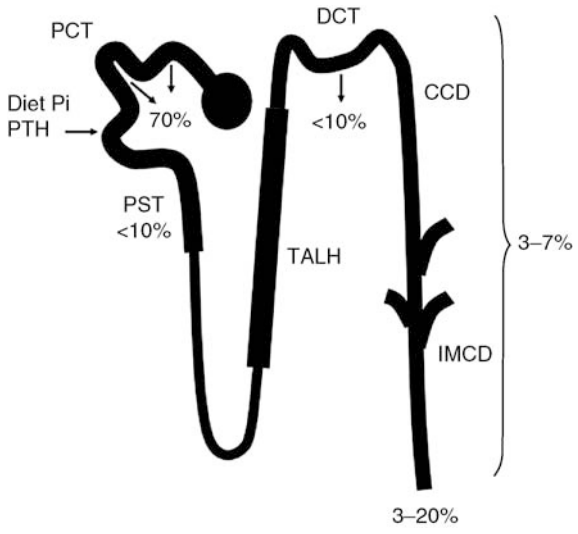
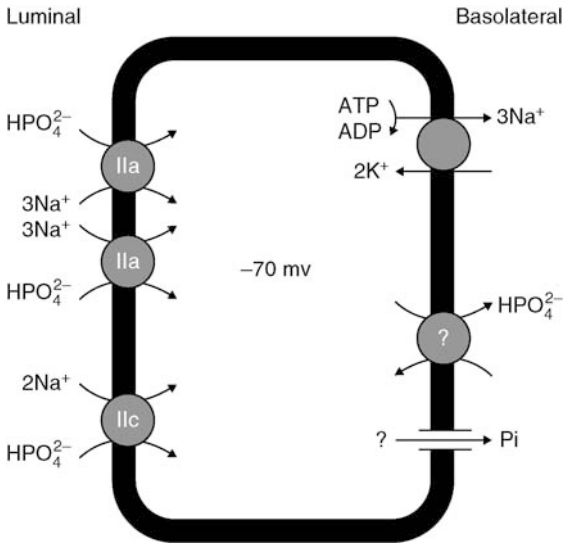


Figure 10-9

Location of identified and postulated type II Na^+-dependent Pi cotransporters in the proximal tubule cell. Available data indicate that most proximal tubule Pi reabsorption occurs via type IIa (NPT2a) and type IIc (NPT2c) cotransporters, which are localized to the brush border membrane and are the major target for physiologic regulation of renal Pi reabsorption. (Modified from (402)).



this value is equal to the ratio, TmP/GFR, and closely approximates the normal fasting serum Pi concentration. Thus, the renal reabsorptive capacity for Pi is the principal determinant of the serum Pi concentration.

Proximal tubule. Approximately 70% of the filtered load of Pi is reabsorbed by the proximal convoluted tubule (PCT). Reabsorption rates in early convolutions (S1 segment) normally are as much as four times greater than those in late convolutions (S2 segment) and the pars recta (S3 segment) (261–263). Due to this axial heterogeneity in Pi transport, most of the Pi reabsorption by the PCT occurs within the first 25% of its length. Internephron heterogeneity in Pi reabsorption also is present in the proximal tubule, with proximal tubules of juxtamedullary nephrons having a greater capacity to both reabsorb Pi (264–267) and adapt to changes in its filtered load (268, 269), as compared with proximal tubules of superficial nephrons. In the absence of PTH, up to an additional 10% of filtered Pi can be reabsorbed in the proximal straight tubule (PST) (*Fig. 10-8*).

Henle's Loop, distal convoluted tubule and connecting tubule. Little or no transport of Pi is thought to occur in Henle's Loop except for the PST segment (238). Up to 10% of the filtered Pi load is reabsorbed by the DCT in the absence of PTH, with the possibility of an additional 3–7% being reabsorbed beyond the accessible late DCT, presumably by the connecting tubule (238).

Collecting tubule. Although some investigators have failed to demonstrate Pi reabsorption in isolated perfused cortical collecting tubules (270), others have demonstrated a small but significant net efflux of Pi in this nephron segment (271, 272).

Cellular and Molecular Aspects

Transepithelial Pi transport in the nephron is essentially unidirectional and involves uptake across the BBM, translocation across the cell, and efflux at the basolateral membrane (*Fig. 10-9*). Pi uptake at the apical cell surface is the rate-limiting step in overall Pi reabsorption, the major site of its regulation, and is mediated by Na/Pi cotransporters that depend on the basolateral membrane-associated Na^+/K^+-dependent ATPase. Na/Pi cotransport is either electrogenic (NPT2a) or electroneutral (NPT2c) and is sensitive to changes in luminal pH, with 10- to 20-fold increases observed when the pH is raised from 6 to 8.5. Little is known about the translocation of Pi across the cell except that Pi anions rapidly equilibrate

with intracellular inorganic and organic Pi pools. There are little data regarding the mechanisms involved in the efflux of Pi at the basolateral cell surface. It has been proposed that in the proximal tubule, a Na^+-dependent electroneutral anion exchanger is at least partially responsible for Pi efflux (273).

Three classes of Na/Pi cotransporters have been identified by expression and homology cloning. The type I Na/Pi cotransporter (NPT1, *SLC17A1*) is expressed predominately in BBMs of proximal tubule cells (274). The NPT1 transporters are approximately 465 amino acids in length with seven to nine membrane spanning segments. NPT1 exhibits broad substrate specificity and mediates the transport of Cl^- and organic anions as well as high affinity Na/Pi cotransport. Its pH profile differs significantly from that of the pH-dependence of Na/Pi cotransport in isolated renal BBM vesicles. Furthermore, conditions that physiologically regulate proximal tubule Pi transport such as dietary Pi or PTH do not alter type I Na/Pi cotransporter protein or mRNA expression. Thus, the physiological role of NPT1 will require further study. The human gene encoding the type I Na-Pi cotransporter (*SLC17A1*) is located on chromosome 6p21.3-p23.

The type II family of Na/Pi cotransporters, whose cDNA shares only 20% homology with that of NPT1 (275,276), is comprised of three highly homologous isoforms: type IIa (NPT2a, *SLC34A1*) and type IIc (NPT2c, *SLC34A3*) (277,278), which are expressed exclusively in the BBM of the renal proximal tubule (*Fig. 10-9*), and type IIb (NPT2b, *SLC34A2*), which is expressed in several tissues including small intestine and lung, but not in kidney, and is responsible for intestinal absorption of Pi (246). Human NPT2a and human NPT2c are comprised of 635 and 599 amino acids, respectively; both proteins are predicted to have eight membrane-spanning segments (*Fig. 10-10*). The human genes encoding NPT2a and NPT2c are located on chromosomes 5q35 and 9q34, respectively (279). NPT2a-mediated Na/Pi cotransport is electrogenic and involves the influx of three Na^+-ions and one Pi-anion (preferentially divalent) (280). NPT2b-mediated cotransport also is electrogenic, whereas the NPT2c isoform mediates electroneutral transport of two Na^+-ions with one divalent Pi-anion.

In the mouse, Npt2a and Npt2c are detected exclusively in the BBM of proximal tubular cells. At the mRNA level, NPT2a is approximately one order of magnitude more abundant than NPT2c. The abundance of Npt2c mRNA and protein are both significantly higher in kidneys of 22-day-old rats than in those of 60-day-old rats, suggesting that Npt2c has a particularly important role during early post-natal development (277). However, several different homozygous and compound heterozygous mutations in the gene encoding NPT2c, *SLC34A3,* have been found in patients affected by hereditary hypophosphatemic rickets with hypercalciuria (HHRH) (281–283), indicating that at least in humans, this cotransporter has a more prominent role than initially thought. Hybrid depletion studies suggested that Npt2c accounts for approximately 30% of Na/Pi co-transport in kidneys of Pi-deprived adult mice (278).

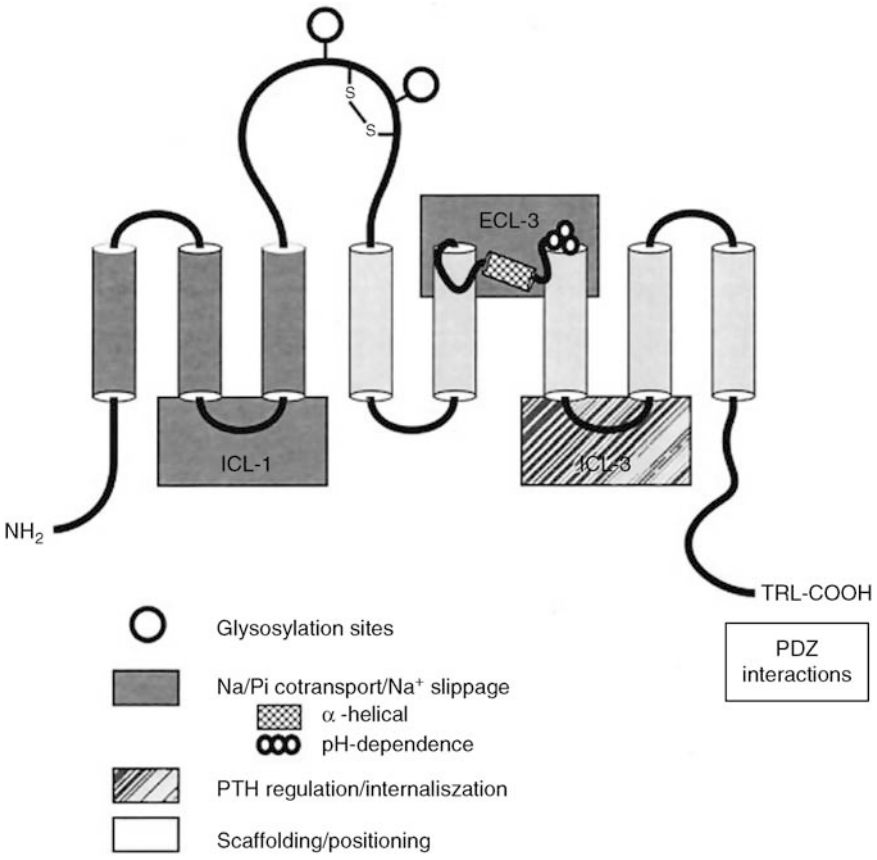
Type III Na/Pi co-transporters are cell-surface retroviral receptors [gibbon ape leukemia virus (Glvr-1, Pit-1, *SLC20A1*) and murine amphotropic virus (Ram-1, Pit-2, *SLC20A2*)] that mediate high affinity, electrogenic Na/Pi cotransport when expressed in oocytes and mammalian cells (257, 284). Glvr-1 and Ram-1 show no sequence similarity to NPT1 or NPT2a. Both Glvr-1 and Ram-1 proteins are widely expressed in mammalian tissues including the kidney and have been considered to function as "housekeeping" Na/Pi cotransporters to maintain cellular Pi homeostasis. However, recent studies report immunohistochemical evidence that Pit-2 is localized to the BBM of the rat proximal tubule, and its protein abundance is strongly up-regulated by dietary Pi restriction, but with a slower adaptation rate compared to Npt2a (285). The authors suggest that Pit-2 is a novel mediator of Pi reabsorption in the proximal tubule, and that its role in overall renal Pi handling should be re-evaluated (285).

Regulation of Renal Phosphate Transport

Regulation of NPT2a and NPT2c. Regulation of renal Pi reabsorption has been the subject of intense investigation. The major regulators of renal Pi reabsorption are thought to be dietary Pi intake, PTH, and FGF-23, although many hormonal and non-hormonal factors also are known to regulate this process (*Table 10-4*). Both NPT2a and NPT2c are the targets of regulation. Dietary Pi, PTH, and FGF-23 regulate renal Pi reabsorption primarily by inducing alterations in the abundance of NPT2a protein in the BBM of proximal tubular cells, which is accomplished either by insertion of existing transporters into the membrane or retrieval of transporters from the membrane with subsequent lysosomal degradation. Dietary Pi, PTH, and FGF-23 also regulate the abundance of NPT2c protein in the BBM (286); however, in contrast to NPT2a, NPT2c does not appear to undergo lysosomal degradation but instead may get recycled and re-inserted into the apical membrane. The mechanisms underlying

Figure 10-10

Model of the secondary structure of the rat type IIa Na-Pi cotransporter, derived from a variety of analytical approaches (reviewed in (256)). A large extracellular loop, stabilized by a disulfide bridge, separates the transporter into two domains. There is intramolecular homology within the ICL1 and ICL3 domains, and these domains are thought to form an important part of a "permeation pore" that participates in both "cotransport" and "Na^+-leak" function. Three amino acid residues between the fifth and sixth transmembrane domain are suggested to determine the pH dependence of the transporter. Two basic amino acid residues in ICL3 are important for PTH-dependent internalization. The COOH terminus contains information important for brush border membrane expression, i.e., a terminal PDZ-binding motif and a membrane internalization signal. (Reprinted with permission from (256)).



membrane trafficking of NPT2a and NPT2c proteins are complex and involve interaction of the transporters with various scaffolding and signaling proteins such as NHERF1, NHERF2, NHERF3 (PDZK1), NHERF4 (PDZK2), and Shank2E (287, 288).

Dietary Phosphate. Dietary intake of Pi is a key physiologic determinant of renal Pi handling. An increase or decrease in dietary Pi predictably induces an increase or decrease, respectively, in urine Pi excretion; with severe Pi restriction, urine Pi is negligible. This adaptation is independent of changes in the filtered load of Pi, in ECF volume, plasma calcium, growth hormone, vitamin D status, or parathyroid activity, and appears to reflect changes in the rate of Pi reabsorption by the proximal tubule, specifically, an increase or decrease in the Vmax of Na/Pi cotransport activity. The adaptation can be demonstrated both in vivo and in isolated perfused PCT segments and BBM vesicles taken from animals maintained on differing dietary intakes of Pi (289–294). Renal tubular adaptation to changes in either Pi intake or plasma Pi concentration occurs rapidly; an increase in BBM vesicle Na/Pi cotransport was observed after 2–4 h of Pi restriction in the rat (295–297); conversely, a decrease in Pi transport was induced after 1 h of Pi infusion (298). Similarly, exposure of cultured renal epithelial cells (LLC-PK1) to a low Pi concentration in the medium

Table 10-4

Factors affecting renal phosphate excretion

Factor	Urine Pi excretion	Mechanism/nephron site
Dietary		
Volume expansion	↑	↑ Filtered load of Pi ↓Proximal and distal reabsorption
High Pi intake	↑	↓ Proximal reabsorption
Phosphorus restriction	↓	↑ Proximal reabsorption
Metabolic		
Acidosis	↑	↓ Tubular reabsorption
Alkalosis	↓	↑ Tubular reabsorption
Hormones		
PTH	↑	↓ Proximal and distal reabsorption
$1,25(OH)_2D$ (chronic)	↑	↓ Proximal reabsorption
FGF-23, FGF-7, sFRP4 (Phosphatonins)	↑	↓ Proximal reabsorption
Calcitonin	↑	↓ Tubular reabsorption
Growth hormone, IGF-1	↓	↑ Proximal reabsorption, ↑ GFR
Thyroid hormone	↓	↑ Tubular reabsorption
Insulin	↓	↑ Proximal reabsorption
Diuretics		
Mannitol, loop diuretics, thiazides	↑	↓ Tubular reabsorption, site varies
Other		
Glucose	↑	Osmotic diuresis, ↓reabsorption PCT
Glucocorticoids	↑	↓ Proximal reabsorption
Immaturity	↓	↑ Proximal and distal reabsorption

induced both short-term (minutes) and long-term (hours) adaptations in Na/Pi cotransport (299–301).

Such dietary Pi-induced regulation of Pi reabsorption is achieved primarily by alterations in the abundance of type IIa Na/Pi cotransporter protein in the BBM of proximal tubule cells. Short-term (hours) exposure of rats to a Pi restricted diet induced an increase in both BBM Na/Pi cotransport activity and NPT2a protein abundance but no change in NPT2a mRNA (302). Chronic (days) restriction of dietary Pi in mice, rats, and rabbits leads to an adaptive increase in BBM Na/Pi cotransport and in the abundance of NPT2a protein and mRNA (303–309). The acute increase in Pi transport induced by Pi deprivation is mediated by microtubule-dependent recruitment of existing NPT2a protein to the apical membrane (302). In contrast, exposure to high dietary Pi leads to internalization of cell surface NPT2a protein into the endosomal compartment by a microtubule-independent mechanism (302). Internalized NPT2a protein is then delivered to the lysosome by a microtubule-dependent process, for degradation (310).

A Pi response element (PRE) was identified in the mouse *NPT2a* promoter by DNA footprint analysis (311). The PRE was shown to bind a mouse transcription factor, TFE3, and the renal expression of TFE3 is increased in response to Pi deprivation (311). On the basis of these results, it was suggested that TFE3 participates in transcriptional regulation of the NPT2a gene by dietary Pi.

NPT2c also is regulated by dietary Pi. Dietary Pi restriction induces an increase in NPT2c immunoreactive protein in the apical membrane of proximal tubule cells, whereas feeding a high Pi diet induces a decrease (286, 312). Internalization of NPT2c was slightly delayed relative to that of NPT2a after acute exposure to high dietary Pi. Internalized NPT2c is, however, not degraded in the lysosomes.

Parathyroid Hormone. PTH is a major hormonal regulator of renal Pi reabsorption (166, 313). PTH acts directly on proximal tubular cells to inhibit Na/Pi cotransport through mechanisms that involve rapid internalization of cell surface NPT2a protein (314) and its subsequent lysosomal degradation (315). A prolonged increase in

PTH also can induce a decrease in type II Na/Pi cotransporter mRNA abundance (314).

PTH binding to the PTH/PTHrP receptors on the basolateral membrane activates PKA- and/or PKC-dependent signaling pathways, whereas PTH binding to apical receptors activates the PKC pathway (316). The extracellular signal-regulated kinase (ERK)/MAPK pathway also participates in PTH-induced signaling (317), and recent studies have shown that the PKA and PKC signaling pathways converge on the ERK/MAPK pathway to internalize NPT2a protein (318). Although the downstream targets for ERK/MAPK-mediated phosphorylation remain unknown, changes in the phosphorylation state of NPT2a are not associated with its PTH-induced internalization (319). Rather, it has been postulated that the phosphorylation of proteins that associate with NPT2a may determine its regulation. AKAP79, an A kinase anchoring protein (320), and RAP, a receptor-associated protein (321), were shown to participate in the PTH-mediated retrieval of NPT2a from the plasma membrane of proximal tubular cells. In opossum kidney (OK) cells, AKAP79 associates with NPT2a and the regulatory and catalytic subunits of PKA, and this process is necessary for PKA-dependent inhibition of Na/Pi cotransport (320). In RAP-deficient mice, PTH-induced internalization of NPT2a is significantly delayed whereas its regulation by dietary Pi is not affected (321).

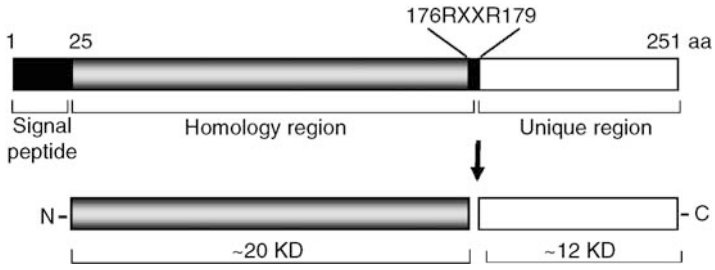
PTH-dependent regulation of NPT2c is less well understood. In response to PTH, NPT2c disappears from the surface of the BBM at a much slower rate than does NPT2a, and NPT2c does not seem to undergo lysosomal degradation (286, 322–324). Indeed, preliminary evidence suggests that NPT2c may be recycled and re-inserted into the BBM (325). In rats fed a low Pi diet, NPT2a and NPT2c undergo different regulation by PTH. PTH(1–34) failed to decrease NPT2a expression in BBM vesicles from rats on a low Pi diet, consistent with the blunted phosphaturic effect of PTH observed in hypophosphatemic humans or rodents (326). In contrast, PTH was able to efficiently reduce the expression of NPT2c (323). Furthermore, whereas NPT2a is expressed in segments S1 through S3 of the proximal tubule, NPT2c is expressed only in the S1 segment.

Fibroblast Growth Factor 23. FGF-23 is a newly discovered, bone-derived circulating peptide that plays an important role in regulating Pi and vitamin D metabolism. Through a positional cloning approach, FGF-23 was first identified as the gene disrupted in patients with autosomal dominant hypophosphatemic rickets (ADHR) (327–329), a disorder characterized by hypophosphatemia due to renal Pi wasting, inappropriately low or normal serum concentrations of $1,25(OH)_2D$, and rickets or osteomalacia (330). Affected patients harbor mutations that alter the peptide's furin cleavage site (residues 176 through 179), thereby preventing the normal proteolytic processing of FGF-23 (331) and resulting in its accumulation in the plasma. Excess FGF-23 also is implicated in the pathogenesis of two related hypophosphatemic disorders, tumor-induced osteomalacia (TIO) (332–334), and X-linked hypophosphatemia (XLH) (330). FGF-23 is abundantly expressed in tumors that cause TIO (335, 336), and serum concentrations of FGF-23 are greatly increased in TIO patients and also in some patients with XLH (337, 338). With surgical removal of the tumor, FGF-23 concentrations decrease to normal values and the disorder resolves. Extracts from these tumors inhibit Pi transport in renal proximal tubule cells in vitro (339, 340), consistent with the notion that FGF-23 is responsible for this inhibition.

The human *FGF-23* gene consists of three exons spanning 10 kb of genomic sequence that encode a 251 amino acid precursor protein comprising a hydrophobic amino acid sequence (residues 1 through 24), which likely serves as a leader sequence (*Fig. 10-11*). Unlike most other fibroblast growth factors, FGF-23 appears to be efficiently

Figure 10-11

Schematic depiction of the mature FGF-23 protein and the sizes of the two fragments derived from cleavage by a subtilisin-like proprotein converstase. Threonine at position 178 within the RXXR motif undergoes O- linked glycosylation.



secreted into the circulation. FGF-23 binds, albeit with relatively low affinity, to most of the different splice variants of the known FGF receptors (341). However, in the presence of Klotho, a membrane bound protein with β-glucuronidase activity, FGF-23 can bind with high affinity to FGFR1(IIIc) (342, 343), suggesting that Klotho plays an important role in mediating the actions of FGF-23. Thus, it is currently thought that FGF-23 acts through known FGFRs but only in those tissues in which Klotho is also expressed, including kidney (344, 345).

FGF-23 acts on the kidney to impair Pi reabsorption and inhibit the synthesis of $1,25(OH)_2D$. In mice, administration of FGF-23 induces a decrease in serum Pi concentration, increased renal Pi excretion, inhibition of BBM Na/Pi cotransport, and decreased renal Npt2a expression (336, 346–349). Mice transplanted with cell lines stably expressing FGF-23 or transgenic mice that over-express FGF-23 display hypophosphatemia due to renal Pi wasting, low serum $1,25(OH)_2D$ concentrations, and abnormal bone development (336, 348, 350–352). FGF-23 suppresses the renal production and serum concentration of $1,25(OH)_2D$ by suppressing 25-hydroxyvitamin D-1α-hydroxylase mRNA and protein expression in vivo and in vitro and stimulating 24-hydroxylase mRNA expression (336, 347, 349, 352). Findings opposite to those in Fgf-23 transgenic animals were observed in mice homozygous for ablation of the Fgf-23 gene (*Fgf23*-null). These animals develop hyperphosphatemia and increased serum $1,25(OH)_2D$ concentrations, abnormal skeletogenesis, and they die prematurely, partly due to renal failure secondary to renal calcification (353–355). The findings in *Fgf23*-null mice overlap significantly with those in *Klotho*-null mice (356–360), even though *Klotho*-null animals show greatly increased serum levels of biologically active FGF-23 (357). These observations provide further evidence that Klotho plays an important role in mediating the actions of FGF-23.

Other Hormonal Regulators: Administration of $1,25(OH)_2D$ to vitamin D-deficient rats induces an increase in BBM Na/Pi cotransport that is accompanied by an increase in renal NPT2a mRNA and protein abundance (361). While these results are consistent with direct effects of $1,25(OH)_2D$ on NPT2a-mediated renal Na/Pi cotransport, the effects may result from a $1,25(OH)_2D$-dependent decrease in PTH levels. However, the finding that $1,25(OH)_2D$ increased the activity of a NPT2a promoter-luciferase reporter gene construct suggests a direct effect of this hormone on NPT2a gene transcription (361). In vitamin D receptor (VDR)-null mice, in which serum levels of PTH and $1,25(OH)_2D$ are greatly increased, the abundance of NPT2a protein in renal BBM vesicles was significantly decreased, whereas the abundance of NPT2c protein was unaffected (362). This finding suggests that $1,25(OH)_2D$ has little direct effect on NPT2c expression.

Growth hormone acts to increase renal Pi reabsorption, independently of PTH (363, 364). In growth hormone-deficient subjects, the serum Pi concentration and the TmP/GFR are reduced; both increase with administration of growth hormone (365, 366). In patients with acromegaly, serum Pi concentrations are increased (367). Growth hormone stimulates proximal tubular Na/Pi cotransport (368–371) which is mediated, at least in part by increased production and release of insulin-like growth factor 1 (IGF-1) (363, 372). Receptors for growth hormone are present on the basolateral membrane of proximal tubule cells and appear to activate the phospholipase C pathway. Receptors for IGF-1 also have been identified in proximal tubule membranes and their effects may involve tyrosine kinase activity (363).

Fibroblast growth factor 7 (FGF7), which is produced by a TIO-causing tumor, was recently shown to inhibit Pi uptake in OK cells, thus suggesting that FGF7 can also cause phosphaturia and may be responsible for TIO in those patients who have no elevation in circulating FGF23 levels (373). Secreted frizzled-related protein 4 (sFRP4), which, like FGF-23, is highly expressed in tumors from patients with TIO (374), has also been tested for its phosphaturic action. sFRP-4 induced a specific increase in the renal fractional excretion of Pi and hypophosphatemia when infused in rats and inhibited Na/Pi cotransport in vitro when added to OK cells (374).

Stanniocalcin is a peptide hormone that counteracts hypercalcemia and stimulates Pi reabsorption in bony fish, and is also produced by humans. Infusion of stanniocalcin in rats stimulates renal Pi reabsorption and BBM Na/Pi cotransport (375), suggesting a role for stanniocalcin in the maintenance of Pi homeostasis in mammals as well as fish. 5-hydroxytryptamin (5-HT) is synthesized in the kidney, and locally generated 5-HT was shown to interfere with PTH-mediated inhibition of renal Na/Pi cotransport (376), suggesting that 5-HT is a paracrine modulator of renal Pi transport. The increase in BBMN Na/ Pi cotransport induced by thyroid hormone is associated with an increase in NPT2a mRNA (377), whereas both hypercalcemia (378) and epidermal growth factor (379) decrease NPT2a mRNA abundance. Neither thyroid hormone nor hypercalcemia has an effect on NPT2a promoter-reporter gene expression (see (380)), suggesting that transcriptional mechanisms are not involved. Other factors which inhibit Pi reabsorption are: PTH-related peptide, calcitonin, atrial natriuretic factor,

epidermal growth factor, transforming growth factor-β, and glucocorticoids (for review see (238, 255, 260)).

Effect of Npt2a and NPT2c Gene Disruption. The critical role of NPT2a in the maintenance of Pi homeostasis has been clearly demonstrated in mice in which the *Npt2a* gene (lower case refers to the mouse gene) was knocked out by targeted mutagenesis (381). Mice that are null for *Npt2a* exhibit decreased renal Pi reabsorption, an ~80% loss of BBM Na/Pi cotransport, hypophosphatemia, an appropriate adaptive increase in the renal synthesis and serum concentration of $1,25(OH)_2D$ (246, 381) and associated hypercalcemia, hypercalciuria, and hypoparathyroidism, and an age-dependent skeletal phenotype (381, 382). Dietary Pi intake and PTH were without effect on renal BBM Na/Pi cotransport in *Npt2a*-null mice (383, 384), demonstrating that NPT2a is a major regulator of renal Pi handling. In the BBM of Npt2a null mice, the abundance of Npt2c protein was shown to be increased significantly (385), likely accounting for at least some of the residual Na/Pi cotransport in the mutant mice.

In preliminary studies, mice that are null for *Npt2c* exhibit, at different ages, only a small increase in blood ionized calcium and some increase in urinary calcium excretion, but not hypophosphatemia or increased urinary Pi excretion (386), suggesting that Npt2c may be of limited functional significance in rodents. However, the combined ablation of Npt2a and Npt2c exhibits a more severe phenotype than the ablation of Npt2a alone, suggesting that the Npt2c is likely to have more significant role than suggested by the Npt2c-null animals.

Non-Hormonal Regulators*: *Volume Status. Expansion of the extracellular fluid volume results in an increase, and volume contraction a decrease, in urine Pi excretion (*Table 10-4*) (166, 238). The effect can be attributed in part to changes in the filtered load of Pi and rate of Pi reabsorption by the proximal tubule, and to changes in plasma ionized calcium, the latter affecting secretion of PTH. A direct effect of volume expansion on tubular Pi reabsorption also has been reported.

Acid–base Status. Changes in acid–base status can significantly effect renal handling of Pi (166,313). Acute respiratory acidosis results in a decrease in renal Pi reabsorption; this effect may depend on an increase in pCO_2 tension but does not depend on an increase in filtered Pi load, expansion of the extracellular fluid volume, or change in PTH or blood bicarbonate concentration (387). Conversely, acute respiratory alkalosis induces an increase in renal Pi reabsorption and resistance to the phosphaturic action of both PTH and cAMP; these effects may depend on changes in pCO_2 tension but are independent of changes in plasma Pi concentration (388).

Although acute metabolic acidosis has minimal effects on urine Pi excretion (389), chronic metabolic acidosis can impair renal Pi reabsorption, independently of PTH and even when dietary Pi is severely restricted (390, 391). The suppressive effect of metabolic acidosis on Na/Pi cotransport is observed in BBM vesicles and is attributed to a decrease in Vmax of the transporter (391, 392). In rats fed ammonium chloride for 10 days, the 60% decrease observed in BBM Na/Pi cotransport activity was associated with a threefold decrease in BBM Npt2 protein abundance and a twofold decrease in mRNA (393). With a shorter duration of acidosis (<24 h), the changes were of lesser magnitude, with no change in Npt2 mRNA observed with very acutely-induced (6 h) acidosis. The inhibitory effect of metabolic acidosis on Pi transport was independent of endogenous PTH activity but was greatly attenuated in rats fed a low (0.1%) Pi diet (393). In recent studies of mice fed ammonium chloride, the renal abundance of Npt2a mRNA was reduced at 2 but not 7 days of acidosis, whereas Npt2c mRNA was reduced at both time points (394). However, the protein abundance of Npt2a and Npt2c in BBM were both paradoxically increased (394), suggesting that with acidosis, phosphaturia might be induced by direct interactions between protons and the type IIa and IIc Na/Pi cotransporters, thereby reducing transport activity (394). Acute metabolic alkalosis induced by infusion of sodium bicarbonate reduces Pi reabsorption when the prior dietary intake of Pi is high, but increases Pi reabsorption when dietary Pi is normal (166, 395–398). Chronic metabolic alkalosis predictably increases renal Pi reabsorption (313).

Growth and Development. As noted above, the serum Pi concentration is considerably higher in newborn infants and young children than in older children and adults. This finding is not due to a limitation of the immature kidney's ability to excrete Pi but rather reflects a higher rate of tubular Pi reabsorption in infants and immature animals, as demonstrated by their higher values for TmP/GFR compared with values in adults (399). In newborn animals as compared with adult animals, Pi reabsorption is greater, both in the early PCT and in more distal nephron segments, presumably the pars recta and segments beyond the DCT. In BBM vesicles, the Vmax for Na/Pi cotransport is higher in newborn than in adult guinea pigs, a finding that cannot be accounted for by differences in plasma Pi concentrations, ionized calcium, PTH, thyroxine, or calcitonin. The higher capacity for Pi reabsorption by the immature kidney also may reflect its relatively greater number of juxtamedullary nephrons, which have a higher

capacity to reabsorb Pi. Newborn animals demonstrate a blunted phosphaturic response both to Pi loading and to administration of PTH, the latter despite a normal increase in urine cAMP. In Pi restricted rats, the adaptive increase in Pi reabsorption is much greater in immature than in adult animals. Growth hormone may play an important role in mediating the increased renal reabsorption of Pi during development. Also, as noted above, the higher abundance of Npt2c mRNA and protein in the kidneys of young rats might contribute to their higher rate of Pi transport as compared to that in older animals (277). Thus, through a variety of mechanisms, Pi handling by the immature kidney is regulated such that Pi retention is promoted, presumably to meet the increased needs for Pi of the growing organism (399, 400).

Diuretic Agents. Although their mechanisms and sites of action differ, the following diuretic agents predictably induce phosphaturia: mannitol, acetazolamide, thiazide diuretics, and loop diuretics. Urine phosphorous excretion is little affected by amiloride, spironolactone, and triamterene (238).

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11 Genetic Disorders of Calcium and Phosphate Homeostasis

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Introduction

The regulation of calcium and phosphate homeostasis involves several different hormones that act on the kidney, intestine, and bone. Parathyroid hormone (PTH) is the primary regulator of extracellular calcium ion concentration; while fibroblast growth factor 23 (FGF-23) is likely to be the most important regulator of extracellular phosphate concentration. In response to a decrease in the extracellular calcium concentration, secretion of PTH from the parathyroid glands is increased. PTH acts on the distal renal tubules to decrease excretion of calcium and it stimulates the production of the 1,25-dihydroxy vitamin D ($1,25(OH)_2D$) in the proximal tubules. The biologically active $1,25(OH)_2D$ in turn acts on the intestine to enhance the absorption of calcium and phosphorus. Together with PTH, this steroid hormone furthermore acts on the bone to increase the release of calcium and phosphorus into the extracellular fluid. PTH and $1,25(OH)_2D$ thus help maintain extracellular calcium concentration within the normal limits, but also increase the extracellular phosphorous concentration, which can be a problem, particularly in patients with chronic kidney disease. To limit the PTH- and $1,25(OH)_2D$-dependent increase in blood phosphorous level, PTH enhances the urinary excretion of phosphate by reducing the expression levels of two sodium-dependent phosphate co-transporters, NPT2a and NPT2c, in the proximal renal tubules.

Disorders with an abnormal regulation of calcium homeostasis can be classified according to whether they arise from an excess or deficiency of PTH, defect in the PTH-receptor (i.e., the PTH/PTHrP receptor), or insensitivity to PTH that is caused by defects down-stream of the PTH/PTHrP receptor. Recent advances in understanding the biological importance of key proteins involved in the regulation of PTH secretion and the responsiveness to PTH in target tissues has led to the identification of molecular defects in a variety of disorders, and thus have enabled the characterization of some of the mechanisms involved in the regulation of parathyroid gland development, parathyroid cell proliferation, PTH secretion, and PTH-mediated actions in target tissues.

Recent progress has also provided important new insights into the regulation of phosphate homeostasis. In particular, the molecular definition of rare inherited disorders of phosphate homeostasis has resulted in the identification and characterization of several proteins that contribute to the normal regulation of phosphate homeostasis; these include fibroblast growth factor 23 (FGF-23), phosphate-regulating protein with homologies to endopeptidases on the X chromosome (PHEX), dentin matrix protein 1 (DMP-1), FGF receptor 1c (FGFR1c), the longevity factor Klotho, the glycosyltransferase GALNT3 (which is responsible for initiating mucin-type O-linked glycosylation), and the two sodium-dependent phosphate co-transporters, NPT2a and NPT2c. In addition, proteins such as matrix extracellular phosphoglycoprotein (MEPE), soluble frizzled-related protein 4 (sFRP4), and FGF7 may have a direct or indirect role in the regulation of phosphate homeostasis. It remains largely unknown, however, whether and how the different phosphate-regulating proteins interact with each other. Furthermore, it is almost certain that additional molecules contribute to these regulatory events, and that molecular genetic studies will continue to be of pivotal importance for the identification of genes encoding novel regulators of phosphate homeostasis.

Regulators of Calcium and Phosphate Homeostasis

Parathyroid Hormone (PTH) and PTH-related Peptide (PTHrP)

The mature secreted form of PTH peptide comprises 84 amino acids, which is derived from a longer pre-pro peptide (for review see (1)). The gene encoding PTH gene is located on chromosome 11p15. PTH gene transcription (as well as PTH peptide secretion) is regulated by the extracellular concentration of calcium and phosphate

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(2, 3), and through a vitamin D response element upstream of the transcription start site (4, 5). After secretion, PTH is cleared from the circulation with a short half-life of about 2 minutes, via non-saturable hepatic and renal uptake (6).

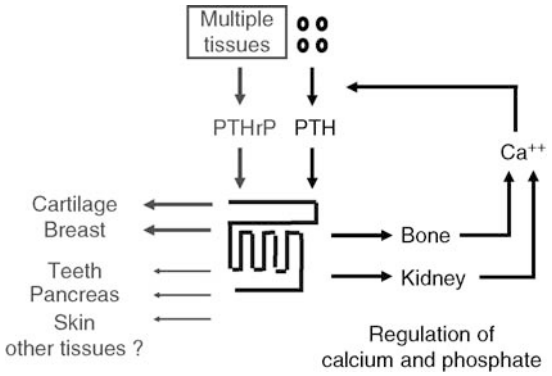
PTH secretion by the parathyroid glands is regulated through the calcium-sensing receptor (CaSR) and even small perturbations in blood calcium concentration lead to remarkable changes in PTH secretion. Besides being expressed in the chief cells of the parathyroid glands, the CaSR is expressed in the kidneys and in several other tissues, albeit at a lower abundance, which are not directly involved in the regulation of calcium homeostasis. In addition to regulating PTH secretion, the CaSR plays an important role in regulating renal divalent mineral transport by both direct and indirect mechanisms (7).

The PTH-related peptide (PTHrP also known as PTHrH, PTH-related hormone), which was first isolated from tumors that cause the humoral hypercalcemia of malignancy syndrome, shares significant amino acid sequence homology with PTH (8–10). The human PTHrP gene is located on chromosome 12p12.1–11.2, which is a region analogous to that containing the human PTH gene (11, 12). PTH and PTHrP, although distinct products of different genes, exhibit considerable functional and structural similarities, including equivalent positions of the boundaries between some of the coding exons and of the adjacent introns; both may have evolved from a shared ancestral gene (11, 12). PTHrP is a larger, more complex protein than PTH and it is synthesized in different organs and tissues. It functions as an autocrine/paracrine rather than an endocrine factor with a little influence on calcium homeostasis except when secreted in large concentrations (13). One of its most prominent functions is the regulation of chondrocyte proliferation and differentiation, and consequently of bone elongation and growth (14).

PTH and PTHrP both mediate their actions through a common receptor (15, 16) (*Fig. 11-1*). This PTH/PTHrP receptor is a member of a subgroup of G protein-coupled receptors; its gene is located on chromosome 3p21.3 (17, 18). The PTH/PTHrP receptor is abundantly expressed in the kidney and the bone, where it mediates the endocrine actions of PTH. However, the most abundant expression of the PTH/PTHrP receptor occurs in chondrocytes of the metaphyseal growth plate where it mediates predominantly the autocrine/paracrine actions of PTHrP, i.e., it delays the hypertrophic differentiation of growth plate chondrocytes (6, 14, 19). A second receptor, the PTH2 receptor, shares more than 50% homology with the PTH/PTHrP receptor (20). The human, but not the rodent PTH2 receptor is activated by PTH. The PTH2 receptor is not activated by PTHrP and its primary ligand is TIP39 (tubuloinfundibular peptide of 39 residues), appears to be involved in nocireception and reproduction (21–24).

Figure 11-1

Parathyroid hormone (PTH) mediates its endocrine actions through the PTH/PTHrP receptor expressed in the kidney and the bone to regulate mineral ion homeostasis and bone metabolism. In numerous other tissues, this G protein-coupled receptor mediates the paracrine actions of PTHrP; particularly important is its role in the growth plate.



Vitamin D and Its Metabolites

Most of the vitamin D in healthy individuals is derived via cutaneous synthesis by ultraviolet light from the precursor 7-dehydrocholestrol (25, 26). Vitamin D (D_2 and D_3) is a prohormone, which is stored in muscle or fat. It undergoes hydroxylation in the liver to form the 25-hydroxyvitamin D metabolite (25(OH) D). In the proximal convoluted tubular cells of the kidney, 25(OH)D is further hydroxylated by the enzyme 25-hydroxyvitamin D, 1α hydroxylase (1α hydroxylase) to yield the biologically active metabolite 1,25 dihydroxyvitamin D ($1,25(OH)_2D$) (25, 26). The activity of the 1α hydroxylase is regulated by extracellular concentrations of ionized calcium, inorganic phosphate, PTH, and FGF-23 (27). $1,25(OH)_2D$ binds in target organs (e.g., intestine, bones, kidneys and parathyroids) to the intracellular vitamin D receptor (VDR) (28), and thereby activates the transcription of genes in the bone, kidney and enterocytes that help increase gut absorption of calcium, reduce urinary calcium losses, and increase bone resorption, thereby ensuring adequate extracellular concentration of calcium and phosphate (26, 28). Mutations in the genes encoding the 1α hydroxylase and the VDR are associated with rickets (25, 26).

Fibroblast Growth Factor 23 (FGF-23) and Other Proteins with Phosphaturic Properties

Fibroblast growth factor 23 (FGF-23) belongs to a large family of structurally related proteins. Its role in the regulation of blood phosphorous homeostasis was first predicted when a positional cloning approach to determine the cause of autosomal dominant hypophosphatemic rickets (ADHR) led to the identification of several different, heterozygous mutations in the gene encoding FGF-23 (29). FGF-23 mRNA and protein were furthermore found to be markedly overexpressed in tumors that cause oncogenic osteomalacia, and in vivo findings indicated that this hormone promotes, either directly or indirectly, renal phosphate excretion (30, 31). The FGF-23 gene consists of 3 exons that encode a 251 amino acid precursor protein comprising a hydrophobic leader sequence (residues 1 through 24), thus allowing its secretion into the blood circulation (*Fig. 11-2*). FGF-23 protein undergoes O-linked glycosylation and it is proteolytically cleaved between Arg^{179} and Ser^{180} by the subtilisin-like proprotein convertase SPC2 (32); the intact FGF-23 appears to be the biologically active hormonal form (33).

FGF-23 is most closely related to fibroblast growth factors 21, 19, and 15, but shows limited homology also with other fibroblast growth factors (29, 31). FGF-23 mRNA could not be detected by Northern blot analysis in normal tissues, but it has been identified by reverse transcriptase (RT)-PCR in heart, liver, thymus, small intestine, and brain (29, 31). However, the most abundant expression of FGF-23 occurs in bone cells, particularly in osteocytes, which represent the largest population of bone cells (34–36). Its regulation remains not thoroughly understood, but recent studies in humans and mice that are null for DMP1 have suggested that this bone matrix protein reduces expression of FGF-23 (37, 38). However, the 57 kDa C-terminal fragment of DMP1 appears to be sufficient for this regulatory role (39).

In the presence of Klotho, a protein associated with longevity (40, 41), FGF-23 binds with high affinity to the FGFR1 splice variant FGFR1c (41, 42) (*Fig. 11-3*). Consistent with the role of Klotho in the regulation of phosphate homeostasis, mice that are "null" for Klotho develop severe hyperphosphatemia due to diminished urinary phosphate excretion. Furthermore, these animals show elevated 1,25 $(OH)_2D$ levels i.e., findings that are similar to those observed in the FGF-23-null mice (35, 36, 43). Distinct from the latter animals, however Klotho-null mice have dramatically elevated FGF-23 levels (42). In the absence of Klotho, FGF-23 can bind, albeit with low affinity, to several other FGF receptors, but the biological consequences of these interactions remain to be determined (44).

Mice receiving the recombinant FGF-23 intraperitoneally and nude mice transplanted with cell lines stably expressing FGF-23 develop hypophosphatemia due to increased urinary phosphate excretion, which is caused by a reduction in the expression of the sodium-dependent phosphate cotransporters NPT2a and NPT2c (31, 33, 45–48). PTH and FGF-23 thus have similar effects on the apical expression of these two important renal transporters, but the time courses of these hormonal effects appear to differ. Besides hypophosphatemia, animals with increased circulating FGF-23 levels show an increase in alkaline phosphatase activity, a marked increase of unmineralized osteoid, and a significant widening of growth plates leading to deformities of weight bearing bones. Furthermore, there is a

Figure 11-2

Schematic presentation of the FGF-23 precursor, which comprises a signal peptide for efficient secretion (amino acid residues 1–24; stripped area). The mature FGF-23 is glycosylated at amino acid residue 178 (and most likely other residues), and it undergoes cleavage at the RXXR through subtilisin-like protein convertases to generate fragments that appear to be devoid of phosphaturic activity.

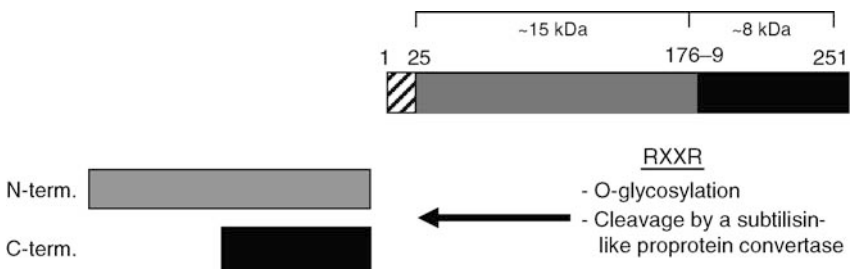
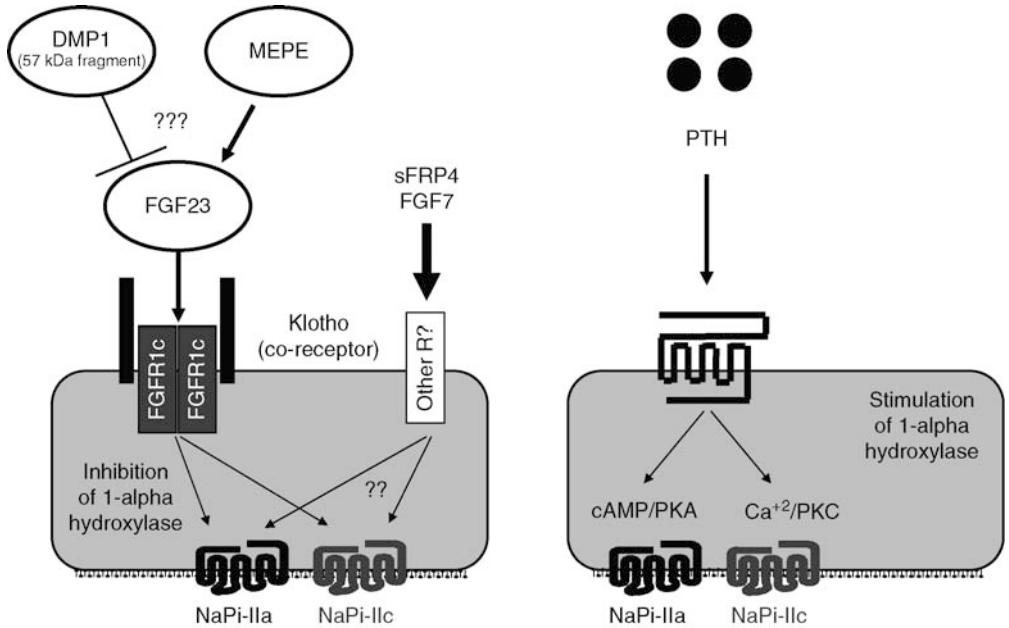


Figure 11-3

Production of fibroblast growth factor 23 (FGF-23) by osteocytes is regulated by matrix extracellular phosphoprotein (MEPE) and dentin matrix protein 1 (DMP1). Parathyroid hormone (PTH) is produced by the parathyroid glands. Both hormones activate their specific cognate receptors, FGFR1c with co-receptor Klotho and PTH/PTHrP receptor (PTHR1), respectively, on the proximal renal tubules to reduce the apical expression of the sodium-dependent phosphate co-transporters NaPi-IIa (NPT2a) and NaPi-IIc (NPT2c); PTH increases expression of the 1-alpha hydroxylase, while FGF-23 inhibits this mitochondrial enzyme. The potential role of other factors, such as soluble frizzled-related peptide 4 (sFRP4) and fibroblast growth factor 7 (FGF7), in the renal regulation of phosphate homeostasis remains to be investigated further.



FGF-23-dependent reduction in the activity of the 1-α hydroxylase in the proximal renal tubules leading to a reduction in serum $1,25(OH)_2D$ levels (49–51).

In contrast to the findings made in animals with increased FGF-23 levels, Fgf-23-null mice (*Fgf-23*$^{-/-}$) develop hyperphosphatemia and elevated serum $1,25(OH)_2D$ concentration, and they die prematurely, secondary to renal failure because of glomerular capillary calcifications (35, 43, 52). *Fgf-23*$^{-/-}$ animals furthermore show reduced bone turnover, an unexpected increase in osteoid, diminished osteoblast and osteoclast number and activity (43).

Besides FGF-23, MEPE, sFRP4, and FGF-7 were also shown to be overrepresented in cDNA libraries derived from tumors that cause oncogenic osteomalacia (53, 54). All three proteins have been implicated in phosphate handling in vivo and/or in vitro, suggesting that "phosphatonins" other than FGF-23, may be involved in the renal regulation of phosphate homeostasis; however, specific receptors mediating their actions have not yet been identified (54–56) (see *Fig. 11-3*).

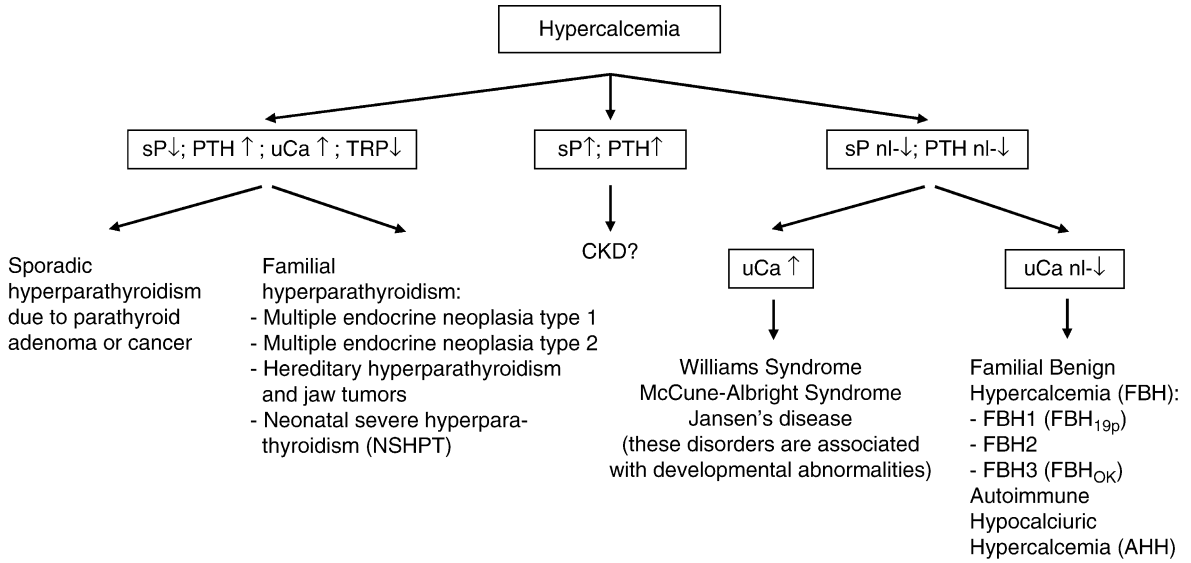
Hence to summarize, protein purification and molecular cloning techniques, and particularly the exploration of rare genetic disorders through positional cloning or candidate gene approaches have provided important new insights and unique molecular tools, which will help in determining the pathogenesis of common and uncommon disorders associated with an abnormal regulation of calcium and phosphate.

Hypercalcemia and Hypophosphatemia due to Increased Parathyroid Gland Activity

Hypercalcemia can be observed in several different sporadic or familial disorders, which requires besides physical examination and a careful review of the family history, the evaluation of several additional laboratory parameters (*Fig. 11-4*).

Figure 11-4

Flow-diagram for the work-up of patients with hypercalcemia.



In children, parathyroid tumors are rare (57), but as in adults two principal defects can lead to the development of parathyroid tumors: (1) a heterozygous mutation that enhances the activity of a gene (gain-of-function mutation), which is therefore referred to as a proto-oncogene, or (2) homozygous loss-of-function mutation in a tumor-suppressor gene, which is therefore referred to as a recessive oncogene. Parathyroid tumors usually occur as an isolated and sporadic endocrinopathy, or as part of inherited tumor syndromes (58) such as the multiple endocrine neoplasias (MEN) or hereditary hyperparathyroidism with jaw tumors (59, 60). Sporadic parathyroid tumors can be caused by single somatic mutations that lead to the activation or overexpression of proto-oncogenes such as PRAD1 (*pa*rathyroid *a*denoma *1*) or RET (mutations of which result in MEN2), or by mutations in tumor suppressor genes - predicted to be located on several different chromosomes, e.g., 1P, the location of RIZ1 and 11q13, which is the location of the MEN1 gene - that allow for the clonal expansion of a single parathyroid cell and its progeny (61) for detailed review of this topic in adults).

Chronic overstimulation of the parathyroid glands frequently occurs in patients with chronic kidney disease (CKD), which may lead to the development of tertiary hyperparathyroidism due to the clonal expansion of one or several parathyroid cells, as described for adults (62), through a process possibly involving the reduced expression of different cyclin-dependent kinase inhibitors (63). Furthermore, the development of hyperparathyroidism may be enhanced by frequently dramatically increased levels of circulating FGF-23, particularly in patients with CKD stage V. However, even in patients with earlier stages of CKD, FGF-23 levels can be elevated considerably, presumably due to intermittent hyperphosphatemia, leading to a reduction in $1,25(OH)_2D$ levels, which may contribute the development of secondary hyperparathyroidism and possibly the clonal expansion of some parathyroid cells (51, 64, 65). Recently, it was furthermore shown that FGF-23 can inhibit PTH secretion by normal parathyroid glands (66, 67), but it remains uncertain, why the dramatically elevated FGF-23 levels in CKD stage V cannot prevent the development of hyperparathyroidism.

PTH structural changes as the cause of hyperparathyroidism appear to be rare. However, recently an adult patient was reported, to have had hypercalcemia and hypophosphatemia, yet undetectable levels of PTH in the circulation. A single parathyroid adenoma was identified that secreted a mutant PTH molecule, which is truncated after amino acid residue 52, thus explaining why different two-side immunometric PTH assays had been unable to detect elevated circulating levels of this hormone (68). After surgical removal of the adenoma, clinical symptoms and biochemical abnormalities were resolved. These findings raise the question whether the lack of the C-terminal portion of PTH contributed to the development of the parathyroid adenoma and whether

this part of the molecule has unknown tissue-specific effects that remain to be characterized.

Hypercalcemic Disorders with Normal Parathyroid Gland Activity

Disorders of the Calcium-Sensing Receptor (CaSR)

CaSR is a G-protein coupled receptor located on chromosome 3q21.1 (69). Three hypercalcemic disorders are caused by mutations in the gene encoding the calcium-sensing receptor (CaSR) (70–76). There appears to be a gene-dosage effect associated with many of the inactivating CaSR mutations. Heterozygous mutations cause familial benign hypercalcemia (FBH), also referred to as familial hypocalciuric hypercalcemia (FHH), with mild hypercalcemia, while homozygous mutations result in neonatal severe hyperparathyroidism (NSHPT), a severe phenotype characterized by hypercalcemia early in life, bone demineralization and failure to thrive. Another form of hypocalciuric hypercalcemia is caused by acquired autoantibodies against the CaSR (AHH) and is associated with other autoimmune defects (see *Fig. 11-4*).

Familial Benign Hypercalcemia (FBH) and Neonatal Severe Hyperparathyroidism (NSHPT)

FBH may be inherited as an autosomal dominant trait, although patients may often not have a family history as they could have developed a *de novo* mutation. Affected patients are usually asymptomatic or have non-specific symptoms such as fatigue, weakness, painful joints and headache, and the diagnosis is often only suspected after a routine biochemical screening showing high calcium levels.

Mutational analyses of the humans have revealed different mutations that result in a loss-of-function of the CaSR in patients with FBH and NSHPT (70–75). Many of these mutations cluster around low affinity calcium-binding sites (that are similar to calsequestrin) containing aspartate- and glutamate-rich regions (codons 39–300) within the extracellular domain of the receptor (76, 77). Extracellular calcium has a steep inverse sigmoidal relationship to PTH secretion; the 'set point' of parathyroid cells is defined as the calcium concentration at which PTH secretion is half-maximal. Approximately two thirds of the affected members of investigated FBH kindreds were found to have unique heterozygous mutations that cause a loss of CaSR function thus increasing the set point (70, 75, 78, 79). Patients with severe neonatal hyperparathyroidism usually have homozygous or compound heterozygous CaSR mutations that are inactivating. However, recently a novel heterozygous mutation (F180C, TTC > TGC) was described in exon 4 of the CaSR gene, which led in the affected individuals to symptomatic hyperparathyroidism, yet low urinary calcium excretion. Vitamin D deficiency was documented in these patients, which probably impaired CaSR expression, thus leading to increased PTH secretion; consistent with this conclusion, vitamin D supplements resulted in the normalization of PTH levels (80). Another CaSR mutation (L137P) previously identified in FBH families was recently found to be involved in the development of chronic pancreatitis, when combined with a mutation (N34S) in the pancreatic secretory trypsin inhibitor gene (*SPINK1*). In fact, numerous patients with chronic pancreatitis, who carry the N34S mutation in *SPINK1,* were found to also have CaSR mutations suggesting that mutations in the latter gene can increase the susceptibility for pancreatitis (81).

Some FBH families in whom a mutation within the coding region of the CaSR could not be demonstrated may either have an abnormality in the promoter of the gene or a mutation at one of the other two FBH loci that have been revealed by family linkage studies. One of these FBH loci is located on chromosome 19p and is referred to as FBH_{19p}. Studies of another FBH kindred from Oklahoma that also suffered from progressive elevations in PTH, hypophosphatemia and osteomalacia (82, 83) demonstrated that this variant, designated FBH_{OK}, was linked to chromosome 19q13 (84). Numerous different CaSR mutations related to FBH, NSHPT, or ADH or to *de novo* disease have been reported (188 missense, 17 nonsense, six insertion and/or deletion, one silent and one splice mutation) (for more details see: *http://www.casrdb.mcgill.ca*).

NSHPT occurring in the offspring of consanguineous FBH families has been shown to be due to homozygous CaSR mutations that usually result in a complete loss of CaSR function causing severe hypercalcemia due to parathyroid bone disease within the first 6 months of life, often necessitating parathyroidectomy (70, 71, 73, 85–87). Infants with NSHPT may exhibit polyuria, dehydration, hypotonia, and failure to thrive (88). However, some patients with sporadic neonatal hyperparathyroidism have been reported to carry *de novo* heterozygous CaSR mutations (72), thereby suggesting the involvement of factors other than mutant gene dosage (85). A novel heterozygous *de novo* mutation (R551K) was shown recently to

cause NSHPT, which gradually reverted to asymptomatic FBH without the need for surgical intervention (89).

Autoimmune Hypocalciuric Hypercalcemia (AHH)

Some patients with clinical features of FBH, who lack CaSR mutations, may have AHH, which is an acquired disorder with circulating antibodies to the extracellular domain of the CaSR. Some of these antibodies stimulate the release of PTH when tested with dispersed human parathyroid cells in vitro, probably by inhibiting the activation of the CaSR by extracellular calcium (90). For patients, in particular who have hyperparathyroidism in combination with other autoimmune disorders, AHH should be considered (90, 91). Such an autoimmune PTH-dependent hypercalcemia was initially described in four individuals from two unrelated kindreds. Of these, three patients had anti-thyroid antibodies and one had celiac sprue with anti-gliadin and anti-endomyseal antibodies (90).

Recently, an IgG4 blocking autoantibody against CaSR has been isolated and reported to cause AHH, which is phenotypically similar to familial hypocalciuric hypercalcemia but is not associated with any mutations in the CaSR gene. The autoantibody inhibits CaSR signaling pathways, inositol phosphate (IP) accumulation and ERK phosphorylation, by binding to specific CaSR sites. AHH patients with this antibody presented with autoimmune dysregulation, including psoriasis, adult-onset asthma, Coombs-positive hemagglutination, rheumatoid arthritis, ureitis and autoimmune hypophysitis and symptoms regressed following glucocorticoid treatment. These findings suggested that the blocking autoantibody against CaSR was in fact responsible for the AHH in this patient (91).

CaSR is known to activate two signal transduction mechanisms, the phospholipase C and ERK1/2 phosphorylation pathways dependent on Gq and Gi coupling respectively. An autoantibody, isolated from a patient with AHH, was shown to enhance calcium-stimulated IP accumulation but inhibited calcium-stimulated ERK1/2 phosphorylation, suggesting that the CaSR when incubated with the patient's autoantibody adopts a distinct active conformation favoring coupling to Gq and uncoupling from Gi. This autoantibody did not interact with representative epitopes in the N-terminal domain of the CaSR, but shifted the concentration-response curve for Ca^{2+} to the left indicating that it causes allosteric changes at a site in close proximity to the binding site for calcimimetics and requires Ca^{2+} for its effect (92).

Jansen's Metaphyseal Chondrodysplasia (JMC)

JMC is an autosomal dominant disease that is characterized by short-limbed dwarfism caused by an abnormal regulation of chondrocyte proliferation and differentiation in the metaphyseal growth plate (*Fig. 11-5*), and associated usually with severe hypercalcemia and hypophosphatemia, despite normal or undetectable serum levels of PTH or PTHrP (93). These abnormalities are caused by heterozygous mutations in the PTH/PTHrP receptor that lead to constitutive, PTH- and PTHrP-independent receptor activation (94–96). Since the PTH/PTHrP receptor is most abundantly expressed in the kidney and the bone, and in the metaphyseal growth plate, these findings provided a likely explanation for the abnormalities observed in mineral homeostasis and for the associated defects in the growth plate

Figure 11-5

Patient with a severe form of Jansen's metaphyseal chondrodysplasia. (From Frame and Poznanski (370), with permission).

development. Three different, heterozygous mutations of the PTH/PTHrP receptor have been identified in the severe form of JMC; these involve codon 223 (HisArg), codon 410 (Thr→Pro), and codon 458 (Ile→Arg). Expression of the mutant receptors in COS-7 cells resulted in constitutive, agonist-independent accumulation of cAMP, while the basal accumulation of inositol phosphates was not measurably increased; the H223R mutation appears to be the most frequent cause of JMC (94–96). Transgenic mice in which expression of a PTH/PTHrP receptor carrying the H223R mutation was targeted to the growth plate by the rat alpha1(II) collagen promoter showed a significant delay in chondrocyte differentiation supporting the conclusion that the defect in endochondral bone formation in JMC patients is caused by the constitutively active mutant receptor (97). The slowed differentiation of growth plate chondrocytes was associated with an upregulation of cyclin- and E2F-dependent gene expression, indicating that the PTH/PTHrP receptor controls the timing of cell cycle exit and the onset of differentiation of chondrocytes (98). Another heterozygous PTH/PTHrP receptor mutation, Thr410Arg, was recently identified in a small Middle Eastern kindred, in which the three JMC patients showed less pronounced skeletal and laboratory abnormalities than previously described individuals affected by this disease, i.e., only mild skeletal dysplasia and relatively normal stature, high-normal plasma calcium concentration associated with normal or suppressed serum PTH levels and with hypercalciuria leading to nephrolithiasis in two individuals (*Fig. 11-6*) (99). In comparison to PTH/PTHrP receptors with the Thr410Pro mutation, the Thr410Arg mutation showed less pronounced agonist-independent cAMP accumulation in vitro (95, 100).

Williams Syndrome

Williams syndrome (WS) (also referred to as Williams-Beuren Syndrome, WBS) is an autosomal dominant disorder characterized by supra-valvular aortic stenosis, hypertension, elfin-like faces, psychomotor retardation and occasionally infantile hypercalcemia. It can be caused by different hemizygous deletions involving 25–30 genes on chromosome 7q11.23. Genetic analyses in four familial and five sporadic cases of WS have demonstrated hemizygosity at the elastin locus in over 90% of patients with the classical Williams phenotype (101), and a series of 235 WS patients revealed submicroscopic deletions detectable by FISH involving the elastin gene in 96% of the investigated individuals (102); however, the presence of two copies of the elastin locus does not exclude WS (103). Interestingly, ablation of the elastin gene in mice results in vascular abnormalities similar to those observed in WS patients (104). Other microdeletions were identified genes that are expressed in the central nervous system, including LIM-kinase (105), the cytoplasmic linker protein-115 (CYLN2), and the transcription factors GTF2I and GTF2IRD1(106), and these may well contribute to some of the distinct neurological and cognitive deficits observed in WS patients. In addition, aneuploid neighboring genes some of which are located several megabases away from the deletion, may contribute to the phenotypic variation observed in WS patients (107, 108). Moreover, deletion of *NCF1* may protect against hypertension by reducing angiotensin II-mediated oxidative stress (109). This gene encodes the p47 (phox) subunit of the NADPH oxidase and is involved in superoxide anion production and protein nitrotyrosination. Hypercalcemia during infancy occurs in about 15% of the children with WS. Although it is clinically not severe in most cases, some affected individuals require therapeutic interventions with bisphosphonates (110). The calcitonin receptor gene, located on chromosome 7q21, is not involved in the deletions identified in WS (111). It is therefore likely that the hypercalcemia observed in some WS patients is caused by another as yet unknown gene within the contiguously deleted region.

Hypocalcemia and Hyperphosphatemia due to Reduced Parathyroid Hormone Activity

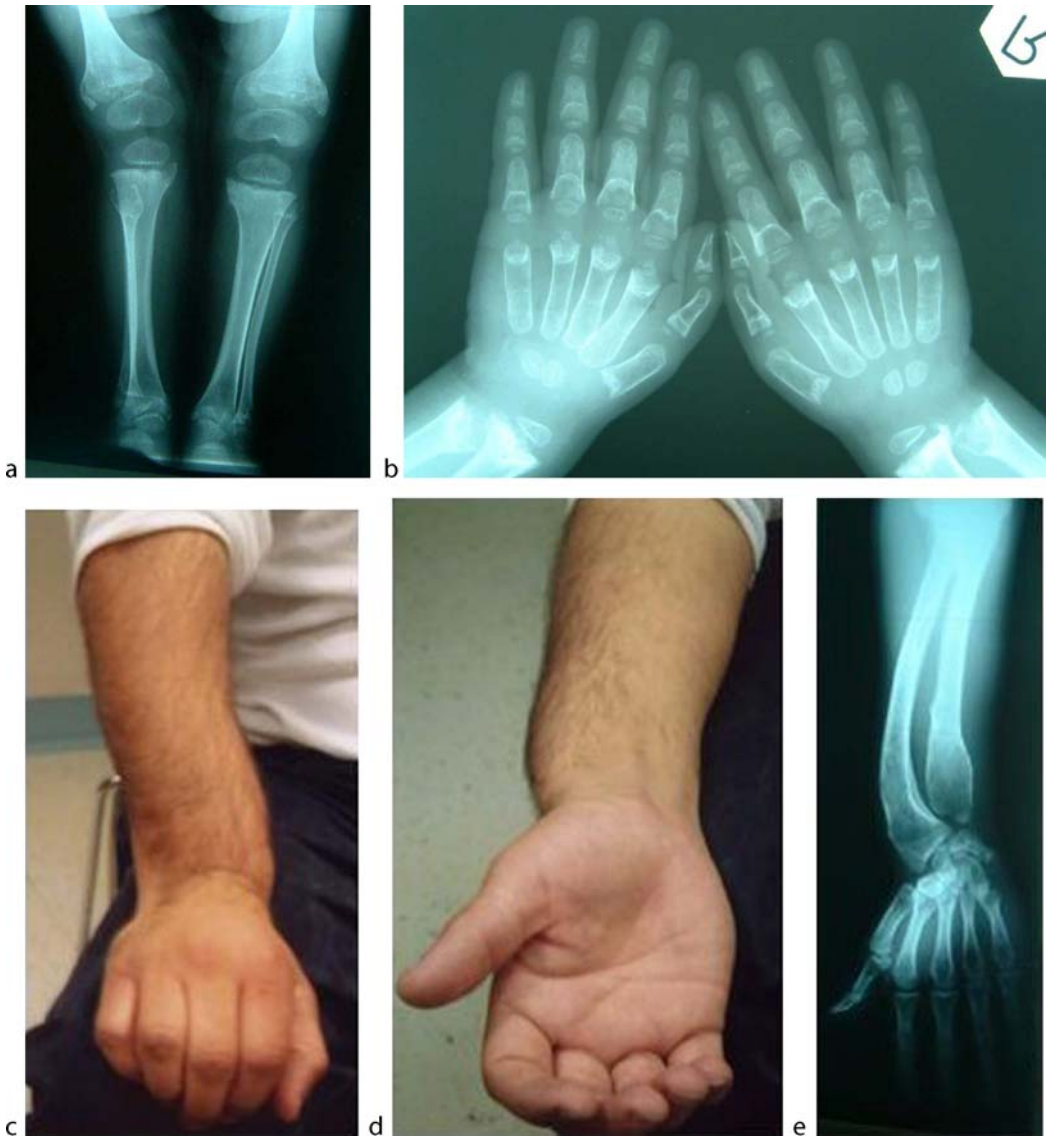
As for hypercalcemic disorders, patients who present with hypocalcemia require careful clinical evaluation, as well as the assessment of laboratory parameters in serum and urine, and a detailed review of the family history (*Fig. 11-7*).

Hypoparathyroidism

Hypoparathyroidism comprises a heterogeneous group of disorders, which includes both acquired and inherited causes, each presenting clinically with hypocalcemia. Idiopathic hypoparathyroidism may be sporadic or familial, and it may occur as an isolated defect or as a component of a disorder with additional manifestations, such as pluriglandular autoimmune disorder or various developmental abnormalities, e.g., DiGeorge syndrome

Figure 11-6

Radio- and photographs of patients with a relatively mild form of Jansen's disease (From Bastepe et al. (99), with permission).



(DGS). However, for the majority of hypoparathyroid patients no genetic mutation has yet been identified.

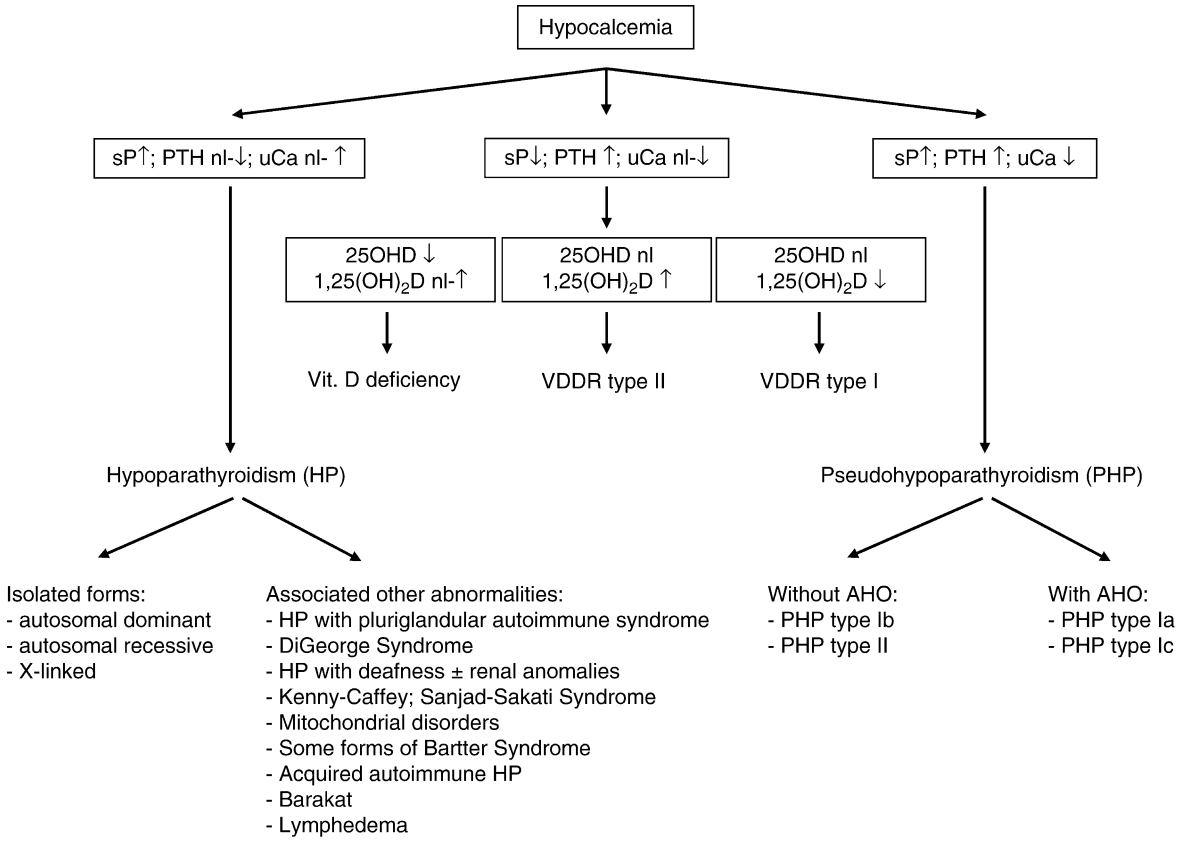
Parathyroid Hormone (PTH) Gene Abnormalities

Preproparathyroid hormone (preproPTH), the PTH precursor, contains a typical 25-residue amino-terminal signal sequence followed by a 6-residue pro-specific peptide and the mature hormone. The hydrophobic core of the human preproPTH signal peptide is composed of 12 contiguous uncharged amino acids (residues -5 to -16 of the signal peptide).

The coding regions, 5′ flanking regions, and splice junctions of the gene encoding PTH were sequenced in a patient with autosomal dominant familial isolated hypoparathyroidism (FIH). The mutant allele differed from the normal allele at only one nucleotide. This single base substitution (T→C) resulted in the substitution of

Figure 11-7

Flow-diagram for the work-up of patients with hypocalcemia.



arginine (CGT) at position 18 in the signal peptide for cysteine (TGT) thus disrupting the hydrophobic core of the signal sequence, which is required by the secreted proteins for efficient translocation across the endoplasmic reticulum. The charged mutant protein showed impaired processing from preproPTH to proPTH (112). Another patient with FIH was shown to have a single point mutation at the -8 position of the signal peptide changing a cysteine to an arginine. The mutant protein interfered with the normal targeting and processing of other secretory proteins, including the normal PTH precursor, suggesting that the mutant gene product exerts a dominant negative effect in vitro by trapping the hormone intracellularly, predominantly in endoplasmic reticulum (ER) (113), thereby causing stress-induced cell death (114). In another family, a single base substitution (T→C) involving codon 23 of exon 2 was detected. This resulted in the substitution of proline (CCG) for the normal serine (TCG) in the signal peptide (115). This mutation at the –3 position of the preproPTH protein cleavage site most likely disrupts cleavage of the mutant precursor molecule and prevents the efficient formation and secretion of PTH (115). The affected individuals of one other kindred with autosomal recessive, isolated hypoparathyroidism showed a single base transition (G→C) at position 1 of intron 2 of the gene encoding PTH. This mutation resulted in the deletion of exon 2, which encodes the initiation codon and the signal peptide, thereby causing parathyroid hormone deficiency (116).

GCMB Abnormalities

GCMB (*g*lial *c*ells *m*issing B), which is the human homologue of the Drosphilia gene Gcm, and of the mouse Gcm2 gene, is expressed exclusively in the parathyroid glands, suggesting that it may be a specific regulator of parathyroid gland development (117). Mice that were homozygous (−/−) for deletion of Gcm2 lacked parathyroid glands and developed the hypocalcemia and hyperphosphatemia as observed in hypoparathyroidism. However, despite their lack of parathyroid glands, Gcm2 deficient (−/−) mice did not have undetectable serum

PTH levels, but instead had levels indistinguishable from those of normal (+/+, wild-type) and heterozygous (+/−) mice. However, this endogenous level of PTH in the Gcm2 deficient (−/−) mice was too low to correct the hypocalcemia, but exogenous continuous PTH infusion could correct the hypocalcemia (117). Interestingly, there were no compensatory increases in PTHrP or $1,25(OH)_2$ vitamin D_3. These findings indicate that Gcm2 mice have a normal response (and not resistance) to PTH, and that the PTH in the serum of Gcm2 deficient mice was active. The auxillary source of PTH was identified to be a cluster of PTH-expressing cells under the thymic capsule. These thymic PTH-producing cells also expressed the CaSR, and long-term treatment of the Gcm2 deficient mice with $1,25(OH)_2$ vitamin D_3 restored the serum calcium concentrations to normal and reduced the serum PTH levels, thereby indicating that the thymic production of PTH can be down-regulated.

The gene encoding the human homolog of mouse Gcm2, namely *GCMB*, is located on chromosome 6p23–24. A large homozygous intragenic *GCMB* deletion was identified in the proband of extended kindred with an autosomal recessive form of isolated hypoparathyroidism (118). In addition, a homozygous mutation consisting of the substitution a glycine residue with a serine at position 63 (G63S) in the DNA binding domain of GCMB has been described; this mutation appears to cause a loss of transactivation capacity and was associated with some residual hormone secretion (119). Another homozygous GCMB mutation changes arginine at position 47 to leucine, which reportedly leads to the disruption of GCMB binding to the DNA binding site (120).

More recently, two closely related, heterozygous GCMB mutations were identified in two families in which hypoparathyroidism follows an autosomal dominant mode of inheritance (121, 122). Both mutations lead to a shift in the open reading frame and the replacement of a putative transactivation domain within carboxyl-terminal region by unrelated amino acid sequence, and both mutant proteins have a dominant negative effect on the wild-type GCMB protein. For a large number of patients with isolated hypoparathyroidism, however, no disease-causing mutation has yet been identified (123) making it likely that mutations in additional, as-of-yet unidentified genes can also cause hypoparathyroidism.

Anomalous expression of GCMB by some nonparathyroid cancers may be necessary for maintenance of the differentiated phenotype that allows sustained ectopic production of PTH. Intrathymic parathyroid adenomas that express GCMB are more likely to be derived from true parathyroid cells rather than from thymic epithelial cells as the thymus in humans does not serve as an auxiliary source of PTH, which is different from the findings in mice (124). Furthermore, GCMB expression was dramatically elevated in a parathyroid adenoma expressing a mutant PTH that is truncated after amino acid residue 52 (68). Conversely, compared to the findings in parathyroid adenoma, hyperplasia, and cancer, GCMB mRNA expression was lower in normal parathyroid glands and GCMB mRNA expression was down-regulated by lowering the extracellular calcium concentration (125). These findings indicate that the transcription factor GCMB may be directly or indirectly involved in mediating the effect of calcium on parathyroid hormone expression and/or secretion.

X-Linked Recessive Hypoparathyroidism

X-linked recessive hypoparathyroidism has been reported in two related multigenerational kindreds (126, 127). Relationship of these two kindreds was established by demonstrating an identical mitochondrial DNA sequence, inherited via the maternal lineage, in the affected males from both families (128). Affected males suffered from infantile onset of epilepsy and hypocalcemia (129). Linkage studies utilizing different X-chromosomal polymorphic markers localized the mutant gene to chromosome Xq26-q27 (130). This region mapped a 906-kb region on Xq27 that contains 3 genes (ATP11C, U7snRNA, and SOX3) but had no mutations were revealed. Further analysis of this region identified a novel molecular deletion – insertion [del(X) (q27.1) inv ins (X; 2) (q27.1; p25.3)], which involves a loss of 23–25 kb of noncoding Xq27.1 sequence and an inverted insertion of 305–340 kb from chromosome 2p25.3 to Xq27 and is approximately 67 kb downstream of SOX3 in X-linked recessive HPT patients resulting in an effect on the position on SOX3 expression. SOX3 may have a role in the embryonic development of the parathyroid glands. Identification of this deletion-insertion highlights the important role for genetic abnormalities that involve non-coding regions in causing disease, a feature that is likely to be of significance in the search for the molecular basis of other Mendelian inherited diseases for which coding region abnormalities have not been identified (131).

Autoimmune Pluriglandular Hypoparathyroidism Type 1 (APS1)

Autoimmune pluriglandular syndrome type 1 (APS1) or autoimmune polyendocrinopathy candiasis ectodermal

dystrophy (APECED) is an autosomal recessive disease with a high incidence in isolated subpopulations in Central and Eastern Finland (132). The diagnosis requires the presence of at least two of the three major components: hypoparathyroidism, candidiasis, and adrenal insufficiency. Hypoparathyroidism may, however, be the only manifestation of the syndrome. Data from 68 patients from 54 families in Finland reported hypoparathyroidism in 54 patients (79%) (133). In addition, the disorder has been reported to have a high incidence among Iranian Jews, although the occurrence of candidiasis is less common in this population (134).

Linkage studies of Finnish families mapped the APECED gene to chromosome 21q22.3 (135). Further positional cloning approaches led to the identification of AIRE (Auto Immune Regulator), a novel gene on chromosome 21q22.3 which encodes a 545 amino acid protein. Specific domains of AIRE protein indicate that it is involved in transcriptional processes, these domains include: (1) an amino-terminal HSR domain, (2) a nuclear localization signal (NLS), (3) a SAND domain, (4) two plant homeodomain (PHD) type zinc fingers, and (5) four LXXLL motifs (136). To date, more than 50 different APS1-causing mutations have been established in affected patients. The mutations are distributed throughout the coding region of the gene. Most of the mutations are, however, located in the amino-terminal HSR domain. The R257X mutation in SAND domain is the most prevalent mutation among Finnish patients, accounting for 83% of disease alleles (136–138). This particular mutation is also frequently found in Central and Eastern European and Northern Italian populations, indicating an early introduction of the mutation into Caucasian populations (137, 139). Another frequent mutation, the 979del13 bp is the most common mutation in North American, British and Norwegian APS1 patients (140–142). The Y85C mutation is typical for Iranian Jews (143) and R139X is found among Sardinian APS1 patients (144).

AIRE has been shown to regulate the elimination of organ-specific T cells in the thymus, and thus APECED is likely to be caused by a failure of this specialized mechanism for deleting forbidden T cells, and thus failure to establish immunologic tolerance (145) by disrupting nuclear organization (146). In an Italian kindred with a unique G228W variant in the SAND domain, an autosomal dominant effect was documented. [G228]AIRE appeared to inhibit wild-type AIRE from reaching the sites of active transcription in medullary thymic epithelial cells. This resulted in a failure to delete T cells reactive against antigens specific for the thymus leading to autoimmunity. Thus, the AIRE mediated dominant negative effect may cause autoimmune predisposition to phenotypes distinct from APS (147). Further, in APS1 patients with hypoparathyroidism activation of the CaSR by antibodies directed to this receptor can decrease PTH secretion leading to hypocalcaemia (148). The NACHT leucine-rich-repeat protein 5 (NALP5), which is predominantly expressed in the cytoplasm of parathyroid chief cells, is the other autoantigen, and antibodies against this protein may be responsible for hypoparathyroidism. For NALP5-specific autoantibodies were detected in almost half of the patients with APS1 and hypoparathyroidism, but were absent in all APS1 patients without hypoparathyroidism (149). As the APS1 syndrome arises from a loss-of-function in the AIRE gene, mutations can be scattered throughout the entire gene and over 50 mutations in the gene have been described in APS1 subjects (150) therefore identification of the causative mutation in an individual patient may require the labor intensive process of sequencing the entire gene. Recently, high titer neutralizing IgG autoantibodies were found reactive to most IFN-α subtypes in 76 Scandinavian APS1 patients with high specificity for those patients with known AIRE mutations. APS1-specific high-titer neutralizing autoantibodies against type I interferons were also found in 100% of Finnish and Norwegian patients, who carry two prevalent AIRE truncations (151). These antibodies can be used as an additional criteria to establish the diagnosis of APS1 (152).

DiGeorge Syndrome

DiGeorge syndrome (DGS) is associated with a spectrum of malformations, including absence or hypoplasia of the thymus and the parathyroid glands, cardiovascular anomalies, and mild craniofacial dysmorphia (153). Most DGS cases that result from deletion of 22q11.2 are designated DGS type 1 (DGS1) (154). In some patients, deletions of another locus, which resides on chromosome 10p have been observed in association with DGS (155) and this syndrome is now referred to as DGS type 2 (DGS2). Approximately 17% of patients with the phenotypic features of DGS have no detectable genomic deletion (156). Mapping studies of the DGS1 deleted region on chromosome 22q11.2 have defined a 250 kb to 3,000 kb critical region that contains approximately 30 genes (157, 158). Studies of DGS1 patients have reported deletions of several of the genes (e.g., rnex40, nex2.2 – nex 3, UDFIL and TBX1) from the critical region (154, 159–161) and ablation of some genes in mice. (e.g., Udf1l, Hira and Tbx1) are associated with developmental abnormalities

of the pharyngeal arches (162–164). Interestingly, mice with deletion of Tbx1 have a phenotype that is similar to that of DGS1 patients (164). However, point mutations in DGS1 patients have only been detected in the TBX1 gene (165), which is therefore considered to be the gene causing DGS1 (166). TBX1, a DNA binding transcriptional factor of the T-Box family, has an important role in organogenesis and pattern formation. Tbx1-null mutant mice ($Tbx1^{-/-}$) had all the developmental anomalies of DGS1 (i.e., thymic and parathyroid hypoplasia; abnormal facial structures and cleft palate; skeletal defects; and cardiac outflow tract abnormalities), whilst Tbx1 haploinsufficiency ($Tbx1^{+/-}$) was associated only with defects of the fourth brachial pouch (i.e., cardiac outflow tract abnormalities). The spectrum of DGS1 malformations is thus elicited in a dose-dependent manner (167), suggesting that the Tbx1 dose, modified by specific genes, may cause the phenotypic variability observed in VCFS/DGS patients. Mice that are null for these modifying genes have similar defects as those observed in $Tbx1^{-/-}$ mutant animals (168).

Tbx1 is expressed in the ectoderm, mesoderm, and endoderm. In the mesoderm, Tbx1 activates the different members of fibroblast growth factor family as well as Pitx 2, a bicoid homeobox gene, which is co-expressed with Tbx1 in the secondary heart field and is required for establishing right-left asymmetry. $Pitx2^{+/-}$; $Tbx1^{+/-}$ double heterozygous embryos showed cardiovascular defects, albeit with reduced penetrance. Gbx2, a homeobox-containing transcription factor activated by Tbx1, interacts with Fgf8 during pharyngeal arch and cardiovascular development (169). In addition, Tbx1 together with Crkl negatively regulates activation of retinoic acid signaling pathways in all three germ cell layers. The gene encoding Crkl is located within the deletion DGS1 region. It encodes an adaptor protein of different activated Fgf8-Fgf receptor complexes, which enhances intracellular signaling in response to Fgf8 (170, 171). $Crkl^{+/-}$; $Fgf8^{+/-}$ mice have DGS1-related defects and compound heterozygosity for the loss of *Crkl* and *Tbx1* resulting in increased penetrance and expressivity of DGS1-related defects compared with $Tbx1^{+/-}$ or $Crkl^{+/-}$ mice (172). Furthermore, $Tbx1^{+/-}$; $Fgf8^{+/-}$; $Crkl^{+/-}$ triple heterozygous mice have more severe defects than double heterozygous mutants.

Genetic manipulation of retinaldehyde dehydrogenase 2 (RALDH2), a member of aldehyde dehydrogenase family, which converts retinaldehyde into retinoic acid were performed in mice. These experiments showed that animals carrying one hypomorphic allele of this enzyme and one null allele for RALDH2 ($Raldh2^{neo/-}$) also display the DGS1 phenotype (173). Retinoic acid effects the expression of Tbx1 and thus regulates the expression of Fgf8. Further, reducing retinoic acid signaling in $Tbx1^{+/-}$; $Crkl^{+/-}$ mice reduced the penetrance of thymic hypoplasia. Other genetic modifiers of DGS1 region are sonic hedgehog (Shh) and chordin. Shh binds to an upstream regulatory region in the Tbx1 locus (174) and it regulates Tbx1 expression through Fox family of transcription factors whereas chordin causes decreased expression of Tbx1and Fgf8 in the endoderm. Vascular endothelial growth factor (Vegf) has an important role in vasculogenesis and angiogenesis and mice expressing the $Vegf^{120/120}$ isoform exhibit DGS1-related cardiovascular malformations by reducing Tbx1 expression (175). Inactivation of transforming growth factor beta (Tgf-ß) by conditional inactivation of Tgf-ß receptor type II gene also resulted in DGS1-related defects by affecting Crkl phosphorylation in neural crest cells (176).

Individuals with 22q11.2 microdeletions show behavioral and cognitive defects and are at high risk of developing schizophrenia. An engineered mouse strain (Df(16)$A^{+/-}$) carrying a hemizygous 1.3 Mb chromosomal deletion spanning a segment syntenic to human 22q11.2 locus had abnormal brain structure and microRNA expression (177). Initially, prepulse inhibition (PPI) deficits caused by Tbx1 and *Gnb1l* haploinsufficiency has been reported in Df1/+ mice and in the affected members of a family with major depression and Asperger syndrome (178). In addition, altered expression of several 22q11 mitochondrial genes, particularly during early post natal cortical development may disrupt neuronal metabolism or synaptic signaling (179, 180).

Hence to summarize, most DGS1 patients have the same 1.5–3 Mb hemizygous deletion of chromosome 22q11.2. Loss of the *Tbx1* gene in the deleted region is likely to be responsible for the etiology of the syndrome. However, despite having the same size deletion, most patients exhibit significant clinical variability secondary to stochastic, environmental and genetic modifiers.

Hypoparathyroidism, Deafness and Renal Anomalies (HDR) Syndrome

The combined inheritance of hypoparathyroidism, deafness and renal dysplasia (HDR) as an autosomal dominant trait was reported in one family in 1992 (181). Patients had asymptomatic hypocalcemia with undetectable or inappropriately normal serum concentrations of PTH, and normal brisk increases in plasma cAMP in response to the infusion of PTH. The patients also had bilateral, symmetrical, non-progressive sensorineural

deafness involving all frequencies. The renal abnormalities consisted mainly of bilateral cysts that compressed the glomeruli and tubules, and lead to renal impairment in some patients. Cytogenetic abnormalities were not detected and abnormalities of the PTH gene were excluded.

However, cytogenetic abnormalities involving chromosome 10p14–10pter were identified in 2 unrelated patients with features that were consistent with HDR. These 2 patients suffered from hypoparathyroidism, deafness, and growth and mental retardation; one patient also had a solitary dysplastic kidney with vesicoureteric reflux and a uterus bicornis unicollis and the other patient, who had a complex reciprocal, insertional translocation of chromosomes 10p and 8q, had cartilaginous exostoses. Neither of these patients had immunodeficiency or heart defects, which are key features of DGS2 (see above), and further studies defined two non-overlapping regions; thus, the DGS2 region was located on 10p13–14 and HDR on 10p14–10pter. Deletion mapping studies in two other HDR patients further defined a critical 200 kb region that contained GATA3 (182), which belongs to a family of zinc-finger transcription factors that are involved in vertebrae embryonic development. DNA sequence analysis in other HDR patients identified mutations that resulted in a haploinsufficiency and loss of GATA3 function (182–184). GATA3 has 2 zinc-fingers, and the C-terminal finger (ZnF2) binds DNA, whilst the N-terminal finger (ZnF1) stabilizes this DNA binding and interacts with other zinc finger proteins, such as the Friends of GATA (FOG) (185). HDR-associated mutations involving GATA3 ZnF2 or the adjacent basic amino acids were found to result in a loss of DNA binding, whilst those involving ZnF1 either lead to a loss of interaction with FOG2 ZnFs or altered DNA binding affinity (184). These findings are consistent with the proposed 3-dimensional model of GATA3 ZnF1, which has separate DNA and protein binding surfaces (184, 186, 188, 189, 190, 191). Electrophoretic mobility shift assays (EMSAs) revealed three classes of GATA3 mutations: those that lead to a loss of DNA binding (over 90% of all mutations); those that lead to a loss of the carboxylterminal zinc finger and result in reduced DNA-binding affinity; and those (e.g., Leu348Arg) that do not alter DNA binding or the affinity but likely induce conformational change in GATA3 (191). No mutations were identified in patients with isolated hypoparathyroidism indicating that GATA3 abnormalities are more likely to result in two or more of the phenotypic features of the HDR syndrome and not in isolated disease affecting only the regulation of calcium homeostasis (191).

The HDR phenotype is consistent with the expression pattern of GATA3 during human and mouse embryogenesis in the developing kidney, otic vesicle and parathyroids. However, GATA3 is also expressed in the developing central nervous system (CNS) and the haematopoietic organs in man and mice, and this suggests that GATA3 may have a more complex role. Indeed, homozygous GATA3 knockout mice have defects of the CNS and a lack of T-cell development. The heterozygous GATA3 knockout mice were initially reported to have no abnormalities (187). However, further studies have revealed that the latter mice have hearing loss that is associated with cochlear abnormalities, which consist of a significant progressive morphological degeneration that starts with the outer hair cells at the apex and eventually involves all the inner hair cells, pillar cells and nerve fibres (187a, 187b). These studies have shown that hearing loss in GATA3 haploinsufficiency commences in the early postnatal period and is progressive through adulthood, and that it is peripheral in origin and is predominantly due to malfunctioning of the outer hair cells of the cochlea (187a, 187b). It is important to note that HDR patients with GATA3 haploinsufficiency do not have immune deficiency, and this suggests that the immune abnormalities observed in some patients with 10p deletions are most likely to be caused by other genes on 10p. Similarly, the facial dysmorphism, growth and developmental delay, commonly seen in patients with larger 10p deletions were absent in the HDR patients with GATA3 mutations, further indicating that these features were likely due to other genes on 10p (182). These studies of HDR patients clearly indicate an important role for GATA3 in parathyroid development and in the etiology of hypoparathyroidism.

A *de novo* heterozygous missense mutation resulting in a non-conservative change of a single amino acid (R276P) in the GATA3 ZnF1 domain, which revealed reduced binding affinity to the GATA motifs, but normal interaction with FOG, was recently described in an HDR patient (188). Furthermore, a novel insertional mutation (405insC) in the GATA3 gene disrupting dual zinc fingers as well as one transactivating domain has been described (189). Three different *GATA3* mutations were furthermore described in several affected members of different Chinese HDR families. These are a single nucleotide deletion in codon 160 (478delG) and a donor splice site mutation at the exon 4/intron 4 boundary (IVS4 + 2 T to GCTTACTTCCC); both result in a shift in the open reading frame leading to a truncated GATA3 protein that lacks both N- and C-terminal zinc-containing fingers. The third missense mutation, R353S, is predicted to

disrupt the helical turn (190). In addition, 21 patients affected by HDR and 14 patients affected by isolated hypoparathyroidism were screened for GATA3 abnormalities. Thirteen different heterozygous germline mutations were identified in the HDR patients, and these consisted of three nonsense mutations, six frame-shift mutation leading to deletions, two frame-shifting insertions, one missense (Leu348Arg) mutation and one acceptor splice site mutation. Electrophoretic mobility shift assays (EMSAs) revealed three classes of GATA3 mutations; those that lead to a loss of DNA binding (over 90% of all mutations), those that lead to a loss of the carboxyl-terminal zinc finger, and those that result in reduced DNA-binding affinity. Additional mutations (e.g., Leu348Arg) do not alter DNA binding or the affinity but likely induce conformational change in GATA3. No mutations were identified in patients with isolated hypoparathyroidism indicating that GATA3 abnormalities are more likely to result in two or more of the phenotypic features of the HDR syndrome and not in isolated disease affecting only the regulation of calcium homeostasis (191).

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Mitochondrial Disorders Associated with Hypoparathyroidism

Hypoparathyroidism has been reported to occur in three disorders associated with mitochondrial dysfunction: the Kearns-Sayre syndrome (KSS), the MELAS syndrome and a mitochondrial trifunctional protein deficiency syndrome (MTPDS). Both the KSS and MELAS syndromes have been reported to occur with insulin dependent diabetes mellitus and hypoparathyroidism (192, 193). A point mutation in the mitochondrial gene tRNA leucine (UUR) has been reported in one patient with the MELAS syndrome who also suffered from hypoparathyroidism and diabetes mellitus (77). Large deletions, consisting of 6741 and 6903 base pairs and involving $>$ 38% of the mitochondrial genome, have been reported in other patients who suffered from, hypoparathyroidism and sensorineural deafness (194). Rearrangements and duplication of mitochondrial DNA have also been reported in KSS. Mitochondrial trifunctional protein deficiency (MTPDS) is a disorder of fatty-acid oxidation that is associated with peripheral neuropathy, pigmentary retinopathy, and acute fatty liver degeneration in pregnant women who carry an affected fetus. Hypoparathyroidism has been observed with trifunctional protein deficiency (195, 196). The role of these mitochondrial mutations in the etiology of hypoparathyroidism remains to be further elucidated.

Kenny–Caffey and Sanjad–Sakati Syndrome

Hypoparathyroidism has been reported to occur in over 50% of patients with the Kenny-Caffey syndrome which is associated with short stature, osteosclerosis and cortical thickening of the long bones, delayed closure of the anterior fontanel, basal ganglia calcification, nanophthalmos and hyperopia (197). Initial description described female and male sibs, born of normal consanguineous parents, with typical findings of Kenny-Caffey syndrome making inherited characteristics autosomal dominant in most cases. Parathyroid tissue could not be found in a detailed post-mortem examination of one patient (198) and this suggests that hypoparathyroidism may be due to an embryological defect of parathyroid development. A recessive form of Kenny-Caffey syndrome was convincingly demonstrated in 16 affected children in 6 unrelated sibships, born to healthy, consanguineous parents of Bedouin ancestry (199). In 8 consanguineous Kuwaiti kindreds, linkage to a locus in the 1q42-q43 region was found for the autosomal recessive form of Kenny-Caffey syndrome (200).

In the Sanjad-Sakati syndrome, hypoparathyroidism is associated with severe prenatal and post natal growth failure and dysmorphic features and this has been reported in twelve patients from Saudi Arabia (201). The presenting complaint in all patients were hypocalcemic tetany or generalized convulsions, usually detected

in the first few days or weeks of life. Consanguinity was noted in 11 of the 12 patients' families, the majority of which originated from the Western province of Saudi Arabia. This syndrome, which is inherited as an autosomal recessive disorder has also been identified in families of Bedonin origin and homozygosity and linkage disequilibrium studies located this gene to chromosome 1q42-q43 (202). Sanjad-Sakati syndrome resembles the autosomal recessive form of KCS with similar manifestations but lacking osteosclerosis. Eight Sanjad-Sakati families from Saudi Arabia were genotyped with polymorphic short tandem repeat markers from the SSS/KCS critical region. A maximum multipoint LOD score of 14.32 was obtained at marker D1S2649, confirming linkage of Sanjad-Sakati syndrome to the same region as autosomal recessive Kenny-Caffey Syndrome. Haplotype analysis refined the critical region to 2.6 cM and identified a rare haplotype present in all the Sanjad-Sakati syndrome disease alleles, indicative of a common founder. In addition to the assignment of the Sanjad-Sakati syndrome in Saudi families and of the Kenny-Caffey syndrome in Kuwaiti families to overlapping genetic intervals, comparison of the haplotypes unexpectedly demonstrated that the diseases shared an identical haplotype. This finding, combined with the clinical similarity between the two syndromes, suggests that the two conditions are not only allelic but are also caused by the same ancestral mutation (203). Molecular genetic investigations led to the conclusion that mutations of the Tubulin-specific chaperone (TBCE) are associated with both syndromes (204). TBCE encodes one of several chaperone proteins required for the proper folding of α-tubulin subunits and the formation of α−β tubulin heterodimers. In addition, deletion and truncation mutations in the gene encoding a tubulin-specific chaperone cofactor E (TBCE) have been shown to cause the hypoparathyroidism, mental retardation and facial dysmorphism (HRD) syndrome which is associated with extreme growth failure. However, cryptic translational initiation at each of three out-of-frame AUG codons upstream of the genetic lesion can rescue a mutant HRD allele by producing a functional TBCE protein (208). The defect in the tubulin folding and assembly pathway also has grave consequences on growth and PMN functions (209).

Additional Familial Syndromes

Single familial syndromes in which hypoparathyroidism is a component have been reported. The inheritance of the disorder in some instances has been established and molecular genetic analysis of the PTH gene has revealed no abnormalities. Thus, an association of hypoparathyroidism, renal insufficiency and developmental delay has been reported in one Asian family in whom autosomal recessive inheritance of the disorder was established (205). An analysis of the PTH gene in this family revealed no abnormalities (205). The occurrence of hypoparathyroidism, nerve deafness and a steroid-resistant nephrosis leading to renal failure, which has been referred to as the *Barakat syndrome* (206), has been reported in 4 brothers from one family, and an association of hypoparathyroidism with congenital lymphoedema, nephropathy, mitral valve prolaps and brachytelephalangy has been observed in 2 brothers from another family (207). Molecular genetic studies have not been reported from these two families.

Calcium-Sensing Receptor (CaSR) Abnormalities

CaSR abnormalities are associated with 3 hypocalcemic disorders. These include autosomal dominant hypocalcemic hypercalciuria (ADHH), Bartter syndrome type V (i.e., ADHH with a Bartter-like syndrome), and a form of autoimmune hypoparathyroidism (AH) due to CaSR autoantibodies.

Autosomal Dominant Hypocalcemic Hypercalciuria (ADHH)

ADHH, although rare, in index cases may comprise a sizeable fraction of cases of idiopathic hypoparathyroidism, perhaps representing as many as one-third of such cases (210). CaSR mutations that result in a loss-of-function are associated with familial benign (hypocalciuric) hypercalcemia (FBHH) (70–76). It was therefore postulated that gain-of-function mutations in CaSR lead to hypocalcemia with hypercalciuria, and the investigation of kindreds with autosomal dominant forms of hypocalcemia have indeed identified such CaSR mutations (76, 211–215). The hypocalcemic individuals generally had normal serum intact PTH concentrations and hypomagnesemia, and treatment with vitamin D or its active metabolites to correct the hypocalcemia resulted in marked hypercalciuria, nephrocalcinosis, nephrolithiasis and renal impairment. Patients with this condition carry an activating CaSR mutation that changes the set-point of Ca^{2+}-regulated PTH secretion to the left and lowers renal tubular calcium re-absorption. Soon after the cloning of

the CaSR, investigators showed linkage of ADHH to a locus on chromosome 3q13 (212), i.e., the same locus as for the gene encoding the CaSR. Shortly afterwards, a heterozygous missense mutation, Q127A, was identified as a cause of ADHH (211). The majority (>80%) of CaSR mutations that result in a functional gain are located within the extracellular domain (76, 211–215), which is different from the findings in other disorders that are the result of activating mutations in G-protein coupled receptors. In addition, two deletion mutations have been described. Most ADHH patients are heterozygous for the activating mutation. In one family, a homozygous mutation was described but it was not associated with a more severe phenotype (216) and although there is a spectrum of phenotypic severity for a given genotype, the symptoms present in affected members of the same family tend to be similar.

Bartter Syndrome Type V

Bartter syndrome is a heterozygous group of autosomal recessive disorders of electrolyte homeostasis characterized by hypokalemic alkalosis, renal salt wasting that may lead to hypotension, hyper-reninemic hyperaldosteronism, increased urinary prostaglandin excretion, and hypercalciuria with nephrocalcinosis (217, 218). Mutations of several ion transporters and channels have been associated with Bartter syndrome, and 5 types are now recognized (218). The CaSR-related cases of Bartter's syndrome identified to date have been inherited in an autosomal-dominant manner, unlike other subtypes that are inherited as autosomal-recessive traits.

Bartter syndrome type V is due to activating mutations of the CaSR. Activating mutations of the *CaSR* gene in three patients; these mutations involved amino acid residues L125P, C131W and A843E. The mutant CaSR proteins inhibited the activity of the ROMK channel provided the missing link that explains why some activating mutations of CaSR can cause the Bartter's syndrome phenotype. Patients with Bartter syndrome type V have the classical features of the syndrome, i.e., hypokalemic metabolic alkalosis, hyper-reninemia and hyperaldosteronism (219, 220). In addition, they develop symptomatic hypocalcemia can, and an elevated fractional excretion of calcium leading to nephrocalcinosis (219, 220). Another recent report described monozygotic twins with a K29E mutation in the extra cellular domain of the CaSR; these patients presented with mild hypokalaemia, minimal aldosterone and renin production, absent alkalosis but notable hypocalcaemia (221). The K29E mutation also leads to cause agonist-independent activation of the CaSR (222) and buttresses previous observations that the phenotype of Bartter syndrome can be variable and is not directly related to the in-vitro potency of the known genetic changes associated with this syndrome (223).

Autoimmune Acquired Hypoparathyroidism (AH)

Twenty per cent of patients, who had acquired hypoparathyroidism (AH) in association with autoimmune hypothyroidism, were found to have autoantibodies directed against the extracellular domain of the CaSR (90, 91, 224). The CaSR autoantibodies did not persist for long; 72% of patients who had AH for less than 5 years had detectable CaSR autoantibodies; whereas only 14% of patients with AH for more than 5 years had such autoantibodies (224). The majority of the patients who had CaSR autoantibodies were females, a finding that is similar to that found in other autoantibody-mediated diseases. Indeed a few AH patients have also had features of autoimmune polyglandular syndrome type 1 (APS1). These findings establish that the CaSR can be an autoantigen in AH (90, 224).

Pseudohypoparathyroidism (PHP)

The term pseudohypoparathyroidism (PHP) was first introduced to describe patients with hypocalcemia and hyperphosphatemia due to PTH-resistance rather than PTH-deficiency (225). Affected individuals show partial or complete resistance to biologically active, exogenous PTH as demonstrated by a lack of increase in urinary cyclic AMP and urinary phosphate excretion; this condition is now referred to as PHP type I (226–228). If associated with other endocrine deficiencies and characteristic physical stigmata, now collectively termed Albrights's hereditary osteodystrophy (AHO), the condition is referred to as PHP type Ia. This latter syndrome is caused by heterozygous inactivating mutations within exons 1 through 13 of *GNAS* located on chromosome 20q13.3, which encode the stimulatory G protein ($G_s\alpha$) (for review see (229)). These mutations were shown to lead to an approximately 50% reduction in $G_s\alpha$ activity/protein in readily accessible tissues, like erythrocytes and fibroblasts, and explain, at least partially, the resistance towards PTH and other hormones that mediate their actions through G protein-coupled receptors (226–228). A similar reduction in $G_s\alpha$ activity/protein is also found in patients with

pseudopseudohypoparathyroidism (pPHP), who often show the same physical appearance as individuals with PHP-Ia, but lack endocrine abnormalities (226, 230–235). However, contrary to previous reports, recent studies have shown that not all AHO features are observed in pPHP patients. For example, obesity, which was thought to be a hallmark of both PHP-Ia and pPHP, was not evident in a large number of pPHP patients (236).

Patients affected by PHP-Ia or pPHP are typically found within the same kindred, but not within the same sibship. Furthermore, hormonal resistance is parentally imprinted, i.e., PHP-Ia occurs only if the defective gene is inherited from a female affected by either PHP-Ia or pPHP; pPHP occurs only if the defective gene is inherited from a male affected by either form of the two disorders (237, 238). Observations consistent with these findings in humans were made in mice that are heterozygous for the ablation of exon 2 of the *Gnas* gene. Animals that had inherited the mutant allele from a female showed undetectable $G_s\alpha$ protein in the renal cortex and decreased blood calcium concentration due to resistance toward PTH. In contrast, the offspring that had obtained the mutant allele lacking exon 2 from a male showed no evidence for endocrine abnormalities (239). Tissue- or cell-specific $G_s\alpha$ expression is thus almost certainly involved in the pathogenesis of PHP-Ia and pPHP. This provides also a reasonable explanation for the finding that heterozygous *GNAS* mutations result in a dominant phenotype.

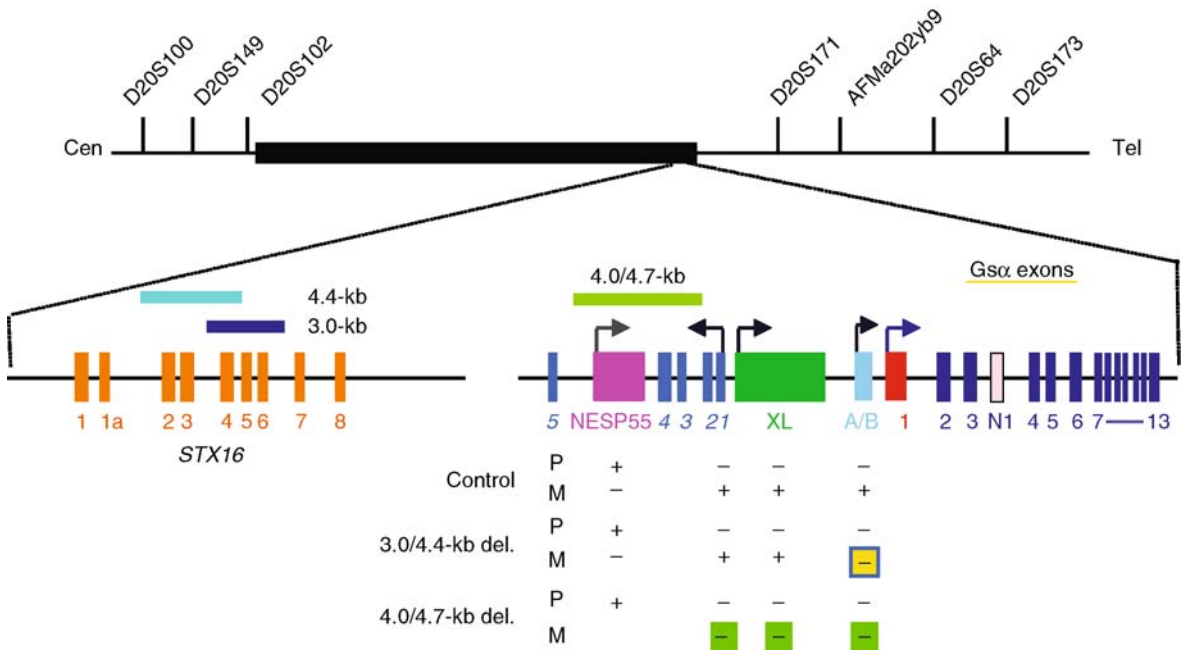
Progressive osseous heteroplasia (POH) was shown to be caused also by heterozygous inactivating mutations in the *GNAS* exons encoding $G_s\alpha$ (240–243). Interestingly, POH became only apparent when the $G_s\alpha$ mutation was inherited from a male, while inheritance from a female appears to have resulted in AHO, i.e., pseudopseudohypoparathyroidism (pPHP). This aspect of the findings was surprising since maternal inheritance of inactivating $G_s\alpha$ mutations usually leads to pseudohypoparathyroidism (PHP-Ia), i.e., AHO with hormonal resistance. However, PTH and TSH levels were not reported (242), and it is therefore conceivable that mild hormonal resistance may have been present in patients with maternally inherited $G_s\alpha$ mutations.

Mutations in the *GNAS* gene encoding $G_s\alpha$ have not been detected in patients with PHP type Ib (PHP-Ib), a disorder in which affected individuals show PTH-resistant hypocalcemia and hyperphosphatemia, but lack developmental defects. This variant of PHP was therefore thought to be caused by PTH/PTHrP receptor mutations; however, mutations in its gene and mRNA could not be identified (244–247). Recently, it was shown that there is an increased incidence of TSH resistance in PHP-Ib (227, 228, 248, 249), and that some patients affected by this disease variant show some shortening of the fourth metacarpals suggesting some overlap between the developmental features of PHP-Ia and PHP-Ib (250, 251).

A genome-wide search to identify the location of the "PHP-Ib gene" mapped the PHP-Ib locus to chromosome 20q13.3, which contains the *GNAS* locus (252), and it was furthermore shown that the genetic defect is parentally imprinted, i.e., it is inherited in the same mode as the PTH-resistant hypocalcemia in kindreds with PHP-Ia and/or pPHP (237, 238). Subsequently, it was shown that patients affected by PHP-Ib show a loss of methylation on the maternal allele, which is usually restricted to *GNAS* exon A/B (253, 254). In most families with the autosomal dominant form of PHP-Ib with parental imprinting (AD-PHP-Ib), the affected individuals and the healthy carriers were shown to carry a 3-kb deletion located with the syntaxin 16 (*STX16*) gene, which occurs between two 391-bp repeats about 220 kb up-stream of exon A/B (255–258) (*Fig. 11-8*). Affected members of one additional AD-PHP-Ib kindred, who show a loss of A/B methylation alone, were furthermore shown to have a 4.4 kb deletion within *STX16*, which overlaps with the 3-kb deletion by 1286 bp (259). In affected individuals with either mutation, the deletion is always found on the maternal allele, while it occurs on the paternal allele in unaffected healthy carriers. The affected members of two small families with broader methylation changes within the *GNAS* locus were shown to carry two distinct approximately 4 kb deletions on the maternal allele that remove *GNAS* exon NESP55 and the antisense exons 3 and 4 (260). Although indistinguishable broad methylation changes were observed in most patients with sporadic PHP-Ib, no deletions or point mutations have yet been identified in these individuals (261). Taken together these findings suggest that several different deletions upstream or within the *GNAS* locus lead to indistinguishable clinical and laboratory findings. However, it remains uncertain how the deletion affecting *STX16* results in a loss of exon A/B methylation alone, while deletion of NESP55 results in a broader loss of methylation. Furthermore, it remains uncertain how the different deletions affect signaling through the PTH/PTHrP receptor in the proximal renal tubules, but not in most other tissues. Mice lacking the murine homolog of exon A/B were recently shown to have biallelic and thus increased $G_s\alpha$ transcription (262). Loss of exon A/B methylation on the maternal allele allowing active transcription from this promoter therefore seems to have a prominent role in suppressing $G_s\alpha$ expression.

Figure 11-8

Location of the *GNAS* locus on chromosome 20q13.3 and of the microdeletions leading to autosomal dominant pseudohypoparathyroidism type Ib (AD-PHP-Ib). *GNAS* gives rise to multiple transcripts, some of which show allele-specific methylation in their promoters (P, paternal; M, maternal) and are expressed exclusively from the non-methylated allele (−). The $G_s\alpha$ specific promoter (exon 1) does not show differential methylation, and transcripts encoding this signaling protein are therefore biallelically expressed in most tissues. However, $G_s\alpha$ expression appears to occur predominantly from the maternal *GNAS* allele in the renal proximal tubules and a few other tissues. In AD-PHP-Ib kindreds, maternal inheritance of microdeletions affecting *STX-16* (gene encoding syntaxin 16; 3.0 or 4.4-kb deletion) are associated with loss of methylation at exon A/B alone, while deletions affecting NESP55 and two of the antisense exons (4.0 or 4.7-kb deletion) lead to the loss of all maternal methylation imprints; paternal inheritance of either deletion is not associated with imprinting defects and individuals carrying these deletions on the paternal allele are healthy. It has been suggested that in the renal proximal tubules a lack of exon A/B methylation and/or active transcription of A/B mRNA, both of which are normally seen on the paternal *GNAS* allele, mediate, *in cis*, the silencing of $G_s\alpha$ transcription. The maternal loss of exon A/B methylation in AD-PHP-Ib is therefore predicted to cause a marked reduction in $G_s\alpha$ expression and consequently resistance to PTH (and perhaps to few other hormones).

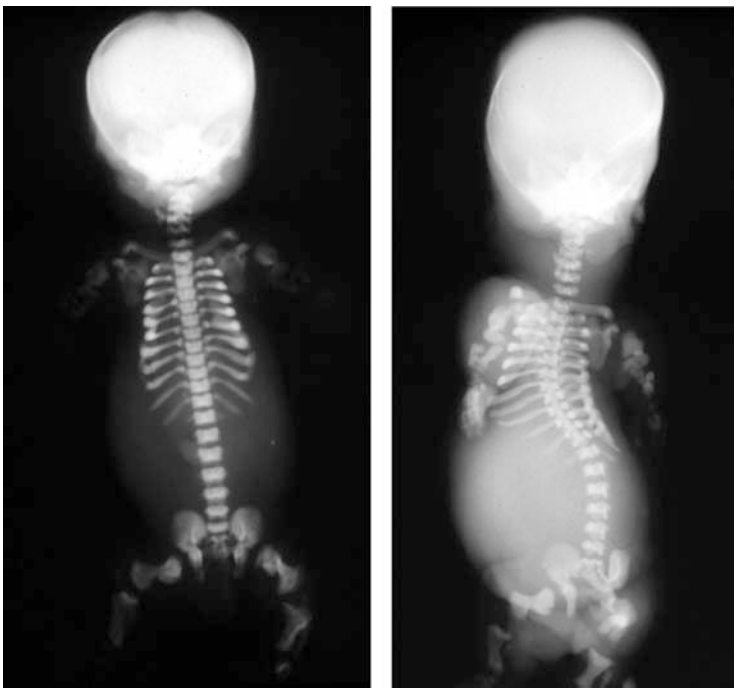


Blomstrand's Disease

Blomstrand's chondrodysplasia is an autosomal recessive human disorder characterized by early lethality, dramatically advanced bone maturation and accelerated chondrocyte differentiation (263). Affected infants are typically born to consanguineous healthy parents (only in one instance did unrelated healthy parents have two affected offspring) (264–268), show pronounced hyperdensity of the entire skeleton (*Fig. 11-9*) and markedly advanced ossification, particularly the long bones are extremely short and poorly modeled. Recently, PTH/PTHrP receptor mutations that impair its functional properties were identified as the most likely cause of Blomstrand's disease. One of these defects is caused by a nucleotide exchange in exon M5 of the maternal PTH/PTHrP receptor allele, which introduces a novel splice acceptor site and thus leads to the synthesis of a receptor mutant that does not mediate, despite seemingly normal cell surface expression, the actions of PTH or PTHrP; the patient's paternal PTH/PTHrP receptor allele is, for yet unknown reasons, only poorly expressed (269). In a second patient with Blomstrand's disease, the product of a consanguineous marriage, a nucleotide exchange was identified that

Figure 11-9

Radiological findings in a patient with Blomstrand's disease. (From (266), with permission). Note the markedly advanced ossification of all skeletal elements, and the extremely short limbs, despite the comparatively normal size and shape of hands and feet. Furthermore, note that the clavicles are relatively long and abnormally shaped.



changes proline at position 132 to leucine (270, 271). The resulting PTH/PTHrP receptor mutant showed, despite reasonable cell surface expression, severely impaired binding of radiolabeled PTH and PTHrP analogs, greatly reduced agonist-stimulated cAMP accumulation and no measurable inositol phosphate response. Additional loss-of function mutations of the PTH/PTHrP receptor have recently been identified in three unrelated patients with Blomstrand's disease. Two of these mutations led to a frame-shift and a truncated protein due either to a homozygous single nucleotide deletion in exon EL2 (272) or a 27 bp insertion between exon M4 and EL2 (273). The other defect, a nonsense mutation at residue 104 also resulted in a truncated receptor protein (274). Affected infants show besides the striking skeletal defects, abnormalities in other organs, including secondary hyperplasia of the parathyroid glands (presumably due to hypocalcemia). In addition, analysis of fetuses with Blomstrand's disease revealed abnormal breast development and tooth impaction, highlighting the involvement of the PTH/PTHrP receptor in the normal development of breast and tooth (275).

Hyperphosphatemic Disorders with Reduced Secretion of Biologically Active FGF-23

Tumoral Calcinosis With/Without Hyperphosphatemia

At least three variants of tumoral calcinosis have been described; an autosomal dominant form (276) and two apparently more common autosomal recessive forms that can be caused by mutations in two different genes. Patients affected by the autosomal dominant form usually have elevated serum 1,25-dihydroxyvitamin D levels, though classic findings of tumoral calcinosis may not always be present. The teeth are hypoplastic with short, bulbous roots and almost complete obliteration of pulp cavities, but they have fully developed enamel of normal color. The molecular defect of this autosomal dominant form of the disorder is not known.

The autosomal recessive forms of tumoral calcinosis can be severe, sometimes fatal disorder, and are typically characterized by hyperphosphatemia and often massive

calcium deposits in the skin and subcutaneous tissues; in some patients, however, only few minor abnormalities are noted (278). Recently, Topaz et al. mapped the gene causing one form of the disease to 2q24-q31 and revealed homozygous or compound heterozygous mutations in *GALNT3* (277), which encodes a glycosyltransferase responsible for initiating mucin-type O-glycosylation. Interestingly, the concentrations of carboxyl-terminal FGF-23 were significantly elevated in affected individuals. These findings implied that defective post-translational modifications of FGF-23 could be responsible for the abnormal regulation of phosphate homeostasis (see *Fig. 11-2*). Another form of tumoral calcinosis is caused by homozygous mutations in FGF-23, and patients affected by this disorder also showed dramatically elevated circulating concentration of carboxyl-terminal FGF-23, while the concentration of the intact protein were within normal limits (32, 279, 280).

Hyperostosis with Hyperphosphatemia

The combination of hyperostosis with hyperphosphatemia was first described in 1970 (281). Besides recurrent painful swelling of long bones, which can have features of tumoral calcinosis, affected patients present with elevated blood phosphate levels, yet normal renal function and usually normal serum calcium, 1,25-dihydroxy vitamin D, and PTH concentrations (282). Most cases appear to be sporadic, but consanguineous parents were described for some patients, implying that the disease can be recessive; the underlying molecular defect is not yet known. Recently, *GALNT3* mutations were also identified in the recessive form of this disease, indicating that one of the two forms of tumoral calcinosis and hyperostosis with hyperphosphatemia are allelic variants (283). As in patients with the autosomal recessive forms of tumoral calcinosis, carboxyl-terminal FGF-23 concentration appear to be significantly elevated (283).

Hypophosphatemic Disorders

The different forms of hypophosphatemia represent the most common cause of hereditary rickets, which can be divided into two main groups according to the predominant metabolic abnormality (284, 285). In the first group, hypophosphatemia is the result of a renal tubular defect, which may consist of either a single (isolated) defect in renal phosphate handling, as it occurs in the X-linked and autosomal dominant forms of hypophosphatemic rickets (XLH and ADHR, respectively), or of multiple tubular defects affecting phosphate, amino acids, glucose, bicarbonate and potassium handling as occurs in Fanconi syndrome's. In the second group, vitamin D metabolism is abnormal, either because of a defect in the 1α-hydroxylase enzyme or because of defects in the 1,25-dihydroxy vitamin D_3 receptor (VDR) leading to end organ resistance. The application of molecular genetic approaches has helped to elucidate some of the mechanisms underlying these disorders of hereditary hypophosphatemic rickets. Thus, XLH has been shown to be due to inactivating mutations of PHEX (*PH*osphate-regulating gene with homologies to *E*ndopeptidases on the *X* chromosome) (286, 287); Lowe's syndrome (oculocerebrorenal syndrome; X-linked recessive) is caused by mutations that result in a deficiency of a lipid phosphatase, which most likely controls cellular levels of the metabolite, phosphatidyl inositol 4, 5 bisphosphate ($P1P_2$) 5-phosphatase (288, 289); Dent's disease (X-linked recessive) results from loss of function mutations of a member of the voltage-gated chloride channel family, CLC-5 (290); vitamin D-dependent rickets type (VDDR type I; autosomal recessive) results from a deficiency of the renal 1a-hydroxylase enzyme (291, 292), which is a cytochrome P450 enzyme that forms part of the superfamily of hem-containing proteins that are bound to the membranes of microsomes and mitochondria and serve as oxidation-reduction components of the mixed-function oxidase system; and VDDR type II (autosomal recessive) is caused by mutations involving the VDR, which is closely related to the thyroid hormone receptors and represents another member of the transacting transcriptional factors that include the family of steroid hormone receptors. Furthermore, the molecular defect leading to ADHR was identified (29), which helped elucidate the role of a novel member of the fibroblast growth factor family, namely FGF-23, in the regulation of normal phosphate homeostasis (see *Figs. 11-2* and *11-3*) and in different acquired and inherited disorders of affecting the regulation of blood phosphate concentration (31, 33, 37, 38, 64, 293, 294).

Hypophosphatemic Disorders with Increased FGF-23 Activity

Autosomal Dominant Hypophosphatemic Rickets (ADHR)

ADHR is characterized by low serum phosphate concentrations, bone pain, rickets that can result in deformities of the legs, osteomalacia and dental caries (clinical and laboratory findings can be variable). ADHR and XLH

(see below) thus have marked clinical similarities but differ in their modes of inheritance. Genetic linkage studies mapped the ADHR locus to chromosome 12p13.3 (295) and defined a 1.5 Mb critical region that contained 12 genes. Mutational analyses of 6 of these 12 genes revealed the occurrence of missense mutations involving a new member of the fibroblast growth factor (FGF) family FGF-23 (29). Three missense FGF-23 mutations (see *Fig. 11-2*) have been identified in 4 unrelated ADHR families affecting codons 176 and 179. The affected members in two unrelated ADHR families have an identical mutation involving codon 176, in which the normal positively charged arginine residue is replaced by a polar but uncharged glutamine residue (Arg176Gln). The other two mutations involve codon 179, and in one ADHR family the normal arginine residue is replaced by a nonpolar tryptophan (Trp) residue (Arg179Trp) and in the other ADHR family, it is replaced by a glutamine residue (Arg179Gln). The clustering of these ADHR missense mutations that alter arginine residues has lead to the speculation that they may cause a gain of function. Mutational analysis of FGF-23 in 18 patients, who had hypophosphatemic rickets but did not have *PHEX* mutations, revealed no abnormalities, suggesting a role(s) for other genes in these hereditary disorders of hypophosphatemic rickets.

Oncogenic Osteomalacia (OOM)

OOM (also referred to as tumor-induced osteomalacia, TIO) is a rare disorder characterized by hypophosphatemia, hyperphosphaturia, a low circulating 1,25-dihydroxy-vitamin D_3 concentration and osteomalacia that develops in previously healthy individuals (296). Thus there are considerable similarities between OOM, XLH and ADHR. OOM is caused by usually small, often difficult to locate tumors, most frequently hemangiopericytomas. The clinical and biochemical abnormalities resolve rapidly after the removal of the tumor, whereas in XLH and ADHR these abnormalities are life long. However, the similarities between OOM, ADHR and XLH suggested that they may involve the same phosphate-regulating pathway, and it is important to note that OOM tumors do express PHEX (297, 298), which is mutated in XLH (see below). The possibility that FGF-23, which is mutated in ADHR, may be expressed in OOM tumors and that FGF-23 may be a secreted protein was therefore explored (30, 53, 294, 299). Indeed, OOM tumors were found by Northern and Western blot analysis respectively, to contain high levels of FGF-23 mRNA and protein. Consistent with this finding, FGF-23 plasma concentrations can be increased considerably in OOM patients, until successful tumor removal (64, 294, 300, 301).

Tumors responsible for oncogenic osteomalacia produce two molecular forms of FGF-23 (~32 and ~12 kDa), and both variants were also observed when assessing conditioned medium from cell lines, such as OK-E, COS-7 and HEK293 cells, expressing full-length, wild-type FGF-23 (30). When conditioned medium from cells expressing [R176Q]FGF-23 or [R179Q]FGF-23 was investigated by Western blot analysis only the larger protein band was observed (30, 33, 45). This implies that the known ADHR mutations, which affect a consensus cleavage site for furin-type enzymes, impair FGF-23 degradation thus enhancing and/or prolonging its biological activity.

X-Linked Hypophosphatemia (XLH)

XLH is the most frequent, inherited phosphate-wasting disorder. Just like ADHR, it is characterized by hypophosphatemia, hyperphosphaturia, low circulating 1,25-dihydroxy-vitamin D3 concentration and osteomalacia. This disorder is caused by inactivating mutations in PHEX, a gene located on Xp22.1 (286, 287). PHEX, which is expressed in kidney, bone and other tissues, shows significant peptide sequence homology to the M13 family of zinc metallopeptidases, which include neutral endopeptidase neprilysin (NEP), endothelin converting enzyme 1 (ECE-1) and 2 (ECE-2), and the Kell antigen. All of these are type II integral membrane glycoproteins that have endopeptidase activity and consist of a short N-terminal cytoplasmic domain, a single transmembrane hydrophobic region and a large extracellular domain. Thus, NEP functions as a membrane bound ectoenzyme that proteolytically inactivates a number of peptides that include atrial natruretic peptide, enkephalin, substance P and bradykinin, whilst ECE proteolytically activates endothelin. The substrate(s) for PHEX remains to be established, but several possible candidates can be considered. These are FGF-23, matrix extracellular phosphoglycoprotein (MEPE) (56, 302), and secreted frizzled related protein 4 (sFRP4) (53, 55). Amongst these proteins, FGF-23 was thought to be a likely substrate for PHEX, and consistent with this conclusion, serum FGF-23 concentrations were found to be elevated in about two third of patients with XLH (64, 249, 294) and they are unequivocally elevated in all Hyp mice, i.e., the murine homolog of XLH (52, 303). PHEX-dependent cleavage of FGF-23 was observed in one study in vitro (304), while another study failed to confirm these findings (32). However, genetic ablation of Fgf-23 in male Hyp mice, i.e., animals that are

null for Fgf-23 and Phex, leads to blood phosphate levels that are indistinguishable from those in mice lacking Fgf-23 alone (35, 36), indicating that FGF-23 resides genetically down-stream of PHEX/Phex. Furthermore, recent studies have shown that Hyp mice normalize their blood phosphate concentration and heal their rachitic changes, when injected with inactivating antibodies to FGF-23, indicating that FGF-23 is indeed the phosphaturic principle in XLH (305).

Autosomal Recessive Hypophosphatemia (ARHP)

Hypophosphatemic rickets in consanguineous kindreds was previously reported, suggesting an autosomal recessive form of hypophosphatemia (ARHP) (306–308). The clinical study of patients affected by ADHR or XLH, including rickets, skeletal deformities, and dental defects, show that affected individuals develop osteosclerotic bone lesions and enthesopathies later in life (see *Table 11-1*). Hypophosphatemia, which results from increased renal phosphate excretion, is accompanied by normal or low 1,25 $(OH)_2D$ levels and increased alkaline phosphatase activity. Patients affected by ARHP have FGF23 levels that are either elevated or inappropriately normal for the level of serum phosphorous (37, 38).

ARHP is caused by homozygous mutations, which affect the gene encoding dentin matrix protein 1 (DMP1). DMP1 belongs to the SIBLING protein family, which includes osteopontin, matrix extracellular phosphoglycoprotein, bone sialoprotein II, and dentin sialoprotein; the genes encoding these proteins are all clustered on chromosome 4q21. DMP1 is a bone and teeth specific protein (309), which is involved in the regulation of transcription in undifferentiated osteoblasts (310, 311). DMP1 undergoes phosphorylation during the early phase of osteoblast maturation and is subsequently exported into the extracellular matrix where it regulates the nucleation of hydroxyapatite. It undergoes post-translational modifications that yield a 94 kDa mature protein that is cleaved into a 37 kDa and a 57 kDa fragment. Preliminary studies suggested that the 57 kDa DMP1 fragment alone is sufficient to reverse the phenotype of *Dmp1*-null animals and to suppress FGF23 secretion (39).

Of the several different DMP1 mutations identified thus far, one mutation alters the translation initiation codon (M1V), two mutations are located in different intron-exon boundaries, and three are frame-shift mutations within exon 6. These mutations appear to be inactivating, suggesting that the loss of DMP1 results in hypophosphatemia. Accordingly, *Dmp1*-null mice show severe defects in dentine, bone, and cartalage, as well as hypophosphatemia and osteomalacia (312, 313). Furthermore, FGF23 levels in osteocytes and in serum are drastically elevated in these animals (38). Based on these findings, another role of DMP1 appears to be in the inhibition of FGF23 expression, thereby regulating phosphate homeostasis. Given the established importance of DMP1 in osteoblast function, loss of DMP1 actions in osteoblasts and extracellular matrix may also contribute to the phenotype of patients with ARHP. Consistent with this hypothesis, a high calcium/phosphate diet capable of rescuing osteomalacia in VDR-null mice does not seem to prevent the bone and dentine mineralization defect observed in Dmp1-null mice (39).

To summarize therefore, different molecular mechanisms, i.e., overexpression/production of FGF-23 by the tumors responsible for oncogenic osteomalacia, generation of a mutant FGF-23 that is resistant to cleavage in patients with ADHR, homozygous inactivating DMP1 mutations resulting in failure to suppress FGF-23 secretion, and inactivating mutations in PHEX that increase FGF-23 secretion through yet unknown mechanisms can all lead to renal phosphate-wasting and the resulting bone changes. FGF-23 thus has undoubtedly a central role in several hypophosphatemic disorders.

Hypophosphatemic Disorders with Normal or Suppressed FGF-23 Activity

Nephrolithasis and Osteoporosis Associated with Hypophosphatemia

Two different heterozygous mutations (A48P and V147M) in *NPT2a*, the gene encoding a sodium-dependent phosphate transporter, have been reported in patients with urolithiasis or osteoporosis and persistent hypophosphatemia due to decreased tubular phosphate reabsorption (314). When expressed in *Xenopus laevis* oocytes, the mutant NPT2a protein showed impaired function and, when co-injected, dominant negative properties. However, these in vitro findings were not confirmed in another study using oocytes and OK cells, raising the concern that the identified NPT2a mutation alone cannot explain the findings in the described patients (315). On the other hand, additional heterozygous NPT2a variations (in-frame deletion or missense change) have recently been identified upon analyzing a large cohort of hypercalciuric stone-forming patients in kindreds; however, these genetic variations do not seem to cause functional abnormalities (316).

Table 11-1

Biochemical findings in several inherited hypo- and hyperphosphatemic disorders and underlying genetic defects

	FGF-23	TRP or TmP/GFR	1,25(OH)2D	PTH	Serum Calcium	Urinary Calcium	Mutant gene
Hypophosphatemic disorders							
XLH	increased/ inappropriately normal	Low	Low/ inappropriately normal	Normal/ increased	Normal	Normal	PHEX
ADHR	increased/ inappropriately normal	Low	Low/ inappropriately normal	Normal	Normal	Normal	FGF23
ARHP	increased/ inappropriately normal	Low	Low/ inappropriately normal	normal	Normal	Normal	DMP1
HHRH	Low/normal	Low	High	Low	Normal	High	NPT2c
Hyperphosphatemic disorders							
Tumoral calcinosis	Intact: low C-terminal: very high	High	Normal-High	Low	Normal/ Increased	Increased	FGF23 or GALNT3 (glycosyltransferase)
	Extremely high (intact and C-terminal)	High	Normal-High	Elevated	Normal/ Increased	Increased	Klotho
Isolated hypoparathyroidism	Normal-increased	Elevated	Low-normal	Low-normal	Low	Increased or inappropriately normal	Calcium-sensing receptor, PTH, or GCMB, and unknown genetic defects
Pseudohypopara-thyroidism type Ia (PHP-Ia) or Ib (PHP-Ib)	Normal-increased	Elevated	Low-normal	High	Low	Low	PHP-Ia: *GNAS* exons encoding Gsα: microdeletions within or up-stream of *GNAS*

Hereditary Hypophosphatemic Rickets with Hypercalciuria (HHRH)

The homozygous ablation of *Npt2a* in mice ($Npt2a^{-/-}$) results, as expected, in increased urinary phosphate excretion leading to hypophosphatemia (317). Due to the hypophosphatemia $Npt2a^{-/-}$ mice show an appropriate elevation in the serum levels of 1,25-dihydroxyvitamin D leading to hypercalcemia, hypercalciuria and decreased serum parathyroid hormone levels, and increased serum alkaline phosphatase activity. These biochemical features are typically observed in patients with hereditary hypophosphatemic rickets with hypercalciuria (HHRH), which was presumed to bean autosomal recessive disorder affecting renal tubular phosphate reabsorption (318). HHRH patients develop rickets, have short stature, and increased renal phosphate clearance (TmP/GFR is usually 2 to 4 standard deviations below the age-related normal range), hypercalciuria despite normal serum calcium levels, increased gastrointestinal absorption of calcium and phosphorus due to an elevated serum concentration of 1,25-dihydroxyvitamin D, suppressed parathyroid function, and normal urinary cyclic AMP excretion. Long-term phosphate supplementation as the sole therapy leads, with the exception of persistently decreased TmP/GFR, to reversal of the clinical and biochemical abnormalities (318). Unlike HHRH patients, $Npt2a^{-/-}$mice do not have rickets or osteomalacia. Instead, they have poorly developed trabecular bone and retarded secondary ossification, and in older animals there is a dramatic reversal and eventual overcompensation of the skeletal phenotype. Consistent with these phenotypic differences, mutations in *SLC34A1*, the gene encoding the sodium-phosphate co-transporter NPT2a were excluded in several kindreds, including the one in whom this syndrome was first described (318, 319). However, subsequent studies have led to the identification of homozygous or compound heterozygous mutations in *SLC34A3*, the gene encoding the sodium-phosphate co-transporter NPT2c, in patients affected by HHRH (320–322). These findings indicate that NPT2c has a more important role in phosphate homeostasis than initially thought (323).

Vitamin D-Dependent Rickets (VDDR)

Patients with VDDR type I show clinical and laboratory findings that are similar to those observed in patients with vitamin D-deficient rickets. However, unlike in vitamin D-deficiency, patients with VDDR type I do not respond to treatment with vitamin D and treatment with 1,25 dihydroxy vitamin D is required instead. VDDR type I was therefore named *pseudovitamin D deficiency rickets* (324). Clarification of the abnormal vitamin D metabolism (325) led to the recognition that VDDR type I was due to a defect in the renal 1α-hydroxylase enzyme; consequently serum 1,25 dihydroxy vitamin D concentration is low. Subsequently another condition was recognized and called vitamin D-dependent rickets type II (VDDR type II). In this condition, which is due to end organ resistance to 1,25 dihydroxy vitamin D, the serum 1,25 dihydroxy vitamin D concentration is markedly elevated.

1-α Hydroxylase Deficiency (VDDR Type I)

Patients affected by VDDR type I (autosomal recessive) show almost all the clinical and biochemical features of vitamin D-deficient rickets. Typically, the child is well at birth and within the next two years develops hypotonia, muscle weakness, an inability to stand or walk, growth retardation, convulsions, frontal bossing and the clinical and radiographic signs of rickets - rachitic rosary, thickened wrists and ankles, bowed legs and fractures. A history of an adequate intake of vitamin D is usually obtained. Trousseau's and Chvostek's signs may be present. The permanent teeth show marked enamel hypoplasia, a feature not seen in X-linked hypophosphatemic rickets (326).

The pathogenesis of VDDR type I was first elucidated by studying vitamin D metabolism in affected patients, and it is shown that massive doses of vitamin D_3 and high doses of 25-hydroxy vitamin D_3 but only small doses of 1,25 dihydroxy vitamin D_3 were required to correct the clinical and biochemical abnormalities (325). This provided indirect evidence that the condition was due to an inborn error of vitamin D metabolism, i.e., a defect in the renal 1α-hydroxylase enzyme, the enzyme that converts 25-hydroxy vitamin D_3 to 1,25 dihydroxy vitamin D_3. The serum 25-hydroxy vitamin D_3 concentration was normal in untreated patients and high in patients treated with vitamin D whereas the serum concentration of 1,25 dihydroxy vitamin D_3 was low in untreated patients (327) and remained low or below-normal in patients treated with vitamin D_3 (328). The low serum concentrations of 1,25 dihydroxy vitamin D_3 despite the normal or high serum 25(OH)D_3 can be explained by a deficiency in the renal 1α-hydroxylase (328–330), and molecular genetic studies have later confirmed this conclusion. Indeed, genetic linkage studies in affected French-Canadian families mapped VDDR type I to a region on chromosome 12q13.3 (331), which comprises the gene encoding the 1α-hydroxylase. DNA sequence analysis of patients

affected by VDDR type I have identified more than 20 different mutations of this enzyme (291, 332–335) in 26 kindreds. All patients with VDDR type I were found to carry homozygous or compound heterozygous mutations, whilst their parents are healthy obligate carriers. Most mutations lead to a complete lack of the 1α-hydroxylase; only two mutations have been identified that maintain partial enzyme activity in vitro. These mutations were found in the two patents with only mild laboratory abnormalities, suggesting that such mutations contribute to the phenotypic variation observed in patients with 1α-hydroxylase deficiency suggesting that not all 1α-hydroxylase mutations lead to the development of the "classicial" laboratory findings in VDDR type II (336).

End Organ Resistance (VDR Mutations, VDDR Type II)

Vitamin D-dependent rickets type II (VDDR type II; autosomal recessive) is an autosomal recessive disorder caused by end-organ resistance to 1,25 dihydroxy vitamin D_3 (337–339). The laboratory and radiographic features of VDDR type II are similar to those found in VDDR type I, with one major exception; patients with VDDR type II have markedly elevated circulating concentrations of 1,25 dihydroxy vitamin D_3. The disease varies in its clinical and biochemical manifestations, which suggested heterogeneity in the underlying molecular defects (339). Most of the patients have an early onset of rickets but the first reported patient was a 22 year old woman who had skeletal pain for seven years (337) and another patient presented himself at the age of 50 (years) following five years of this symptom (340). Alopecia totalis occurs in some patients and it was suggested that the therapeutic response to the 1α-hydroxylated derivative of vitamin D_3 in patients with normal hair growth was better than in those with alopecia totalis (341), but this was not found to be a constant predictive sign (342). The severity of resistance to 1,25 dihydroxy vitamin D_3 is variable and some patients have improved following therapy with very large doses of vitamin D (343) or 1,25 dihydroxy vitamin D_3 (344–346). In patients who are refractory to vitamin D therapy, alternative treatments with oral calcium supplements have been tried with limited success. However, long term nocturnal intravenous calcium infusions followed by oral calcium supplementation have successfully healed rickets and promoted bone mineralization in VDDR II patients (347), though there are considerable practical difficulties with this therapy.

The elevated serum concentrations of 1,25 dihydroxy vitamin D_3 in patients with VDDR II suggested an abnormality in the mode of action of 1,25 dihydroxy vitamin D_3 within target tissues. The functions of 1,25 dihydroxy vitamin D_3 are mediated by an intracellular receptor that binds DNA and concentrates the hormone in the nucleus (348), analogous to the classical steroid hormones (349). The interactions between 1,25 dihydroxy vitamin D_3 and its intracellular receptor have been studied using cultured skin fibroblasts from control subjects and patients with vitamin D-dependent rickets type II (350, 351). Several defects were identified, including absent receptors, a decreased number of receptors with normal affinity, a normal receptor-hormone binding but a subsequent failure to translocate the hormone to the nucleus, and a post-receptor defect, in which normal receptors are present but there is a deficiency in the induction of the 25-OHD-24 hydroxylase enzyme in response to 1,25 dihydroxy vitamin D. Thus, the heterogeneity suggested from clinical observations in VDDR II patients could be demonstrated at the cellular level with various combinations of defective receptor-hormone binding and expression. The 1,25 dihydroxy vitamin D_3 receptor (VDR), which is closely related to the thyroid hormone receptors and represents another member of the trans-acting transcriptional factors including the steroid and thyroid hormone receptors is an intracellular protein, which has a molecular weight of 60,000 daltons. The binding site for 1,25 dihydroxy vitamin D_3 resides in the C-terminal part of the protein while the N-terminal part of the molecule possesses the DNA-binding domain (352). Zinc and other divalent cations are important in maintaining the DNA binding function of the receptor, possibly by determining the conformation of the protein and giving rise to processes that can interdigitate between the helices of DNA. This hormone-receptor complex binds to a DNA region, which is located upstream of the promoter of genes encoding calcium-binding proteins and other proteins.

The availability of cDNAs encoding the avian and human VDR (353) helped to clarify the molecular basis of VDDR type II (354). Nucleotide sequence analysis of genomic DNA revealed that the human VDR gene consists of 9 exons; exons 2 and 3 encode the DNA-binding domain, while exons 7, 8 and 9 encode the vitamin D-binding domain. The gene is located on chromosome 12q12-q14 in man (331), i.e., in a region that comprises the gene encoding the 1α-hydroxylase. Mutational analysis of the VDR gene in VDDR II patients demonstrated the presence of nonsense and missense mutations affecting different parts of the receptor. Expression of these

mutations in COS-1 monkey kidney cells demonstrated that these mutations result in a reduction or a loss of VDR function similar to the heterogeneous effects observed in cultured fibroblasts from VDDR II patients. Furthermore, null mutant i.e., "knockout" mice for VDR produced by targeted gene disruption (355, 356), were found to have growth retardation, skeletal deformities and an earlier mortality, and adult mice developed alopecia. In addition, biochemical investigations revealed that the VDR mutant mice were hypocalcemic and hypophosphatemic, with markedly elevated serum 1,25 dihydroxy vitamin D_3 concentrations. Thus, the VDR-null mutant mice have the features consistent with those observed in patients with VDDR type II.

Other Hypophosphatemic Disorders

There are several other genetic disorders associated with hypophosphatemia and often with other defects in proximal tubular function. These include, Dent's disease, an X-linked recessive disorder, which is caused by mutations in *CLCN5* encoding the voltage-gated chloride channel CLC-5 (290, 357) and Lowe syndrome (oculo-cerebro-renal syndrome) (358), another X-linked recessive disorder that is caused by mutations in OCRL1 (359, 360). Furthermore, Fanconi-Bickel syndrome, which is caused by homozygous or compound heterozygous mutations in GLUT2, can be associated with severe hypophosphatemia, but this feature is often not very prominent (361). Other rare hypophosphatemic diseases are osteoglophonic dysplasia (OGD) (362), an autosomal dominant disorder, which was recently shown to be caused by different heterozygous missense mutations in the FGFR1 (363, 364), linear nevus sebaceous syndrome (LNSS), also known as epidermal nevus syndrome (ENS) or Schimmelpenning-Feuerstein-Mims syndrome, in which elevated FGF-23 were observed (365, 366), and fibrous dysplasia, which is caused by heterozygous activating, post-zygotic mutations in exon 8 of *GNAS*, the gene encoding the alpha-subunit of the stimulatory G protein ($G_s\alpha$) (367, 368), that lead in the dysplastic regions to a cAMP-dependent increase in the production of FGF-23 by osteoblasts/osteocytes and fibrous cells (34, 369).

Concluding Remarks

Remarkable advances have been made in identifying key proteins that are involved, either directly or indirectly, in the regulation of calcium and phosphate homeostasis, the hormones that are involved in these mechanisms and receptors that mediate these hormonal actions in the different target tissues. Furthermore, the identification of mutations in several of these proteins provided a plausible molecular explanation for a variety of familial and sporadic disorders of mineral ion homeostasis and/or bone development. In addition to advances in further defining the biological role(s) of known proteins, genetic loci and/or candidate genes have been identified for many of the inherited disorders associated with an abnormal regulation of calcium and phosphate homeostasis. It is likely that the definition of these familial disorders at the molecular level, which is greatly aided by the rapid progress in the Human Genome Project, and the exploration of the underlying cellular mechanisms will provide further important insights into the regulation of calcium and phosphate.

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12 Nutrition and Metabolism

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Introduction

The effects of nutrition on the health and well-being of individuals is well described. The adverse effects of poor nutrition on children are amplified by their influence on the child's ability to grow and develop appropriately. Because of the kidneys' function on elimination of waste products from metabolism, the correlation between kidney disease and nutritional imbalance is well established, and this is most obvious in children with chronic kidney disease (CKD). Poor nutrition often complicates CKD and inevitably adversely affects weight gain, growth and development of these children. Conversely, abnormalities or reduction of renal function frequently lead to abnormalities of nutritional intake or metabolism, resulting in a complex inter-relationship of renal function and nutrition.

Nutrition in Children with CKD

The etiology of growth delay and cachexia in pediatric patients with CKD is thought to be multifactorial with inadequate caloric intake, uremic toxicity, anemia and metabolic and endocrine abnormalities among the leading causes (1, 2). Given the numerous causes of poor growth in CKD, identifying children with inadequate calorie and protein intake can be challenging. There is evidence that most infants and young children with CKD have suboptimal caloric intake (1). Foreman et al. found that mean caloric intake was 80 ±23% of the recommended dietary allowance (RDA) for age in these children (3); however, they found no correlation between caloric intake and height velocity. Calcium, vitamin B6, zinc, and folate intakes were also low. The mean protein intake in these children was 153 ±53% of the RDA. The same study suggested that the amount of energy per unit body weight (kcal/kg/day) consumed by children with CKD is comparable to that of age-matched healthy children, but because of their small size, children with CKD consume fewer total calories and have a lower %RDA for age (3). Nutritional supplementation in children with inadequate intake improves growth to some extent, but does not restore normal growth velocity. Further growth improvements have not been seen when energy intakes exceed 75% of the RDA (4, 5), suggesting that adequate calorie and protein intake are only part of a very complex mechanism involved in the etiology of poor growth and cachexia associated with CKD.

Current evidence suggests that dysregulation of hormones, particularly growth hormone, insulin, leptin and ghrelin, as well as inflammatory cytokines have a large impact on metabolism, body composition and growth (6–37). As a result, these compounds have been the focus of much recent research on the nutritional aspects of CKD. The abnormal regulation of multiple hormones and compounds creates an unfavorable state for growth and development. It is a great challenge for the medical team to promote adequate weight gain and growth given the powerful catabolic effects of many hormones and cytokines. These compounds have substantial impact on body composition and metabolism in CKD.

CKD creates a state of glucose intolerance (6). The anabolic effect of insulin is blunted, including the glucose metabolism, as are the amino acid uptake into cells and the lipoprotein lipase (LPL) activity. Clinically, this state usually consists of fasting euglycemia but abnormal glucose tolerance with a delayed decrease in blood glucose in response to insulin and hyperinsulinemia. The mechanisms responsible seem to be multiple but the most prominent metabolic disturbance in uremic patients is insulin resistance mainly due to a postreceptor defect (7, 8). Chronic metabolic acidosis (CMA), frequently associated with CKD, contributes to insulin resistance and its treatment increases insulin sensitivity (9). In addition to insulin resistance, there is abnormal insulin release attributable to reduced adenosine triphsophate content in the pancreatic islets, induced partially by high intracellular calcium, secondary to augmented parathyroid hormone (PTH)-induced calcium entry into the cells (7, 8). The abnormalities with insulin and CMA have negative effects on body composition and growth. Insulin resistance, acidosis and inflammation activate the ubiquitin-proteasome system (UPS), which degrade muscle protein leading to a catabolic state (10–14).

The dysregulation of growth hormone plays a major role in growth velocity and development in children. The

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GH/insulin-like growth factor plays a fundamental role in the growth process during childhood. Although serum GH and IGF-1 levels are usually normal or high in growth-retarded children with CKD (15), uremic serum has a high IGF-1 blinding capacity, resulting in low IGF-1 bioactivity (16, 17). The mechanisms for this increased IGF-1 binding capacity seem to be related to decreased renal clearance of IGFBP (IGF binding proteins), because serum levels of IGFBP-1, -2, -4 and -6 were shown to correlate inversely with GFR (18, 19). In addition, uremia raises hepatic mRNA levels for IGFBP-1 and -2 by an unknown mechanism (20).

Secondary hyperparathyroidism has significant effects on longitudinal growth. High PTH levels are associated with mobilization of calcium from bone. PTH also causes proliferation of the fetal growth plates chrondrocytes, inhibits the differentiation of these cells into hypertrophic chondrocytes, and stimulates accumulation of cartilage-specific proteoglycans that are though to act as inhibitors of mineralization (21). However despite the high serum PTH level, there is "PTH resistance" at the level of growth plates, owing to intertwined factors affecting the PTH/PTH-related peptide receptors. This receptors mRNA expression was found to be downregulated in the kidney and growth plate of uremic rates, in the osetoblasts of patients with ESRD (22), and as a result of high-dose intermittent calcitriol therapy (23, 24). On the other hand, PTH/PTH-related peptide expression is upregulated by GH and physiologic doses of calcitriol (23).

Chronic metabolic acidosis may affect growth independently of uremia because diseases such as renal tubular acidosis are associated with growth defects, and catch-up growth can be achieved when patients are given alkali therapy. When the blood pH is less the 7.25, length gain is diminished earlier than weight gain. A reduction of weight gain is observed only for more severe acidosis with pH of less than 7.20 (25). This finding suggests that longitudinal growth of bone is more sensitive to acidosis than are such factors as protein synthesis, which influences body weight. The mechanisms appear to be profound in vitro-negative effect of CMA on the GH/IGF-1 axis, mainly through downregulation of the receptors for both hormones (26) plus a stimulation of osteoclastic and suppression of osteoblastic activity (27).

It was not until recently that the effects of cytokines and the hormones ghrelin and leptin have been understood. Cachexia and poor growth associated with kidney disease may be largely caused by the effects of proinflammatory cytokines and hormones (29–33). An understanding of this mechanism is the first step in the development of strategies to prevent or treat the symptoms of wasting in kidney disease. As kidney function declines, plasma levels of inflammatory cytokines increase. These elevated cytokines exert their effects on a number of sites throughout the body and likely contribute to cachexia in multiple ways (25–34).

Although the exact mechanism remains uncertain, studies have found that cytokines suppress appetite through actions on the central nervous system. Three cytokines whose effects on body composition have been well studies are interleukin 6 (IL-6), tumor necrosis factor alpha (TNF α and interleukin 1β (IL-1β)). IL-6 is present in both adipose tissue and the hypothalamus. It is the primary cytokine responsible for the activation of the acute phase response. The energy demands of the acute phase response are great, requiring large amount of essential amino acids. This overwhelming need for amino acids stimulates muscle breakdown (28). Animal studies have shown that infusion of IL-1β, and TNF-α lead to anorexia, weight loss and muscle wasting (4). In addition, IL-1β produces a delay in gastric emptying that may further contribute to poor nutrition. Cytokines also disrupt carbohydrate metabolism causing peripheral insulin resistance, which further contributes to wasting (28). Peripheral insulin resistance results in the transport of glucose away from muscle to the liver forcing skeletal muscle to use nonessential amino acids for energy. This process leads to negative nitrogen balance.

Leptin is a satiety hormone produced by adipose tissue that may have a large impact on appetite and weight in kidney disease (4, 35). In healthy individuals, leptin circulates in proportion to the amount of body fat. It inhibits appetite and food intake and increases energy expenditure. In healthy individuals, leptin circulates in proportion to the amount of body fat (4, 35). It reduces appetite and food intake and increases energy expenditure. During times of fat loss and decreased calorie intake, leptin levels decrease, which results in an increase in appetite and more efficient metabolism. Leptin functions as a regulatory hormone under healthy conditions and tends to preserve body composition at a relatively stable state. The kidneys are the main sites for clearing circulating leptin levels. As kidney function declines, excess leptin accumulates and becomes greatly out of proportion with the amount of body fat (30). It has been suggested that inflammation may also contribute to elevated leptin levels but this association is not clear. One study showed a significant association between C-reactive protein levels and leptin in patients with chronic kidney disease (36). However a more recent study showed no association between the two markers (36). One study even showed a positive correlation between plasma leptin and normalized protein

catabolic rate (nPCR), subjective global assessment score and midarm muscle circumference (37). This suggests that higher leptin levels may be a marker of good nutritional status.

Ghrelin is a second regulatory hormone that functions to maintain fat stores and body composition is. Ghrelin is produced primarily in the stomach but is also produced in smaller amounts in other tissues including the kidney. Ghrelin is elevated after fasting and its concentration is highest in lean people and lowest in obese people (29, 32). It stimulates growth hormone release, increases food intake, and promotes weight gain, particularly from fat. Ghrelin may make metabolism more efficient because increased body fat is seen in patients with hyperghrelinemia without extra calorie intake (29, 32). As kidney function deteriorates, ghrelin levels tend to increase. CKD patients often experience changes in body composition that lead to muscle wasting and preservation of fat mass. It is of interest that despite the already elevated ghrelin levels seen in patients with kidney disease, recent studies have found that treatment with exogenous ghrelin and ghrelin receptor antagonists actually improve body composition (34). A recent study performed on uremic rats has found that treatment with exogenous ghrelin and ghrelin receptor antagonists improves lean body mass, increased random GH levels and may decrease inflammation (34).

Nutritional Assessment and Interpretation of Anthropomteric Data in Children with CKD

It is important for the nutritional status and growth of children with even mild CKD to be assessed regularly by a skilled registered dietitian. The frequency of monitoring children with stage 2–5 CKD is dependent on the age of the child, stage of CKD and how well an individual child is thriving. Closer monitoring is indicated in children with growth delay, poor intake or low Body Mass Index. Nutritional assessments should include a method of estimating nutrient intake and measurement of growth parameters as well as growth velocity.

The two most clinically practical ways to assess energy and nutrient intake are the three-day food diary and 24-h recall (38, 39). For younger children, assessment of dietary intake can be done through a 3-day diet diary. Diet diaries have shown to provide accurate estimates of nutrient intake in normal weight children under the age of 10 (39). Similar accuracy has not been seen with adolescents keeping food records because they tend to underreport what they have consumed (40). A 24-h recall may be more suitable for adolescents who are not as inclined to comply with other methods of diet recording. This method has the advantage of being quick to carry out and can provide detailed information about specific foods.

The extent to which nutrition influences growth is highly dependent on the age of the child. The greatest benefit from correction in nutritional deficits is seen in infants. Growth occurs at the most rapid rate during the infancy stage and is driven predominately by nutrition (41). Infants use a much larger percentage of their daily energy for growth as compared with older children and adolescence. The first 6–12 months of life is normally considered the infancy stage, however in CKD the transition to the childhood phase is often delayed until 2–3 years of age (41, 42). Infancy is the most critical time for nutrition intervention in children with CKD. Infants with significant renal impairment frequently present with poor intake. Any signs of inadequate growth or weight gain are an indication for immediate initiation of nutritional supplements (*Table 12-1*) (41). Infants also may require gastrostomy tubes to meet their energy and fluid needs.

Growth retardation is also common in older children with CKD. However growth in those children is driven mainly by growth hormone during childhood and sex hormones during adolescence. Nutritional supplements have been shown to improve growth in infants but similar results have not been seen consistently in older children (41, 43–45). Poor growth in older children and adolescents has historically been attributed to poor calorie and protein intake; but given that numerous other factors are now known to be involved in inadequate growth and development including the dysregulation of hormones and cytokines, this conclusion is likely not accurate. The most recent KDOQI guidelines suggest that in older children poor intake may be the result of inadequate growth and not the cause (41). Determining whether inadequate growth is due to true undernutrition or another cause is crucial in providing the appropriate intervention.

Anthropometric measures, such as weight, height, head circumference and growth velocity should be monitored routinely. Infants and children with CKD should be measured and plotted on the length- or height-for-age curves and SD score calculated (41). In infants, length should be measured on a length board and in older children height should be assessed with a stadiometer. Growth velocity, defined as change in length per unit time should be assessed routinely. In children over age 2 years of age, height velocity is accurately assessed with intervals of 6 months of greater. Adequate linear growth is a good indicator of long-term nutritional status. However, weight

Table 12-1

Nutrition formulas for children with renal disease

	Manufacturer	Kcal/ml	Protein (g/L) sources	Fat (g/L) sources	Carbohydrates (g/L sources)	Na/K (mEq/L)	Ca/P (mg/L)	mOsm/Kg water
Similac PM 60/40	Ross products	0.67	16 (whey, caseinate)	38 (soy, coconut)	69 (lactose)	7/15	380/190	250
Amin-aid	R and D laboratories	2	19 (free amino acids)	46 (partially hydrogenated soybean oil	365 (maltodextrin, sucrose)	<15/<15	–	700
Suplena	Ross product	1.8	44 (caseinates)	95 (high oleic, safflower and soy oil	199 (maltodextrin, sucrose, cornstarch)	33/28	1,060/700	600
Renalcal	Nestle nutrition	2	34 (essential L amino acids, select nonessential amino acids, when protein concentrate)	82 (MCT oil, canola oil, corn oil, soy lecithin)	290 (maltodextrin, modified cornstarch)	–	–	600
NeproCarb Steady	Ross products	1.8	81 (caseinates)	96 (high oleic safflower oil and soy oil)	167 (corn syrup, sucrose, FOS)	46/27	1,060/700	585

loss or poor weight gain may precede growth stunting (41). In the early stages of undernutrition, linear growth often continues at a normal rate until severe wasting occurs. Therefore, other indicators of nutritional status should be used. Assessing weight for age (in children 2 years of age and under) and BMI in older children can help determine the degree of wasting. BMI is determined as a ratio of weight to height and is calculated by dividing weight in kg by the square of the height in meters (BMI = Wt {kg}/[ht {M)}]^2. In healthy children, BMI is compared with reference values for a particular gender and age. Because many children and adolescents with kidney disease have delayed sexual maturation and linear growth, the most recent KDOQI guidelines recommend that plotting BMI against actual age may be misleading (41, 46). Body composition changes are related to pubertal growth as well as to age. Plotting BMI against actual age may falsely classify some patients as undernourished and it can be misleading for patients who are consuming adequate calories and protein but have delayed growth or sexual maturation due to non-nutritional causes. Therefore, plotting BMI against a particular child's height age (the age at which the height falls along the 50th percentile) may be more reasonable. These methods can be very accurate in assessing nutritional when fluid overload is not an issue. Extreme fluid overload as is seen with children with chronic nephrotic syndrome can make determining dry weight difficult and can mask weight loss (41). Estimating dry weight regularly (by means of physical exam and other clinical markers) is important to accurately track a child's weight gain.

Sophisticated body composition data are generally used for research purposes only, owing to the difficult of standardization (47). For determination of fat-free mass, the following methods can be used: total body water, total body potassium, total body nitrogen and calcium, bioelectrical impedance and electromagnetic scanning. Determination of body fatness can be done by densitometry, inert gas absorption or near-infrared interactance. Neuron activation or dual-energy x-ray absorptiometry can be used to determine the bone mineral content (48).

In the clinical setting, accurately detecting and treating malnutrition has been a challenge. Until recently, there has not been a clear or uniform definition of undernutrition for the CKD population. The definition of undernutrition set by the World Health Organization (WHO) for the general population is not suitable for children with CKD (41). The WHO defines undernutrition as weight-for-age, height-for-age, and weight-for-height $\geq$2 SD below the CDC reference range (41, 49). These guidelines are based on the fact that undernutition in a healthy child may lead to low weight-for-height as

well as low height-for-age (growth stunting). As discussed earlier, in children with CKD, many factors influence growth and stunting will often occur even with adequate nutrient intake. Therefore this definition of malnutrition should not be applied to children with CKD. Practitioners should be cautious in labeling a child with CKD as malnourished. Careful evaluation of height, weight, ratio of height to weight and BMI should be considered before a diagnosis of malnutrition is made. If a child is small for age (weight-for-age <5th percentile and height-for-age <5th percentile) but BMI for age of ≥50 percentile, and diet evaluation suggests adequate intake, it is reasonable to conclude that the growth delay is due to non-nutritional factors.

Table 12-2

Equations to estimate energy requirements for children at healthy weights

Age	Estimated energy requirement (EER) (kcal/d) = Total energy expenditure + Energy deposition
0–3 mo	EER = (89 × weight (kg) − 50) + 175
4–6 mo	EER = (89 × weight (kg) − 50) + 56
7–12 mo	EER = (89 × weight (kg) − 50) + 22
13–35 mo	EER = (89 × weight (kg) − 50) + 20
3–8 y	Boys EER = 88.5 – 61.9 × age (y) + PA × (26.7 × weight (kg) + 903 × height (m)) + 20
	Girls EER = 125.3 – 30.8 × age (y) + PA × (10 × weight (kg) + 934 × ht (m)) + 20
9–18 y	Boys EER = 88.5 – 61.9 × age (y) + PA × (26.7 × weight (kg) + 903 × height (m)) + 25
	Girls EER = 135.3 – 30.8 × age (y) + PA × (10 × weight (kg) + 934 × height (m)) + 25

Adapted from (41)

Table 12-3

Equations to estimate energy requirements for children ages 3–18 years who are obese

Age	Weight maintenance total energy expenditure (TEE) in overweight children
3–18 y	Boys: TEE = 114 − (50.9 × age (y) + PA × (19.5 × weight (kg) + 1,161.4 × ht (m))
	Girls: TEE = 389 − (41.2 × age (y) + PA × 15.0 × weight (kg) + 701.6 × height (m))

Adapted from (41)

Nutritional Support for the Child with CKD 1–4

Macro- and micro-nutrient guidelines for children with CKD before dialysis vary depending on the age of the child and stage of kidney disease (*Tables 12-2–10*). Equations for estimating energy needs differ with overweight versus normal weight children as well as activity level (*Tables 12-2–4*). Studies show that disturbances in nutritional intake, bone biochemistry and growth occur early in CKD and suggest the need for joint medical and dietary intervention in children even with mild and moderate CKD (50). A thorough nutrition assessment will ensure that poor growth or weight gain is detected and addressed early. To achieve the greatest benefit from nutrition intervention, it is imperative that it be started early.

Calorie and protein supplementation either orally or via a gastrostomy tube may be necessary if the child is unable to meet his energy needs through food. Vitamin and trace element supplementation may also be indicated the child's estimated intake is considerably less than the RDA. Iron supplementation is usually required in the predialysis period when depletion of iron stores is documented by laboratory studies. Patients receiving erythropoietin generally require iron supplementation owing to increased turnover of red blood cells and require regular

Table 12-4

Physical activity coefficients for determination of energy requirements in children ages 3–18 years

Gender	Sedentary	Low active	Active	Very active
	Typical activities of daily living (ADL) only	ADL + 30–60 min of daily moderate activity (e.g., walking 5–7 km/h)	ADL + ≥60 min of daily moderate activity	ADL + ≥60 min of daily moderate activity + an additional 60 min of vigorous activity or 120 min of moderate activity
Boys	1.0	1.13	1.26	1.42
Girls	1.0	1.16	1.31	1.56

Adapted from (41)

Table 12-5

Protein recommendations for children with CKD stages 3–5

Age	DRI (g/kg/day)	Recommendation for CKD Stage 3 (g/kg/day) 100–140% DRI	Recommendation for CKD Stages 4–5 (g/kg/day) (100–120% DRI)	Recommended for HD (g/kg/day)	Recommended for PD (g/kg/day)
0–6 mo	1.5	1.5–2.1	1.5–1.8	1.6	1.8
7–12 mo	1.2	1.2–1.7	1.2–1.5	1.3	1.5
1–3 y	1.05	1.05–1.5	1.05–1.25	1.15	1.3
4–13 y	0.95	0.95–1.35	0.95–1.15	1.05	1.1
14–18 y	0.85	0.85–1.2	0.85–1.05	0.95	1.0

Adapted from (41)

Table 12-6

Sodium guidelines for children with CKD stages 2–5

<table>
<tr><th>Indications for Na supplementation</th><th colspan="2">Indications for Na restriction</th></tr>
<tr><td>Infants and children with stage 3–5 CKD with polyuria</td><td colspan="2">Restriction should be considered for children with stage 2–5 CKD with HTN or pre-HTN</td></tr>
<tr><td>All infants receiving PD therapy</td><td colspan="2" rowspan="3">Suggested restriction 1–2 mmol/kg/day (adequate intake (AI) levels of Na for healthy children is a reasonable restriction for children with CKD</td></tr>
<tr><td>Suggested supplement dose: 4–7 mmol/kg/day</td></tr>
<tr><td>Adjust according to blood chemistry tests</td></tr>
<tr><td rowspan="4"></td><td>Age (y)</td><td>AI for Na (mg)</td></tr>
<tr><td>1–3</td><td>1,000</td></tr>
<tr><td>4–8</td><td>1,200</td></tr>
<tr><td>9–18</td><td>1,500</td></tr>
</table>

Information based on (41)

Table 12-7

Fluid guidelines for children with CKD stages 2–5

Indications for fluid supplementation	Indications for fluid restriction
Supplement free water in Infants and children with polyuria	Children with stage 3–5 CKD who are oligoanuric
For infants 0–12 months, fluid needs are often 150–200 ml/kg	Daily fluid restriction = insensible losses + urine output + amount to replace additional losses (e.g., vomiting, diarrhea, enterostomy output) − amount to be deficited

Information based on (41)

checking of serum iron levels. Zinc administration is not routinely administered to children during the predialysis phase, but because foods rich in zinc are often limited due to poor appetite, supplementation by pharmacologic means on the basis of RDA standards may be recommended if there is reason to suspect zinc deficiency. Fluoride supplementation is provided only when the water supply is not satisfactory (51).

Nutritional Support for the Child Treated with Maintenance Hemodialysis

Chronic hemodialysis (HD) results in dialysate loss of amino acids and water-soluble vitamins. In addition, the HD procedure itself has catabolic effects (52). In adult studies, up to 8 g of free amino acids lost per HD session have been reported. This loss is further influenced by

Table 12-8

Recommended phosphorus intake for children with CKD

Age	DRI (mg/d)	High PTH and normal phosphorus	High PTH and high phosphorus
0–6 mo	100	≤100	≤80
7–12 mo	275	≤275	≤220
1–3 y	460	≤460	≤370
4–8 y	500	≤500	≤400
9–18 y	1,250	≤1,250	<1,000

Adapted from (41)

Table 12-9

Recommended calcium intake for children with CKD stages 2–5 and dialysis

Age	DRI	Upper limit (for healthy children)	Upper limit for CKD stages 2–5
0–6 mo	210	ND	≤420
7–12 mo	270	ND	≤540
1–3 y	500	2,500	≤1,000
4–8 y	800	2,500	≤1,600
9–18 y	1,300	2,500	≤2,500

Adapted from (41)

Table 12-10

Vitamin recommendations for children with CKD: indications for supplementation

Stages 2–5	Stage 5, dialysis
The supplementation of vitamins and trace elements should be provided to children with CKD stage 2–5 if dietary intake alone does not meet 100% of the DRI	Children on maintenance dialysis should receive a water soluble vitamin supplementation

Information based on (41)

choice of membrane: Newer synthetic polyacrylonitrile membranes have decreased losses relative to cellulose membranes (53).

Children treated with maintenance HD require sufficient calorie intake not only for weight gain and growth but also to avoid the use of proteins as an energy source. Current recommendations based on KDOQI guidelines are that children treated with maintenance HD should receive a calorie intake based on the Estimated Energy Requirement (EER) plus an activity factor (*Tables 12-2–4*). This is based on the fact that there is no evidence to suggest that children on HD have higher calorie needs than healthy children (2). Ongoing adjustments should be made, depending on the child's growth velocity and rate of weight gain (54).

Recommendations regarding the dietary protein intake (DPI) in patients on maintenance HD are that DPI should be based on the DRI for chronologic age plus an additional increment of 0.1 g/kg/day to compensate for losses in the dialysate (*Table 12-5*) (41).

Children on maintenance HD often have insufficient intake of oral nutrients because of anorexia and dietary restrictions. The first attempt at improving energy intake is through oral supplementation (55) (*Table 12-1–3*). As discussed previously, nutrition is most important during periods when energy demands are the greatest- in the first 2 years of life and also during adolescence (56). When oral supplementation fails, enteral feeding through a gastrostomy tube is common and successful at achieving catch-up growth in infants and young children (57, 58).

In cases where the GI tract is not functional for any reason and the patient is at risk for developing malnutrition, intradialytic parentaeral nutrition (IDPN) can be initiated (59). The effectiveness of IDPN in children and adolescence is controversial and few clinical trails are available in this population. Several adult studies revealed morbidity and mortality were lower in patients receiving IDPN (60, 61). However one study failed to demonstrate a beneficial effect of IDPN (62). A study evaluating the use of IDPN in the pediatric population demonstrated a dramatic increase in oral caloric intake and eventual weight gain after 3 months secondary to improved dietary intake.

Pediatric IDPN indications and appropriate compositions are not well defined but involve the infusion of 5–6 mg/kg/min of glucose, 1.2–1.4 g/kg/day of protein, and possibly the addition of intralipids (63).

Possible side effects of IDPN are hyperglycemia associated with glucose infusion or rebound hypoglycemia when the infusion is suddenly terminated. A frequent complaint in patients receiving IDPN is painful cramps in the arm containing the fistula, an effect thought to be due to the rapid infusion of the hyperosmolar solution causing rapid fluid shifts from muscle cell to interstitium. A long-term complication of IDPN is the possible development of abnormal liver function tests due to fatty deposits in the liver and cholestasis. Therefore, patients on IDPN require close monitoring of glucose control, hepatic function and lipid profile (59).

IDPN provides minimal overall supplementation, usually between 500 and 1,500 kcal and is administered only three times per week. In addition, only approximately 70% of the infused amino acids are retained owing to rapid clearance by HD (59). In patients who are severely malnourished and expected to rely on parenteral nutrition for an extended period of time, IDPN would be unable to provide sufficient nutrients. In such cases, total parenteral nutrition (TPN) may be indicated. However, in patients with moderate malnutrition who are unable to be given enteral supplementation, a short course of IDPN may improve nutritional status (63).

Thus, short-term IDPN can be a safe and effective nutritional intervention in children treated with HD who are unable to receive sufficient nutrition from enteral feedings. However, IDPN is quite expensive, and generally restrictive criteria prevent reimbursement. As soon as the patient can tolerate an increase in oral intake or becomes a candidate for tube feedings, enteral supplementation should be initiated.

The practice of prescribing water-soluble vitamins has not been rigorously tested but probably does little harm. There are losses of water soluble vitamins in the dialysate fluid, particularly ascorbic acid (64). Vitamin B6 and folate are important supplements especially because they are useful in reducing the homocysteine levels (65). In view of the reports of peripheral neuropathy and hyperoxalemia with high dose vitamin B6 and vitamin C supplementation, megavitamin therapy with water-soluble vitamins should be given only when there is a clear indication because of the risk of toxicity. Vitamin A levels are invariably increased in the plasma of ESRD patients because of retinol-binding protein is increased in uremia (66).

Trace elements are indispensible components of many enzymes, and abnormalities are primarily the result of uremia and the dialysis procedure. Plasma trace element concentrations in adult HD patients are distinctly different compared to those of healthy controls. Elements such as cesium, magnesium, molybdenum, and rubidium are reduced and cadmium, and lead are accumulated in HD patients (67). Adequate water treatment, including reverse osmosis, prevents the accumulation of the majority of trace elements in HD patients. Zinc supplementation may be recommended for patients with proven zinc deficiency, but its use in all HD patients is questionable (68, 69). Selenium deficiency is to be suspected in dialyzed patients, and supplementation may be beneficial by increasing glutathione peroxidase activity, cardioprotective effect, and immunostimulatory properties (70).

Nutritional Support for the Child Treated with Maintenance Peritoneal Dialysis

Malnutrition in children receiving chronic peritoneal dialysis (PD) has specific etiologies and treatments (71). Benefits of this modality, such as more constant control of uremia, a liberalization of dietary restrictions, and an additional source of calories from the dialysate glucose absorption, are counterbalanced by losses of proteins, amino acids, vitamins and trace elements in the dialysate, anorexia possibly related to the pressure effect of dialysate in the abdomen, and to the hyperglycemia effects of absorption of glucose from dialysate (72). Finally, there is a catabolic effect induced by episodes of peritonitis (73, 74).

Adequate dialysis may be an important factor for obtaining and maintaining adequate nutritional status and growth (75, 76). The delta height velocity of children with a mean age at initiation of PD of 28.5 months was found to be significantly correlated with total creatinine clearance, residual global filtration rate (GFR), and Kt/V urea (77). On the other hand on a cautionary note, Schaefer et al. have also described an important inverse correlation between growth rates and overall clearance in children on PD perhaps attributable to dialytic losses of an essential factor (78). The analysis of change in height SDS over 18 months in children on PD revealed that high transporter state and total dialysate volume had a negative effect, whereas higher dialytic creatinine clearance had a positive effect (79). Alternatively, a Kt/V greater than 2.75 in PD patients had no effect on nutrition but resulted in increased albumin losses (80). Malnutrition in PD patients is multifactorial. Increased losses of amino acids, water-soluble vitamins and trace elements occur, whereas protein losses are inversely related to the patient's weight and peritoneal membrane total area (72). During peritonitis, the permeability of the peritoneum for proteins and amino acids increases significantly by 50–100%. Between 100 and 300 mg of protein/kg/day are lost in the drained peritoneal dialysate, which translates to up to 10% of total protein intake (72, 74). The main protein lost is albumin, but there are also losses of immunoglobulins, transferrin, opsonins, and water-soluble vitamins, such as vitamin B6, vitamin C, and folic acid (81, 82).

In addition the nonspecific factors contributing to decreases caloric intake in CKD, there are specific factors related to PD, such as abdominal fullness from the dialysate and the absorption of glucose from the dialysate. Whereas the glucose absorbed from the dialysate provides calories, it also contributes to the anorexia seen in PD patients (55, 83). The number of calories provided by

dialysate glucose absorption may be predicted by an equation developed from a study of adults (84). Pediatric studies reported dialysate glucose absorption ranging from 9 to 18 kcal/kg/day, representing 7–15% of the total daily caloric intake respectively (85).

Current recommendations for energy intake in children treated on PD should follow the RDA for chronological age, including calories derived from the dialysate glucose, adjusted accordingly (54). Malnourished children however may require additional "catch-up" energy supplementation. Supplemental G-tube feeding facilitates weight gain in infants and older children receiving PD, and arrests the decline in height SDS traditionally observed in infants with ESRD (86, 87). Gastrostomy feedings via a button in children on PD significantly improves BMI (88).

Current recommendations regarding DPI in patients on PD are also based on the DRI for chronological age, to which a supplement of 0.15–0.3 g/kg/day is added to compensate for peritoneal losses (*Table 12-5*) (41). Dietary recommendations for children on PD could be further defined using a series of nitrogen balance studies. The correlation between estimated DPI and nitrogen balance indicates that a DPI of more than 140% RDA and a total energy intake of more than 85% are required to obtain an estimated nitrogen balance of at least 50 mg/kg/day, which is considered adequate for metabolic needs in children (89). High biologic value protein (e.g., meat, milk, eggs) should constitute 60–70% of the DPI (85).

The use of special amino-acid based dialysis solutions in children on PD may compensate for losses into the dialysate (90). Furthermore, the potential complications related to the dialysate glucose load, such as hyperlipidemia, excessive weight gain, and glucose intolerance, could be ameliorated (90, 91). Usually, one exchange per day is replaced with the amino acid solution consisting of both essential and nonessential amino acids with electrolyte composition similar to that of standard dialysis solutions. Between 50 and 90% of the amino acids are absorbed without any changes in ultrafiltration compared with standard dialysis solutions (92, 93). Side effects include a rise in blood urea nitrogen, metabolic acidosis, anorexia, and nausea (94, 95). This nutritional intervention is still rather expensive and should be reserved for malnourished patients who fail more conservative nutritional support.

Dietary intake of water-soluble vitamins is lower than the RDA in the majority of children on PD and supplementation results in intakes that exceed the RDA (96–98). Hyperhomocysteinemia, an independent risk factor for cardiovascular disease in adults (99), is associated with deficiencies of folate, vitamin B6, and vitamin B12 (100). Elevated plasma homocysteine levels in pediatric patients on PD were significantly reduced after administration of 2.5-mg folic acid daily for 4 weeks (101). Supplementation of trace elements such as zinc is reserved for specific deficiencies (71, 102).

The Use of Albumin and Prealbumin and Normalized Protein Catabolic Rate in the Nutrition Assessment

Laboratory markers historically used to evaluate nutritional status (albumin and prealbumin) are now shown to be unreliable (41, 102–108). While albumin is strongly correlated with morbidity and mortality and remains an important part of the overall clinical picture, it is not an accurate measure of nutritional status. Albumin and prealbumin are known to be skewed in states of fluid overload or proteinuria. However, even in the absence of edema or proteinuria, albumin and prelabumin fail to be accurate markers of nutritional status. The decrease in albumin seen with chronic disease has been found to be related to the illness itself and is independent of calorie or protein intake. Albumin and prealbumin are negative acute phase proteins and decrease with inflammation and infection. There has been an increasing trend to shift away from the use of albumin as a nutritional marker (41, 102–108). The most up-to date pediatric KDOQI guidelines address this issue. There continues to be much confusion surrounding the term malnutrition, which is distinct from wasting or cachexia (41). To help alleviate some of confusion surrounding malnutrition and inflammation in the kidney disease population, the International Society of Renal Nutrition and Metabolism (ISRNM) met to establish uniform guidelines for wasting in chronic kidney disease (109). The term malnutrition implies abnormalities caused by insufficient calorie, protein or micronutrient intake. Since disruptions in body composition in patients with kidney disease are often driven by inflammation and metabolic changes, the term malnutrition can be misleading. The ISRNM panel recommends the use of the term "protein-energy wasting" (PEW) for loss of body muscle mass and reserve. This is distinct from energy wasting or malnutrition in that PEW might occur despite adequate nutrition and cannot be corrected by increasing energy intake alone. The diagnosis of protein energy wasting requires three criteria – low levels of serum albumin, transthyretin, or cholesterol, reduced fat mass and reduced muscle mass (109).

In malnutrition that is not complicated by a disease state, such as anorexia nervosa, albumin and prealbumin

levels usually remain normal (105). The loss of body mass that occurs in starvation can be corrected simply by increasing nutrients in the diet. PEW in kidney disease involves an increase in proinflammatory cytokines, tumor necrosis factor alpha and interleukin-6. It is this inflammatory response that results in poor protein anabolism and a decrease in albumin and prealbumin. Increasing calorie and protein intake alone will not be enough to reverse the catabolism associated with the inflammation. Albumin, prealbumin C-reactive protein can be used to assess degree of inflammation or illness. They should not be used as an indicator of nutritional status or as a means to assess adequacy of calorie or protein intake.

In adolescent and adult HD patients, the normalized protein catabolic rate (nPCR) may serve as a predictor of nutritional status (41). The nPCR is calculated as the change in BUN between dialysis treatments as an estimation of the urea generation rate. The most recent KDOQI guidelines recommend that the target nPCR for adolescents and adults receiving HD be set at $\geq$1 g/kg/day (41). This recommendation is based on research that demonstrated a direct relationship between nPCR values less than 1 g/kg/day and weight loss over a 3 month period (41, 110). To date studies on nPCR values have not been shown to predict weight loss in younger children (41). The exact reason for this is unclear but may be related to differences in protein catabolism and growth rate in younger children (41). However, given that regular measurement of nPCR is not of increased cost and does not pose any risk to the patient, it should be monitored monthly in all children receiving HD (41). In theory, changes in nPCR may be reflective of nutritional status.

Inflammation and poor intake often overlap and inflammatory cytokines can induce anorexia. Negative effects on health and body composition can stem from both poor intake and inflammation. Treating both of these problems will result in the best outcome for the patient.

Therapies for Treatment of Protein Energy Wasting Currently Under Investigation

In recent years, new research has brought a clearer understanding of the causes of poor growth and wasting in chronic and end-stage kidney disease. It is well documented that simply increasing calories and protein in the diet has limitations and is often ineffective (111). The challenge now is to develop ways to treat the inflammation and metabolic disturbances that may in turn improve body composition, growth and appetite. The etiology of growth delay and loss of lean body mass in kidney disease is very complex and likely includes many mechanisms. Therefore, it has been suggested in recent literature that wasting should be treated with multiple therapies including diet and pharmacologic components for the maximum benefit (111).

Much attention has been given to certain functional foods because evidence suggests they may have strong benefits in mediating the inflammatory response. Two of the dietary therapies currently being investigated for use in the chronic kidney disease and dialysis population are omega-3 fatty acids and soy protein. Omega 3 fats (DHA and EPA) found in fish have been shown to have antihypertensive, antiatherogenic, antithrombotic and anti-inflammatory properties in the general population (112). It is suspected that omega-3 levels in blood and tissue of dialysis patients are inadequate (113). Several current studies have investigated effects of omega-3 fatty acids or fish intake on specific makers of inflammation in dialysis patients. A pilot study by Saifullah et al. found that supplementing hemodialysis patients with 340 mg of EPA and 170 mg of DHA significantly decreased CRP values compared with controls (114). One perspective study of interest found that patients who reported regular fish consumption had higher serum albumins at baseline (112). The same study found patients who reported fish consumption were 50% less likely to die over a 3-year period (112). A double blind placebo controlled trail looking at the combined effects of gamma tocopherol and DHA on dialysis patients found significant reductions in interleukin-6 in the treatment group compared with controls (115). A recent quasi experimental study by Moriera et al. examined the effects of omega-3 fatty acids in the form of sardines on CRP levels in dialysis patients (116). CRP levels were stratified into tertiles. There was a significant reduction in CRP found only in study participants at the highest tertile of CRP. Still another study showed no benefit in administration of omega-3 fats on dialysis patients (117). Clearly more randomized placebo controlled trails in both the adult and pediatric kidney disease population are needed to better assess efficacy, safety and dosing before omega-3 fatty acids supplements can be recommended. Preliminary data of omega-3 supplementation does suggest positive clinical benefits however. The main risk associated with this treatment appears to be increased bleeding times with doses in excess of 3 g per day (113).

Soy protein is another compound that has been found to have protective effects on the kidney and may also have anti-inflammatory properties (118). Soy contains phytoestrogens, which may block inflammatory gene

expression (119). A recent study found an inverse correlation between blood levels of soy isoflavones and inflammatory markers in hemodialysis patients (120). In East Asian countries where consumption of soy products is significantly higher, the prevalence of inflammation and vascular disease among dialysis patients is lower than in Western countries (121). Asian diets are higher in fiber and fish, which may also offer benefits in reducing inflammation (111). Prebiotiics and probiotics may also play a role in regulating the immune response and protecting the gut barrier (111). These studies suggest that the nutrition therapies should focus more attention on the specific foods that are supplying energy in the diet and not just the number of calories, protein and fat being consumed. Although these therapies are in the early stages of investigation, they warrant discussion, as they will likely be the treatments of the future.

In addition to diet interventions to reduce inflammation, pharmacologic treatments may also have a role (111). Drugs that may have anti-cytokine properties include cyclooxygenase (COX)-2 inhibitors, which have been shown to reduce tumor mediated wasting. Other anti-inflammatory drugs under investigation include anti-TNF drugs such as etanercept and infliximab, which have recently been shown to be effective in rheumatoid arthritis and inflammatory bowel disease (122). studies in rats have shown that anti- TNF drugs improve both appetite and weight (123). These findings suggest a potential role for these drugs in inflammation associated with kidney disease as well.

Developing ways to treat inflammation is likely the cornerstone to improving body composition and linear growth in children with kidney disease. Standard nutritional supplementation is seldom effective when inflammation is present. In addition, the presence of inflammation may diminish the action of growth hormone.

Influences of Nutrition Disorders on Renal Function

Influences of Obesity on Renal Function

Obesity has been found to be an important risk factor for a certain number of diseases, including cardiovascular diseases, hypertension (124), lipid and lipoprotein abnormalities (125). The kidney is an organ that can be directly affected by obesity. In this section only those kidney problems that are considered to be direct effects of obesity will be discussed. Other kidney conditions that can also be associated with obesity, but related more to obesity co-morbidities, such as hypertension and type 2 diabetes will be discussed in other chapters.

The prevalence and severity of obesity is increasing in the pediatric population and this is becoming a severe health hazard. As a consequence, the prevalence of the sequelae of obesity is expected to be increasing, as well. Indeed, the rates of obesity among US children defined as BMI $\geq$95th percentile for age and sex, have increased dramatically, with a threefold increase since 1976 (126). The National Health and Examination Surveys (NHANES) have shown steady increases from the late 1970s to 2004 in the prevalence of overweight (BMI $>$95th percentile of the US growth reference) and at risk of overweight (a BMI between the 85th and 95th percentile) among children and adolescents. In 2004, 17.1% of American children and adolescents were overweight, and an additional 16.5% were at risk of overweight. Nearly 14% of 2–5-year old children and 19% of 6–11-year old children were overweight (127).

The association of obesity with proteinuria has been well described in adults as early as the 1970s and 1980s (128, 129). Obesity-associated proteinuria in adults is associated with focal segmental glomerulosclerosis (FSGS) (123). In addition, it is interesting to note that non-obese patients with increased BMI due to elevated muscle mass are also at risk of developing a secondary form of FSGS that resembles obesity-related glomerulopathy (130). However, the FSGS diagnosed in obese and non-obese patients with high BMI shares clinical features distinctive from those usually seen with primary FSGS: albumin levels tend to be higher, often above 3.0 g/dL, total cholesterol levels are only mildly elevated, often $<$300 mg/dL, proteinuria is moderate, blood pressure tends to be normal or only mildly elevated and edema may be minimal to absent (131). Histologic features of obesity-associated proteinuria in adults include focal segmental sclerotic changes often in a hilar location, mesangial proliferation and hypertrophy, foci of hyalinosis, and glomerulomegaly. In obesity-associated FSGS, on electron microscopy, the foot process fusion is often minimal and focal rather than diffuse, as opposed to idiopathic FSGS (123).

Despite the prevalence of childhood obesity, an association between obesity and renal abnormalities such as proteinuria had not been clearly reported until very recently. In one study seven obese African American adolescent patients had FSGS and significant proteinuria, but no edema, serum albumin levels were slightly low and total cholesterol was normal or mildly elevated. The histologic features included glomerular hypertrophy, FSGS, increased mesangial matrix and cellularity, relative preservation of foot process morphology, and absence of

evidence of inflammatory or immune-mediated processes (132). These clinical and histological characteristics resemble the descriptions of obesity-related FSGS found in the adult studies. In addition, even clinically healthy nondiabetic obese children and young adults have a higher degree of microalbuminuria than normal weight children, indicating early glomerular dysfunction as a consequence of childhood obesity (133, 134).

There is clearly a predisposition for African American children to develop obesity-related FSGS (132). Idiopathic primary FSGS is also much more common in African American children and carries a more severe prognosis compared with other races (135, 136). Epidemiologic data also suggest that there is a racial genetic component to susceptibility to renal injury as a complication of both chronic hypertension (137, 138) and type 2 diabetes (139) in African Americans compared with Caucasians. The pathophysiology of obesity-related FSGS is considered to be multifactorial. Potential etiologic factors seem to be hyperfiltration, hyperlipidemia, renal venous hypertension, and glomerular hypertrophy.

Based on the above clinical and pathological characteristics, the patients with obesity-related FSGS seem to share features with a very diverse group of patients whose initiating factor is a reduction in renal mass. The reduction in renal mass can be due to reflux nephropathy (140), solitary kidney (141), unilateral nephrectomy (142), or the more recently described oligonephropathy of prematurity (143). These reduced numbers of glomeruli are subjected to hemodynamic stress by taking on the filtration of a constant volume of plasma. This sets up a cascade of proteinuria, mesangial cell gene expression, and focal scarring. Similarly, in case of obesity there is increased plasma volume and cardiac output that translates into a relative deficit in the number of glomeruli (144). In severe obesity, renal plasma flow and GFR levels exceeded those of lean controls by 31% and 51%, respectively (145). In addition, obesity is associated with hypertension. Significant weight gain is also accompanied by excess renal sodium resorption, leading to increased systemic arterial pressure and glomerular hyperfiltration (146). All these changes result in glomerular capillary wall stress, leading to glomerular cell proliferation, matrix accumulation and glomerular sclerosis by the following mechanisms. Glomerular hyperperfusion, hyperfiltration, and hypertension lead to stretching of the capillary wall with subsequent injury of endothelial, epithelial, and mesangial cells. A high transforming growth factor-β (TGF-β) concentration in the glomeruli could result from cyclic stretching of the mesangium, as well as from elevated levels of angiotensin II (147).

Hyperlipidemia is another potential contributing factor, since the Zucker obese rat, a model in which FSGS develops, is hyperlipidemic and reduction in serum lipids appears to decrease or prevent development of FSGS (148). A clinical study performing autopsies in obese adults noted that those with FSGS had higher lipid levels and showed lipid deposits in renal tubular epithelial cells (149). In support of the importance of hyperlipidemia in the pathophysiology of obesity-related FSGS, it was recently suggested that low-density lipoprotein apheresis may be useful in maintaining long-term remission in adult (150) as well as pediatric FSGS (151).

Renal venous hypertension has been incriminated as another contributing factor to obesity-related FSGS. One study demonstrated that adult obese patients had increased plasma volume and increased right atrial pressure, which decreased with weight reduction along with parallel decreases in urinary protein losses (128). Other conditions also associated with increased right atrial pressure and presumed renal venous hypertension such as tricuspid atresia, constrictive pericarditis, and pulmonary hypertension have been associated with proteinuria and nephrotic syndrome (152, 153).

The glomerular hypertrophy theory postulated a sequence of changes that may occur in the genesis of obesity related FSGS from glomerular hypertrophy, stimulated in part by angiotensin II and other growth factors, leading to production of excess amounts of extracellular matrix in mesangial areas and the development of obesity related FSGS (154). Enhanced formation of angiotensin II additionally promotes tissue injury via inflammation, oxidative stress, and profibrotic actions (155).

Recent studies showed the connection between adipose tissue and kidney at molecular level mediated by leptin, a small peptide mainly produced in adipose tissue, therefore directly reflecting the amount of body fat. In glomerular endothelial cells, leptin stimulates cellular proliferation, (TGF-β) synthesis, and type IV collagen production. Conversely, in mesangial cells, leptin upregulates synthesis of the TGF-β type II receptor and stimulates glucose transport and type I collagen production through signal transduction pathways involving phosphatidylinositol-3-kinase. These data suggest that leptin triggers a paracrine interaction in which glomerular endothelial cells secrete TGF-β, to which sensitized mesangial cells may respond. Both cell types increase their expression of extracellular matrix in response to leptin. Infusion of leptin into normal rats produces the development of FSGS and proteinuria (155).

There is clear evidence that the treatment of obesity-related FSGS should include weight reduction. This

has been first shown in the 1980s in an animal model of obese Zucker rats, when a reduction in FSGS lesions occurred with reduced dietary intake and subsequent reduced weight (148). Several studies reported that extensive reduction of body weight, without use of any therapy, markedly reduced or eliminated proteinuria in obese adult patients (156–158). In the pediatric study, one patient showed a dramatic reduction in proteinuria in response to weight reduction (132). In general, patients who lost weight successfully showed marked decreases in proteinuria (159). However, unfortunately studies have shown that very few patients actually achieved a sustained weight loss, in which case the prognosis of obesity-related FSGS was poor, with almost one-half of patients developing advanced renal failure (160). This is why it was suggested that for refractory cases of obesity-associated renal disease in adults, when weight loss is not achieved by conventional methods such as diet and exercise, anti-obesity medication or bariatric surgery to be tried. Rimonabant, a new anti-obesity medication, showed beneficial potential effect in treating clusters of metabolic syndrome, which may ultimately suggest potential benefit in treating obesity-related glomerulopathy (161). Bariatric surgery has been evaluated in adults with extreme obesity and at 24 months after surgery, obesity-related renal alterations considerably improved (162). The effects of neither antiobesity medications, nor bariatric surgery have been studied in pediatric patients with obesity-related kidney diseases. The other treatment modality is the use of medications that have negative effects upon the renin-angiotensin system, such as angiotensin converting enzyme (ACE) inhibitors or angiotensin receptor blockers. In an animal model of obese Zucker rats, the use of an ACE inhibitor normalized proteinuria, cholesterol levels, glomerular lesions, and podocyte morphology (163). One study done in obese adults reported that patients with proteinuria who were given captopril had reductions in proteinuria similar to those seen with weight reduction alone (164). It is not clear whether the action of ACE inhibitors in such cases is related to hemodynamic changes in efferent arteriolar resistance or to non-hemodynamic factors. In two pediatric studies, patients with obesity-related FSGS who were given ACE inhibitors had significant reduction of proteinuria (132, 165).

Influences of Malnutrition on Renal Function

Extensive data already showed in the 1960s and 1970s that renal development is influenced by many factors, including nutrition. Maternal diet may influence nephrogenesis, with the result that individuals whose mothers have low protein intake during gestation may have a relatively lower total number of nephrons at birth (166, 167). After birth, the nutritional intake of an infant is a major stimulus to both renal growth and functional maturation. In early infancy, protein and amino acid intake can influence renal growth. GFR increases more rapidly in premature infants receiving a relatively high-protein diet than in those who do not. However, a high protein diet has been reported to be associated with metabolic acidosis, failure to thrive and even evidence of renal injury (168).

Later in life, renal function alterations are similar with diverse forms of malnutrition such as primary protein-calorie malnutrition, iatrogenic malnutrition or anorexia nervosa. These changes include decrease in GFR, decrease in renal plasma flow, poor urinary concentrating ability, decreased ability to excrete sodium and impaired acid excretion. In case of malnutrition, GFR can be as low as 50%. A low calorie diet and also a low protein diet can independently produce a fall in GFR or renal plasma flow (169). These changes are usually reversible, not associated by renal structural damage, and are not explained by hypoproteinemia or edema which can occur in these states (170).

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13 Fluid and Electrolyte Therapy in Children

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Introduction

The prescription of fluid and electrolyte therapy is a common task for the pediatric clinician. The clinical situations requiring such therapy are myriad and range from the urgent in cases of children with circulatory collapse to the more mundane in children with mild dehydration from gastroenteritis. Superimposed on the simple provision of any set amount of water and salts are the changes in normal physiology and homeostatic mechanisms that accompany both acute and chronic illness. Recognition of each child's individual clinical situation and each situation's ultimate goal in respect to hydration or volume resuscitation is crucial for the provision of the correct combination of fluid and electrolytes in the proper amount of time.

In the eighteenth century, attempts to replace the profound enteral losses of salt and water from cholera infection led to an initial understanding of the morbidity and mortality that accompanies significant perturbations in fluid and electrolyte balance (1). The recognition that affected patients improved with repeated infusions of a saline solution became an impetus to define fluid and electrolyte needs in healthy individuals and to develop clinical parameters for fluid and electrolyte therapy. The understanding gained from clinical observations in epidemics of cholera and other diarrheal illness came to be generalized to other conditions in which there was an element of dehydration or poor circulation and ultimately helped to define the threshold for the minimum daily provision of fluid and electrolytes – so-called maintenance requirements – as well as a threshold of maximal tolerance.

By the early twentieth century, clinicians began more frequently to use such techniques as intraperitoneal injection of saline or intravenous infusion of isotonic solutions to try to restore circulation in children with volume compromise (2, 3). Additionally, spearheaded by Gamble and colleagues, fluid spaces in terms of intracellular and extracellular compartments were defined and the kidney's role in the regulation of overall body volume and specific gradients of solute between these fluid spaces were elucidated (4, 5). These early studies established the basis for all modern fluid and electrolyte therapies.

By the middle of the twentieth century, Holliday and colleagues devised simplified equations to link average daily metabolic rate to daily fluid requirements, and the practice of calculating daily fluid and electrolyte needs for an ill child based on continuing or "maintenance" needs and past and current losses or "deficits" was taught as the gold standard to minimize complications and improve clinical outcomes (6). Although the need to make exceptions to this approach in situations with reduced urine output or non-osmotic stimulated antidiuretic hormone activity was clearly stated, emphasis on these empiric equations led to relatively formulaic hydration protocols regardless of specific clinical condition. Moreover, in children requiring rehydration, a tradition of fluid therapy grounded on the so-called "deficit therapy" approach became widespread (7, 8).

Reassessment of this traditional approach, again spawned in large measure by Holliday's work, has come to appreciate that such elaborate maneuvers are often unnecessary and that reassessment of a child's response to any initial fluid therapy is crucial to the assessment of its adequacy and applicability (9). Moreover, increasing recognition that the use of oral rehydration solutions are a simple, safe, and efficacious alternative for most children in need of fluid and electrolyte therapy has also impacted this tradition of precisely calculated intravenous fluid volumes (10).

Nonetheless, there continues to be a place for such careful assessment and prescription of fluid and electrolyte therapy, especially in children with non-diarrheal illness (11). In more complex disorders of fluid and electrolyte pathophysiology, such as seen in critically ill children with sepsis, burns, trauma, or postoperatively, an understanding of the distribution of body fluids, usual fluid and electrolyte requirements, and the effect of disturbances in normal homeostatic balance remain vital to the correct prescription of therapy and the proper assessment of response. Such an understanding resonates

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louder when approaching the care of a child with renal disease. Frequently, the presence of a preexisting renal condition or the development of acute kidney injury complicates the fluid and electrolyte management of the child. Standard approaches to fluid therapy assume that normal renal homeostatic mechanisms will come into play with the provision of adequate water and electrolytes. Such approaches are ill advised for the child with renal disease in whom such regulation may be deranged. Similarly, these approaches may have limited value with the critically ill child in whom normal fluid and electrolyte homeostasis may also be altered. In these circumstances, the clinician needs to approach fluid and electrolyte therapy systematically and with attention to individual clinical circumstance. Otherwise, in the absence of a customized prescription, the possibility arises that a therapeutic intervention may be deleterious, given preexisting reduced tolerance to alterations in body fluid volume, composition, or distribution.

Lastly, with improvements in long-term management strategies, many children with severe chronic disease are living longer lives, often surviving into adulthood. As a result, there is also an increasing population of children and adolescents with conditions such as chronic pulmonary disease, complex congenital cardiac disease, and chronic kidney disease who may require fluid and electrolyte therapy for support through an acute illness or during medical or surgical procedures when usual oral or enteral hydration may be contraindicated. For these children with more fragile clinical states, hydration must also be tailored to individual need and tolerance, and clinicians cannot rely on the formulaic prescription of water and electrolytes. In this regard, these children with significant chronic disease pose a special challenge to a medical community that often wishes to simplify or standardize approaches to care across a spectrum of patients. In addition, they also demonstrate why a well grounded understanding of volume balance and appreciation of both normal and aberrant physiology is critical to the clinician who is regularly prescribing fluid and electrolyte therapy.

Total Body Water

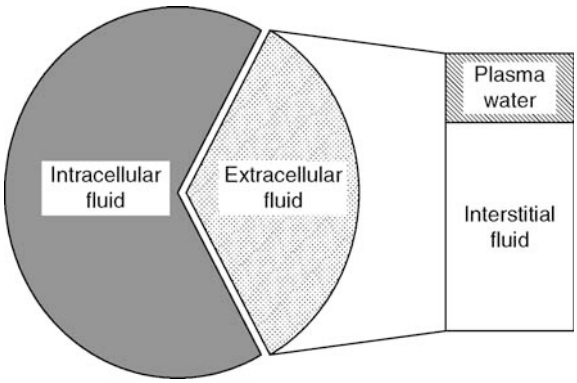
Extracellular and Intracellular Fluid Compartments

Although water makes up a large component of body weight, the exact proportion in each child varies with age, body size, and body composition. Early in gestation, 90% of the weight of the developing fetus may be water. By the first weeks of the third trimester, total body water (TBW) of the developing fetus approximates 80% of body weight in kilograms. This percentage falls to 70–75% in term infants, 65–70% in toddlers and young children, and eventually to 60% in older children and adolescents. Lean individuals have more body water than obese individuals and adolescent boys with increasing muscle mass tend to have more total body water than less muscular adolescent girls. Although 60% is the usual benchmark for estimating TBW in older children and adults, the actual percentage may be lower, especially in less well-conditioned individuals and the elderly (12).

Body water is found in both intracellular and extracellular compartments (*Fig. 13-1*). The intracellular compartment consists of the water within the cells of the body, comprising about two-thirds of TBW or 40% of body weight. The extracellular compartment comprises one-third of TBW or 20% of body weight. The extracellular compartment is divided into the interstitial fluid that bathes all cells and the plasma water that is carried intravascularly. The increased TBW seen in young children is the result of a relatively increased surface area as compared to body weight that accounts for an overall increased extracellular compartment (13).

The boundary between the intracellular and extracellular compartments is the cell membrane. Input or output from the body proceeds via some interface with the extracellular compartment. For instance, intravenous electrolyte solutions are infused into the extracellular intravascular space and their subsequent delivery intracellularly depends

Figure 13-1
Total body water: Fluid compartments and solute composition.



on a host of factors that influence transport across the cell membrane.

Since most cell membranes are readily permeable to water, the distribution of water between the intracellular and extracellular spaces reflects osmotic forces. In each body space there is a solute that is primarily sequestered within that compartment and that maintains its osmotic gradient (14, 15). For instance, activity of the sodium-potassium pump found in cell membranes leads to an increased concentration of potassium intracellularly and an increased concentration of sodium in the interstitial fluid. Thus, sodium serves as the effective osmole interstitially and potassium intracellularly. Similarly, plasma proteins, most notably albumin, exert an osmotic force to maintain water intravascularly. Hydrostatic perfusion pressure counterbalances this osmotic force by pushing water across the capillary from the lumen to the interstitium. Changes in the distribution of effective osmoles can result in redistribution of water between intracellular and extracellular spaces.

A dynamic equilibrium exists between the intracellular and extracellular spaces. Diffusional gradients, osmotic forces, and the activity of cellular transporters all combine to establish the composition differences between body compartments. Since the intracellular space cannot be directly accessed, its composition can be altered only by affecting the extracellular space and its subsequent communication with the cell. Any intake by ingestion or infusion into the extracellular space will result in a new equilibrium being established with the intracellular space as solute and fluid comes to be exchanged. Ultimately, the final equilibrium is a result of complex biochemical, electrical, and physical interactions.

Communication between the cellular spaces can be bidirectional. In other words, there can be exchange from the intracellular space to the extracellular space allowing for transfer or release of cell metabolites. In addition, since the extracellular space can communicate with the external milieu, output from the extracellular space to the external milieu results in effective excretion from the body. No direct communication exists, however, between the intracellular space and the external milieu. Any output from the cells themselves is mediated via the cell's direct ability to interface with the interstitial fluid or the plasma water.

Any impairment in the patient's normal homeostatic mechanisms regulating fluid and electrolyte balance will have a striking impact on the patient's total body water and its extracellular and intracellular constituents. An example of this disruption of normal balance is the development of hypertension frequently seen in individuals with progressive chronic kidney disease. As glomerular filtration rate falls, the ability of the kidney to excrete free water (CH_2O) also declines. Frequently, this change is in the setting of a decreasing number of effectively functioning nephrons with concomitant impairment of overall tubular solute excretion, most notably salt. Superimposed on this baseline tendency for dysregulation of solute and water balance may be clinical factors such as circulatory failure and decreased effective arterial volume leading to further renal salt and water retention. This salt and water overload leads to chronic expansion of TBW and mediates systemic hypertension with expansion of the extracellular volume compartment. Appropriate therapy in this instance would include use of diuretics to reduce the total body burden of salt and water and to restore the total body water to a more physiologic state. In this instance, failure to appreciate the preexisting expansion of the total body water because of chronic salt and water overload could prove deleterious to the patient if management did not include some measure to reduce the salt and water overload. This example also underscores the concept that fluid and electrolyte therapy may involve the removal of solute and water as well as the more usual notion that it is solely concerned with the correction of deficits of electrolytes and volume.

Effective Circulating Volume

Effective circulating volume is a more abstract concept than the division of body water into intracellular and extracellular fluid compartments. As the vascular volume circulates, oxygen and nutrients get delivered to the intracellular space and cellular metabolites get cleared from the intracellular space. The effective circulating volume refers to that portion of the extracellular vascular space that actually perfuses the tissues and accomplishes such an exchange.

Any compromise in this exchange proves deleterious to usual cell homeostasis and, as a result, the body constantly senses and regulates effective perfusion of fluid through the intravascular space. Homeostatic feedback mechanisms include baroreceptors that respond to the stretch of specialized areas of the carotid arteries and the atrium. Hypoperfusion of these areas decreases stimulation of the stretch receptors, triggering the secretion of vasopressin that increases water reabsorption in the most distal nephron and expands the vascular volume.

Similarly, in response to glomerular hypoperfusion, there is not only decreased afferent arteriolar stretch but also decreased glomerular filtration and delivery of sodium to the macula densa. These stimuli both can lead to the secretion of renin from the juxtaglomerular cells of the afferent arteriole. Renin release initiates a cascade resulting ultimately in increased aldosterone-mediated sodium and water reabsorption from the kidney as well as increased angiotensin-mediated vasoconstriction and sodium and water uptake.

Effective circulating volume should be considered the product of multiple factors, not the least of which include the size of the vascular space and the influence of various regulatory hormones. As a component of the extracellular body water, the size of the vascular space often parallels the size of the extracellular space. The size of the vascular space and the adequacy of the effective circulating volume do not, however, always vary coordinately. The extracellular space may be replete or expanded and the actual effective circulating volume decreased. For instance, children with significant liver disease are often edematous, due to sodium retention and expansion of the interstitial component of the extracellular space. The intravascular component of their extracellular space may also be expanded as a result of factors resulting in avid salt and water reabsorption by the kidney. But, because of portal hypertension, splanchnic vessel congestion, and multiple arteriovenous spider angiomas that are seen with this condition, much of the expanded intravascular volume is ineffective – it does not serve to perfuse the tissues and accomplish effective cellular exchange. Thus, these children act as if they are volume depleted: they avidly reabsorb any filtered sodium and excrete small volumes of urine and they vigorously continue to expand their already over expanded extracellular space by reabsorbing even more salt and water in response to the effects of renin and ADH. Similarly, this paradoxical state of sodium avidity and ADH-mediated water reabsorption characterizes children with nephrotic syndrome or with cardiac failure despite their preexisting expansion of the extracellular space.

In managing all aspects of a patient's fluid and electrolyte therapy, the clinician must accurately assess both the patient's current extracellular volume status and effective circulating volume and reconcile these with potential causes of volume loss. At all times, it is crucial to maintain an effective circulating volume and to make therapeutic decisions based on the unique clinical circumstances facing the patient. Such management may require rather disparate therapeutic interventions. For instance, expansion of the extracellular volume with vigorous rehydration may be called for in a child with poor perfusion secondary to gastroenteritis-induced dehydration whereas another child with equally poor perfusion due to cardiodynamic compromise may be intravascularly replete and require the initiation of pressor therapy and another child with edema from relapsed nephrotic syndrome may actually require fluid and salt restriction. These examples underscore that loss of effective circulating volume generally arises as a result of one or more broad perturbations in the extracellular fluid compartment that impacts effective perfusion (*Table 13-1*). In hospitalized children where there may be both aberrant disease related physiology and iatrogenic derangements of regulatory response, the causes of effective volume perturbations may be even more complex.

Clinical signs and symptoms of effective circulating volume loss may be subtle. At times, there may be preservation of effective circulating volume in the face of an overall depleted extracellular fluid compartment. Failure to initiate appropriate fluid and electrolyte therapy in such a circumstance may result in eventual compromise of the effective circulating volume. Important initial clinical signs to assess in any patient being evaluated for fluid therapy include pulse rate and capillary refill. Tachycardia and sluggish refill generally precede more obvious signs of ineffective circulation such as hypotension and oliguria. Clinical symptoms may also be non-specific and include fatigue and lethargy that are often attributed to an underlying illness rather than volume depletion. Proper restoration of effective circulating volume or extracellular fluid compartment depletion requires an understanding of baseline fluid and electrolyte needs as well as consideration of any extenuating clinical circumstances unique to the patient in question.

Table 13-1

Alterations in effective circulating volume

Cause	Mechanism
Contracted extracellular fluid space	Water or sodium chloride deficit
Massive vasodilatation	Loss of vascular tone sustaining perfusion pressure
Loss of intravascular osmotic pressure	Osmotic fluid losses into interstitium
Overfill of the intravascular space	Hydrostatic fluid losses into interstitium
Hemorrhage	Direct loss of blood and plasma water

Water and Electrolyte Requirements

Maintenance Therapy

The concept of maintenance therapy refers to that amount of water and electrolytes required to replace usual daily losses and to maintain an overall net balance of no water or electrolytes gained or lost. Such needs are a function of homeostatic and environmental factors and vary from day to day and from child to child. In the average child with adequate access to water and food, these maintenance needs are generally readily met (6, 16). In the ill or hospitalized child who requires therapeutic intervention and in whom there may be ongoing aberrant physiology, these needs must be considered when clinicians prescribe fluid therapy.

To assist in estimating maintenance needs, fluid and electrolyte requirements are typically calculated based on weight or surface area, but individual clinical circumstance must be considered when making such calculations (17). For instance, the 20-kg child who is well will require a far different "maintenance" quantity of fluid and electrolytes than the 20 kg child who is tachypneic and febrile or the 20 kg child who is anuric and on a ventilator in the pediatric intensive care unit. Careful clinical assessment of the patient's volume status and close attention to the balance of overall daily input and output will prove more useful at arriving at a correct estimate of daily fluid and electrolyte needs than merely using mathematical equations without clinical correlation. With these caveats in mind, it is nonetheless a common clinical practice to make certain empirical assumptions regarding daily needs for water and the major electrolytes.

Historically, daily maintenance water needs have been estimated based on energy expenditure (*Table 13-2*) (6, 16). For each kilocalorie of energy expended daily, 1 mL of water must be provided. Based on the computed energy expenditure of the average hospitalized patient, for the first 10-kg of body weight, 100 mL of water per kg is provided daily. For the next 10 kg of body weight, 50 mL of water per kg is provided daily, and for every kg of body weight in excess of 20 kg, 20 mL of water per kg is provided daily. In addition, in the process of oxidation of carbohydrate and fat, approximately 15 mL of water is generated for every 100 kcal of energy produced. This water of oxidation contributes significantly to overall water balance.

Maintenance water losses occur from urine output and from insensible sources that are almost exclusively evaporative and respiratory losses. In the child with average metabolic demands, for every 100 kcal of energy expended, 100 mL of water must be ingested. Oxidative metabolism generates 15 mL of water in the course of producing the 100 kcal of energy. Of this 115 mL of water, 40 mL is lost insensibly and 75 mL is lost as urine output. Overall, net water balance, composed of 100 mL ingested, 15 mL generated and 115 mL excreted, becomes equilibrated.

Table 13-2
Relationship of body weight to metabolic and maintenance fluid needs

Body Weight (kg)	Metabolic needs[a] (kcal/day)	Maintenance fluid needs[b]	
		mL/day	mL/h
5	500	500	20
10	1,000	1,000	40
15	1,250	1,250	50
20	1,500	1,500	60
30	1,700	1,700	70
40	1,900	1,900	80
50	2,100	2,100	90
60	2,300	2,300	95
70	2,500	2,500	105

[a]Based on 100 kcal/kg for first 10 kg of body weight + 50 cal/kg for next 10 kg of body weight + 20 cal/kg for next 50 kg of body weight
[b]Based on need of 1 mL of water to metabolize 1 kcal of energy
As described in (6)

Clinical factors can have a striking impact on insensible water losses (*Table 13-3*). Fever increases insensible losses by more than 10% per degree Celsius. Premature infants with relatively increased surface areas for size can have insensible losses two to threefold higher than baseline, especially if they are on open warmers or under phototherapy. On the other hand, children on ventilators providing humidified oxygen may have half the insensible losses of a non-ventilated child.

Similarly, urinary water output can vary tremendously. A child with a renal concentrating defect or ADH unresponsiveness may have urinary water losses of several liters per day, whereas an oligoanuric child will have no appreciable urinary water losses. In any child with normal renal function, even in the setting of maximal ADH stimulation, there is a minimal volume of urinary water obligatory to excrete the osmotic load ingested by the diet and generated by basal metabolism. As a result, even the child concentrating urine to 1,200–1,400 mOsm/L will lose nearly 25 mL of urinary water per 100 kcal of energy expended (6).

Table 13-3

Factors affecting insensible water losses

Increased losses	% Change	Decreased losses	% Change
Prematurity	100–300	Enclosed incubator	25–50
Radiant warmer	50–100	Humidified air	15–30
Phototherapy	25–50	Sedation	5–25
Hyperventilation	20–30	Decreased activity	5–25
Increased activity	5–25	Hypothermia	5–15
Hyperthermia	12%/°C		

Recent reports contend that the relationships between energy expenditure and water requirements demonstrated by hospitalized children at bed rest do not apply to anesthetized or critically ill children (18). For instance, in infants and children studied during general anesthesia, energy expenditure was half that of awake children at bedrest. On the other hand, water needs for cell metabolism was increased over baseline by about 60%, leaving the overall relationship between water needs and caloric expenditure similar in both situations. In some critically ill children, maintenance volumes may need to be reduced by 40–50% to prevent positive water balance (19).

Serum Osmolality

Water homeostasis maintains a stable serum osmolality. Serum concentrations of sodium, glucose, and urea nitrogen determine serum osmolality (20). Serum osmolality is estimated by the equation: (2 × serum Na) + (serum glucose/18) + (BUN/2.8), where the serum sodium is measured in mEq/L and the glucose and BUN in mg/dL. In the majority of children with no functional renal impairment and normal glucose metabolism, the contributions of BUN and glucose to the effective osmolality are small and the serum osmolality can be estimated by doubling the serum sodium concentration (21, 22). Thus, most children have a serum osmolality between 270 and 290 mOsm/L, corresponding to serum sodium values of 135–145 mEq/L.

Chemoreceptors in the hypothalamus constantly sense serum osmolality and respond to even small variations towards either limit of normal by adjusting ADH release from the posterior pituitary. Changes in osmolality in the setting of hypovolemia augment ADH release further. ADH effect on water permeability of the collecting tubule is a principal influence on the regulation of water balance.

Alterations in water intake or excretion result in the development of hypo- or hyperosmolality as the usual ratio of extracellular solute to water is perturbed. Since sodium is the largest component of extracellular osmolality, its concentration can be influenced profoundly by changes in water metabolism. An understanding of this link between water regulation and serum sodium values is crucial when prescribing fluid and electrolyte therapy. Most importantly, the clinician must recognize that hypo- or hypernatremia is usually a manifestation of impaired water regulation and that therapy must address regulation of water balance rather than alterations in body sodium stores.

Water Homeostasis in Acutely Ill Children or During the Perioperative Period

In ill children, there are multiple causes of both physiologic and aberrant vasopressin effect as listed in *Table 13-4* (23). As a result, if these children receive hypotonic intravenous fluids for prolonged periods of time or in volumes exceeding those generally recommended, there is the risk of acute hyponatremia. After volume resuscitation with isotonic fluids, most hospitalized children have traditionally been provided hypotonic fluids for their maintenance therapy. Given the tendency for ill children to have vasopressin effect independent of the usual osmotic and volume related stimuli, over the last decade some have suggested that isotonic fluids may be safer alternatives and should be continued as the source of maintenance fluid even after acute volume repletion (24).

Similarly, some have called for using isotonic saline as the intravenous fluid of choice whenever a maintenance infusion is needed in setting like the perioperative period when oral hydration has been held and when high ADH levels may come to be expected because of pain or anxiety. In these instances, children could receive intravascular volume expansion with an initial infusion of 20–40 mL/kg over a period of a few hours and then continued on isotonic saline as dictated by clinical circumstance, rather than the transition to fluids with more free water content.

Table 13-4
Common causes of vasopressin effect in hospitalized children

Category	Specific etiology
Physiologic	Hyperosmolar state, hypovolemia
Pulmonary	Pneumonitis, pneumothorax, asthma, bronchiolitis, cystic fibrosis
Drug effect	Narcotics, barbiturates, carbamazepine, vincristine, cyclophosphamide
Metabolic	Hypothyroidism, hypoadrenalism, porphyria
CNS	Infection (meningitis or encephalitis), tumor, trauma, hypoxia, shunt malfunction, nausea, pain, anxiety

Proponents of this routine believe it would decrease the overall numbers of hospitalized children who develop hyponatremia and prevent hyponatremia-related central nervous system damage (25).

Others have claimed that long term maintenance infusions of isotonic saline to all ill or perioperative patients may result in sodium loading in children who do not have triggers for water retention (8, 26). Moreover, hypernatremia has been described in some children receiving less sodium than that provided in these maintenance isotonic infusions (27). These cases of hypernatremia are often a result of an underlying renal concentrating defect, related to significant free water loss from sources other than urine, or resulting from aggressive fluid restriction (28). Children with certain renal or cardiopulmonary problems may be especially sensitive to such sodium loads and may more readily develop unintended sequelae of such sodium provision (29).

Several studies have shown that children with acute illness requiring emergency department evaluation or hospitalization do seem to be at risk for hyponatremia. In one study of 103 children admitted to a German pediatric hospital, nearly 80% had elevated serum ADH levels and increased plasma renin activity, independent of the underlying illness. As expected with ADH release, plasma osmolality was reduced significantly in comparison to a group of well children (23). In another report from a Canadian pediatric center, fewer than 5% of children were hyponatremic at presentation to the emergency department, but nearly 10% became hyponatremic after hospital admission, most as a consequence of excess free water provision by aggressive intravenous hydration with hypotonic fluid. Hospitalized children who became hyponatremic received on average three-times the volume of electrolyte free water than their hospitalized colleagues who remained eunatremic and were also three-times more likely to receive fluids at a rate that exceeded recommended maintenance rates (30).

A retrospective analysis of postoperative admissions to a pediatric ICU found that 11% of children manifested hyponatremia (serum sodium <130 mEq/L) during their ICU stay (31). Of those children with hyponatremia, although there was a trend towards an increased incidence in children receiving hypotonic fluids in comparison to children given isotonic solutions, there was no statistical difference demonstrated in the incidence of what the authors termed either moderate (<130 mEq/L) or severe (<125 mEq/L) hyponatremia. No children developed any neurologic sequelae or other morbidity. Children did have serum electrolytes assayed four times daily, however, so this vigilance likely resulted in clinical interventions to prevent exacerbating hyponatremia.

A prospective randomized study of 102 children with gastroenteritis at an Australian children's hospital demonstrated that the risk of developing hyponatremia decreased with provision of isotonic saline (32). Eunatremic children at presentation were most protected from subsequent falls in serum sodium when provided isotonic (0.9% NaCl) versus hypotonic (0.45% NaCl) intravenous solutions. Urinary biochemistry demonstrated that in the face of isotonic fluid provision hyponatremic children were more likely to retain sodium and bring their serum sodium values closer to normal, whereas children with normal serum sodium values already at presentation were able to excrete excess sodium appropriately and prevent hypernatremia.

A meta-analysis of six studies comparing hypotonic and isotonic maintenance intravenous solutions in children suggested that no single fluid composition or rate is ideal for all children but that an isotonic or nearly isotonic solution may prove less likely to cause symptomatic hyponatremia in acute illness and the perioperative period (33). Despite this growing body of literature to suggest that isotonic fluids may be preferable in many situations where non-osmotic ADH effect can be expected, children are still commonly prescribed hypotonic fluid therapy. A recent survey of anesthesiologists in the United Kingdom found that 60% were still prescribing hypotonic fluids for children intraoperatively, with 75% prescribing such fluids in the postoperative period (34).

Maintenance Electrolyte Therapy

Estimates for the maintenance requirements of the major electrolytes sodium, potassium, and chloride can also be

made based on metabolic demands and daily water needs (6). For sodium and chloride, approximately 2–3 mEq/100 mL of daily water requirement is needed with 1–2 mEq of potassium for each 100 mL of daily water need. Again, these estimates require adjustment based on clinical circumstance but the daily intake of most healthy individuals contains more than adequate electrolytes for maintenance needs. Although at times there can be significant electrolyte losses through the skin or the gastrointestinal tract, most electrolyte losses are urinary (17, 35). In the setting of anuric renal failure and no concomitant electrolyte losses from other sources, a much lower level of maintenance electrolyte supplementation is needed and patients may maintain adequate electrolyte balance with water supplementation alone to provide insensible fluid losses.

As with provision of water, in children who require electrolyte therapy their electrolyte prescription must be tailored to their individual needs. For the provision of sodium and chloride, this requires careful assessment of the extracellular fluid space, especially the effective circulating volume. Providing too little sodium chloride results in volume contraction and circulatory compromise; providing too much causes volume overload and sequelae such as hypertension and edema.

Similarly, inappropriate potassium supplementation may have significant clinical ramifications. In children with diminished renal function or who are at risk of hyperkalemia for other reasons, it is usually appropriate to forego any maintenance potassium supplementation. When supplementation is given acutely, it is important to monitor serum potassium values closely. When chronic potassium supplementation is needed, there should continue to be periodic assessments. A course of supplemental potassium administered orally is safer than a bolus intravenous injection; intravenous potassium supplements rarely need to exceed 0.5 mEq/kg/h (20).

Perturbations in Fluid and Electrolyte Homeostasis

Alterations in Water Balance, Serum Sodium, and Cell Volume

As discussed above, the regulation of osmolality is achieved by alterations in water intake and excretion. Serum sodium levels often vary with these alterations in water balance. Generally, serum sodium is kept regulated at levels between 135 and 145 mEq/L. A serum sodium value below 130 mEq/L or above 150 mEq/L is out of the range of normal homeostasis and most often indicates a problem with water balance.

Since sodium is the major extracellular osmole, alterations in serum sodium can result in water flux between the intracellular and extracellular spaces. Because significant water movement into or out of cells could prove deleterious to cell function, cell volume is closely regulated to minimize such shifts (36). For instance, with hyponatremia there is decreased effective osmolality in the extracellular space. As a result, water can shift from the plasma into the intracellular space and cell swelling will occur. To counterbalance such swelling, the cell acutely regulates its volume by transporting electrolytes, especially potassium, from the intracellular space into the extracellular space, thereby decreasing the osmotic gradient for water transfer. Over several days, when faced with chronic hyponatremia or chronic hypoosmolality, the cells will also achieve effective volume regulation by losing organic osmolytes such as taurine and inositol, thereby further diminishing the osmotic gradient for water transfer into the cell (37).

With hypernatremia, the osmotic gradient favors water movement out of the cells and into the extracellular space and, without protective mechanisms, cells will shrink. Again, there are both acute and chronic mechanisms minimizing these changes in cell volume. Acutely, there is transport of electrolytes intracellularly, whereas with time there is stimulation of the production of organic idiogenic osmoles (37, 38). Together, these act to blunt the loss of intracellular volume that would otherwise occur.

Changes in serum sodium values evolving slowly and reflecting chronic processes tend to be better tolerated clinically than acute alterations. Such slow changes allow for maximal counter-regulation and fewer clinical sequelae. On the other hand, profound sudden alterations – serum sodium values falling below 120 mEq/L or above160 mEq/L – are often accompanied by dramatic neurologic complications directly related to the acute changes in cell volume in the central nervous system.

These regulatory mechanisms must also be kept in mind when formulating specific therapeutic intervention. Acute perturbations can be corrected more rapidly than chronic conditions because the full gamut of responses to the imbalance has yet to come into play.

Hyponatremia: Initial Approach

Hyponatremia is usually defined as a serum sodium value less than 130 mEq/L. Depressed serum sodium values are likely to be the result of persistent ADH effect and a

relative surfeit of water for solute in the extracellular space; hyponatremia uncommonly arises secondary to depleted salt stores alone.

Infrequently, pseudohyponatremia may be seen. Pseudohyponatremia is not a depletion of sodium stores but a change in the usual make-up of the extracellular space such that the relative concentration of sodium is now depressed because of the pathologic elevation of another solute. Common etiologies of pseudohyponatremia include hypergylcemia, hyperlipidemia, and hyperproteinemia. In these clinical conditions, serum sodium values tend to be only modestly depressed. Since there is no underlying true anomaly in sodium or water stores in these conditions, the serum sodium need not be addressed with any therapeutic maneuvers. With the introduction of ion sensitive electrodes for the measurement of plasma sodium concentration, pseudohyponatremia related to the presence of confounding factors in the laboratory assay is much less commonly encountered.

To clarify the etiology of the hyponatremia, the clinician should assess the patient's extracellular volume status and determine if it is decreased, normal, or increased. Then, by measuring urine sodium excretion and determining the renal response to the hyponatremia, it becomes easier to determine if the patient should receive sodium and water or if sodium and water restriction is the appropriate therapy (*Table 13-5*).

Table 13-5
Etiology of hyponatremia

Circulating volume	Urinary Na (mEq/L)	
	≤ 20	≥ 20
Decreased	Burns	Adrenal insufficiency
	Cystic fibrosis	Diuretics –early
	Diuretics – late	Salt wasting
	Gastroenteritis	
Normal or Increased	Cardiac failure	Renal failure
	Hepatic cirrhosis	SIADH
	Nephrotic syndrome	Water intoxication

Hyponatremia: Decreased Volume Status and Urine Na ≤ 20 mEq/L

Decreased circulating volume is usually seen with states of significant sodium loss. The most common site of this loss is the gastrointestinal tract as a result of vomiting, diarrhea, or tube drainage. The renal response to the decreased effective circulating volume involves increased activity of the renin-angiotensin axis and relatively high levels of aldosterone and angiotensin. As a result, urine sodium values are generally low (<20 mEq/L) and water reabsorption in the distal nephron is facilitated by high levels of ADH. In the face of continuing sodium losses exceeding intake, this state of vigorous ADH effect leads to a relative excess of water and concomitant hyponatremia.

A similar clinical state of hyponatremia can also be seen in cystic fibrosis where there are increased skin losses of sodium and chloride, with bleeding, with burns, and with certain losses of fluid from the intravascular space of the extracellular fluid into the interstitial space ("third-spacing") as can occur post-operatively, in conditions of vascular leak such as sepsis, or with peritonitis. Such hyponatremia can also be seen following a period of diuretic therapy. In response to chronic diuretic-mediated volume contraction, the mechanisms outlined above come into play. Thiazide diuretics, especially in combination with a loop diuretic such as furosemide, are particularly prone to inducing such hyponatremia.

The appropriate therapeutic response to these conditions is the provision of sodium and water either by use of intravenous saline solutions or oral electrolyte solutions. This results in restoration of sodium balance and volume expansion.

Hyponatremia: Decreased Volume Status and Urine Na ≥ 20 mEq/L

Decreased circulating volume with a random urinary sodium excretion >20 mEq/L is indicative of renal salt wasting, either from an intrinsic tubulopathy or from early diuretic effect. Less commonly, adrenal insufficiency can cause sodium wasting from the cells of the distal nephron. Such a deficiency can arise from an intrinsic endocrine defect such as congenital adrenal hyperplasia related to 21-hydroxylase deficiency, from some secondary impairment of adrenal function caused by infection, bleeding, or malignancy, or from pharmacologic adrenal suppression without adequate replacement therapy. In the setting of adrenal insufficiency, provision of appropriate adrenal hormone replacement as well as adequate sodium and water proves therapeutic. With renal salt wasting, supplementation with sodium and any other electrolytes exhibiting impaired renal reabsorption is useful.

Hyponatremia: Normal or Expanded Volume and Urine Na ≤ 20 mEq/L

Normal or increased circulating volume and random urine sodium excretion <20 mEq/L can be seen in conditions where there is an excess of both total body water and total body sodium. The three major disorders that cause this type of hyponatremia are the nephrotic syndrome, hepatic failure related to cirrhosis, and cardiac failure. In all these conditions, there is a state of sodium and water avidity related to high levels of ADH and aldosterone. Most commonly, this is in the setting of preexisting total body sodium overload as evidenced by edema. In all of these conditions, despite the increased extracellular or circulating volume, the effective circulating volume is often depressed. As a result of this ineffective perfusion of the tissues, sodium and water avidity is only heightened by stimulation of the renin-aldosterone-angiotensin axis, further exacerbating the total body excess of salt and water. Appropriate therapy includes striking a balance between interventions promoting the maintenance of effective circulating volume and restricting the provision of excess water and sodium which will only contribute to further total body water and sodium overload.

Hyponatremia: Normal or Expanded Volume and Urine Na ≥ 20 mEq/L

Hyponatremia in the setting of normal or increased effective circulating volume is always related to persistent ADH effect (15). If the random urine sodium value is >20 mEq/L, the most common clinical scenario is the syndrome of inappropriate antidiuretic hormone secretion or SIADH. SIADH can arise from disparate clinical conditions including the postoperative child, the child with significant pain, or the child with pulmonary disease. As described earlier, ill children are at risk for both inappropriate ADH secretion and inappropriate ADH effect (39). In SIADH, despite a state of hypo-osmolality, the urine is inappropriately concentrated as a result of an inability to suppress ADH secretion and block ADH-mediated water reabsorption from the distal nephron. Appropriate therapy for SIADH includes restricting water intake and attending to any underlying clinical factors predisposing to this syndrome. Provision of fluids containing higher concentrations of sodium will not necessarily increase serum sodium without attention to concomitant water restriction.

Normal or increased extracellular volume and high urine sodium concentration can also be seen in the setting of renal failure as both glomerular filtration and water clearance falls while the fractional excretion of sodium rises. A more unusual cause of this form of hyponatremia is polydipsia, usually psychogenic in nature. Such water intoxication is rare in children but can occasionally be seen with emotional or psychiatric illness in older children or with infants inappropriately provided large volumes of water or very hypotonic fluid in a repetitive fashion by a caretaker. In both these circumstances, restriction of the volume of free water ingested on a daily basis may be beneficial.

Hypernatremia

Hypernatremia is defined as a serum sodium value greater than 150 mEq/L. Generally, even higher serum sodium values can be tolerated with the most significant clinical and neurologic effects not occurring until sodium values exceed 160 mEq/L. As with hyponatremia, it has long been recognized that hypernatremia is more commonly a reflection of a problem with water balance rather than a sodium imbalance (40). In most instances, the patient has a relative deficiency of water for normal extracellular solute content.

Since sodium is the major determinant of plasma osmolality, as serum sodium levels rise, serum osmolality concomitantly increases. Increases in serum osmolality are sensed by hypothalamic osmoreceptors, triggering ADH release from the posterior pituitary as serum osmolality increases over 280 mOsm (41). Increased serum osmolality also causes a sensation of thirst. Thus, in the normal state, as serum osmolality increases, there is increased fluid intake mediated by thirst in the setting of high ADH levels. This results in increased reabsorption of free water from the cortical collecting duct and re-equilibration of serum sodium levels and serum osmolality before clinically significant hypernatremia or hyperosmolality occurs.

Outside infancy, hypernatremia as a result of sodium excess or salt poisoning is infrequent. Its major cause is improper preparation of powdered or liquid concentrated formula resulting in a hypertonic, hypernatremic solution. Since infants do not have free access to water, they cannot respond to their increasing sense of thirst as they develop such hypernatremia. As caregivers continue to provide the same incorrectly prepared formula for further feedings, there is further salt loading. In addition, young

infants are unable to excrete sodium loads as efficiently as older children and this limits the intrinsic renal response. Iatrogenic sodium loading can also be seen in children who receive large doses of sodium bicarbonate because of persistent acidosis, during a resuscitation, or in children who have been given inappropriate amounts of sodium in peripheral nutrition. Iatrogenic sodium loading can also be seen in the child who has received repeated large volumes of blood products, generally isotonic or sodium-rich solutions.

Children who have hypernatremia from sodium excess should exhibit the physical signs and symptoms of an expanded extracellular space. They frequently have peripheral edema and may have hypertension or symptoms of pulmonary edema. These children can respond to therapy aimed at augmenting sodium elimination. The use of diuretics and the provision of adequate free water decrease the total body sodium burden. Rarely, dialysis may be necessary when the hypernatremia must be corrected rapidly (42, 43).

Hypernatremia as a result of salt loading is rare and the pediatric clinician is much more likely to see hypernatremia stemming from a free water deficit or a combined water and sodium deficit where the water losses exceed the sodium losses. Hypernatremia secondary to a water deficit arises in the setting of inadequate access to water or some impairment in ADH release or response. It is uncommon to see hypernatremia secondary to poor water intake except in infants or young children who cannot get water for themselves in response to their sense of thirst (44). As for ADH-related anomalies, there are many causes of central or nephrogenic diabetes insipidus (45). Again, given normal access to water, it is rare for the older child to develop hypernatremia even with an impairment in the ADH axis because of the strong drive to drink in response to thirst (46). In very young children with diabetes insipidus, however, the issue of access to water arises and hypernatremia may be a concern. Such hyponatremia can also be seen in the postoperative state in children with concentrating defects who are not allowed to drink by mouth and who are receiving a prescribed volume of fluid based on a presumed ability to concentrate urine and conserve water.

The most common etiology of hypernatremia in children is the loss of hypotonic fluid, that is fluid with a relative excess of water for its sodium content. In these situations, the total body water is decreased more than the total body sodium. The usual clinical scenario leading to such a condition is viral diarrheal illness in the setting of poor water intake or persistent vomiting. In this condition, there is loss of stool with a sodium content generally <60 mEq/L. These children tend to excrete small volumes of concentrated urine with urine sodium content <20 mEq/L, underscoring the fact that they are conserving both water and sodium. Their hypernatremia is not a manifestation of a total body excess of sodium but a depletion of sodium that is overshadowed by a larger relative depletion of body water. Therapy is aimed at restoring water and sodium balance by providing back the hypotonic fluid which was initially lost either by the use of intravenous saline solutions or with oral electrolyte therapy.

Fluid Replacement Therapy

Most commonly, the prime goal of fluid replacement therapy is restoration of an adequate effective circulating volume. In its absence, significant metabolic derangements can occur that then exacerbate perturbations in fluid and electrolyte homeostasis. The volume of fluid replacement required varies with the extent and etiology of the compromised circulation. In many children with acute illness, there may be an element of decreased effective volume that is mild and difficult to appreciate by clinical examination. Expansion of extracellular volume with infusion or ingestion of 20–40 mL/kg of fluid over a few hours often results in better perfusion and improved clinical appearance from presentation. Urine output tends to remain normal in these situations speaking against persistent non-osmotic ADH activity that would encourage volume overload (47).

Children with mild dehydration, manifesting with <5% weight loss, will usually respond to 30–50 mL/kg of fluid. In the setting of more significant compromise of effective volume such as with loss of vascular tone in sepsis or a systemic inflammatory response, more than 200 mL/kg may be needed to achieve hemodynamic stability and effective perfusion. In most situations other than outright shock, the clinician can approach fluid resuscitation with either intravenous or oral rehydration therapy.

Assessment of Volume Depletion

In estimating the severity of dehydration, a change in weight from baseline is the most objective measure (48). As rehydration proceeds, following weights on a serial basis becomes an important adjunct in assessing the efficacy of fluid repletion. If no baseline weight is known, the clinican

will use various parameters based on history and physical examination to judge the severity of dehydration (*Table 13-6*). Children with mild dehydration will have minimal clinical signs and only a modest decline in urine output. As dehydration becomes more significant, more classical findings such as dry mucous membranes, tenting skin, sunken eyes, and lethargy become prominent. With profound dehydration, there is anuria, marked alterations in consciousness, and hemodynamic instability.

A capillary refill time greater than 2 s has long been touted as a useful physical finding pointing towards effective volume depletion (49). Unfortunately, delayed capillary refill is neither a sensitive nor specific marker of dehydration (50, 51). It may be most useful if normal, as this does seem to exclude reliably severe dehydration. In a prospective cohort study of dehydrated Egyptian children between 3 and 18 months of age, the best correlation between clinical assessment of degree of dehydration and actual volume depletion came in children who had clinical parameters of significant dehydration such as prolonged skinfold tenting, a dry mouth, sunken eyes, and altered sensorium (52). Similarly, in a review of pre-school children with dehydration, the best clinical indicators of volume depletion – decreased skin turgor, poor peripheral perfusion, and Kussmaul breathing – accompanied more significant dehydration, underscoring the difficulty with which mild degrees of dehydration may be estimated by the clinician without access to prior weights (53).

A study of 97 American children given intravenous fluids in an emergency department for rehydration underscored the difficulty in assessing accurately even severe dehydration by standard clinical estimates (54). Physicians' initial estimate of dehydration compared to the actual percent loss of body weight varied dramatically, with a sensitivity of 70% for severe dehydration (>10% loss) but only 33% for moderate dehydration (6–10% loss). This study suggested that adding a serum bicarbonate level to the assessment may be useful, increasing the sensitivity of the clinical scales to 100% in severe dehydration and 90% in moderate dehydration if standard clinical features and a serum bicarbonate <17 mEq/L were found.

Other studies have found that laboratory studies by themselves are poor indicators of the degree of dehydration. In 40 children receiving intravenous fluids for dehydration, pre-hydration assessment of serum BUN, creatinine, uric acid, anion gap, venous pH, and venous base deficit were made as well as assessment of urinary specific gravity, urinary anion gap, and fractional excretion of sodium. Only the serum BUN/Cr ratio and serum uric acid significantly correlated with increasing levels of dehydration, but both lacked sensitivity or specificity for detecting more than 5% dehydration (55). Similarly, in a retrospective review of 168 dehydrated children, elevated serum urea levels and depressed serum bicarbonate levels were found to be useful adjuncts to clinical evaluation in accurately assessing the degree of dehydration but were not by themselves predictive (56).

In fact, with viral gastroenteritis, the most common cause of dehydration in children, there rarely is a significant laboratory anomaly despite clinically detected

Table 13-6

Clinical assessment of dehydration

	Degree of dehydration		
	Mild	Moderate	Severe
Vital signs			
Pulse	Normal	Rapid	Rapid and weak
Blood pressure	Normal	Normal to slightly low	Shock
Weight loss			
Infant	<5%	10%	>15%
Older child	<3%	6%	>9%
Mucous membranes	Tacky	Dry	Parched
Skin turgor	Slightly decreased	Decreased	Tenting
Eye appearance	Normal tearing	Decreased tearing ± sunken	No tears + very sunken
Capillary refill	Normal	Delayed (>3 s)	Very delayed (>5 s)
Urine output	Decreased	Minimal	Anuric

volume depletion, as underscored by a cohort of children from the United Kingdom admitted for rehydration due to viral gastroenteritis in whom only 1% of admitted children had an electrolyte derangement (57–59).

In children with volume depletion accompanying trauma, sepsis, surgery, or underlying renal dysfunction, it would be more likely to find perturbations in electrolyte and acid-base status. Thus, in the absence of a straightforward case of mild to moderate diarrheal dehydration, it is general consensus that blood should be obtained for assessment of electrolytes, bicarbonate, and renal function to help guide specific fluid and electrolyte therapy (60, 61)

As the child is volume resuscitated, it is important to reassess the child's clinical status. Initial estimates of degree of dehydration may need to be adjusted if the child is not showing progressive improvement. Most clinicians follow parameters such as general appearance and sensorium, change in weight from initiation of rehydration, and urine output and urine osmolality. In children with some types of renal dysfunction, there may often be an underlying chronic urinary concentrating defect. In these children, relatively dilute urine flow may be maintained even in the face of clinical dehydration and markers other than urine output and osmolality should be followed.

Oral Rehydration Therapy

Although oral rehydration with electrolyte solutions is a safe, efficacious, and convenient way to treat mild to severe volume depletion, parenteral fluid and electrolyte therapy has been the mainstay of medical treatment for most children presenting with fluid and electrolyte imbalances (62–65). Especially underutilized in North America, oral therapy has proved successful in clinical settings worldwide in resuscitating children of all ages with profound fluid and electrolyte anomalies, and short of significant circulatory compromise, can be used as first line therapy in all fluid and electrolyte aberrations (66, 67). An example of a situation calling for oral rehydration in the following clinical scenario:

A healthy 4-year old girl presents to her pediatrician's office following 3 days of a febrile illness. For most of this time, her appetite has been severely depressed and her parents estimate that she has only had a few cups of fluid in the last 12 h. Yesterday, she vomited once and today has had an additional episode of emesis. She has continued to urinate, although less frequently and with smaller volumes. On physical examination, the girl looks unhappy but non-toxic and alert. Initially, her pulse is 120 but, after acclimation to the examination, it has decreased to 100 beats per min. Her sitting blood pressure is 80/50 and she will not cooperate with attempts at orthostatic vital signs. Her mucous membranes are somewhat dry and her weight today is 15 kg, exactly the same as her weight at a well child examination 6 months previously. The parents are concerned that she is becoming dehydrated.

Primary care and emergency department physicians face such clinical scenarios regularly. An otherwise healthy child with an apparent viral illness causing mild to moderate dehydration will frequently be treated by intravenous therapy with the contention that oral rehydration will rarely succeed, will be too labor intensive, or will take too much time. In fact, such children are excellent candidates for oral rehydration. In most developed countries where a viral disease is thought to be the etiology of the dehydration and there is little concern about a cholera-like enteritis, solutions with sodium contents from 30 mEq/L to 90 mEq/L have been shown for years to be efficacious for oral rehydration (68–71).

With this girl, given the history and physical examination, it is unlikely that any clinically significant electrolyte perturbations will be found, so there is little indication for assaying electrolytes or renal function prior to starting oral rehydration (57–59). The family is given a commercially available oral rehydration solution containing 75 mEq/L Na, 20 mEq/L K, 30 mEq/L citrate, and 2.5% glucose. They are asked to provide 1 L of fluid (50 mL/kg) to the child over the next 4 h. The child should be offered small aliquots of fluid very often – 5 mL every 1–2 min at initiation. If this regimen is tolerated with no vomiting, the aliquots may be gradually increased in volume and the frequency reduced, aiming to deliver at least the prescribed total volume over about 4 h. After her rehydration, the child should subsequently continue to be provided free access to fluid and resume an age-appropriate diet. If, on the other hand, there are any further episodes of vomiting, then for each episode of emesis an additional 120–240 mL of oral rehydrating solution should be given, again with the goal to complete rehydration and resume usual fluid intake and nutrition.

The initial provision of oral fluid given often in small volumes is far more likely to be well tolerated by the dehydrated child than larger aliquots. If families are unwilling to provide the fluid in this manner, a nasogastric tube may be placed for continuous infusion of rehydrating solution.

Although occasional children may fail this approach and require intravenous rehydration, most children with mild to moderate rehydration can be rehydrated orally.

A guide for the volumes of fluid to provide and the duration of rehydration can be found in Table 13-7.

The first oral rehydration solutions were developed in the 1940s at academic medical centers. Within 10 years, a commercial preparation formulated as a powder meant to be mixed with water was available, but its use became associated with an increased incidence of hypernatremia (72). Several factors contributed to the development of this problem: the preparation was sometimes incorrectly administered as the powder itself or improperly diluted with too little water; when correctly reconstituted, the solution had a final carbohydrate concentration of 8% predisposing to an osmotic diarrhea; and it was a common practice at that time for parents to use high solute fluids such as boiled skim milk as an adjunctive home remedy. Taken together, these early experiences contributed to reluctance to use oral rehydration solutions on the part of many clinicians.

Over time, there came to be a better understanding of the physiology of water and solute absorption from the gut. Of prime importance was the recognition that many substances actively transported across intestinal epithelium had an absolute or partial dependence on sodium for absorption and that sodium itself was actually better reabsorbed in their presence (73–75). This led to the routine introduction of glucose into oral rehydration solutions in a fixed molar ratio of no more than 2:1 with sodium. Moreover, it became clear that the sodium/glucose cotransporter remained intact not only in the face of enterotoxic-gastroenteritis such as seen with cholera or *Eschericia coli* but also in more common viral and bacterial enteritides (66, 67).

The World Health Organization (WHO) and the United Nations Children's Fund have championed the use of a rehydrating solution that includes: Na 90 mmol/L, Cl 80 mmol/L, K 20 mmol/L, base 30 mmol/L, and glucose 111 mmol/L (2%). This WHO solution has proved useful in many clinical trials rehydrating children and has also been shown to reduce the morbidity and mortality associated with diarrheal illness regardless of its etiology (68, 69).

Most commercially available oral rehydration solutions differ from WHO solution in that they have a somewhat higher carbohydrate content, a lower sodium content, and a higher carbohydrate to sodium ratio (Table 13-8). Some preparations are available as powder and other as ready to drink formulations. Some manufacturers have also used rice solids as a carbohydrate source instead of glucose.

Many of these formulation changes arose from concerns that using an oral rehydrating solution with a sodium content >60 mmol/L would prove problematic in developed countries where most gastroenteritis is viral in nature and has a lower sodium content than the secretory diarrheas seen in less developed areas. Some clinicians

Table 13-7

Oral rehydration for previously healthy, well-nourished children

Type of dehydration	Rehydration phase	Rehydration duration	Replacement of ongoing losses	Nutrition
Mild (<5%)	30–50 mL/kg ORT	3–4 h	60–120 mL ORS for each diarrheal stool or episode of emesis[a]	Continue breast milk or resume age appropriate usual diet
Moderate (5–10%)	50–100 mL/kg ORT	3–4 h	As above	As above
Severe (>10%)	100–150 mL/kg ORT	3–4 h	As above with use of nasogastric tube if needed	As above
Evidence of shock	20 mL/kg 0.9% NaCl or Lactated Ringers IV	Repetitive infusions until perfusion restored then transition to ORT 100 mL/kg over 4 h	As above with use of nasogastric tube if needed or consideration of further IV therapy	As above
Accompanying Hypernatremia	Per type of dehydration	At least 12 h for ORT. Monitor fall in serum Na	As above	As above

ORT, Oral Rehydration Therapy with fluid containing 45–90 mmol/L Na, 90 mmol/L glucose, 20 mmol/l K, 10–30 mmol/L citrate; IV, Intravenous infusion

[a]For children >10 kg, aliquots for replacement of ongoing losses should be doubled to 120–240 mL rehydration solution for each stool or emesis

Table 13-8

Oral rehydration solutions

	Concentration (mmol/L)					
Product						
	Na	Sugar	K	Cl	Base	Osmolality (mOsm/L)
WHO ORS[a]	90	111	20	80	30	311
CeraLyte 90[a]	90	220[b]	20	80	30	275
Low-Na ORS[a]	75	75	20	65	30	245
Rehydralyte	75	140	20	65	30	300
CeraLyte 70[a]	70	220[b]	20	60	30	230
CeraLyte 50[a]	50	220[b]	20	40	30	200
CeraLyte 50 lemon	50	170[b]	20	40	30	200
Enfalyte	50	170	25	45	34	167
Pedialyte	45	140	20	35	30	254

[a]Provided as powder. Needs to be reconstituted with water
[b]Contains rice-syrup solids substituted for glucose

feared that, if minimally dehydrated children losing small amounts of sodium in their stools were exclusively provided WHO solution, hypernatremia might ensue without provision of excess free water. A few early studies did document iatrogenic hypernatremia related to such rehydration techniques (76). In cases of mild dehydration stemming from causes other than secretory diarrhea, solutions with lower sodium contents may be as useful and, in fact, solutions with sodium content ranging from 30 to 90 mmol/L have proved quite effective in this setting (70, 71, 77). A more recent meta-analysis of studies focused on the safety and efficacy of oral rehydration solution in well nourished children living in developed countries documented little evidence that WHO solution was more likely to cause aberrations in serum sodium than lower sodium containing oral rehydration solutions (78). Why ingestion of lower tonicity oral rehydration fluids would be less problematic than infusion of similar tonicity intravenous fluid is not clear, but does again underscore the safety of oral rehydration.

Oral Rehydration with Fluids Other than ORS

Despite the efficacy and widespread availability of commercial oral rehydration solutions and the ease with which other electrolyte solutions can be mixed at home with recipes requiring few ingredients other than water, sugar, and salt, there are many children who are still given common household beverages in attempts at rehydration. In children with dehydration and electrolyte losses from vomiting or diarrhea, most common beverages do not contain adequate sodium or potassium supplementation. Moreover, the base composition and the carbohydrate source are often sub-optimal for the dehydrated child, especially in the setting of diarrheal illness (*Table 13-9*). Similarly, most beverages marketed as sports drinks for "rehydration" following exercise are also deplete of sufficient electrolytes given that the electrolyte composition of sweat is many fold lower than the composition of gastrointestinal fluid. In prescribing oral rehydration to children in an ambulatory setting, the clinician should be specific to the family as to the appropriate fluid and volume for the child to ingest, emphasizing the need to use a fluid with appropriate electrolye content if there is concern about evolving imbalances in sodium, potassium, or bicarbonate homeostasis.

Oral Rehydration and Serum Sodium Abnormalities

Although oral rehydration is often considered for children with modest dehydration and no presumed electrolyte anomalies, oral rehydration with WHO or WHO-like solution has also been used in cases of dehydration accompanied by hyponatremia or hypernatremia (79, 80). Although most children with severe hypernatremia (>160 mEq/L) can be successfully rehydrated orally, there have been reports of seizures, generally as a result of too rapid correction of serum sodium stemming from the

Table 13-9

Composition of common oral fluids[a]

Fluid	Na (mEq/L)	K (mEq/L)	Source of base	Carbohydrate (g/100 mL)
Apple juice	<1	25	Citrate	12
Orange juice	<1	55	Citrate	12
Milk	20	40	Lactate	5
Cola	2	<1	Bicarbonate	10
Ginger ale	4	<1	Bicarbonate	8
Kool-Aid	<1	<1	Citrate	10
Gatorade	20	2.5	Citrate	6
Powerade	10	2.5	Citrate	8
Jello	25	<1	Citrate	14
Coffee	<1	15	Citrate	<0.5
Tea	2	5	Citrate	10

[a]Adapted in part from data found in (74)

provision of supplemental water in addition to the glucose-electrolyte solution (79–81). In those cases, the average serum sodium fell by 10–15 mEq/L over 6 h rather than over 24 h as advised. In follow-up studies, no seizure activity was seen in a similar cohort of hypernatremic children who received 90 mmol/L Na rehydration solution alone at a rate calculated to replace the infant's deficit over 24 h (79). It is important for the practitioner to remember that once peripheral perfusion has been stabilized with initial volume expansion, there is no benefit to correcting any deficit rapidly and taking 24–48 h may be a more prudent course in the face of significant electrolyte anomalies.

Oral Rehydration Schemes

Several oral rehydration schemes have been shown to be quite effective and well tolerated. In one approach used extensively in developing countries, the patient's volume deficit is calculated on the basis of weight loss and clinical appearance (82). The volume deficit is doubled; this is the target rehydration volume to be given over 6–12 h. Two-thirds of this volume is given as a glucose-electrolyte solution containing 90 mmol/L Na over 4–8 h; once this has been ingested, the remaining volume is provided as water alone over 2–4 h. In cases of suspected or confirmed hypernatremia with serum sodium exceeding 160 mEq/L, the volume deficit would not be doubled and would be administered as 90 mmol/L Na glucose-electrolyte solution alone over 12–24 h. Patients who refuse to take fluids by mouth have nasogastric tubes placed. With this approach, successful oral rehydration is the rule; 95% of children are fully rehydrated without the need for intravenous therapy.

An alternative approach has been to have the child begin by taking 15 mL/kg/h of a 60–90 mmol/L Na rehydration solution by mouth or nasogastric tube (83). The solution is given in small frequent quantities and increased up to 25 mL/kg/h until hydration has improved at which point solid feedings are reintroduced and volumes of 5–15 mL/kg of rehydration solution offered after feeds until the volume deficit has been delivered.

Over two decades ago, the American Academy of Pediatrics issued guidelines for the treatment of fluid and electrolyte deficits with oral rehydration solutions (66). Children with acute dehydration and extracellular volume contraction were to be provided 40–50 mL/kg of a glucose-electrolyte solution containing in each liter 75–90 mmol Na, 110–140 mmol glucose (2–2.5%), 20 mmol potassium, and 20–30 mmol base. This volume was to be administered over 3–4 h and then once there has been amelioration of the extracellular volume contraction, the child would be changed to a maintenance solution with 40–60 mmol/L Na at half the rate. If the child was still thirsty on this regimen, there should be free access to supplemental water or low-solute fluid such as breast milk.

Based on much of this published clinical experience, an evidence-based guideline for treating dehydration in children from industrialized European countries was created in the late 1990s, recommending oral rehydrating solution containing 60 mmol/L of sodium, 90 mmol/L of glucose, 20 mmol/L of potassium, and 10–30 mmol/L

of citrate with rehydration occurring over 3–12 h utilizing from 30 to 150 mL/kg of fluid depending on the degree and type of dehydration (48).

In 2004, the American Academy of pediatrics updated its oral rehydration recommendations and endorsed guidelines promulgated by the Center for Disease Control and Prevention (84, 85). Minimal dehydration in children weighing less than 10 kg was to be treated with provision of 60–120 mL of oral rehydration fluid for each watery stool or each episode of vomiting. In larger children, twice this volume would be provided. For children with more moderate dehydration, 50–100 mL/kg of oral rehydration solution would be given over 2–4 h to account for estimated fluid deficit and ongoing losses would be treated with the 60–240 mL per stool or emesis depending on size. Nursing babies would continue to receive breast milk as desired and formula fed babies would be provided age appropriate diet as soon as they had been rehydrated. For severely dehydrated children, a combination of intravenous hydration with isotonic fluids and prompt transition to oral rehydration solution by mouth or nasogastric tube was recommended. Overly restricted diets were to be avoided during gastrointestinal illness and attention to adequate caloric intake emphasized (see *Table 13-7*).

Use and Acceptance of Oral Rehydration Solutions

Despite the availability of guidelines for oral rehydration and their endorsement by professional organizations, a minority of American academic pediatricians, private practitioners, and pediatric house staff acknowledged utilizing oral rehydration (70). Although oral rehydration schemes have been shown to be used significantly more frequently by emergency room physicians who were familiar with the American Academy of Pediatrics recommendations, even in this group oral therapy was underutilized in children with all degrees of dehydration. Worldwide, WHO estimates that fewer than 25% of patients who could benefit from oral rehydration are actually treated with such therapy (86). Moreover, even in areas of the world such as Bangladesh, where oral rehydration has been championed by both local and international medical agencies for decades, its use is still suboptimal (87, 88). Among practitioners, there may also be generational differences in the use of oral rehydration therapy. In a national survey of Amercian pediatric emergency department physicians, although there was no difference in the baseline knowledge of published data in this field or acceptance of its validity, recent graduates of training programs were much more likely to use oral rehydration for more advanced cases of clinical dehydration than their older colleagues (89).

When utilized according to these schemes, oral rehydration has been demonstrated to be almost universally successful in achieving some degree of volume repletion (90). Other advantages to oral therapy include the safety and stability of the product despite lengthy shelf storage, its ability to be administered readily by the child's caretaker in nearly any locale, and avoidance of the discomfort and potential complications associated with intravenous catheter placement (91). In developed countries, there has been the concern that some powdered ORS formulations may not be looked upon by parents as a convenient hydration solution since they involves preparation. In fact, in a randomized controlled trial of an urban pediatric clinic and a suburban medical practice in the United States, parents were as equally satisfied with the ease of administration and effectiveness of a powdered solution as a commercially prepared ready to drink solution (92).

Oral rehydration is somewhat less successful in hospitalized children than in children treated in an ambulatory setting (90). This difference may be directly related to the degree of dehydration or other complicating clinical issues leading to hospital admission. Moreover, the relatively labor intensive slower approach to oral rehydration may be problematic in medical facilities with time constraints or space limitations (91, 93).

Frozen flavored oral rehydration solutions may be more readily accepted than conventional unflavored liquid electrolyte solutions. Their use resulted in higher rates of successful rehydration in children with mild to moderate dehydration, even if these children initially failed conventional oral rehydration (91). Frozen flavored rehydration solution is now commercially available in many parts of the world, as is a variety of flavored rehydration solutions.

Another potential issue with oral rehydration is that its use does not alter the natural course of the child's illness. For instance, in gastroenteritis with dehydration, by far and away the most common illness requiring rehydration in children, oral rehydration does not lower stool output or change the duration of diarrheal illness (94). As a result, caretakers may abandon oral rehydration because the child continues to have symptoms, failing to appreciate the benefits of ongoing hydration. Oral rehydrating solutions have been formulated with lower electrolyte composition and different carbohydrate moieties with the goal to reduce the osmolarity of solutions and potentially augment fluid absorption from the small intestine (78). The rice based oral solutions have been studied most

extensively. In these solutions, glucose is substituted with 50–80g/L of rice powder. In a meta-analysis of 22 randomized clinical trials comparing rice based solution to conventional glucose containing solutions, stool output dramatically decreased in children with cholera given rice based hydration but did not change in children with other bacterial or viral enteritides (95).

There are some reports that suggest that providing children with non-cholera enteritis with reduced osmolarity rehydration solution may be beneficial. A study of 447 boys less than 2 years of age admitted for oral rehydration were assigned to either receive WHO solution (osmolarity 311 mmol/L) or a solution containing less sodium and chloride (osmolarity 224 mmol/L). Children who received the lower osmolarity solution had reduced stool output, reduced duration of diarrhea, reduced rehydration needs, and reduced risk of requiring intravenous fluid infusion after completion of oral hydration (96). A meta-analysis of 9 trials comparing WHO solution to reduced osmolarity rehydration solution concluded that children admitted for dehydration had reduced needs for intravenous fluid infusion, lower stool volumes, and less vomiting when receiving the reduced osmolarity solution (97).

Intravenous Therapy

Although absolute indications for parenteral intravenous therapy are limited, they do include significantly impaired circulation or overt shock. In addition, there are occasional children who are truly unable to sustain an adequate rate of oral fluid intake despite concerted effort or have such persistent losses that parenteral therapy comes to be necessary. The mainstays of fluid therapy in children are saline or buffered saline crystalloid solutions. Isotonic versions of these crystalloids are used for volume resuscitation and hypotonic saline solutions may be used in addition to provide supplemental maintenance hydration. In addition to crystalloid solutions, there are several colloid fluids that are also used by many clinicians. *Table 13-10* lists the electrolyte content of some of the more common intravenous solutions used for pediatric fluid therapy.

Table 13-10

Composition of common intravenous fluids

Fluid	Osmolarity (mOsm/L)	Na (mEq/L)	K (mEq/L)	Cl (mEq/L)	Buffer (source) (mEq/L)	Mg (mEq/L)	Ca (mEq/L)	Dextrose (g/L)
Chrystalloids								
0.9% Saline	308	154	0	154	0	0	0	0
Lactated ringers	275	130	4	109	28 (lactate)	0	3	0
D5 0.45% Saline	454	77	0	77	0	0	0	50
D5 0.22% Saline	377	38	0	38	0	0	0	50
5% Dextrose water	252	0	0	0	0	0	0	50
Normosol	295	140	5	98	23 (gluconate) 27 (acetate)	3	0	0
Plasma-Lyte	294	140	5	98	23 (gluconate) 27 (acetate)	3	0	0
Colloids								
5% Albumin	309	130–160	<1	130–160	0	0	0	0
25% Albumin	312	130–160	<1	130–160	0	0	0	0
Fresh frozen plasma	300	140	4	110	25 (bicarbonate)	0	0	0
3.5% Hemaccel	301	145	5	145	0	0	6	0
6% Hetastarch	310	154	0	154	0	0	0	0
Dextran 40 or 70	310	154	0	154	0	0	0	0

Choice and Volume of Parenteral Fluid

Children with significant extracellular volume contraction (greater than 10% acute weight loss in an infant or 6% weight loss in an older child) should receive an isotonic crystalloid solution such as 0.9% saline (154 mEq/L NaCl) or Lactated Ringer's (130 mEq/L NaCl) at a rate of 20 mL/kg over 30–60 min. In some children, even more rapid infusions or serial provision of such aliquots may be necessary to restore effective volume. Children with less pronounced dehydration may not exhibit signs or symptoms of volume contraction. In certain situations, however, it may be clinically warranted to provide them with an initial rapid intravenous bolus to initiate rehydration therapy.

Concomitant with the placement of intravenous access, blood should be obtained for determination of serum electrolytes, osmolality, and renal function. Given that dehydrated children often have high levels of vasoactive hormones and high vasopressin levels, it is most circumspect to establish baseline electrolyte levels since it is possible to alter electrolyte balance rapidly with intravenous therapy. In the face of inadequate tissue perfusion, a parenteral fluid infusion should begin immediately prior to the return of any pertinent laboratory results. If hemorrhagic shock is suspected, resuscitation with packed red blood cells is optimal. In cases of severe volume depletion, if the child does not improve with the initial 20-mL/kg crystalloid bolus, this should be repeated up to two additional times. In children who have not improved despite administration of 60 mL/kg of total volume over an hour or in children in whom underlying cardiac, pulmonary, or renal disease may make empiric aggressive rehydration more problematic, consideration should be given to placement of a central monitoring catheter to more accurately assess intravascular volume and cardiac dynamics (98). In some instances of profound ineffective circulating volume, such as might accompany certain cases of sepsis, initial volume resuscitation may require sequential infusions of fluid ultimately exceeding 100 mL/kg.

Within minutes of infusion of a crystalloid fluid, it becomes distributed throughout the extracellular space. Since this involves equilibration of the fluid between the two components of the extracellular space – the intravascular and interstitial spaces – actually only one-third to one-quarter of infused crystalloid stays in the blood vessels (99). This accounts for the need to give large volumes of crystalloid in the setting of circulatory collapse and leads some to suggest that colloid solutions such as 5% or 10% albumin should play a role in resuscitation (100, 101).

Colloid Solutions and Volume Resuscitation

The use of colloid solutions for volume resuscitation is controversial. Colloids were once included in a number of widely promulgated guidelines for the care of patients in emergency facilities and intensive care units both for hemorrhagic shock prior to the availability of blood and for non-hemorrhagic shock as an adjunct to crystalloid use (102). Types of colloid utilized included 5% albumin, fresh frozen plasma, modified starches, dextrans, and gelatins. These guidelines, generally aimed toward the fluid resuscitation of adults, were composed despite the prior publication of a systematic review of randomized controlled trials that demonstrated no effect on mortality rates when colloids were used in preference to crystalloids (103). Moreover, there is a distinct cost disadvantage to using colloid solutions.

Subsequent systematic reviews have looked at this issue anew. In one meta-analysis of 38 trials comparing colloid to crystalloid for volume expansion, there was no decrease in the risk of death for patients receiving colloid (102). In the other review, albumin administration was actually shown to increase mortality by 6% compared to crystalloid (104). Proposed mechanisms contributing to this worse outcome include anticoagulant properties of albumin (105) and accelerated capillary leak (106).

A drawback of all these systematic reviews, however, has been the limited number of studies that included children other than ill premature neonates. As a result, generalization of these results from ill adults may not be germane to all critically ill volume depleted children. For instance, a report of 410 children with meningococcal disease suggests that albumin infusion in this population may not have been harmful, as case fatality rates were lower than predicted (107). Overall, however, there seems to be no substantive data to support the routine use of colloid to complement or replace crystalloid in fluid resuscitation. Rather, repetitive infusions of large volumes of crystalloid seem to be well tolerated in volume depleted children, do not seem to predispose to excessive rates of acute respiratory distress syndrome or cerebral edema, and in some conditions such as sepsis, play an important role in improved survival (108). A recent survey of pediatric anesthesiologists in western Europe reported that colloid solutions are being used less frequently in infants and older children and suggested that familiarity with some of the issues raised in these systematic reviews are affecting practice patterns (109).

Repetitive infusions of crystalloid may also prove problematic in some children. Most notably, if very large volumes of 0.9% saline are used acutely for volume

resuscitation, it is not unusual for children to develop a hyperchloremic metabolic acidosis. This occurs as acidotic peripheral tissues begin to reperfuse and already depleted extracellular bicarbonate stores are diluted by a solution with an isotonic concentration of chloride (98, 110). This acidosis can be ameliorated by supplemental doses of bicarbonate as well as the addition of supplemental potassium as needed. There is sometimes a tendency for clinicians to react to the hyperchloremic metabolic acidosis with further saline bolus infusions. In the face of corrected hypoxia or hypovolemia, however, such maneuvers may only exacerbate the chloride driven acidosis (111). This hyperchloremic acidosis is seen less frequently when Ringer's lactate solution is used as the resuscitation fluid because of the metabolic conversion of lactate to bicarbonate. In the setting of significant preexisting acidosis or underlying hepatic dysfunction preventing the metabolism of lactate, infusion of Ringer's lactate solution may, however, exacerbate an acidosis.

With the recent suggestion that some children with acute illness or following surgery may benefit from a prolonged period of isotonic fluid infusion given high levels of ADH and the possibility for hyponatremia developing with hypotonic fluid therapy, some have expressed concern that a hyperchloremic acidosis may develop in these children. In a prospective randomized study of more than 100 children with gastroenteritis given isotonic intravenous rehydration and maintenance therapy, there was no tendency for the development of hyperchloremic acidosis even after a day of isotonic fluid provision. Although serum chloride levels did tend to increase in these children, serum bicarbonates also improved, potentially related to improved effective volume and subsequent better tissue perfusion (112). Similarly, in children post cardiac surgery who were given isotonic solutions for their maintenance fluid needs, although there was a tendency for a hyperchloremic acidosis to develop, there seemed to be no significant clinical ramifications and long-term outcomes were similar to children who did not develop hyperchloremic acidosis (113).

Large volume infusion of blood may also predispose to electrolyte anomalies as well as manifestations of citrate toxicity. If aged whole blood is infused, there is the possibility that a large potassium load will be delivered to the patient as potassium over times migrates down its concentration gradient from less viable erythrocytes into plasma. Since most patients receive packed red blood cells instead of whole blood, this potential problem is minimized since little plasma is infused and, thus, the relatively small amount of infused potassium can be accommodated by intracellular movement.

Citrate is used as the anticoagulant in stored blood. Since citrate complexes with calcium, there can be a fall in ionized calcium levels if large volumes of blood are infused rapidly or if there are concomitant perturbations in calcium homeostasis. Similarly, citrate may complex with magnesium and magnesium depletion may occur. The liver usually metabolizes infused citrate into bicarbonate. Alkalosis may, thus, occur if large volumes of citrate are metabolized. In the setting of hepatic dysfunction, however, citrate will not be metabolized and serves as an acid load and will help create an acidosis or exacerbate any underlying acidosis.

Regardless of the initial infusion with either colloid or crystalloid, once sufficient volume to restore circulatory integrity has been infused, less rapid volume expansion is necessary. During this phase, the rapidity of fluid repletion is most probably not a concern unless there are severe underlying aberrations in the serum sodium or serum osmolality. In the absence of these derangements or profound volume deficit, if the child has improved significantly with the initial parenteral volume expansion, attempts should be made to reinstitute oral rehydration. Prolonged intravenous therapy rarely should be necessary.

Rapid Rehydration

Over the last decade, a scheme of rapid intravenous resuscitation and follow-up oral rehydration has been adopted by many pediatric emergency departments to treat children with up to 10% dehydration secondary to vomiting and gastroenteritis (114). After infusion of 20–30 mL/kg on intravenous crystalloid, the child is allowed to take up to several ounces of a standard oral rehydration fluid, and if this intake is tolerated without vomiting for 30–60 min, then discharged home to continue rehydration, initially with a prescribed volume of standard rehydration solution.

If the child does not tolerate oral rehydration or if there are such significant electrolyte anomalies that there are concerns regarding potential adverse CNS sequelae of too rapid rehydration, then intravenous rehydration may be the best route for continued hydration. It is rare for children to become symptomatic from serum sodium aberrations until levels less than 120 mEq/L or greater than 160 mEq/L are reached. Children who have had very sudden fluxes in electrolytes may become symptomatic earlier. On the other hand, children whose severe sodium abnormalities are thought to be more chronic in nature must be treated in a more controlled fashion since they are at higher risk for developing CNS symptoms during treatment.

The vast majority of children treated in emergency facilities for volume repletion do well with such rapid rehydration. These children are generally healthy with normal cardiac and renal function and have developed extracellular volume depletion relatively rapidly. As a result, they suffer no ill effects from rapid rehydration. In fact, the clinical success of this aggressive restoration of extracellular volume underlies the calls to reexamine the traditional deficit therapy approach to rehydration with its tedious calculations of fluid and electrolytes losses and requirements (9, 115).

Symptomatic Hyponatremia

In the setting of symptomatic hyponatremia, especially if the child has seizures, it is important to raise the serum sodium approximately 5 mEq/L acutely. Generally, this results in stabilization of the clinical situation and allows for further evaluation and treatment of the child in a less urgent fashion. This is one of the few situations in which hypertonic saline (3% saline) should be utilized.

To calculate the proper volume of 3% saline to infuse, the child's TBW must be multiplied by the 5 mEq/L-desired increase in serum sodium to determine the amount of sodium (in mEq) to infuse. Since every mL of 3% saline contains 0.5 mEq of sodium, doubling the number of mEq of sodium needed results in the proper milliliter volume of 3% saline to infuse. Thus, in the 20 kg child, the TBW is approximately 12 L (0.6 L/kg × 20 kg) and the desired sodium dose would be 60 mEq (12 L × 5 mEq/L). If 120 mL of 3% saline were infused, the serum sodium would be expected to rise by approximately 5 mEq/L. The infusion should be given at a rate to increase the serum sodium by no more than 3 mEq/L/h and is often given more slowly over the course of 3–4 h (116). If the child continues to be symptomatic from hyponatremia after this infusion, additional 3% saline may be given until the symptoms improve or the serum sodium is in the 120 to 125 mEq/L range. At that point, further correction of the hyponatremia should consist of a slower infusion of more dilute saline to cover the sodium deficit, the sodium maintenance needs, and any volume deficit. Consideration of the role of ADH and prior excess free water provision should also be considered in determining the volume and tonicity of fluid to be provided.

Asymptomatic Hyponatremia

If a child has severe hyponatremia but is not symptomatic, there is no need to administer hypertonic saline based solely on a laboratory anomaly. With or without symptoms, in cases of severe hyponatremia the child should be carefully evaluated as to the etiology of the hyponatremia, keeping in mind that hyponatremia tends to result from an imbalance of water regulation. If this is the case, free water should be restricted and appropriate supplementation with intravenous saline solutions begun to provide maintenance sodium requirements of approximately 2–3 mEq/kg/day and any ongoing losses of sodium.

Besides these maintenance sodium needs, if the child has an element of dehydration, every kilogram of body weight lost from baseline represents a 1 L deficit of nearly normal saline from the total body water as well. These losses are often referred to as isotonic losses. These account for a sodium deficit of 154 mEq/L that also must be included in the calculations for sodium replacement.

In the setting of hyponatremic dehydration, there have been additional sodium losses as well. Generally, these occur as viral diarrheal stool losses with a sodium content $\leq$60 mEq/L are replaced with fluids with a lower sodium content. To estimate these sodium losses, the difference between the child's desired serum sodium and current serum sodium is multiplied by the child's estimated TBW. This product represents the hyponatremic sodium losses that must be added to the maintenance sodium needs, any ongoing losses, and the sodium losses that accompanied weight loss. An example of the calculations and therapeutic maneuvers that need to be considered with significant hyponatremia is presented in the following case study:

A girl who normally weighs 10 kg suddenly develops generalized seizures and is brought by ambulance to the emergency department. She has had a week of gastroenteritis, has felt warm to touch, and has been drinking water and apple juice only, refusing any other liquids or any solid food for several days. Intravenous access is placed and lorazepam is administered and the seizure activity stops. The emergency department physician orders serum chemistries and the child is weighed and found to be 8.8 kg. A bolus infusion of 200 mL of 0.9% NaCl is administered over the next 30–60 min after which the girl appears well perfused but she is still lethargic. The serum sodium is then reported to be 112 mEq/L. While further evaluation of the child's overall status is ongoing, it is important to begin correcting the symptomatic hyponatremia.

The child has actually already received approximately 30 mEq of sodium in the 0.9% NaCl bolus given because of her dehydration and poor perfusion. Given her TBW of roughly 5.5 L (wt in kg × 0.6 L/kg), this should result in an increase in her serum sodium by approximately 5 mEq/L.

Since the child has had hyponatremic seizures and is still exhibiting some central nervous system effect with her lethargy, it is prudent to raise the serum sodium by 5 mEq/L so that it will be in the 120–125 mEq/L range. Since she is hemodynamically stable, it is also best not to provide an excess of further volume until the child undergoes imaging to assess for cerebral edema, especially given the history of seizures, lethargy, and hyponatremia. By using a small volume of hypertonic saline, the serum sodium can be raised in a controlled manner while further evaluation of the child continues. It would take about 22 mEq of sodium (TBW × desired increase in serum sodium = 5.5 L × 5 mEq/L) to accomplish the desired elevation. Since each mL of 3% saline contains about 0.5 mEq of sodium, a total of 44 mL of 3% saline could be infused over approximately 3–4 h.

In addition to this acute management to restore initial circulation and perfusion and to raise the serum sodium to a safer level, plans must be formulated to attend to the patient's overall volume and sodium deficit. To prescribe the proper follow-up intravenous fluid, the patient's water and electrolyte deficits at presentation must be reconciled with his therapy thus far.

The child's water deficit is 1.2 L, reflecting the 1.2-kilogram weight loss. She has "maintenance" water needs of an additional 1 L/day based on her normal weight of 10 kg. She is having no other ongoing water losses and has already received nearly 250 mL in intravenous fluid in the form of 0.9% NaCl and 3% NaCl. Her current water needs are thus 1,950 mL.

The child's normal "maintenance" sodium needs are 30 mEq/day (3 mEq/kg/day). She has lost 1.2 kilograms of isontonic fluid in body weight that represents 185 mEq of sodium. In addition, she has hyponatremic sodium losses that have arisen as her diarrheal stool that contained sodium was replaced with water alone. To calculate these needs, her normal total body water needs to be multiplied by the difference in her serum sodium from a normal value of 135 mEq/L. Her TBW is 6 L (TBW = 0.6 L/kg × 10 kg) and the difference in serum sodium is 23 mEq/L (135 mEq/L − 112 mEq/L); her hyponatremic losses are therefore 138 mEq (6 L × 23 mEq/L). Total sodium needs are thus 30 mEq of maintenance, 185 mEq of isotonic losses, and 138 mEq of hyponatremic losses or a total of 353 mEq. She has already received 52 mEq of sodium from the 400 mL of 0.9% NaCl given in the emergency department. Her current sodium needs are thus just about 300 mEq.

To choose the proper solution for this child, the deficit of 1,950 mL of water should contain 300 mEq of sodium. This is best approximated by 0.9% NaCl with its NaCl content of 154 mEq/L NaCl. In the past, it has been suggested that half of the fluid and sodium deficit be replaced over 8 h and the remainder over the ensuing 16 h. Although such a plan can be followed, there is little evidence that more rapid correction of the hyponatremia is harmful except if the patient has been symptomatic with hyponatremia or has profound asymptomatic hyponatremia of chronic duration. In these cases, it is safest to plan to correct the serum sodium by no more than 12–15 mEq/L over 24 h. More rapid correction has resulted in osmotic demyelination injury to the brain with devastating long-term neurologic outcomes (116–118).

Severe Hypernatremia

With hypernatremia, therapy is again guided by the clinical situation and provision of intravenous fluid is usually reserved for those children with very elevated serum sodium values who are not considered candidates for oral rehydration therapy. In cases of hypernatremia due to salt poisoning, there should be signs of overhydration and volume expansion. Excretion of sodium should be enhanced by using a loop diuretic to augment urine sodium losses and by replacing urine output with free water. If the patient has significant underlying renal or cardiac compromise, dialysis and ultrafiltration may be necessary to correct the water and electrolyte imbalance (42, 43). With hypernatremia and volume expansion from salt excess, it will be detrimental to provide further intravenous saline.

In hypernatremia accompanied by volume loss, any significant alterations in effective circulation should be addressed with 20 mL/kg bolus infusions of an isotonic crystalloid solution until effective peripheral perfusion is restored. Then, further provision of water and sodium should be provided based on calculated water and sodium needs. In the majority of cases, with mild elevations in serum sodium and minimal degrees of dehydration, the actual calculation of deficits is probably unnecessary since the child will be hemodynamically stable and a candidate for exclusive oral rehydration. In situations where there is profound hypernatremia or circulatory compromise, it remains necessary, however, to be able to calculate a free water deficit to tailor intravenous rehydration therapy. An example of such a situation is outlined in the following clinical scenario:

After 2 days of refusing to nurse, a 5 kg infant boy with a viral syndrome presents to an emergency department in shock, 15% dehydrated with a weight of 4.25 kg and a

serum sodium of 170 mEq/L. He receives 300 mL of 0.9% NaCl urgently and further therapy is now planned.

The child has lost 750 g of weight. Since this is hypernatremic dehydration, there has been loss of water in excess to salt. Thus, part of the weight loss represents isotonic losses but a larger proportion represents free water loss. The child's free water deficit can be calculated by the equation:

$$[(\text{Serum Na actual})/(\text{serum Na desired}) \times \text{total body water}] - \text{total body water}$$

Substituting the appropriate data for this baby:

$$[(170/145) \times (0.6 \times 4.25)] - (0.6 \times 4.25) = [1.2 \times 2.55] - 2.55 = 0.51\text{L}]$$

Thus, of this baby's 750-mL fluid deficit due to dehydration, 510 mL is free water and 240 mL is normal saline.

Too rapid correction of the baby's serum sodium with free water could result in cerebral edema as the water infused into the extracellular space follows osmotic forces and moves into the intracellular space. In cases of hypernatremia where the serum sodium exceeds 160 mEq/L, it is considered safest to correct the serum sodium by no more than 15 mEq/day. In this boy's case, this would mean that correction to a serum sodium in the normal range would take about 2 days.

If the fluid and electrolyte therapy must be given intravenously, the appropriate prescription again depends on calculation of water and sodium requirements and deficits. His original fluid deficit was 750 mL and his maintenance water needs are estimated at 500 mL/day. Thus, over the next 2 days, the fluid needs to replace the deficit and provide maintenance would be 1,750 mL. Of this volume, sthe baby has already received 300 mL of fluid in the emergency department so a net deficit of 1,450 mL now exists.

The baby has maintenance sodium needs of 15 mEq/day (3 mEq/kg/day). His sodium deficit reflects only the isotonic fluid losses that have been estimated above at 240 mL of normal saline or 37 mEq of sodium. Thus, over the next 2 days, his sodium needs are 67 mEq of which he has already received more than 45 mEq in the emergency department due to initial volume expansion.

Initiating an infusion of 30 mL an hour of free water should result in the slow and steady correction of the hypernatremia over 2 days by providing the nearly 1.5 L of free water that the child requires to replace losses and provide ongoing needs. The serum sodium should be monitored every 4 h initially and if it is falling faster than desired (about 0.5 mEq/hour) then sodium should be added to the rehydration fluid.

Fluid and Electrolyte Therapy with Renal Dysfunction

Impact of Kidney Disease

Children with compromised renal function often manifest a reduced tolerance for changes in total body water as well as changes in the composition or distribution of volume between the intracellular and extracellular body spaces. Similarly, alterations in electrolyte balance are more likely problematic because normal homeostatic mechanisms are frequently perturbed. Especially in the child with marked nephrosis or significant impairment in renal clearance, it becomes vital to approach the provision of fluids and electrolytes with great care.

As far as fluid therapy is concerned, of utmost importance is the recognition that the concept of maintenance fluids or electrolytes pre-supposes normal renal function. Roughly two-thirds of any daily maintenance fluid prescription is to replace urinary water losses. Similarly, urinary electrolyte losses figure prominently in daily electrolyte balance. In the setting of oliguria or anuria, provision of maintenance fluids could contribute to and, potentially, exacerbate volume overload and maintenance electrolyte therapy could result in electrolyte anomalies.

Fluid and electrolyte needs of the child with renal dysfunction are better considered in the context of the child's current volume status and electrolyte needs. For instance, in symptomatic volume depletion with decreased circulatory perfusion, volume expansion would be initiated regardless of urine output. Once volume replete, the child's needs could be reassessed along with his current renal function. The child who is volume overloaded would best be managed by fluid restriction and provision of only insensible losses of approximately 300 mL/m^2. Insensible fluid losses should be considered essentially electrolyte free water. The child who is volume replete should be kept volume replete. This is most readily accomplished by providing a combination of insensible losses as free water and any other volume losses (urine output, diarrheal stool, surgical drain output, emesis) on an additional milliliter for milliliter basis. If there are significant ongoing losses from a single source, the electrolyte composition of this fluid can be assayed so that the replacement fluid may more accurately reflect the electrolyte losses. Otherwise, a solution of 0.45% NaCl can be used initially and altered as the clinical situation continues to develop and further electrolyte determinations are made.

If the child's volume status or the adequacy of renal function is difficult to discern initially, it is best to provide the child with replacement of both insensible and ongoing losses. This approach should maintain the child's current volume status and allow for further determination of the appropriateness of more vigorous hydration or conversely fluid restriction as the clinical situation clarifies. Monitoring the child's weight on at least a daily basis and documenting the child's total fluid intake and output will also assist in arriving at a proper hydration regimen.

Assessing the child's current electrolyte status and monitoring the loss of electrolytes in the urine or in any other source of significant output will help tailor the daily electrolyte prescription. An understanding of the pathophysiology underlying the child's renal dysfunction will also be useful. The child who has profound tubular electrolyte losses will require more sodium on a daily basis than the child who is edematous and total body salt overloaded from his nephrotic syndrome. The child with chronic renal insufficiency and hypertension mediated by long-standing salt and water overload may actually benefit from diuretic therapy to remove salt and water rather than any further volume expansion with saline.

Certainly the provision of supplemental potassium to the child with renal dysfunction must be done judiciously. The oliguric or anuric child should receive no potassium until it is well documented that serum potassium levels are low or that there are extrarenal potassium losses (for instance losses from diarrheal stool). The child with marginal renal function should receive small amounts of potassium (approximately 1 mEq/kg/day) with at least daily assessment of electrolyte balance to determine adequacy and appropriateness of continued potassium supplementation.

Fluid and Electrolyte Therapy in the Pediatric Intensive Care Unit

Critically ill children present a challenge to the clinician attempting to prescribe appropriate fluid and electrolyte therapy. Oftentimes, there may be acute kidney injury or multiorgan failure complicating management decisions. With such children, rote reliance on standard equations or practice guidelines to prescribe fluid and electrolyte therapy may create significant fluid and electrolyte anomalies. Rather than prescribing set maintenance requirement of fluid or electrolytes, the clinician should assess the patient's individual fluid and electrolyte needs in the context of the underlying pathophysiology, the current volume status, the efficacy of tissue perfusion, the current ventilatory requirements, and the current renal function. Whenever there is concern about incipient or exacerbating fluid overload, it is important to review the volume and type of fluids being provided. Maximizing the concentration of continuous medication drips and assessing medication compatibility for simultaneous infusions are important steps in limiting total daily fluid input. Initially, it is crucial in these critically ill children to ascertain that their intravascular space is replete to help maintain hemodynamic stability. Once a patient is felt to be intravascularly replete, maintaining euvolemia by providing insensible water losses as well as replacing any ongoing fluid and electrolyte losses should maintain fluid and electrolyte balance.

Oftentimes, despite a desire to limit fluids in the critically ill child, medication requirements, nutritional needs, and hemodynamic insufficiency may result in very large daily fluid loads. There may also be situations in which increased vascular permeability or "leak" causes a critically ill child to become massively volume overloaded but with a decreased effective circulating volume. In other words, renal and tissue perfusion may be sluggish because fluid has leaked from the intravascular space into the interstitial space. In this setting, there may be need to continue to administer large volumes of fluid to maintain circulatory integrity with the knowledge that such infusions will only exacerbate the total body fluid overload. Aggressive diuretic therapy may prove useful especially if renal function is not compromised. Combination diuretic therapy utilizing agents that work at separate sites along the renal tubule may be necessary. Ultimately, the use of either periodic or continuous ultrafiltration may be beneficial to these patients by allowing ongoing fluid administration but limiting the daily imbalance between fluid intake and output. Ultrafiltration may be accomplished via peritoneal dialysis, by intermittent hemodialysis with ultrafiltration, or by utilizing one of the slow continuous ultrafiltration techniques now known as continuous renal replacement therapy (CRRT)). The recognition that volume overload has a deleterious effect on many aspects of patient management and seems to be a strong prognostic indicator of poor ultimate outcome has led some to suggest that early ultrafiltration should be considered in critically ill patients (119).

If ultrafiltration is initiated, extreme vigilance is necessary to prevent exacerbation of intravascular depletion and the development of pre-renal azotemia or frank renal failure. Special care must be taken with the continuous modalities to insure that ultrafiltration rates are

periodically reassessed and readjusted. Furthermore, because the electrolyte losses that accompany the ultrafiltration of fluid are isotonic, the electrolyte content of infused fluids must be adjusted to match the composition of the ultrafiltrate, especially if there is no component of dialysis ongoing that may blunt the development of serum electrolyte anomalies. As a result, serum electrolyte values need to be followed in a serial fashion with periodic review and readjustment of the composition of supplemental intravenous fluids.

Abnormalities in Serum Sodium Complicated by Kidney Injury

Because of the important contribution of serum sodium to serum osmolality, alterations in serum sodium, especially coupled with alterations in BUN related to renal failure, can complicate the usual approach to a child with fluid and electrolyte anomalies. Generally, there are greater concerns with hypernatremia and renal failure since the need to correct the sodium in a slow fashion can be problematic when renal replacement therapy needs to be initiated for clearance of urea. Balancing the correction of sodium and the hyperosmolar state with the clearance of urea requires a carefully considered plan that is grounded in a firm understanding of fluid and electrolyte homeostasis.

In most cases of hypernatremia related to severe dehydration, some degree of acute kidney injury is present. This renal dysfunction is usually "pre-renal" in nature, a result of a decreased effective circulating volume rather than an intrinsic glomerular or tubular disorder. Most often, in the course of rapid restoration of perfusion and early rehydration, urine output increases and azotemia begins to resolve.

Alternatively, there are occasional cases in which due to intrinsic renal dysfunction or acute tubular necrosis, the renal insufficiency will not respond to volume infusion and, in fact, the provision of excess volume may contribute to significant volume overload. In these cases, there may be need to consider some form of renal replacement therapy to assist in the controlled correction of fluid and electrolyte derangements, especially if the renal failure is oliguric or anuric in nature. Such an example is detailed in the following case study:

A 15-year-old boy presents with several weeks of polyuria, severe weight loss, fatigue, and poor oral intake. He is diagnosed as having diabetes mellitus with ketoacidosis by his pediatrician and referred to an emergency department for management. At this point, his serum sodium is 154 mEq/L, his creatinine is 3.0 mg/dL, and his BUN is 30 mg/dL. In the emergency department, the child receives several bolus infusions of normal saline supplemented with sodium bicarbonate and is started on an insulin drip. He is admitted and continues to receive brisk intravenous hydration with normal saline with bicarbonate supplementation per a practice guideline for treating children with diabetic ketoacidosis. He is noted to be oliguric and this does not improve with several more h of hydration with normal saline following the guideline hydration recommendations. The next morning, laboratory values reveal a serum sodium of 165 mEq/L, a creatinine of 4.5 mg/dL, and a BUN of 50 mg/dL. He has made only 75 mL of urine in the last 8 h and is developing some mild peripheral edema.

In this case, the renal insufficiency and poor urine output have complicated the usual management of diabetic ketoacidosis and has exacerbated an underlying hypernatremia. Given the patient's evolving renal failure, it is not feasible to provide the necessary volume of free water to correct the hypernatremia without contributing to further volume overload. Because of the apparent progressive renal failure, it would also be useful to correct the hypernatremia in case dialysis becomes necessary for urea clearance. By performing controlled ultrafiltration on the patient and replacing back the volume ultrafiltered with free water, the serum sodium could be corrected without exacerbating the volume status.

With a serum sodium of 165 mEq/L and an estimated TBW of 42 L (70 kg $\times$ 0.6 L/kg), this boy has free water needs of 7.5 L to lower his serum sodium to the 140 mEq/L range ([165/140 $\times$ 42]–42). Since the patient is now significantly hypernatremic and has been subject to various fluid and electrolyte shifts as his diabetic ketoacidosis has been treated, it would be prudent to correct his serum sodium by no more than 10–12 mEq/day over the course of 3 days. Thus, if the boy undergoes ultrafiltration with a goal of 2.5 L removed daily, and the ultrafiltration volume each day is replaced back totally as free water, the serum sodium should be in the normal range in 3 days' time. The ultrafiltration goal could be achieved over the course of a few hours each day if the patient were hemodynamically stable or over a more prolonged period of time each day if there were concerns regarding hypotension. Thus, either a conventional hemodialysis set up could be used for relatively rapid ultrafiltration only or a continuous filtration circuit for either rapid or slow filtration.

Since the fluid removed in ultrafiltration is isonatremic to the serum sodium, the sodium concentration of each liter of ultrafiltrate should mirror the serum sodium

concentration at the time of ultrafiltration. Thus, on the initial day of ultrafiltration, each liter of ultrafiltrate would contain a sodium content of 165 mEq/L. By providing back the volume ultrafiltered each day as free water, the serum sodium content could be expected to fall, in this case, by about 8–10 mEq/L/day.

It is important to recognize that free water must be provided back to the patient to make up for the ultrafiltration losses. Otherwise, since the ultrafiltrate is isotonic, there will be no change in the serum sodium concentration and the ultrafiltration may potentially exacerbate the renal failure by depleting the intravascular space and the effective circulating volume.

Moreover, it is also important to recognize that the boy's overall daily fluid needs will be greater than the daily ultrafiltration volume alone since maintenance fluid requirements and any ongoing fluid losses must also be considered. Since the boy is in renal failure, his maintenance fluid needs can be scaled back to insensible losses of 300 mL/m^2/day and, in this case, there are no ongoing losses. Thus, each day for the next 3 days this 70-kg patient needs to receive approximately 500 mL/day of insensible losses and 2,500 mL/day of ultrafiltration replacement or a total of 3,000 mL/day. His maintenance sodium requirements are 3 mEq/kg/day. Although it may seem counterintuitive to provide a hypernatremic patient with maintenance sodium, disregarding these requirements will result in a more rapid correction of the hypernatremia than desired. If the child were to receive a saline infusion of 0.45% NaCl at a rate of 125 mL/h, this will provide just over 3 mEq/kg/day of sodium in a total volume of 3 L/day.

If the child with hypernatremia has profound renal failure and requires dialysis for urea clearance, the dialysis prescription must take into account the need to correct the serum sodium slowly. Normally, regardless of the modality of renal replacement therapy, most dialysate contains sodium isotonic to the normal serum sodium range. It may prove detrimental, however, to dialyze a patient who is very hypernatremic against a dialysate with a sodium concentration 30 mEq/L or more less than the patient's serum sodium concentration. The diffusional gradient during dialysis would lead to more rapid correction of the serum sodium than the desired drop of approximately 1 mEq every 2 h.

Although most hemodialysis machines can be readjusted so that the dialysate produced will have a sodium content as high as the low to mid-150s, this still may not reduce the gradient sufficiently in cases of severe hypernatremia. In those situations, by maximizing the sodium concentration of the dialysate and by performing dialysis for limited amounts of time, one could minimize the drop in serum sodium. Still, there would need to be frequent assessments of the serum sodium concentration, and overall clearance may need to be sacrificed to prevent too rapid correction of the serum sodium and a rapid concomitant decrease in the serum urea that may increase the chances for dialysis dysequilibrium.

Alternatively, a continuous hemodiafiltration technique such as continuous venovenous hemodiafiltration (CVVHDF) could be performed. By asking the hospital pharmacy to increase the sodium content of the dialysate and replacement fluid to within 10–12 mEq/L of the serum sodium concentration, the diffusional gradient for sodium clearance could be minimized. Then, by making appropriate adjustments in the sodium content of the dialysate as the serum sodium falls, the serum sodium levels could be reduced gradually by 10–12 mEq/L/day while at the same time adequate urea clearance and ultrafiltration for most situations would be achieved.

Peritoneal dialysis has also been used in cases of severe hypernatremia (120–122). Again, the concentration of sodium in the dialysate may need to be adjusted upwards in severe hypernatremia to prevent too rapid clearance of sodium. In addition, since the degree of clearance and ultrafiltration may not be as precisely controlled as with hemodialysis or hemodiafiltration, frequent assessment of electrolyte values will be necessary. Manipulation of dwell volumes and dwell times will also influence overall clearance and the use of smaller dwell volumes for longer periods of time will help to minimize sodium clearance.

In contradistinction to hypernatremia, since hyperosmolality is less common with hyponatremia, in some ways it is easier to employ renal replacement therapy in the setting of severe hyponatremia and concomitant renal insufficiency. Again, the focus needs to be on the rapidity of the correction of the serum sodium. In conditions of severe but asymptomatic hyponatremia of some chronicity, the rate of correction of serum sodium should parallel the rate of correction recommended in hypernatremia – approximately 10–12 mEq/L/day. Correction of chronic hyponatremia at a more rapid rate has been associated with the development of central pontine demyelinosis.

All of the manipulations described above for hypernatremia and renal failure can be utilized with hyponatremia and renal failure, with the understanding that the dialysate sodium concentration should now not exceed the serum sodium value by 10–12 mEq/L. Conventional hemodialysis machines can be adjusted to produce dialysate with a sodium concentration as low as the mid-120s. In the very rare situation in which a child with profound hypernatremia (<110 mEq/L) were being hemodialyzed,

brief hemodialysis runs may be necessary initially to prevent too rapid correction of the serum sodium level and the attendant risk of central pontine demyelinosis. If dialysate is being custom prepared for peritoneal dialysis or hemodiafiltration, precise alterations in the electrolyte content can be made more readily to reduce the sodium gradient.

The local resources, the training of ancillary staff, the unique circumstances of each patient, and the comfort of the clinician with different modalities of renal replacement therapy will guide the choice of therapy when faced with renal failure and significant serum sodium anomalies. The actual modality of renal replacement therapy utilized is less important than careful attention to the rate of correction of the electrolyte anomaly, to the rate of urea clearance being achieved, and to the clinical response of the patient to on-going therapy.

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Research Methods

14 Molecular Biology

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Introduction

The field of molecular biology developed initially between the 25 years from 1940–1965, through the efforts of scientists which led to the discovery of the genetic code and the establishment of the role of RNA in the synthesis of proteins (1, 2). Thereafter, efforts of an increasing number of researchers have provided an explosion of key technological innovations allowing recombinant DNA technology to be applied to a wide variety of biologic problems and to enter clinical practice. Major milestones include discovery of restriction nucleases and DNA ligases, development of DNA libraries, DNA cloning procedures, nucleic acid hybridization techniques, the polymerase chain reaction (PCR), rapid sequencing techniques, and the production of transgenic animals. These advances permitted detection, amplification and engineering of DNA sequences, and delineation of the role of genes in cellular physiology and pathophysiology.

This molecular revolution has affected all sciences, including medical and clinical research, and has culminated in the completion of the human genome project (3). It has provided new insight into the complexity of living organisms that are now been studied by functional genomic and proteomic approaches that involve microengineering, computer technology, and bioinformatics.

This chapter illustrates the basic concepts of molecular biology that underlie various strategies that have been developed for studying living cells and organisms and summarizes molecular biology techniques that are used in nephrology research and clinical practice. It is therefore necessarily limited in breadth and depth. For more information, the reader can refer to the many specialized reference textbooks that are available (1, 2).

Basic Concepts

From DNA to Proteins

In the mid-1950s, the "central dogma of molecular biology" was proposed soon after the discovery of the DNA structure in 1953. To date, this dogma remains one of the keystones that guides the study of genetic human diseases.

DNA sequences are transcribed into RNA molecules that carry the flow of genetic information out of the nucleus to be translated into proteins. These processes have been well defined and are detailed in most introductory cell biology texts (1, 2). This simple concept has been however thoroughly revised, as genetic information has been shown to be conveyed in both directions through a complex series of feedback loops.

The link between nucleic acids and proteins is contained in the genetic code. Sixty-one of the 64 codons correspond to amino acids, whereas three correspond to termination codons. The code is said to be "degenerate", as it contains redundancies. Codons corresponding to the same amino acid generally differ by the nucleotide in the third position.

As a practical consequence of the degeneracy of the genetic code, nucleotide substitutions (point mutations) in the third nucleotide of a given codon may not change the primary sequence of a protein and are frequently found in nature, as they are not subject to natural selection. Point mutations in the first and second position result in an amino acid substitution or in a termination codon, either of which can dramatically alter protein structure and function.

In principle, each messenger RNA (mRNA) can be translated in any of the three possible reading frames determined by overlapping triplet codes. With few exceptions, only one reading frame produces a functional protein because stop codons encountered in the other two reading frames terminate translation. As the only punctuation signal is located at the start codon (ATG), the reading frame is set at the beginning of the translation and proceeds until a termination codon is reached. Thus, finding the correct reading frame and locating the start codon are essential steps in the process of cloning genes and defining their protein products. Mutations causing deletion or insertion of one or two nucleotides result in a shift of the reading frame (frameshift mutations) and cause production of aberrant protein products.

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Gene Structure

Complementary strands of chromosomal DNA are arranged in an anti-parallel fashion as dictated by hydrogen bond pairing of nucleotide bases. It is estimated that the human genome is composed of approximately 3 billion base pairs of DNA containing over 30,000 genes, arrayed on 23 pairs of chromosomes (4). Overall, the amount of DNA containing genes comprises a minority of nuclear DNA.

Traditionally, a gene is depicted as a "transcriptional unit" as illustrated in *Fig. 14-1*. The DNA double helix is represented as a line interrupted by rectangular boxes corresponding to exons, with its 5′ end on the left and its 3′ end on the right. Each gene is divided into two major regions, namely the promoter and the coding regions. The promoter region is located upstream near the 5′ boundary of the coding region and contains clusters of short sequences (less than ten base pairs) spread over a few hundred bases that bind transcription factors. These regulatory proteins mediate the attachment and activation of type II RNA polymerase, which mediates transcription along the DNA while unwinding the duplex and adding nucleotides to the growing RNA molecule. DNA sequences in the promoter region that bind to transcription factors are referred as *cis*-acting elements. In some

Figure 14-1

General organization and processing of a eukaryotic gene. A gene consisting of four exons and three introns is shown. The promoter region (black box) is located near the 5′ end of the gene. Transcription of the gene yields a primary mRNA transcript that contains both exon and intron sequences. Differential splicing of this mRNA transcript yields two mature mRNA species by inclusion or exclusion of exon 3 (hashed box). In order to be exported into the cytosol and translated, mRNAs need to be modified by polyadenylation of their 3′ end and capping of their 5′ end with methylated guanosines. Differential splicing results in two different protein isoforms which are encoded by a single gene.

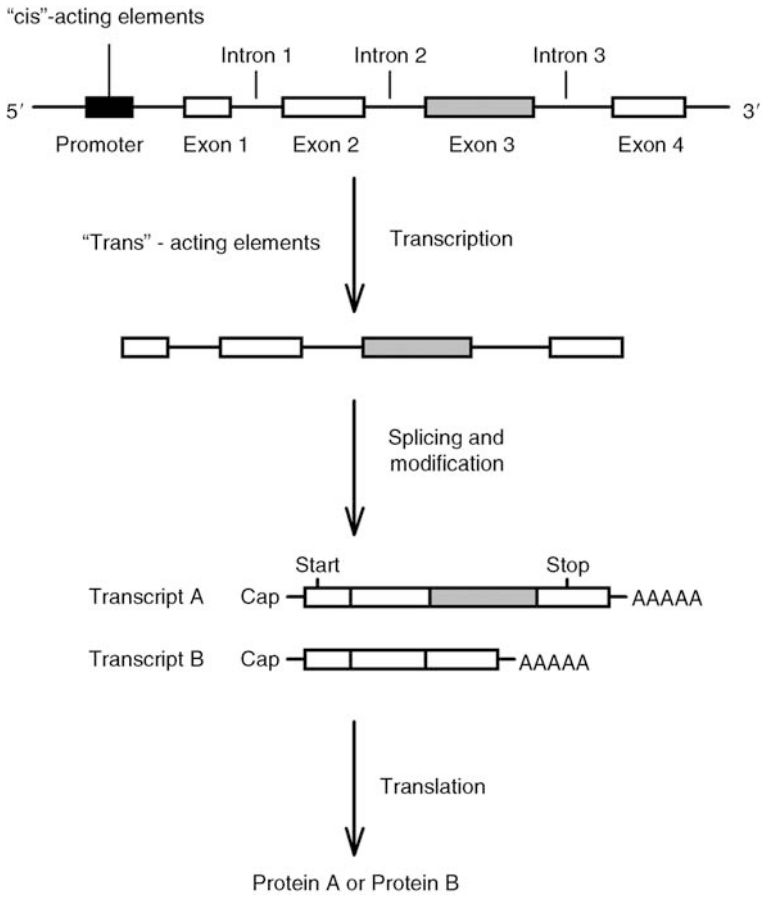
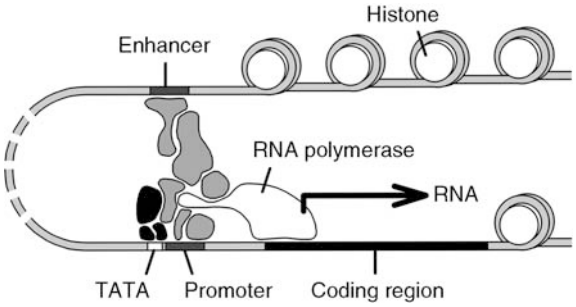


Figure 14-2

Transcriptional regulation in eukaryotes. Different transcription factors bind to the promoter and enhancer regions and position type II RNA polymerase at the starting point of transcription. General transcription factors shown in black interact with the TATA box and hold together the enhancer-promoter-RNA polymerase complex. When not activated, eukaryotic genes are hidden in nucleosomes which are composed of a central core formed by histones. Activation of eukaryotic genes requires remodeling of the chromatin in order to allow the transcriptional apparatus to interact with regulatory sequences.



cases, transcription factors also interact with other *cis*-acting elements (enhancers) that are located at a greater distance (up to a few thousand bases) from the promoter. The physical gap between the enhancer and the promoter explains the need for accessory factors that convey transcriptional signals to the RNA-polymerase through protein-protein interactions (*Fig. 14-2*). All molecules, generally proteins, which are physically involved in the regulation of transcription are referred to as *trans*-acting elements, because their DNA sequence resides in a different location of the genome (1, 2). The coding region contains information for protein synthesis. In this region, most genes are composed of a succession of exons and introns. Exons are coding sequences that ultimately transfer into mature mRNA, whereas introns are edited out of the newly synthesized transcript by a process called splicing.

Control of Gene Expression

As only a fraction of the available genes are expressed in a given cell at a given developmental stage, differential transcription and processing of genes provides for enormous diversity between cells within the same organism.

Although gene expression can be controlled at different levels, transcriptional regulation generally predominates. Information that governs transcription is located in DNA sequences that correspond to *cis*-acting elements or which encode for transcription factors. These sequences occupy a minimal portion of the entire genome but are key determinants of cell organization by ensuring a balanced expression of different genes that preserves phenotypic stability. They also provide for differences among cell types within the same organisms and are at the core of the evolutionary process.

Gene expression in eukaryote organisms is complex. Unlike prokaryotes, genes operating within the same metabolic pathway are not usually physically aligned along the genomic DNA and are often located on different chromosomes. The expression of functionally related genes is achieved by activation of shared classes of transcription factors that act on common structural motifs in DNA-binding domains (enhancers and promoters). Transcription factors are also often involved in developmental regulation. The eukaryotic type II RNA polymerase differs from its prokaryotic ancestor by the complexity of transcription factors that are required for its activation (5) (*Fig. 14-2*). This elaborate modular system allows forflexible and highly coordinated regulation of gene transcription. In transcription, DNA rearranges to allow interaction between transcription factors located at the promoter and at the enhancer site. The TATA box, which contains consensus sequences recognized by general transcription factors (TFIID, TBP, and TAF), is usually located approximately 24 bases upstream from the promoter and acts as a bridge that holds together the enhancer-promoter-RNA polymerase complex and positions the enzyme at the starting point of transcription (6). Although enhancers are not essential, they increase promoter efficiency.

A second characteristic of transcription in eukaryotic cells is related to the association of nuclear DNA with histones, forming nucleosomes. This particular organization prevents interaction between regulatory sequences and transcription factors unless conformational changes of the chromatin permit gene activation (*Fig. 14-2*).

Gene transcription can be chemically blocked in eukaryotic cells by methylation of specific DNA regions located near the promoter. Experimentally, the state of DNA methylation can be determined by inhibition of the nuclease activity of restriction enzymes that recognize non-methylated GC doublets.

Transcription Factors

The major characteristic of transcription factors is to contain specialized domains that allow for interaction with the DNA double helix.

Zinc fingers, helix-turn-helix (HTH) domains, helix-loop-helix domains, and leucine zippers are examples of DNA binding domains that are encountered in more than 80% of transcriptional factors (7). Zinc finger motifs are composed of a Zn ion holding together a peptide loop (the finger) through interaction with two histidines and two or four cysteines. The functional DNA binding domain is located in the amino acid residues at the base of the finger and recognizes specific consensus sequences that are generally located in the enhancer region. Steroid receptors are the most popular examples of this class of transcription factor, including glucocorticoid, mineralocorticoid, androgen, progesterone, thyroid hormone, and vitamin D receptors (8).

Similar DNA binding activity characterizes other classes of transcription factors. HTH domains are often found in proteins that have key roles during morphogenesis such as the homeobox group of transcription factors (9). Members of the helix-loop-helix class include proteins that control myogenesis, such as MyoD, that have been implicated in the transdifferentiation of myofibroblasts, which promote renal fibrosis (10). Leucine-zipper motifs are used by the Jun and Fos proteins. Both are members of the AP-1 heteromeric transcription complex that is involved in cell proliferation and regulation of cell matrix during fibrogenesis (11). An important transcription factor that is often implicated in renal diseases is nuclear factor-κB, which regulates many proinflammatory pathways and is itself under the control of other proteins, such as angiotensin II, known to promote inflammatory reactions in the renal parenchyma (12).

Transcriptional Control during Development

Transcriptional regulation plays a central role during development, by governing complex sequences of events that transform undifferentiated embryonic cells into highly specialized mature cells (1, 2). Three major classes of developmental regulatory genes have been identified. These include maternal, segmentation, and homeotic (Hox) genes. Each are expressed at different stages of cell maturation following complex sequences of activation in which gene products that are expressed at a given stage activate genes at a following stage. This highly sophisticated sequence of events creates a hierarchy according to which maternal, segmentation, and homeotic genes are expressed sequentially. Maternal systems activate expression of specific transcription factors, termed morphogens, that are responsible for the initial patterning of the embryo. Segmentation genes are zinc-finger proteins that control the boundaries of compartments, whereas Hox genes control the differentiation steps of each segment. Hox genes are characterized by a common HTH-type DNA-binding motif at their carboxy- terminus termed homeobox and are organized in the human genome in four different clusters (A,B,C,D) containing up to ten genes each. As the number of identified developmental transcription factors is rapidly increasing, their impact on renal development and involvement in congenital renal anomalies is a field of active research (13). Moreover, during the recovery phase of renal cell injury, cells reactivate several developmental genes in repair and restoration of function (14).

Modification and Splicing of mRNAs

During cleavage and processing of primary RNA transcripts, RNA molecules are capped at their 5′ origin by the addition of methylated G nucleotides, and a segment of poly-A residues is attached near their 3′ end (*Fig. 14-1*). These modifications are necessary to allow export of the transcript from the nucleus, to stabilize mRNA molecules, and to allow interaction with ribosomes. The presence of a poly-A tail is a major characteristic of mRNAs and constitutes a key tool to isolate mRNAs from other RNA molecules, mostly ribosomal, using complementary oligo-dT primers.

Before RNA is exported into the cytosol, splicing of intron segments is performed. As introns facilitate recombination events, they play a major role in the evolution of species and are often targeted in the process of generating knockout animals. Their physical structure may be more important than their actual nucleotide sequence, as these diverge more rapidly between species than exons (15, 16). Introns can range in size from 80 to several thousand nucleotides. They contain specific sequences at their extremities referred as the 5′ splice site (always ending with 5′-GU-3′) and the 3′ splice site (always ending with 5′-AG- 3′) that come together in the process of splicing. During splicing, a large catalytic heteromeric complex, termed spliceosome, is formed by the assembly of different ribonucleoproteins (1, 2). After RNA binding, spliceosomes bridge together two exons and excise their intron segment. Generally, each 5′ splice site pairs with the closest 3′ splice site in the spliceosome, producing only one form of mRNA. In some cases, however, splicing of RNA enables a single gene to produce different mRNA transcripts by jumping from a given 5′ splice site to a more distant 3′ splice site (*Fig. 14-1*). Each of these mRNAs yields different isoforms of the same protein that can be alternatively produced in different types of cells. Genome-wide analysis of

alternative splicing indicates that 40 to 60% of human genes have alternative splice isoforms (16, 17). Splicing of mRNA is an important regulatory step in the production of cell proteins that requires high degree of accuracy. In general, DNA mutations that involve splice sites (splice site mutations) do not prevent splicing but instead cause the normal partner to seek alternative splice sites, producing the synthesis of various abnormal proteins lacking one or more exons, as, for example, in Frasier syndrome (18). Constitution of gene banks containing thousand of gene transcripts has greatly facilitated the recognition of splicing variants of a given gene, which can be easily accessed online: (www.ncbi.nlm.nih.gov/IEB/Research/Acembly/).

Regulation of Proteins Synthesis

Mammalian ribosomes are the site of mRNA translation and composed of two asymmetric subunits - the 40S that binds mRNAs and the 60s that interacts with transfer RNAs. Eukaryotic cells can decrease their rate of protein synthesis in various conditions such as infections or heat shock. One important mechanism that mediates these types of nonselective responses involves phosphorylation of a repressor protein termed elF-2 that interacts with target regions containing the AUG start codon preventing ribosomal binding. A second gene-specific mechanism of translational regulation is termed attenuation and involves the formation of mRNA hairpins that block the translation. Similar mechanisms also regulate mRNA stability and degradation by RNases. Given the fact that each molecule of mRNA can serve as a template for multiple copies of proteins, the rate of mRNA degradation is a major determinant of protein abundance and is often the site of complex regulatory processes. In general, these processes operate primarily on unstable mRNAs (such as cytokines) that are stabilized under specific conditions by their interaction with *trans*-acting elements (19, 20). The stability of transferrin mRNA, for example, increases during iron deprivation, triggering the synthesis of more transferrin molecules (21).

Protein Sorting and Degradation

Newly synthesized polypeptides are processed by a complex network of cellular enzymes and other binding proteins that are arranged in a highly organized fashion in various organelles in the cell. Information resident in the primary amino acid sequence as well as the folded structure of the proteins allow each to be recognized and targeted to its ultimate destination (1, 2). Proteins which are synthesized in free cytosolic ribosomes are normally directed to the nucleus or the mitochondria, whereas membranous ribosomes are the site of synthesis for proteins that enter the reticuloendothelial system (co-translational transport) to be redirected to their final destination after being processed in the Golgi apparatus. An excellent example of this process is highly polarized epithelial cells in renal tubules. In these cells, transport proteins are located specifically on the apical or basolateral plasma membranes. This arrangement enables epithelial cells to perform net transport of solutes and water to either secrete or reabsorb fluid. Some proteins are able to self-assemble by spontaneous interaction among reactive amino acid groups, whereas other proteins, such as the V2 receptor, require assistance of molecules such as the Hsp70 system and chaperonins (22). These molecules control accessibility of reactive groups and maintain the peptide in a relatively flexible state until it reaches its final conformation. The final fate of most cell proteins is degradation into proteosomes. A process called ubiquitination that involves covalent linkage of small peptides called ubiquitins to target proteins precedes this step. Ubiquitination is also involved in important signal-transduction pathways, such as the nuclear factor- κB pathway - in which inhibitory subunits are degraded after stimulatory signals and activate various signaling cascades (23).

Recombinant DNA Technologies

Introduction

Until recently, molecular biology has addressed two major objectives; namely, to identify genes and analyze their function. With the completion of the human genome project, focus is shifting from the first to the second goal and to the more complex task of delineating complex patterns of gene expression. In the following sections, the more common recombinant DNA and protein analysis technologies are described, to illustrate common experimental procedures that are routinely used in molecular diagnostics as well as basic research.

Basic Recombinant DNA Technology

Hybridization and Detection of Nucleic Acids

Pairing of nucleotide bases in DNA and RNA allows a wide variety of specific recognition processes both *in vivo*

and *in vitro*. These not only form the basis of many critical cellular functions, but also provide the molecular biologist with tools to detect and study single genes.

The extraordinary specificity of nucleic acid-base recognition has been exploited in the process of hybridization of complementary DNA or RNA *in vitro*. Under appropriate conditions, a unique nucleotide sequence present within a complex mixture of nucleic acids can be identified with a resolution of greater than one part per million. Using standard techniques, DNA or RNA is isolated from cells or tissues, stripped of proteins, and denatured into single strands. When incubated under conditions favoring renaturation, complementary sequences reassociate. Experimental conditions (commonly temperature and salt concentration) can be altered to allow for only perfect or nearly perfect sequence matches. This is called stringency. Lowering stringency conditions is sometimes desirable to identify close relatives of particular nucleotide sequences. Thus, one can search for a gene or mRNA in kidney that is a close relative of a transcript expressed in other tissues as well as species. Nucleic acids are commonly fractionated by agarose gel electrophoresis. Under these conditions, the agarose acts as a molecular sieve, retarding larger strands while allowing smaller strands to migrate in the electric field placed across the gel. Fractionated DNA or RNA is then eluted from the gel or can be transferred to a filter and exposed to a labeled DNA probe. When the filter contains DNA, this process is called a Southern blot, whereas it is called Northern blot if it contains RNA (1, 2).

This same procedure has also been adapted to tissue sections to localize expression of mRNA transcripts by specific cell types within a complex organ such as the kidney. This technique is called *in situ* hybridization (1).

Restriction Endonucleases

The discovery by Arber in 1962 of bacterial nucleases that cut DNA molecules at specific locations constitutes one of the cornerstone in the development of recombinant DNA techniques (24).

Most restriction enzymes recognize palindromic DNA sequences, meaning that the 5′ to 3′ sequence in the upper strand is identical to the 5′ to 3′ sequence in the lower strand.

To date, nearly 1,000 different restriction enzymes have been purified. Each enzyme can produce defined DNA restriction fragments possessing specific nucleotide sequences at each end from any given DNA sample. With these enzymes, strings of DNA can be isolated, ligated into plasmid or phage genomes, and amplified (*Fig. 14-3*).

Historically, restriction nucleases have allowed construction of the first detailed maps of various genomes (restriction maps). They are commonly used for allelic discrimination by restriction fragment length polymorphism (RFLP). In this technique, DNA mutations which modify the recognition sequence for specific restriction enzymes can be identified by the length of the restriction reaction product.

DNA Amplification Using Procaryotic Systems

The possibility of replicating specific strings of DNA to obtain quantities sufficient for analysis and further manipulation is central to all recombinant DNA technologies. In the early 1970s, work by Boyer and by Cohen provided the first fundamental tools for DNA cloning with the discovery of DNA ligases and the characterization of bacterial plasmids.

Plasmids are circular molecules of DNA that replicate in the cytoplasm of bacterial cells (*Fig. 14-3*). Specific regions of plasmids not vital for vegetative growth under laboratory conditions can be engineered using restriction nucleases for insertion of exogenous DNA fragments. When inserted into *E. Coli* cells, plasmid genes encoding for antibiotic resistance are expressed, allowing selection of bacteria that have been transformed. During this process, the inserted DNA is replicated along with the rest of the bacterial genome. This technology enables amplification and characterization of virtually any DNA string of appropriate size.

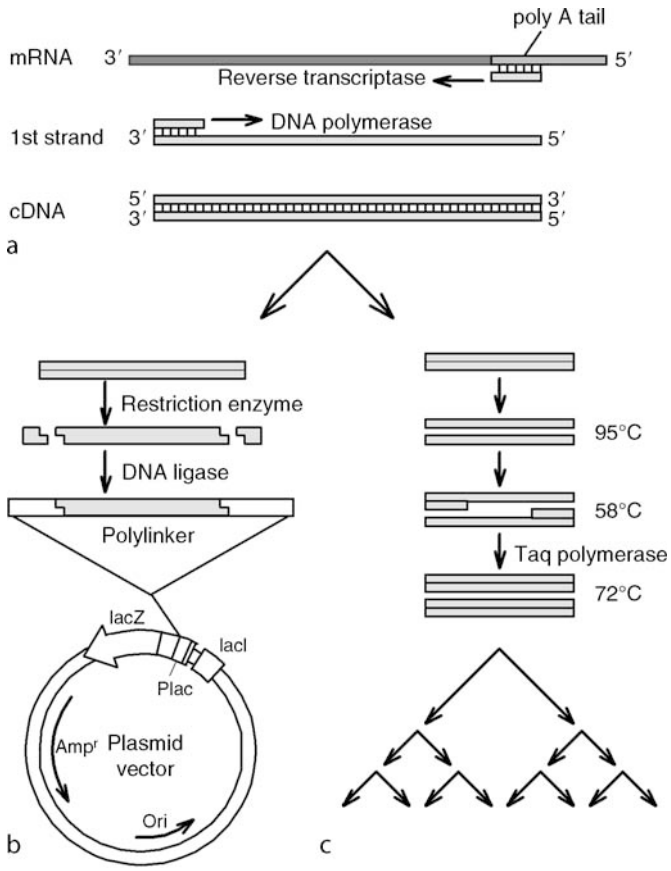
Because bacteria has a mean generation time of approximately 20 min during exponential growth and contains up to several hundreds copies of plasmids per bacterium, virtually limitless amounts of DNA can be grown and harvested routinely. Most available plasmids are engineered to incorporate a polylinker corresponding to a portion of DNA that contains multiple restriction sites that facilitate cloning of DNA fragments generated by various restriction enzymes.

Because of their simplicity, DNA is generally cloned into plasmids. The relatively low efficiency of bacteria transformation with plasmids limits their use however, when generating DNA libraries. In this case, bacterial viruses such as the *bacteriophage* λ, are more advantageously used.

The λ phages are composed of a head that contains the viral genome and a tail that infects bacterial cells with high efficiency. Phages can be packaged *in vitro* after insertion of exogenous DNA and are replicated in

Figure 14-3

Basic enzymes and techniques in recombinant DNA technology. The figure summarizes basic procedures used in routine laboratory experiments. a. mRNA molecules are reverse-trascribed into cDNA using enzyme reverse transcriptase in order to synthesize the 1st complementary DNA strand, which is then used to generate double-stranded complementary DNA (cDNA) using a DNA polymerase. Both enzymes require prior priming with complementary oligonucleotides. b. cDNA and other DNA molecules can be amplified in bacteria after insertion into plasmids using restriction enzymes and DNA ligase. Recombinant plasmids generally contain a polylinker that offers different restriction sites to facilitate insertion of exogenous DNA. In addition, they are engineered to contain antibiotic resistance genes (Ampr) for selection of transformed bacteria and often contain other useful genes like the lacZ in this example, which allows color detection of colonies transformed with "empty" plasmids. c. Alternatively DNA can be amplified by PCR with the Taq polymerase. Repeated cycles of DNA denaturation (95°C), primer annealing (58°C in this example), and DNA synthesis (72°C) allow for exponential replication of DNA strands encompassed by the two primers. When reverse-transcription and PCR are combined, the procedure is referred to as RT-PCR.



bacteria. Both λ phages and plasmids can amplify DNA fragments up to 20 kb. They are ideal for cDNA (see below) and other relatively small DNA molecules, but are insufficient for large strings of genomic DNA. In these cases, other vectors can be used. These include cosmid vectors, containing elements of both plasmids and λ phages that can accommodate up to 45-kb fragments or bacteriophage P1 housing up to 100 kb of exogenous DNA. If larger fragments need to be replicated, plasmid vectors based on the *E.Coli* F vector can incorporate up to 300 or 1,000 kb of DNA. The bacterial artificial chromosome (BAC) is a derivative of the F plasmid and is present in very few copies per *E.Coli* cell. Currently, BACs are the preferred vectors for genomic DNA libraries (1, 2). Yeast artificial chromosomes (YAC) can also be used for the same purpose.

DNA Amplification with Polymerase Chain Reaction

The second breakthrough in DNA amplification was achieved in 1985 by Mullis and coworkers who developed PCR (25).

PCR permits selective amplification of minute quantities of DNA, facilitating every aspect of molecular biology research and diagnostics including site-directed mutagenesis, labeling, and sequencing of DNA. Fixed tissues on slides or small tissue fragments, such as renal biopsy specimens, can also provide sufficient material for PCR amplification.

PCR reaction has nowadays replaced Southern and Northern blotting in most recombinant DNA applications.

PCR relies on the binding of two chemically engineered priming oligonucleotides (primers) that flank a region of DNA, to amplify the region located in between (*Fig. 14-3*). Primers are complementary to the opposite DNA strands. Addition of DNA polymerase results in the synthesis of new DNA. Repeated cycles of denaturation, annealing, and DNA synthesis are performed in a chain reaction such that the newly synthesized strands become templates for further DNA synthesis. This process exponentially increases the number of DNA copies containing the sequence of interest. This allows isolation of a given DNA string from the genomic DNA or from a pool of cDNA molecules (see below).

PCR reactions are performed using thermostable DNA polymerase species derived from the thermophilic bacterium Thermus aquaticus (Taq polymerase), which retain activity after being heated to 95°C. A number of modified enzymes that guarantee reliable DNA duplication or amplification of long DNA strings are commercially available.

Since the complete sequencing of the human genome, *in silico* PCR can now be performed to identify PCR products.

Amplification of mRNA with Reverse Transcription

In several experimental circumstances direct amplification of mRNAs is required.

mRNA transcripts reflect the actual genes that are activated in a cell system and contain nucleotide sequences which can be directly translated into proteins, obviating the tedious task of sorting exon segments from introns when working with genomic DNA.

This process is achieved with a reverse transcriptase derived from retroviruses, which is one major exception to the central dogma, as it harbors their genetic information in RNA molecules that are copied into DNA on infection of host cells.

The DNA obtained by reverse-transcription is called cDNA; it reflects the nucleotide sequences of mRNAs (*Fig. 14-3*). Similar to DNA polymerases, reverse transcriptase requires complementary oligonucleotide priming to begin transcription. Oligo-dT hybridizing to poly-A tails or random primers can be used to reverse-transcribe mRNA molecules (3, 9) in a non-selective manner.

Once converted into cDNA, nucleic acids can be ligated into vectors or directly amplified by the PCR reaction, a process that is referred as reverse transcription polymerase chain reaction (RT-PCR) (*Fig. 14-3*). In other circumstances, gene-specific primers are designed to amplify selected mRNA molecules.

As investigators are progressively turning their attention from genomic analysis to the analysis of gene expression, this process has become extremely important. It permits the generation of collections of "Expressed Sequence Tags" (ESTs), which provide extremely rapid tools to identify genes, evaluate their expression, and construct genome maps. ESTs are small DNA sequences that are generated by RT-PCR reactions and which when pooled together, represent a collection of DNA sequences that are expressed in a given cell or tissue. These "tags" can be used to identify specific portions of the genome that encode for a given gene, "fish-out" similar genes, or identify splicing variants of a given mRNA, using online collections of ESTs.

Sequencing Nucleic Acids

The highly specific binding of small oligonucleotides to DNA also lies at the heart of the dideoxy chain termination sequencing of DNA.

DNA sequences are obtained from a uniform population of DNA, obtained by PCR.

After DNA is denatured, complementary primers are added. In the traditional manual sequencing, synthesis of complementary radioactive DNA strands was initiated by DNA polymerase after addition of ^{35}S-labelled radioactive deoxy-nucleotides in four different reactions containing one of the four dideoxy-nucleotide analogs of G, A, T, or C. In each reaction, chain termination occurs if a dideoxy analog is inserted in the newly formed DNA strand, preventing further extension.

Modern automated sequencing machines apply these basic principles using fluorescence labeled deoxynucleotides. The reaction products are resolved on sequencing gels or electrophoresis capillary. As DNA advance along the electric gradient, a laser beam is used to excite their fluorescence, which is read and analyzed by a computer. Reliable DNA sequences can generally be obtained for more than 500 nucleotides per run, and multiple lanes can be read simultaneously.

Analysis of Gene Expression

A critical issue in normal physiology and renal pathophysiology is the determination of the expression of given genes under different cellular and environmental conditions.

Classically, gene expression analysis is performed by protein detection with specific antibodies in Western blotting or, when antibodies are not available, by measuring the amount of mRNA transcripts. Densitometric methods have been developed to compare the amount of expressed protein or mRNA, with respect to a control preparation. These semi-quantitative techniques however, have severe limitations. They require relatively large amounts of starting material, can only study a limited numbers of genes simultaneously, and require development of specific probes such as antisera. To overcome some of these limitations, other techniques have been developed and are briefly reviewed.

Real-Time RT-PCR

Unquestionably, RT-PCR is more sensitive than the traditional Northern blot analysis to measure levels of mRNA expression, and can be performed from limited amounts of mRNA.

The major difficulty in quantifying mRNA by RT-PCR however, is related to the exponential nature of the method, which tends to amplify differences when comparing levels of expression in different biological conditions or biological systems.

For this reason, competitive RT-PCR was initially developed and was based on the use of internal standards sharing the same priming sequences as the transcript of interest, which were added to the mixture to act as competitors during the reaction (26). This method was however time consuming and has now been replaced by real-time PCR and micro-array analysis when quantitative expression of multiple genes needs to be evaluated.

Real-time RT-PCR allows detection of PCR products as they are being formed (*Fig. 14-4*), using quenched fluorescent dyes linked to the 5′ end of one primer that are released by the 5′ nuclease activity of the Taq DNA polymerase. The emitted light is measured in real time during PCR reaction and is proportional to the amount of PCR product. The number of cycles required to cross a given fluorescence threshold is inversely proportional to the amount of mRNA present in the original reaction mixture. Multiplex real-time RT-PCR represents an extension of this technique and is based on differential fluorescent labeling of primers that amplify for different genes. This permits comparison within the same PCR reaction of the relative amount of up to 3–4 different transcripts (27). Generally, a house-keeping gene that is presumed to be stably expressed, serves as an internal control, allowing correction of the results for the amount of RNA that was loaded in the initial sample reaction.

Multiplex real-time RT-PCR allows determination of gene expression even from extremely small samples such as renal biopsies - and has been developed for example, to detect gene expression in human renal specimens (28) or quantify viral genome copies in biological samples.

Differential mRNA Display Using DNA Microarrays

DNA microarray technology has become a valuable technique for comparative gene expression analysis. Transcriptomic DNA chips are composed of thousands of known expressed sequence tags or synthetic oligonucleotides, which are deposited in gridded arrays by a robotic spotting device on a solid support such as a glass microscope slide or a membrane matrix (29).

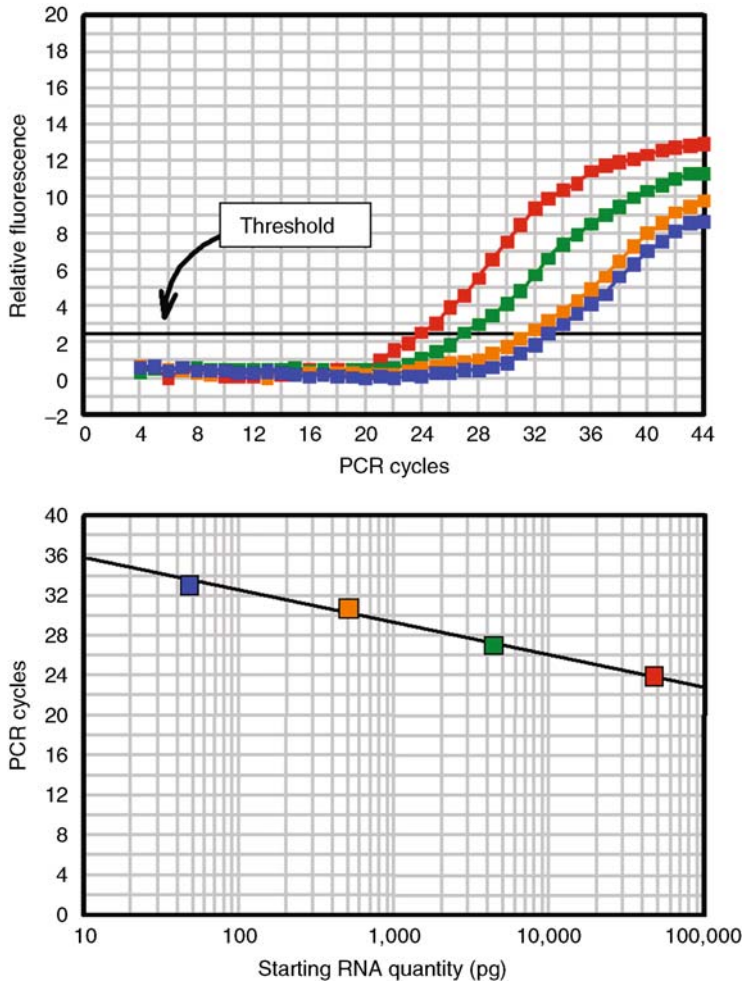
Oligonucleotide sequences are selected from databases such as GenBank, dbEST, or UniGene (30). Several thousand genes can be spotted on a single microscope slide for large screening. Other chips contain clusters of genes that are functionally related, as in oxidation chips or cell-cycle chips, for example. Two populations of mRNA are labeled with different fluorophores, hybridized to the same chip, and analyzed with a laser scanning device (*Fig. 14-5a*).

The intensity of the fluorescence emitted by each dye is proportional to the amount of RNA that has hybridized at a given location, reflecting the level of a gene expression represented by that spot.

Despite increasing developments, this technique has limitations, particularly in terms of chip reproducibility and variability in the efficiency of labeling and hybridization, which generally need to verify the obtained results by standard RT-PCR or real-time RT-PCR.

Figure 14-4

Real-time PCR. Figure illustrates an actin calibration curve. Total RNA was extracted from HK2 cells and loaded in increasing concentrations in the sample reaction. Fluorescence was measured in real-time as primers were incorporated in newly synthesized PCR fragments with an ABI Prism 7,700. The number of cycles required to cross a given fluorescence threshold shown in the upper panel is proportional to the initial amount of loaded mRNA as shown in the lower panel.



The enormous quantity of information generated by expression data from thousands of genes requires sophisticated computer analysis to generate meaningful results (31). Computer-based algorithms have been developed to recognize patterns of gene expression within complex genetic networks such as the human genome. The so-called cluster analysis is a powerful statistical tool, permitting grouping of genes in hierarchical clusters that follow similar patterns of expression (*Fig. 14-5b*). This information can then be used as a molecular fingerprint for diagnosis or monitoring response to therapy. It may allow detection of subtle changes of gene expression and may identify functions and interactions of uncharacterized proteins, by grouping them into clusters of genes whose function is known.

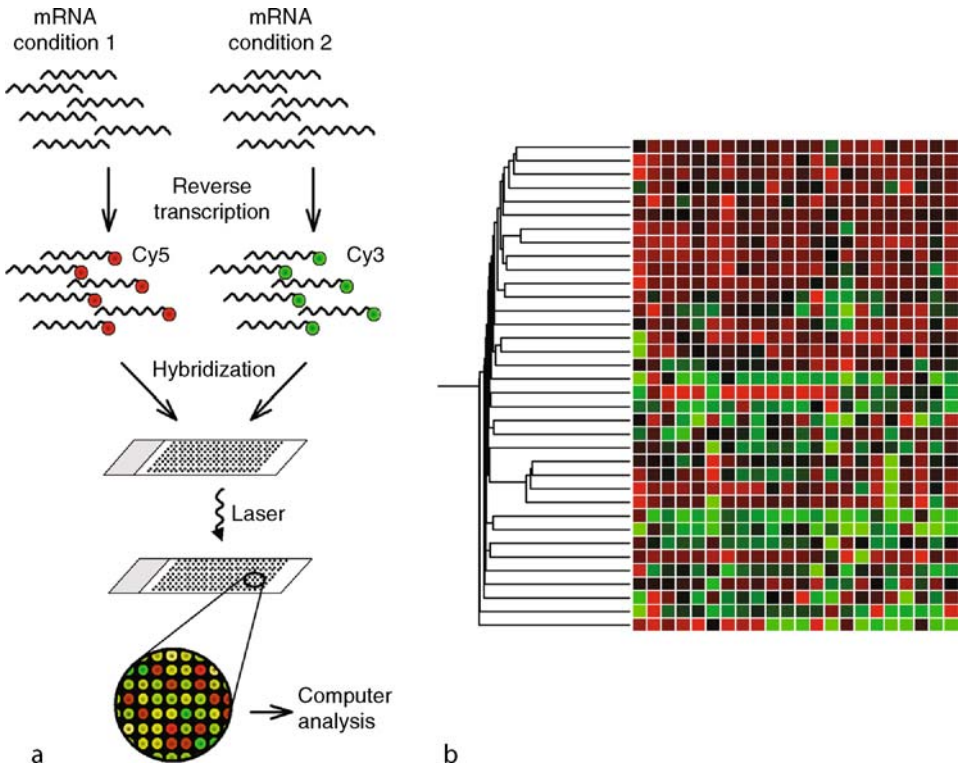
Microarray Technologies for Genomic Analysis

Similar approaches to the transcriptomic chips have also been developed for the analysis of genomic sequences.

In "comparative genomic hybridization" (CGH) for example, genomic gain or loss of particular genes can be detected, permitting identification of small deletions or duplications throughout the genome.

Figure 14-5

Differential display on DNA microarrays. (a) mRNA obtained from two different samples are labeled with two fluorescent cyanine dyes with one round of reverse-transcription. The fluorescent targets are then pooled and hybridized under stringent conditions to the clones on the microarray chip. The emission light is measured with a scanning confocal laser microscope at two different wavelengths that are specific for each dye. Monochrome images are then pseudo-colored, combined, and analyzed with a suitable computer software. (b) Cluster analysis of results obtained in a microarray experiment. Rows represent individual genes whereas columns indicate different experiments. Genes that are the most up-regulated are colored in red, while down-regulated genes are indicated in green. By analyzing patterns of gene expression, genes that behave similarly are grouped together in hierarchical order, to identify patterns of gene expression.



Microarray chips have also been developed to detect gene mutations. In this case, the target is limited to few genes. Sequential small fragments of the genes of interest are arrayed onto the chip, which detects even single nucleotide changes, when hybridized with amplified DNA obtained from patients carrying the mutation.

Likewise, microarrays can also be used to screen for single nucleotide polymorphisms (SNPs). Chips containing over 1.6 SNP markers have now been developed allowing whole-genome genotyping to search for associations of particular SNPs with given clinical conditions. Using this approach for example, genes that are involved in the pathogenesis of Systemic Lupus Erythematosus have been identified (32), and whole genome association studies can be done with large populations.

Gene Cloning and Analysis of Cloned Sequences

Until recently, one of the primary goals in molecular research was to clone genes responsible for diseases. This task has been largely accomplished for monogenic diseases, although several genes, mostly encoding for proteins involved in rare diseases, still need to be identified. Other diseases have a polygenic base of inheritance and a vast majority of these that regulate complex patterns of inheritances still need to be identified.

Cloning of genes responsible for genetic disorders can be achieved without prior knowledge of the molecular nature of the disease by a strategy termed positional cloning, which is based on the genomic localization of

the locus of interest using genetic markers present at a known chromosomal location.

Alternatively, gene cloning has been performed by screening tissue-specific collections of recombinant vectors containing sequences of cDNA, termed cDNA libraries. This basic approach can be refined by the use of subtractive libraries for example, that are obtained by subtracting unwanted mRNA species from the library before screening.

Currently, the process of cloning genes has been completely revolutionized by the creation of electronic databases. More than 100,000 partial ESTs sequences that cover most human genes have been collected (33), and thousand of putative genes that were generated by digitally sorting out and mending together potential exon regions of the human genome have been obtained.

These sequences are collected in databases that can be accessed online, which constitute virtual DNA libraries to be screened electronically using partial DNA or protein sequences. Tags sequences can be obtained from differentially expressed proteins or DNA molecules (identified by microarray analysis for example) or from sequences of related genes. Once a putative gene has been identified, its sequence can be directly amplified from mRNA or from genomic DNA, by RT-PCR or PCR, respectively. As already mentioned, ESTs databases also represent invaluable tools for "serial analysis of gene expression" (SAGE), which is considerably increasing our knowledge of the human transcriptome (33).

Other techniques that are used to identify genes include library screening with antibodies, functional assays, or by protein-protein interaction. Using expression plasmids, peptides encoded by exogenous cDNAs can be directly translated in the bacterial lawn and identified with specific antisera (34). With a similar approach, phage epitope libraries expressing randomly generated oligopeptides can be screened to find domains recognized by antibodies or by other proteins (35). The recognized epitope sequence generally corresponds to a partial amino acid sequence contained in the natural antigen or binding protein. From this sequence, the protein can be identified or cloned. This technique is particularly powerful for identifying autoantibodies or for cloning proteins by their reciprocal interactions such as in receptor-ligand association.

The two-hybrid system is an alternative strategy which permits investigators to fish for clones which code for peptide that interact with other proteins offered as bait in the screening process (1, 2).

The strategy of expression-cloning relies on screening cDNAs that produce functionally active proteins when expressed in a suitable system, such as Xenopus oocytes. Assays using oocytes are usually used to identify and study by electrode impalements, patch clamping, and flux techniques proteins involved in membrane transport. Large quantities of RNA (cRNA) can be synthesized *in vitro* and injected into oocytes (36).

Online programs provide important clues regarding the nature of newly cloned cDNAs and proteins, such as structural aspects, including membrane-spanning domains or antigenicity, presence of specialized amino acid sequences coding for functional domains such as phosphorylation, glycosylation, or targeting domains to specific cell compartments (www.ncbi.nlm.nih.gov/IEB/Research/Assembly/). Sequence alignment in databases helps define relationships with other genes and identify functional motifs, such as DNA-binding and protein-binding domains, that can give important clues to the biologic function of the newly cloned sequence (37). In addition, important information is also contained in the promotor region that can be identified and screened for consensus sequences that help defy the physiological role of the protein in the cell.

Gene Expression and Silencing in Cell Cultures (see also Chapter on In Vitro Metods in Renal Research by Dr. Wilson in this text, Chapter 15)

One important aspect of recombinant DNA technology is the demonstration of the biological role of a selected gene. The easiest and often first approach, is to express or suppress a given gene in cells that are cultured *in vitro*.

Cells can be obtained from tissues after disruption of the extracellular matrix with enzymes, such as trypsin or collagenase. Cells of the same type need thereafter to be purified using different techniques that are based on cell size, resistance to specific conditions, or expression of specific markers. In the latter case, cells are often isolated by fluorescence activated cell sorting (FACS) or with beads that bind to specific antigens. In highly organized tissues, such as the kidney, laser captured microdissection can help isolate fragment of tissues containing only few cell types, which greatly facilitates the following purification steps. These "primary cell cultures" are extremely powerful biological models, because they often retain much of their original phenotype. Unfortunately, they often grow slowly, their preparation is expensive and time consuming, and they tend to stop growing after few passages due to a process termed "replicative cell senecesce". This process is in part caused by the lack of telomerase, which prevents shortening of telomeres at each cell division. Introducing the catabolic sub-unit of the telomerase gene allows in some cases to obtain "immortalized cell lines". In most cases however,

mammalian cells stop dividing as they activate cell-cycle "check-point mechanisms". In order to inactivate these mechanisms, viral pro-oncogene genes are usually inserted to generate "transformed cell lines". These cells proliferate indefinitely, but unfortunately tend to loose their phenotype. To partially circumvent this phenomenon, strategies aimed at turning-off the pro-oncogene can be used. The thermo-sensitive Simian Vacuolating virus 40 T antigen (SV40 Tag) for example, promotes undifferentiated cell proliferation at 33°C, but is turned-off at higher temperatures. Conditionally immortalized cell lines can therefore be grown to confluence at 33°C, but will stop proliferating at 37°C and will, in most cases, recover part of their original phenotype (38).

To study the effects of a given gene, cell cultures and cell lines can be directly obtained from specimens of patients lacking a given gene. Alternatively, genes can be over-expressed or suppressed in cell cultures. Genes are usually inserted into expression vectors under the control of a potent viral promoter, like the CMV promotor. Cells are thereafter "transfected" with these vectors. Transient transfection allows for gene expression for a few days. Stably transfected cell lines can also be obtained by transfecting cells with a linearized vector that will insert itself randomly into the cell genome in few cells. As most of these vectors contain an antibiotic resistance gene, stably transfected cells can be selected and purified.

Vectors harboring mutated genes can also be engineered by site-directed mutagenesis techniques, which allow selective mutatation, deletion or insertion of peptide residues of a given protein. Once expressed, these mutated peptides produce information on the function of various domains of a single protein (39).

Specific cDNAs sequences can also be fused to sequences encoding for fluorescent proteins (GFPs). Once, these vectors are transfected into cells, it will prompt the synthesis of a fusion protein which is composed of the protein of interest and a GFP tag, which helps to follow the protein expression in the cell by fluorescence microscopy.

Similarly, gene regulatory regions such as promoters can be transfected. Their effects on a neighboring reporter gene can then be determined using a gene product that is easily assayed, such as chloramphenicol acetyltransferase or a luciferase.

In some cases, non mammalian cells are more advantageously used. *Xenopus* oocytes are popular systems to express membrane transport proteins. The cystic fibrosis gene was among the first gene to be isolated without knowledge of its actual function. Oocytes were used to demonstrate its function as an epithelial cell chloride channel (40). Other fundamental membrane transport proteins have similarly been cloned using Xenopus oocytes, including Na-H exchangers (41), bumetanide-sensitive Na-K-2Cl and thiazide-sensitive NaCl cotransporters (42), the renal outer medullary adenosine triphosphate–regulated potassium channel (43), multiple aquaphorin water channels (44), and the amiloride-inhibitable epithelial Na + channel, or ENaC (45).

These studies have provided molecular links between epithelial cell transport data and the expression of specific genes within individual kidney epithelial cells.

Detailed knowledge of these transporter proteins has also enabled the identification of specific gene abnormalities in humans that cause inherited disorders of renal tubular function including nephrogenic diabetes insipidus (46) and Bartter's (47, 48), Gitelman's (49), and Liddle's (50) syndromes.

Although gene suppression in animal model is probably the most powerful approach to study the function of a given gene, another approach is to inactivate its mRNA. This is achieved by a technique called RNA interference (RNAi, siRNA), in which double stranded RNA molecules matching the sequence of interest are introduced into cells, where they hybridize with their complementary mRNA, causing its degradation. Fragments of degraded RNA form other double-stranded RNA, which continues to eliminate more targeted mRNA. In addition, some RNA molecules enter the nucleus where they inhibit directly gene transcription by interaction with the targeted genomic sequence (51).

The range of applications of recombinant DNA technologies to protein expression expands well beyond the above mentioned techniques. Bacteria for example, can also be "transformed" with recombinant plasmids containing bacterial promoters that activate transcription of genes fused to specific detection sequences. These fusion proteins can then be purified in large quantities and used for functional studies or as immunogens to raise antisera. As mammalian proteins expressed in bacteria are not post-translationally modified, viral expression systems have been developed, allowing the production of recombinant proteins by viral infection of cultured insect cells (52). These proteins are then properly processed, glycosylated, and phosphorylated. A similar approach can be used in yeast. In fact, yeast cells have in fact become particularly interesting for the study of the cellular effect of given proteins. The 6,000 yeast genes have been fully sequenced, are particularly easy to mutate, and large collections of well characterized mutant strains are available. This is enabling researchers to perform functional genomics and proteomics studies in a simple organism and has

enabled dissection of genomic control mechanisms as well as identification of several proteins that regulate a variety of cell functions, including endocytosis and membrane fusion (53).

Expression and Suppression of Specific Genes in Animal Models

In vitro cell expression systems are very powerful tools, but have limitations when studying the role of genes in multicellular organisms in which a gene's expression or lack thereof has complex effects on an animal's development and physiology. In these cases, genetically engineered animals which are modified in genes that are homologous to their human counterparts, are used advantageously. Whenever the animal phenotype is similar to the human disease, it always represents an extremely powerful tool to understand the disease and test new treatments.

Likewise, scientists have studied genes which are responsible for different animal phenotypes or have been genetically manipulated to modify activity of given genes, for which the human homologue has not been associated with specific diseases. Most of these experiments have been performed in mice, but also in other organisms such as *S. Cervisiae, C. Elegans, Arabidopsis,* and *Drosophila*. This has led to the constitution of a collection of animal mutations that represent invaluable repertoire of candidate genes for human diseases which can be tested based on clinical phenotypes. This approach is referred as "reverse genetics", because instead of identifying a given gene using biological material obtained from patients, scientists begin their search from experimental gene mutation data, to identify by phenotypic homology human diseases.

While engineering animal models of a given disease, different strategies are used depending on the effects of the human mutations ("loss of function" or "gain of function") and the mode of inheritance (recessive, addictive or dominant).

In most cases the removal of a given gene, termed "knock out", is the first approach to reveal the function of its encoded protein. Other strategies are aimed at changing the levels of expressions of a given gene or changing its expression in specific tissues or in time. This latter approach, which is generally based on the use of inducible promoters, is particularly interesting when analyzing genes that are implicated during development or when the mutation is lethal in animals. In this case, the gene of interest can be "turned-off" only after birth, when the animal is fully developed. In some cases, researchers have adopted a dominant-negative strategy, when over-expression of a mutant protein can inhibit by competition, the activity of its wild-type homologue, which continues to be normally synthesized. In general, gene replacement or addition is more complicated and time-consuming than gene knock-out. Regardless of the strategy that is used, all these genetically modified animals are termed "transgenic" and their artificially modified genes are referred as "transgenes".

Introduction and disruption of specific genes into amphibians and insects such as *Xenopus* and *Drosophila* are particularly useful in the analysis of developmental genes and have been used extensively to characterize various developmentally specific transcripts governing tissue-specific differentiation, including in the kidney (1, 2). This research is greatly facilitated by the ability to manipulate easily, cells of the earliest embryo stages and have produced a fundamental understanding of pattern formation in these animals.

For most human diseases, mice have become the animals of choice, because their genes can now be easily manipulated, their genome has been fully sequenced, a full range of techniques have been developed to analyze their phenotype and they can be rapidly bred to produce heterozygous and homozygous mutants or compound transgenic animals, in which more than one gene has been modified (54–56).

In some cases, production of transgenic animals may be performed by injection of the transgene directly into the pronucleus of a one-cell stage embryo so that it can integrate into the genome. In mice, a vector carrying the transgene is introduced in embryonic stem cells (ES) which are allowed to proliferate *in vitro.* The rare cells where homologous recombination with the original gene has occurred are then selected, and injected with a micropipette into the embryo at its early stage. This leads to the formation of a chimeric animal that will carry the mutation in a significant percentage of its germ lines. Mice are then bred to produce heterozygous male and female off-springs, which, once mated together, will produce homozygous animals. Both heterozygous and homozygous animals can then be studied.

Conditional mutants allow for the disruption of genes in specific tissues at given times.

To express a specific protein in podocytes, for example, the nephrin promotor can be used, in order to activate the transcription of a given gene only in cells that can activate this promotor (56). Using this strategy for example, researchers have over-expressed the macrophage migration inhibitory factor (MIF) gene in podocytes, demonstrating the development of mesangial sclerosis in the presence of high levels of MIF (57).

To knock-out genes at a given time, site-specific recombinant systems are used, like the Cre/Lox system. For this purpose, a fully functional gene or a portion of this gene is flanked by small sequences of DNA corresponding to the "lox" sites, which are recognized by the Cre recombinase protein. Transgenic animals are then mated with mice expressing the Cre recombinase under the control of an inducible promotor that excise the gene of interest, when activated (56).

Protein and Peptide Analysis

Principles and Techniques

The principle of protein analysis has considerably evolved over the past years in parallel with key technological advancements, especially in the field of mass spectrometry.

The basic principle in proteomic analyses is that the digestion products of a given peptide create a fingerprint of the original protein, which permits its identification.

In most cases, identification of a protein requires a preparative step, to obtain a purified sample. Two-dimensional (2D) electrophoresis is frequently used for this purpose, while LC-Mass and MALDI-TOF are commonly used for the analysis of digestion products.

Denaturing and Non Denaturing 2D-Electrophoresis

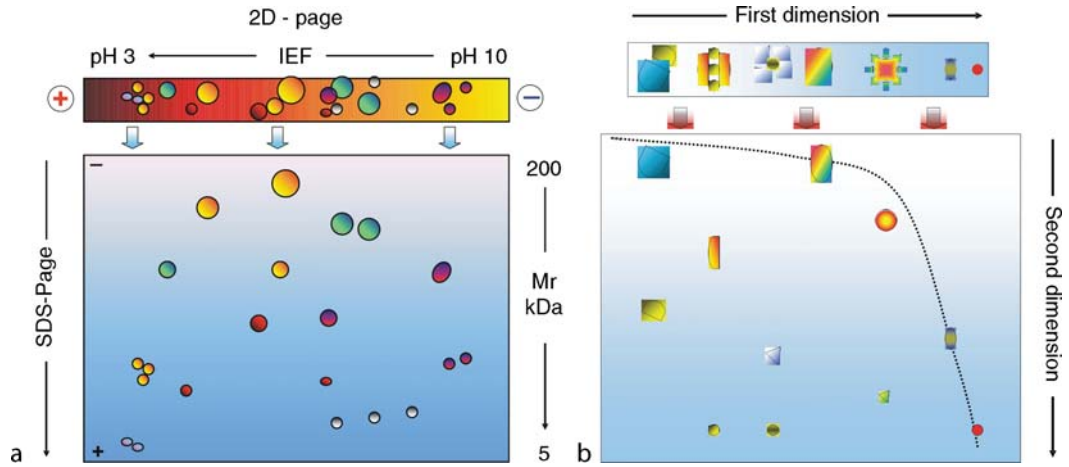
Classic 2D protein electrophoresis is the technique of choice for the analysis of plasma proteins or other complex biological samples. High-resolution is usually achieved by combining separation by charge and separation by size in denaturing conditions (IEF/PAGE) (*Fig. 14-6*) (58, 59). This can then be followed by micro-scale mass spectrometry that allows identification of individual spots. The development of soft gels has considerably improved the resolution of high molecular weight proteins, but requires denaturing conditions, which prevents the analysis of protein-protein interactions (*Fig. 14-6b*) (60). For this reason, new techniques that separate protein mixtures in low denaturing conditions have recently been developed. These include Blue-PAGE, which is performed on membranes, or the Nat/SDS PAGE, which is performed on a polyacrylamide substrate (61–63). This latter system helps to resolve protein aggregates and disclose protein interactions (*Fig. 14-6b*).

Protein Staining

Traditionally protein staining after electrophoresis has been performed with Coomassie R-250 and silver ions. New dyes allow differential protein expression analysis on

Figure 14-6

Schematic representation of a classical 2D-polyacrylamide gel electrophoreis (2D-PAGE) (a) and of a 2D electrophoresis in non- denaturing condition (Nat/SDS-PAGE) (b). In the former approach, proteins are first separated according to their charge in the presence of urea (IEF) and are then run in a polyacrylamide gradient that separates them on the basis of size. In Nat/SDS-PAGE protein complexes migrate unresolved in the first dimension and are then separated in denaturing conditions, in the second dimension.



2D-gels (DIGE). This technique is based on modification of selected aminoacid residues and has become a standard application of quantitative proteomics. Peptides are labeled with matched sets of fluorescent N-hydroxysuccinimidyl ester cyanines (NHS) that have different excitation-emission wave-lengths (64, 65). Use of thiol-based reagents (maleimide, iodoacetamide) increases specificity and reproducibility. Protein mixtures are label separately with different NHS dyes, combined and resolved on a single 2D gel that is analyzed with different fluorescence excitation wavelengths (66). The differential expression of individual proteins is by this means analyzed and quantified.

Mass Spectrometry

Proteins are generally characterized by mass spectrometry. As ionized molecules are accelerated through an electric field, they are separated, reaching the detector at different times, depending on their mass and charge. This "time-of-flight" (TOF) is specific to a given solute, allowing its identification. Whole proteins need to be first ionized by electrospray ionization (ESI) or matrix-assisted laser desorption/ionization (MALDI). The commonly used "MALDI-TOF" procedure indicates that the MALDI sample preparation is followed by TOF mass analysis, while in the Surface Enhanced Laser Desorption and Ionization (SELDI) procedure, proteins are immobilized on solid supports or customized protein chips.

In the so-called "top-down" strategy, intact proteins are ionized and resolved in the mass analyzer. Alternatively, proteins are pre-digested into smaller peptides before mass analysis; a procedure referred to as "bottom-up". In the latter case, the source protein is identified by its pattern of digestion that creates a "peptide mass fingerprinting" (PMF), or by "*de novo* sequencing", tracking back the protein sequence from the mass sequence data using protein databases. Often, both 'top-down" and ''bottom-up" strategies need to be used to optimize protein identification.

Research Applications

Protein-Protein Interaction

One important question in protein analysis is to identify interactions between proteins, as these are at the core of most intracellular signaling pathways and are critical to the assembly of functional peptides.

Recent developments of the Nat/SDS-PAGE technique permit identification of protein-protein interactions in biological fluids (63). If proteins are extracted from cells or tissues, strategies based on binding to targets linked to solid supports are more advantageously used. These approaches can be further refined using recombinant DNA techniques, in order to construct target protein fragments enabling identification of domains that mediate protein-protein interactions.

Alternatively, the yeast two-hybrid system is based on the modular structure of gene activation and may be used for the same purpose. In general, the GAL4 transcriptional activator is exploited. This transcription factor has a DNA-binding domain and an activation domain, both of which must be in close association to activate transcription. By DNA recombinant techniques, two protein sequences acting as bait and target are fused with sequences encoding with one of the GAL4 domains. When expressed in yeast cells, the activation domain and the DNA-binding domain are bridged together when the two proteins interact and promote transcription of a reporter gene. This system helps to study interactions between known molecules and to clone new proteins using cDNAs libraries.

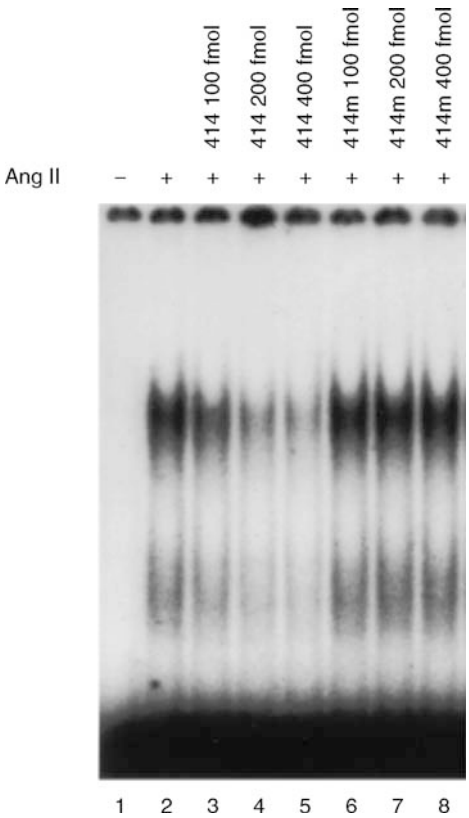
Similar prokaryotic systems have been developed based on the modular structure of bacterial RNA polymerases, in which target and bait cDNAs are fused to either the core enzyme or a Φ factor.

Protein-DNA Interaction

Techniques that analyze the regulation of DNA expression by transcriptional factors have helped to identify consensus DNA binding regions and their regulatory proteins. Most of these studies are based on the principle that protein-DNA complexes have high molecular weights and are therefore retarded when resolved by polyacrylamide gel electrophoresis. In addition, proteins interacting with DNA molecules tend to protect the nucleic acid regions to which they bind, from digestion with DNAse, which allows its identification (67). In *Fig. 14-1* for example, a nuclear extract of proteins obtained from cells stimulated with angiotensin II was co-incubated with strings of DNA that encode for the promoter of type III collagen. As shown, angiotensin II stimulation promotes synthesis of a protein that binds to the ^{32}P-labeled DNA target, forming macromolecular complexes that are retarded in the gel.

Figure 14-7

Gel retardation assay. Figure demonstrates binding of regulatory nuclear proteins to cis-elements in the COL3A1 promoter after angiotensin II (Ang II) stimulation. Nuclear extracts were obtained from Ang II stimulated cells (Ang II+) and incubated with a 32P-labelled oligonucleotide (414) that contains sequences +3 to +20 of the COL3A1 promoter. Ang II stimulates synthesis of a peptide that binds to the target DNA and retards its migration in the gel (lanes 2). This reaction can be competed with increasing amounts of non-labeled wild-type oligonucleotide (lanes 3–5) but not with a cold mutated analogue sequence (414m) (lanes 6–8). In the absence of Ang II stimulation (AngII −) no DNA-protein complex generating gel retardation is observed (lane 1).



The Building of the Podocyte Protein Map

Unquestionably, the completion of the human genome sequencing has expanded considerably our possibilities to understand and study cell molecular processes. Though over 30,000 genes are encoded in the human DNA however, only a portion is expressed in a give cell. Cell- and tissue-specific cDNA libraries have partially overcome this limitation but do not completely reflect the cell protein repertoire and the levels of expression of individual peptides. Recent advances in protein analysis have now helped to build protein maps. A podocyte protein inventory for example, will allow the study in depth of the signaling pathways regulating cell function in these highly specialized cells, which play a key role in many renal diseases. Definition of the podocyte protein map is in progress and should allow, when completed, to study changes observed under pathological conditions (*Fig. 14-8a*). Currently, podocyte cell lines are being used. Future studies may directly use podocyte expanded from kidney biopsies or from urines of patients with glomerular diseases.

Clinical Applications

Proteomic Analysis of Biological Fluids

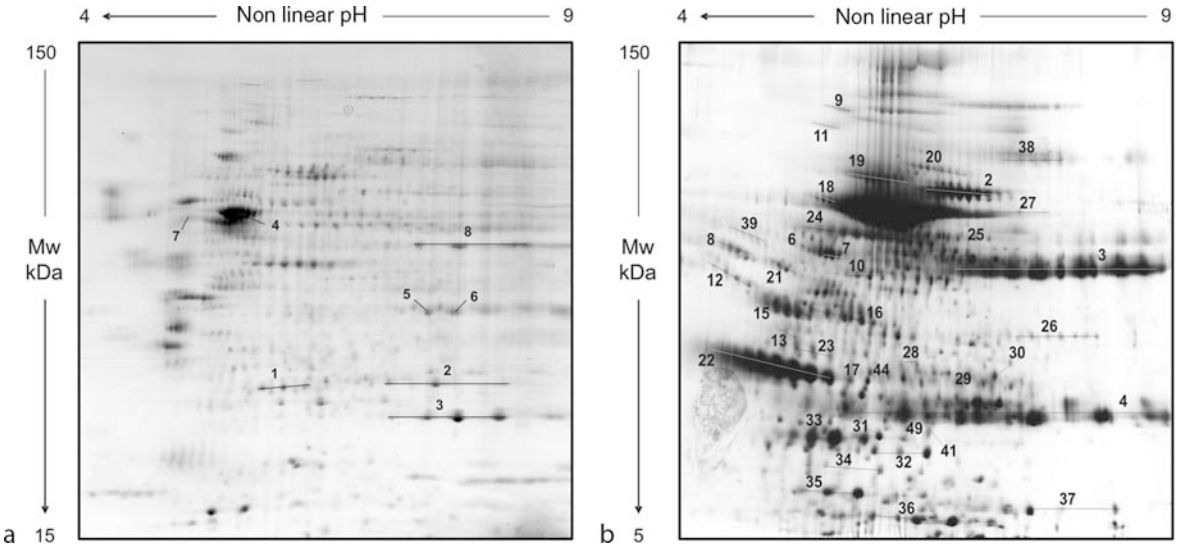
The plasma compartment contains important diagnostic markers and causative molecules of renal diseases. The expression of most plasma proteins cannot be assayed by recombinant DNA technologies, as these are generally not synthesized by circulating cells. Proteomic represents therefore a major tool to study plasma composition. In nephrotic syndrome for example, proteomic has been used to identify glomerular permeability factors and to characterize oxidized protein products. By Nat/SDS PAGE analysis, albumin and other proteins, such as the α1-antitrypsine, have been shown to undergo post-translational modifications that include fragmentation, polymerization, and formation of adducts in nephrotic states (63, 68). Role of these transformed peptide products in the pathophysiology of proteinuria is currently under study.

Likewise, proteomics can also be applied to urine samples (*Fig. 14-8b*). Until recently, urinary proteins were primarily characterized by classic single dimension electrophoresis. Alternatively, the excretion of individual proteins such as albumin, IgG or β2-microglobulin for example, were measured and used as markers for glomerular selectivity or low molecular weight proteinuria.

As the urine proteomic map is nearly completed, researchers are now attempting to use proteomic analysis to create a collection of urinary fingerprints that would allow diagnosis of more specifically renal diseases. Until now, studies in humans have been disappointing and have shown that even distantly related renal diseases can share very similar patterns of proteinuria. The current level of accuracy of urinary biomarkers reliably differentiate

Figure 14-8

Two-dimensional electrophoresis analysis showing a partial normal podocyte proteomic map (a) and a urine proteomic map (b) Identified proteins in Panel A correspond to (1) ubiquitin carboxy terminal; (2) Triosophosphato Isomerase; (3) superoxide dismutase; (4) HSP 60; (5) Glyceraldeyde 3P dehydrogenase; (6) aldose reductase; (7) secernin, (8) alpha enolase. All numbered proteins in Panel B correspond to known proteins that together represent a fingerprint of urinary protein excretion in normal and pathological conditions.



diseases with glomerular and non glomerular involvement, but is inadequate to distinguish between different types of glomerular lesions and to guide their treatment. Experimentally however, candidate disease markers such as haptoglobin in passive heymann nephritis or clusters of proteins in adriamycin nephropathy have been identified (69, 70).

Of notice, urine proteomic analysis also allows to detect factors such as C1qTNF, complement factor Bb or inter-α−trypsin inhibitor chain 4 (spots 50, 23, 52 in *Fig. 14-8b*), which are not detected in plasma because they are readily eliminated in the urine. These proteins also represent potential markers for renal diseases such as primary nephrotic syndrome, IgA nephropathy, or post-transplant proteinuria (71–73).

Peptidome and Degradome

Plasma and urine also contain very small peptides (<5 Kda). Several methodological hurdles still need to be overcome, before applying these analyses to human diseases. Specifically, results obtained by different techniques, such as SELDI and LC-ESI-MS/MS, are often not concordant and lack reproducibility.

Several, if not most of these small urinary peptides, originate from proteolysis of plasma proteins. It is still unclear if their urinary excretion correlates with significant biological events and represent surrogate biomarkers of diseases. Experimentally, a number of them have been shown to have biological activities and most are derived from digestion of urinary albumin.

Few studies have analyzed potentialities of small urinary peptide maps as fingerprints of diseases.

Recently, Decramer et al. have used capillary elctrophoresis followed by tandem mass analysis to characterize the peptide composition of urines obtained from infants born with hydronephrosis secondary to ureteric pelvis junction (74). They could show quantitative changes of a type V pre-procollagen α2 chain fragment that was predictive of the clinical evolution of hydronephrosis and subsequent need for surgery, with a reasonable sensitivity.

Similarly, by combined tandem mass spectrometry, protein chip immunoassay, and SELDI-TOF, O'Riordan et al. have identified two peptides of 4.7 and 4.4 Kda derived from defensin1 and α1antichymotrypsin respectively, and have shown that their ratio correlates with episodes of acute rejection (75). Likewise, they also identified a 4.1 urine peptide that correlates with clinical response in children with nephrotic syndrome (76).

Obviously, most of these findings need to be confirmed with large scale prospective studies. They represent nonetheless one of the forefronts of proteomic research in human diseases and may provide in the near future, less invasive diagnosis and monitoring tools for several renal conditions.

Metabolomics

Similar to proteomics, recent advances in Nuclear Magnetic Resonance (NMR) and Mass Spectrometry (MS) have helped in the search for patterns of recognition of specific conditions by analyzing non-peptidic products of the metabolism. By analogy to "genomics" and "proteomics", these approaches are now referred to as "metabolomics" and their description is beyond the scope of this chapter. The following few examples however suggest that metabolomics may complement proteomics and may provide in the future, new insights into the diagnosis and understanding of renal diseases. In murine models of cis-platinum tubulopathy for example, specific urinary metabolic spectra have been identify that precede the decline in renal function and normalize after treatment (77). Likewise, combined proteomic and metabolomic analysis have identified fingerprints which distinguish between different genetic forms of Fanconi syndrome (78) and specific metabolic profiles have been identified in patients with different glomerular lesions, which may help their diagnosis and monitoring of treatment (79).

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15 *In Vitro* Methods in Renal Research

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Significant advances in the understanding of normal and disease-associated renal cell function, epithelial cell biology, and morphogenesis have been made in recent years by the application of in vitro techniques. The researcher today is presented with a wide choice of in vitro models, and the aim of this chapter is to provide not only an overview of techniques available but also sufficient information to allow insight into the advantages and limitations of each system. This provides the experimental pediatric nephrologist with an appreciation of the range of renal in vitro methods currently available and allows selection of the most appropriate in vitro system to adequately answer the questions posed. For a more complete methodologic review of isolation and culture techniques, the reader is referred to standard tissue culture texts (1–3).

Renal Cell Types

The kidneys are highly heterogeneous, predominantly epithelial organs containing one million nephrons in each human kidney. Each nephron is comprised of a glomerulus and a highly segmented epithelial tubule with specific functions and markers (*Fig. 15-1*; *Table 15-1*), supplied by a specialized vasculature. Detailed, studies of the properties of glomerular epithelia, endothelia and mesangial cells, and of the >15 different epithelial types in the renal proximal, distal and collecting tubules are needed for the fundamental understanding of the function and development of the normal kidney as well as effects of injury and disease. Substantial progress toward achievement of this goal has been made over the last 2 decades.

Specifically, techniques have been developed in mammalian species (predominantly human, mouse, rat and rabbit) for the isolation and culture of glomerular podocytes and mesangial cells; epithelial cells from proximal convoluted and straight tubules of the S1, S2 and S3 segments; medullary and cortical thick ascending limbs of Henle's loop; distal convoluted tubules, principal and intercalated cell types of the cortical, outer medullary and inner medullary (papillary) collecting tubules; as well as interstitial cortical and medullary fibroblasts and renal vascular smooth muscle cells. Recently, progress has also been made towards isolation and culture of specialized areas of the nephron such as the macula densa and juxtaglomerular apparatus (see *Table 15-2*).

Renal Cell Isolation Techniques

The first step and prerequisite for the establishment of a useful and informative renal cell culture system is the development of a reliable isolation technique for the individual renal cell type of interest. Several techniques have been used successfully, including physical microdissection, size discrimination by graded sieving, centrifugation through Ficoll or Percoll gradients or selection by immunoabsorption on to specific antibody or lectin-coated solid surfaces, beads or nanospheres (4, 5, 17, 50–56). Certain fundamental differences between these starting isolates should be stressed. The use of long periods of tissue disaggregation by incubation in collagenase, pronase, or other dissociative enzyme solutions is often detrimental to cell membrane integrity, receptor and adhesive function and thus viability and should thus be kept to a minimum necessary to generate tubule fragments. For these reasons, physical microdissection techniques with minimal or no enzyme pretreatment is often the preferred method. These techniques provide precision of specific segment selection and is suitable for isolation of many segments of the nephron since the ease of dissection can be increased by the use of pathogen-free animals or pre-perfusion of human kidneys in situ with sterile salts solutions. Although physical microdissection of tubules provides the most accuracy, only relatively small numbers of tubules can be obtained from a single isolate because of the limited period of tissue viability. This limitation can be alleviated to some extent by freezing tissue slices in 5% dimethyl sulfoxide-containing medium for subsequent use, but the technique remains time consuming and labor intensive.

If purity is less important than large quantities of cells, tissue mincing with or without mild enzymatic digestion and/or Percoll gradient centrifugation followed by immunoabsorption with cell-type specific antibodies provides potential for generation of large numbers of pure cells. In this case, the quality of the preparation is directly related

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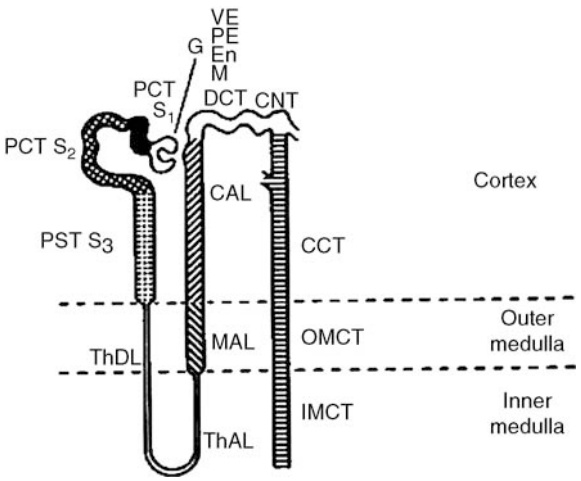
Figure 15-1

Schematic representation of distribution of cell types in the mammalian nephron. All tubule cell types can be isolated by microdissection techniques, and those shaded can be grown as primary culture monolayers in serum-free defined media. Glomeruli can be isolated by sieving and individual cell types grown in culture. Additional cells (not shown) include juxtaglomerular, vascular endothelia and smooth muscle cells, and interstitial fibroblasts of the cortical and medullary zones. CAL, cortical thick ascending limb of Henle's loop; CCT, cortical collecting tubule; CNT, connecting piece; DCT, distal convoluted tubule; En, endothelial cells; G, glomerulus; IMCT, inner medullary collecting tubule; M, mesangial cells; MAL, medullary thick ascending limb of Henle's loop; OMCT, outer medullary collecting tubule; PCT, proximal convoluted tubule; PE, parietal epithelial cells; PST, proximal straight tubule; ThDL, thin descending limb of Henle's loop; ThAL, thin ascending limb of Henle's loop; VE, visceral epithelial cells.



to the quality and specificity of the antibody used. Development of coated beads and nanoparticles offers the potential for significant improvement in separation techniques in the near future.

There are many obvious advantages to long-term study of defined cell populations in vitro. The ability to adapt culture conditions to permit the proliferation of isolated renal cells in vitro has led to a dramatic increase in the understanding of renal cell biology. Such techniques provide a powerful tool for study of renal tubule function, development, and disease. Most glomerular, tubular and interstitial isolation techniques have used adult kidneys as starting material, but increasingly, these basic techniques are being applied and adapted to the isolation, purification and culture of fetal kidney cells (2).

Renal Cell Culture Techniques

The ideal tissue culture method to use depends on the experimental question to be asked. The simplest form of cell culture is conducted in monolayers which can be derived from primary isolates or from immortalized cell lines. More elaborate culture systems may involve co-cultivation of different cell types in stratified culture systems, in 3-dimensional gels, or on synthetic scaffolds. For the system most closely resembling complex multi-cell type interactions, organ culture systems are the most appropriate choice. Each method provides advantages and disadvantages. For instance, primary cultures are likely to closely resemble the properties of the cells of origin *in vivo*, but are labor-intensive to generate and may not be feasible for the study of normal human samples or of rare diseases. Permanently growing cell lines are convenient, but may be transformed and possess abnormal characteristics. In such cases, single cell cloning may be an option to derive a homogeneous cell type with the desired property(ies). Several *in vitro* culture techniques are available to the renal researcher.

Primary Cell Cultures

Traditional explant techniques, in which small pieces of whole kidney (separated into cortex or medulla) are chopped and placed into tissue culture media- containing serum. Cell monolayer outgrowths of explants then studied in primary culture, are of little use in kidney research. The extreme heterogeneity of cell types derived from the explants and the nonselective nature of serum stimulation of growth are major limitations. Such concerns led to the development of cell type-specific isolation techniques which have contributed to significant advances in renal cell biology.

Glomeruli can be readily isolated from mammalian kidney cortex by graded sieving (57), and the differential supplementation of culture media and extracellular matrix to collagenase digests favors the primary outgrowth of epithelial, endothelial, or mesangial cells. Mesangial cells are the easiest to obtain because they grow on uncoated plastic in Roswell Park Medical Institute (RPMI) medium containing high levels (20%) of serum (9). Endothelial cells require gelatin for attachment and endothelial cell growth factor supplementation in the medium (8), whereas epithelial cells require collagen as matrix and supplementation of the medium with transferrin, insulin, dexamethasone, tri-iodothyronine, and prostaglandin E2 (6). The nature and degree of purity of primary cell populations can be determined by rigorous marker

Table 15-1

Markers of differentiated cell types from normal adult kidneys

Glomerulus	
Parietal epithelium	Cytokeratin, VCAM1
Visceral epithelium	Podocalyxin, podocin, nephrin, synaptopodin, WT-1, laminin α5, integrin α3, C3B receptor, angiopoietin,-1, VEGF, o-acetylated ganglioside
Endothelial cells	Tie-2, PDGFB, von Willebrand factor, Factor, Weibel-Palade bodies, angiotensin-converting enzyme, acetylated low-density lipoprotein uptake.
Mesangial cells	PDGF receptor β, angiotensin,II receptor, Actomyosin, contractility, desmin, dystrophin
Tubules	
Proximal	Abundant brush border, alkaline phosphatase, y-glutamyl transpeptidase, isomaltase, leucine aminopeptidase, meprin, aminopeptidase N, dipeptidylpeptidase, villin, megalin (GP330, Heymann nephritis antigen), parathyroid hormone receptors, Na-glucose transporter, Na- amino acid transporter, aquaporin-1, NHE3; GLUT-2, ClC5, CFTR, rBAT, PEX, NBC, hUAT, 1,25-hydroxy 2D3
Thin descending limb of Henle	Aquaporin-1, Ca-ATPase-3
Thick ascending limb of Henle	No brush border, abundant mitochondria, highest Na-K-ATPase activity, Tamm-Horsfall protein (uromodulin), pre-pro-epidermal growth factor (EGF), ROMK channel, NKCC2 (BSC1), NHE3 CaSR, glucocorticoid receptor, osteopontin
Juxtaglomerular apparatus	Renin
Macula densa	Anion exchanger AE2, NHE-2, NHE-4
Distal convoluted tubule	Epidermal growth factor, osteopontin, NHE2, NCC, Ca-ATPase,-2, CaSR, 28Kd CaBP, Na/Ca exchanger, ClC-K1, 2, claudin 16, Ca^{2+}, Mg^{2+}-ATPase, 11-hydroxysteriod dehydrogenase-2, mineralocorticoid receptor, calcitonin response
Connecting tubule	Kallikrein, 28kD CaBP, Na/Ca exchanger, 11-hydroxysteriod dehydrogenase-2, mineralocorticoid receptor, aquaporin-2
Collecting tubule	
Principal cells	Light cytoplasm, few organelles, calcium-binding protein, vasopressin, receptor V2 aquaporin-2, aquaporin-4, CFTR, ENaC 11-hydroxysteriod dehydrogenase-2, mineralocorticoid receptor, AE-1, AE-228kD CaBP, vasopressin receptors
Intercalated cells	Dense cytoplasm, many organelles, Cl-/HCO_3- exchanger (Band 3), H^+-ATPase, carbonic anhydrase II
Fibroblasts	
Cortical	Vimentin, FSP-1 I-CAM-1
Medullary	Vimentin, FSP-1, tenascin

It should be noted that species differences are common in segment distribution of some transporters

Abbreviation: CaSR, calcium sensing receptor; C1C5, chloride channel; ENaC, epithelial sodium channel; GLUT-2, Na-glucose cotransporter; hUAT, urate transporter; PDGF, platelet-derived growth factor; PEX, Na/phosphate transporter; NBC, Na-$HC0_3$ transporter; NCCT, Na^+ Cl^- transporter; NHE3, Na/H^+ exchanger; NKCC2 (BSC1), Na^+ K^+ Cl^{2-} transporter; rBAT, cystine and dibasic amino acid transporter; ROMK, renal outer medullary potassium channel; CAM, cell adhesion molecule 1; VEGF, vascular endothelial growth factor

analysis using immuno-detection techniques using cell-type specific antibodies (see *Table 15-1*).

Primary cultures of defined renal tubule epithelia have been derived from several mammalian species, using a wide variety of techniques. As discussed above, microdissected tubules provide maximum purity and allow discrimination between the S1, S2, and S3 portions of the proximal tubule (10–12, 58) as well as isolation of specialized regions such as the macula densa and the juxta-glomerular apparatus (21, 59–61). Commonly used, reliable techniques are available for the culture of proximal tubules, thick ascending limbs of Henle's loop, and collecting tubules of cortical and medullary origin. These techniques are used for the primary monolayer culture of cells of adult and fetal

Table 15-2

Mammalian renal cell and tissue cultures

Cell type	Species	References
Primary cultures		
Glomerular visceral epithelia (podocytes)	Human, rat, mouse, pig,	(4–7)
Glomerular endothelia	Human, bovine, rat	(8)
Glomerular mesangial cells	Human, bovine, rat	(9)
Proximal convoluted tubule S1, S2	Human, rabbit, mouse	(3, 10–12)
Proximal straight tubule S3	Human, rabbit, mouse	(3, 10–12)
Thick ascending limb of Henle's loop	Human, rabbit	(10, 11, 13, 14)
Distal convoluted tubule	Rabbit, rat, mouse	(14–16)
Collecting tubule	Human, bovine,dog, rabbit	(10, 11, 15, 17)
Intercalated cells	Rabbit	(16)
Principal cells	Rabbit	(18)
Outer medullary collecting tubule	Human, rabbit	(10, 11)
Inner medullary collecting tubule	Human, rabbit, rat	(10, 11, 19, 20)
Macula Densa	Mouse	(21)
Juxtaglomerular cells	Rat	(22–25)
Cortical interstitial fibroblasts	Human, rabbit, rat	(21, 26, 27)
Medullary interstitial fibroblasts	Rabbit, rat	(23, 27, 28)
Renal vascular smooth muscle cells	Rabbit	(24)
Spontaneous permanent cell lines		
Proximal-like: JTC-12, BSC-1, OK, LLC-PK1, PT	Monkey, opossum ,pig, mouse	(29–32)
Distal like: MDBK, MDCK, GRB-MAL, MmTAL-1C	bovine, dog, rabbit, mouse	(33–35)
Fetal kidney	Human	(33)
Immortalized/transformed cell lines		
Glomerular epithelia (podocytes)	Human, rat, mouse	(36, 37)
Proximal tubule	Human,	(38, 39)
Distal convoluted tubule	Mouse	(39, 40)
Cortical collecting tubule	Rabbit	(33, 41)
Inner medullary collecting tubule	Mouse	(29)
Fetal Kidney (HEK 293)	Human	(25)
Metanephric blastema (RSTEM-1)	Mouse (fetal)	(42)
Conditionally immortalized/differentiated cell lines		
Glomerular epithelial (podocytes)	Mouse	(43)
Proximal tubule	Human (fetal)	(44)
Proximal tubules	Human (adult), rat, mouse	(45–47)
Thick ascending limb of Henle	Human (adult)	(44)
Colllecting tubule	Human adult and fetal	(44, 48, 49)
Ureteric bud	Mouse fetal	(42)

Abbreviation: LLC-PK1, pig kidney; MDBK, Madin-Darby bovine kidney; MDCK, Madin-Darby canine kidney; MmTAL, mouse medullary thick ascending limb; OK, opossum kidney; PT, proximal tubule

origin. In addition to epithelial cells, renal interstitial fibroblasts can also be cultured with ease from the cortical or medullary explants (23, 24, 27, 28, 62). These are fairly easy to generate because fibroblasts proliferate rapidly in response to 10% serum stimulation, whereas renal epithelial proliferation is inhibited under these conditions. This means that the renal fibroblasts initially present in mixed cell populations in explants eventually overgrow the cultures. Subculture with trypsin results in the acquisition of pure preparations of fibroblasts by the third passage, because renal epithelia are more sensitive to the destructive effects of trypsin than fibroblasts (62).

To assess the validity and stability of any primary culture technique, rigorous marker analysis is essential and possible because each renal cell type expresses specific protein(s). This permits analysis of both the purity of the initial preparation and the maintenance of the differentiated state through subsequent passages in vitro. If these criteria are fulfilled, primary cultures are particularly useful for the study of normal cell function. Most cell cultures derived from glomerular or tubule preparations isolated from normal adults survive a few (three to five) passages but then die out. This is consistent with normal cell properties and is thought to represent the maximal repair capacity of renal cells in vivo. This contrasts with neoplastically transformed cells, which possess unlimited proliferative potential and produce tumors in vivo and immortal cell lines in vitro.

Permanent Cell Lines of Renal Origin

Renal Cell Lines

Several permanently growing renal epithelial cell lines have been identified and used to study many aspects of renal cell biology (*Table 15-2*). These cell lines can provide a convenient supply of large numbers of cells for the study of polarity, ion transport, hormone receptor interactions, ischemic injury and toxic responses (29–35). However, their limitations must be appreciated. Although LLC-PK1, OK, and JTC-12 cells have properties suggesting proximal tubule origin, and Madin-Darby canine kidney (MDCK) cells have some properties of distal tubules, the precise cells of origin of these spontaneously immortal lines is unknown. In addition, like all immortal cell lines, they have undergone genetic drift because of numerous passages. This has led to the loss of some normal and the acquisition of abnormal, anomalous properties. Some of these limitations can be overcome by recognition of unique subclones (i.e., MDCK-1 and MDCK-2 cells), and single cell cloning and restricted use of cells for study (i.e., with a maximum of 10 passages). For instance MDCK and LLC-PK1 cells have proven to be invaluable for the study of specific transport proteins, and for analysis of mechanisms controlling epithelial cell polarity.

Immortalized, Transformed Renal Cell Lines

An important advance in the generation of cell lines for renal research has been the successful application of immortalization techniques using recombinant viral vectors introduced into cells by infection or transfection. Initial studies used oncogenic viruses to deliver transforming oncogenes to cells in vitro, including Human Papilloma Viral (HPV)-immortalized HK2 cells; adenovirus/simian virus immortalized RCCT-28A cells and the adenovirus-5-transformed human embryonic kidney (HEK) 293 cell line (*Table 15-2*). The latter cell line has become widely used due to its permissiveness to transfection. But it should be cautioned that all of these cells are highly transformed and may therefore not be the cell line of choice for the study of normal cell functions. An additional successful strategy has been to isolate a variety of renal segmental cell lines from the SV40-large T antigen transgenic mouse, including podocytes, Macula densa, juxta-glomerular apparatus (JGA), mouse cortical collecting tubules (mCCT) and widely used mouse inner medullary collecting duct (mIMCD)3, M1 and K2 cells (see *Table 15-2*) (21, 24, 25, 33–41, 63). The advent of these cell lines has greatly improved the efficiency and options for *in vitro* study of renal genes and proteins, since they provide large numbers of cells of a single type amenable to transfection with specific genes. However, like any transformed cells, they suffer from the limitation that the permanently proliferative phenotype is conferred by the presence of the introduced oncogene rendering the cells abnormal. Thus the significant advantage conferred by a constantly renewable proliferative source of cells is counter-balanced by the disadvantage of abnormalities conferred by this transformation.

Conditionally Immortalized Renal Cell Lines

To date, the most successful advance made towards achieving the goal of production of large numbers of

permanently proliferating but normally differentiated renal cell cultures has been the introduction of a temperature-sensitive (tsA58) allele-containing Simian virus (SV-40) T antigen into defined renal cell types (42, 44, 47–49, 64, 65). This has permitted the immortalization by virtue of expression of the integrated SV-40 tsT antigen, and proliferation to confluence without loss of contact inhibition when cells are grown at the permissive temperature of 33°C. However when such cells are switched to a non-permissive temperature($\geq$37°C), they lose T antigen expression, cease proliferation and exhibit a fully-differentiated cellular phenotype. This technique has been widely and successfully applied to generate several conditionally immortalized human, mouse and rat renal segment specific cell lines including podocytes, proximal, thick ascending limb and collecting tubules, ureteric bud epithelial and renal fibroblasts of normal adult and fetal origin (see *Table 15-2*). This has been achieved utilizing two approaches: either by isolating specific renal cell types for culture from the SV-40tsA58 transgenic mouse (Immorto-mouse) (43, 64) or by in vitro retroviral delivery of pZIPtsA58U19 into microdissected primary cell cultures of specific renal cell types followed by selection of transfectants in neomycin (45). The latter technique has been used successfully to conditionally immortalize human renal epithelial cells of normal and diseased origin, to generate conditionally immortalized cell lines of normal human fetal and adult proximal tubules, thick ascending limbs and collecting tubules, as well as epithelial cell lines from patients with cystinosis, autosomal dominant polycystic kidney disease (ADPKD) and autosomal recessive polycystic kidney disease (ARPKD) (44, 48, 49, 65–67). As required for understanding the utility of all cells in culture, these cells have been fully characterized by marker analysis and show maximal differentiation and characteristics of their segment of origin after 5–14 days at 37°C (*Table 15-3*). Conditional immortalization of microdissected primary renal cell cultures provides a homogeneous, renewable system to study specific cell types that closely resemble their cell type of origin, exhibit normal function and polarization of proteins, and are highly transfectable (*Fig. 15-2*). These techniques offer substantial promise for the study of cellular basis of disease and are being applied to many mouse models by crossing mutant diseased mice with the Immorto-mouse to facilitate the generation of conditionally immortalized renal cells after isolation applying micro-dissection, or immunodissection approaches (68).

Role of Extracellular Matrix

Studies in several in vitro systems have elucidated a major role for the extracellular matrix in the regulation of epithelial cell proliferation, adhesion, polarity, differentiation and gene expression (69). *In vivo*, the renal extracellular matrix is comprised of the tubular and glomerular basement membranes as well as peri-tubular interstitial matrix and contains an intricate network of collagens (types IV, I and III), laminin, fibronectin, entactin and heparan sulphate proteoglycans (HSPG). Confluent renal tubule epithelial cells in vitro have also been shown to secrete a basement membrane (10, 70, 71). To promote normal growth and differentiation, renal tubule epithelia are optimally seeded onto tissue culture plastic or membrane filters that have been pre-coated with an extracellular matrix protein or combination of proteins such as collagen type I or type IV; or Matrigel (derived from the EHS sarcoma) This not only facilitates adhesion via normal integrin-based receptors but also promotes proliferation, polarization and differentiation. Although renal tubule and glomerular epithelial cells have a strong preference for exogenous matrix for their optimal culture, this is not the case for renal cells of mesenchymal origin such as mesangial cells, smooth muscle cells or fibroblasts that grow on uncoated tissue culture plastic in the presence of serum.

Role of Growth Factors

Soluble growth factors have long been known to play a major role in the control of cell proliferation. In vitro, serum was first used to induce cell growth. However, serum is a complex mixture of proteins, and its detailed composition has not been fully elucidated. The value of serum in renal cell culture is that it favors the growth of fibroblasts at the expense of epithelia. In mixed epithelial and fibroblast cell populations, this enables the generation of fibroblast cell lines due to their differential survival in vitro. For instance, when human kidney cortex or medulla is grown without exogenous matrix in the presence of 10% serum, after three passages only fibroblasts are present. A significant advance for renal epithelial cell culture was the definition of serum-free growth factor-supplemented culture media that support the proliferation of renal epithelial cells (72). Modifications of the original formulation have led to optimization of the culture conditions for most renal epithelial cell types

Table 15-3

Characterization of conditionally immortalized human renal epithelial cell clonal lines

Protein	HFPT	HFCT	NHPT	NHTAL	NHCT	ADPKD	ARPKD
Keratin	+	+	+	+		+	+
Vimentin	−/+	−	−	−	−	+	+
Alkaline phosphatase		−	+	−	−	+(−)	−
Leucine	−	−	+	−	−	+(−)	−
Aminopeptidase							
Multidrug resistance P-glycoprotein	+	−	+	−	−	+	−
Aquaporin 1	+	−	+	−	−	+(−)	−
NHE3		−	+		−	+	±
Epidermal growth factor receptor	+/−	+(A)	+(B)	−	−	+CA)	+(A)
Erb-B2	−	+(A)	−	−	−	+CA)	+(A)
α2-integrin	+/−	+	+			+	+
α6-integrin	−	−/+	+	+	+	+	
β1-integrin	+	+	+	+	+	+	
Tamm-Horsfall protein	−	−	−	+	−	−(+)	
Aquaporin 2	−	+(−)			+(−)	+(−)	+
H+ATPase	−	−(+)	+	−	+(−)	−(+)	−
Carbonic anhydrase II		−	−	−	+(−)	−(+)	−
ENaC	−	+	−	−	+	+	+
NaK-ATPase -α1	−	+(A)	+(B)	+(B)	+(B)	+(A)	+(A)
NaK-ATPase-β1	−	−	+(B)	+(B)	+(B)	+(C)	−
NaK-ATPase-β2	+(A_	+(CA)	−	−	−	+(A)	+(A)
Polycystin 1	−	+	−	−	+	+(0	+
Protein kinase X	−	+	−	−	−	+	+
WT1	−	−	−		−	+	−
Pax-2	−	+	−	−	−	+	+

Abbreviation: weak signal; + /−, most clones positive, few clones negative; (A), apical; ADPKD, autosomal dominant polycystic kidney disease; ARPKD, autosomal recessive polycystic kidney disease; (B), basal; (C), cytoplasmic; HFCT, human fetal collecting tubule; HFPT, human fetal proximal tubule; NHCT, normal human adult collecting tubule; NHPT, normal adult human proximal tubule; NHTAL, normal human adult thick ascending limb

in serum-free media. All epithelia have an absolute requirement for transferrin and proximal tubules are stimulated to proliferate by combinations of dexamethasone (or hydrocortisone) and insulin, although this requirement can be substituted by epidermal growth factor (EGF). Optimal proliferation of thick ascending limb epithelia requires additional supplementation with tri-iodothyronine, whereas collecting tubules require only dexamethasone and transferrin (12). It is thought that the different balance of growth factor and hormonal supplementation reflects the different complement of membrane transporters and receptors of the different epithelial segments.

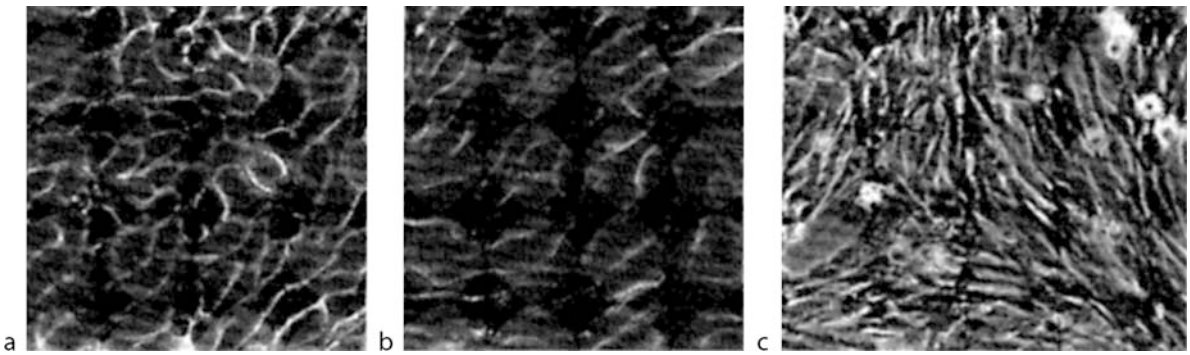
In vitro Techniques for the Analysis of Normal Renal Function, Development, Differentiation and Morphogenesis

Proliferation and Differentiation of Monolayer Cultures

The in vitro system can be utilized with great power to understand the role of individual components involved in normal and abnormal processes by controlled studies in which single components can be deleted or added at a time.

Figure 15-2

Phase-contrast light micrographs of confluent primary cultures. (a) Epithelial monolayer grown from individually microdissected human fetal proximal tubule explanted onto type I collagen-coated plastic plates in Click- RPMI (Roswell Park Medical Institute) medium supplemented with human transferrin, dexamethasone, insulin, and fetal bovine serum (1%). Note polygonal morphology. (b) Epithelial monolayer grown from individually microdissected human fetal collecting tubule expianted onto type I collagen-coated plastic plates in Click-Roswell Park Medical Institute medium supplemented with transferrin, dexamethasone, and fetal bovine serum (1%). Note polygonal morphology. (c) Renal fibroblasts derived from cortical explants plated onto uncoated tissue culture plastic in Dulbecco modified Eagle medium supplemented with 10% fetal bovine serum. Note elongated, spindle-shaped cell morphology.



Regulation by Matrix and Soluble Factors

The interactions of extracellular matrix proteins such as collagens I, III, IV, and V; laminin and proteoglycans with their specific αβ integrin matrix receptors are critically important in development and establishment of epithelial cell polarity as well maintenance of the differentiated state (73–75). In vitro techniques are ideally suited to analyze the roles of specific matrix proteins on proliferation, adhesion, polarization and differentiation of renal cell types and can be used to compare these in adult versus fetal epithelia (71). The action of a variety of soluble factors via their specific growth factor and hormone receptors also play important roles in renal cell proliferation and differentiation in development and maturity which cam also be readily assessed in renal cell culture systems (76).

Establishment of Epithelial Cell Polarity

The physiologic hallmark of the renal tubule epithelia is polarized reabsorption and secretory functions specific to each nephron segment, which is paralleled by and a consequence of the highly polarized and asymmetric distribution of specific ion transporters, channels, and receptor proteins on the apical versus basolateral membranes. Structural polarity is also seen in that only apical membranes elaborate brush borders and basal membranes elaborate a basement membrane. The maintenance of polarity is determined by the integrity of occluding tight junctional complexes that form a continuous belt between epithelial cells at the apical pole of renal epithelial cells. Renal epithelia in vitro also form tight junctions and polarized monolayers when grown on extracellular matrix. The MDCK cell line has been highly utilized to study mechanisms of polarization since it readily forms a tight monolayer. To maximize morphological and physiological polarization of normal renal epithelial cell types, growth on collagen coated membrane filters suspended in tissue culture wells (e.g., Transwell assemblies) is highly recommended. It appears that the feeding of cells from the basal (as well as the apical) cell surface facilitates maximal differentiation and polarization of epithelial cells in vitro. These techniques have been widely applied and are suitable to examine the polarization potential of developing and mature epithelia by marker analysis of specific membrane proteins (see *Table 15-4*). The analysis of cell polarization events in monolayer primary cultures derived from differentiated renal progenitors will in the future provide a temporal and spatial map of sequential events involved in tubule maturation during nephrogenesis.

Marker Analysis

The complex arrangement of the kidney into multiple nephrons comprising a filtering glomerulus and transporting

Table 15-4

Markers of membrane polarity in normal adult tubule epithelia

Apical	Basal and lateral
Influenza hemagglutinin	VSV-G
Alkaline phophatase	Na-K-ATPase
γ-Glutamyl transpeptidase	Ankyrin
5′-Nucleotidase	Fodrin
Meprin	E-cadherin
Diaminopeptidase-IV, meprin	Vasopressin-2 receptors
Aminopeptidases, M, N, P	EGF receptors
Maltase	IGF-1 receptors
H^+- ATPase	Laminin receptors
Trehalase	Type IV collagen receptors
Carcinoembryonic antigen	HSPG receptors
Na-glucose transporter GLUT2	Integrin α6
Na-amino acid transporter	Integrin α2
Na-H exchanger NHE3	Integrin β1
Aquaporin-2	Integrin β4
Na^+ K^+ $2Cl^-$ symporter	CI/HCO3, AEI transporters
Urate transporter	Aquaporin-4

Abbreviation AQP, aquaporin; ATPase, adenosine triphosphatase; HSPG, heparan sulfate proteoglycan

tubular epithelium divided into approximately 15 segments with distinct physiological functions, provides the basis for marker analysis due to the accompanying expression of segment and cell type-specific genes and proteins. As described above, this feature can be used to verify the validity and purity of a cultured cell population. In addition, it can be used to analyze the physiological and developmental regulation of specific proteins. *In vitro* cell systems are ideal to test the effects of potential hormonal agonists and antagonists on specific proteins and is widely used in studies of renal ion transporter and channel regulation. Much less is known about the underlying processes controlling the conversion of undifferentiated, nonadhesive, nonpolarized mesenchymal cells of the metanephric renal blastema into fully polarized, adhesive, highly specialized nephron (77). In vitro cell systems utilizing renal epithelia from fetal through to adult kidneys will elucidate the temporal and spatial characteristics and may shed light on developmental defects in renal function associated with sporadic or genetic malformations. To facilitate study of this complex array of events, a series of developmental cell- and stage-specific markers are becoming available due to studies conducted in developing kidneys (*Table 15-5*).

Table 15-5

Markers of renal development

Cell Type or Structure	Markers
Metanephric blastema (undifferentiated mesenchyme)	Vimentin; syndecan; integrins α1,α9; collagen I, III; laminin β1, y1, fibronectin; N-CAM; IGF-II; GDNF HGF; c-Met; NGFR; midkine; CRABP; BF2; tenascin; WT1, cadherin -11; Hox AII; Hox DII; Six-2; Sca-1;; FGF2; BMP4; PNA+; apoptosis
Condensates	Vimentin, syndecan, α8 integrin, N-CAM, N-myc, WT1, IGF-II, HGF, c-Met, NGFR, midkine, pax-2, wnt4, cadherin 6, E-cadherin
S-shaped bodies	Laminin, α1 integrin, E-cadherin, syndecan, tenascin, N-myc, NGFR, wnt4, Notch-1
Glomerular pole	Vimentin, WT1, α3 integrin, podocalyxin, C3b receptor, IGF-II, nephrin- B2
Tubular pole	γGT, MDR, α6 integrin, α1-dystroglycan
Fetal tubule epithelia	Cytokeratin, laminin α1, collagen IV, α6 integrin, E-cadherin, desmoplakin, HGF, c-Met, LIM-1, α1-dystroglycan
Proximal	Alkaline phosphatase, leucine aminopeptidase, renal peptidase, meprin, MDR, midkine, aquaporin 1, CFTR, cadherin 6
Thin descending limb	Aquaporin 1
Thick ascending limb	Na-K-ATPase-α1 β2, Tamm-Horsfall protein, osteopontin, L-myc, integrin β4, integrin α2, calcium-binding protein, E-cadherin, Brn 4/1
Collecting tubule/ureteric bud	Midkine, Wnt7b, PKD-1, polycystin-1, CFTR, Pax-2, carbonic anhydrase, H-ATPase, aquaporin 2, Hox B7, c-ret, calbindin, Pax-2, E-cadherin, DBA+
Fetal glomeruli	Vimentin; integrin α3, β1; podocalyxin; C3bR; WT-1; ACE
Fetal interstitium/stroma	Vimentin, tenascin, GD-3, ganglioside, IGF-II, FGFa, NGFR, RAR α, RAR β, BF-2

Assay Development in Tissue Culture Plates

Cell culture monolayer systems on multiwell tissue culture plates offer a highly manipulable system with the potential to develop high through-put, multi-variable assays to measure the effects of agonists, inhibitors, or gene modification on cellular responses including proliferation, apoptosis, matrix adhesion, cell-cell adhesion, motility and secretion. These systems are being utilized for analysis of therapeutic small molecule or antibody drugs and for toxicity testing (*Fig. 15-3*).

Transfilter systems are also particularly beneficial for the study of renal epithelial polarity and polarized function (*Fig. 15-3*). By establishment of a gradient of growth factor or ion in question by differential media supplementation in the apical and basal compartments, effects on polarized cell secretion or absorption, chemotaxis or directional cell migration can readily be analyzed. These types of assays have been productively applied to the analysis of altered functions of diseased (polycystic kidney) epithelia (78–80). In addition, important observations have been made to dissect the respective roles of soluble factors versus cell-cell contact in the inductive events leading to metanephric blastemal induction in response to the ureteric bud by separating the two components (81, 82). These types of assays also provide a strong potential for analysis of cell type interactions for instance between epithelial sheets and interstitial cells in normal development, function and disease (83, 84).

Morphogensis in Three-Dimensional Gels

Experimental confirmation of the role of mesenchymal and extracellular matrix induction on normal renal epithelia has also been shown by the ability of collagen or reconstituted basement membrane gels to promote the development of three-dimensional tubule-like structures from mouse inner medullary collecting ducts (mIMCD), dog (MDCK) collecting tubules and pig proximal tubules (LLC-PK1) (85–87). When initially seeded into type I collagen gels, MDCK (and LLC-PK1) cells form inside-out cysts and co-cultures with fibroblasts or the addition of fibroblast-conditioned media or hepatocyte growth factor (HGF) elicits a tubulogenic effect (87, 88) (*Fig. 15-4*). These surrogate tubulogenesis assay techniques are now widely utilized to study the effects of gene modulation by transfection or siRNA, or added soluble co-factors or specific inhibitors in development and disease (89–91). The utility of this system is that it provides a robust three-dimensional assay to analyze the roles of cell-cell, cell-matrix, and cell-soluble factor interactions. It should be cautioned, however, that MDCK cells may not be the ideal cell type to model a developing kidney and the use of ureteric bud or other fetal-derived cells should be explored to answer developmental questions.

Embryonic Organ Culture

The most sophisticated and directly relevant in vitro system to study renal development is organ culture of fetal kidneys. These techniques have the advantage that epithelial-mesenchymal tissue interactions are maintained, although vasculature does not develop as in would in vivo. Nevertheless, the branching morphogenesis of the ureteric bud and the induction and differentiation of glomeruli and nephron tubules can be studied for upto a 14-day period in these cultured organ explants (*Fig. 15-4*). Mouse embryonic kidneys from embryonic day (E) 11–16 embryos are dissected and placed on a stainless steel rafts or filter membranes in a culture well and specific serum-free, growth factor supplemented media is added to the lower compartment to allow a thin film of media to cover the explant. These types of assay have been instrumental in identifying important matrix and growth factors involved in the regulation of normal ureteric bud branching and metanephric blastemal progenitor cell production (81, 92–96).

For studies of renal development and nephrogenesis, the most important modification of organ culture, the transfilter technique, was first introduced by Grobstein (81) and continues to be used extensively. Metanephric blastemas are attached to a filter suspended above a tissue well and tissue fragments of ureter or spinal cord are applied to the undersurface of the filter to induce nephrogenesis. Alternatively, the natural inducer, the ureteric bud maybe removed from one E11 kidney and development compared with that of the paired kidney in which it was left in. Progressively refined analysis of the transfilter organ culture system has provided the basis for much of our present understanding of the cellular and molecular events associated with nephrogenesis. These include cell-cell contact and cell-matrix attachment, allowing regulated migration and cell shape changes as well as appropriate growth factor-receptor interactions that coordinately regulate proliferation, apoptosis, differentiation and epithelial polarization associated with renal tubule maturation.

More recent studies have focused on using these organ culture systems to understand cystic expansion and ureteric bud malformations associated with disease processes by the introduction or modulation of genes by direct

Figure 15-3

(a) Schematic representation of in vitro system for study of differentiation, polarized transport, secretion and directional migration properties of cells. Cells are seeded in the apical compartment of permeable, filter membranes suspended in a well. Cells maybe rendered fluorescent by pre-incubation in calcein AM or by transfection with enhanced green fluorescent protein (EGFP)-containing expression constructs (b).

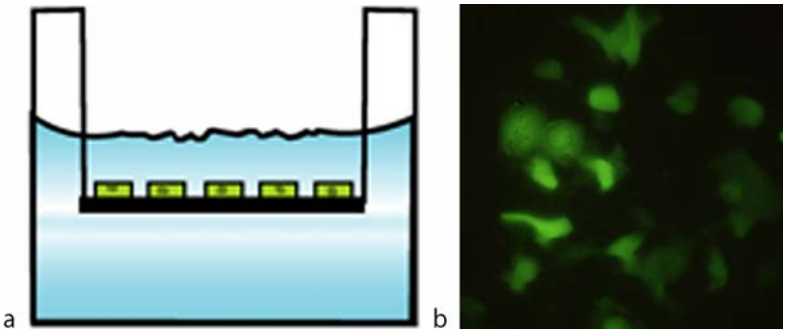
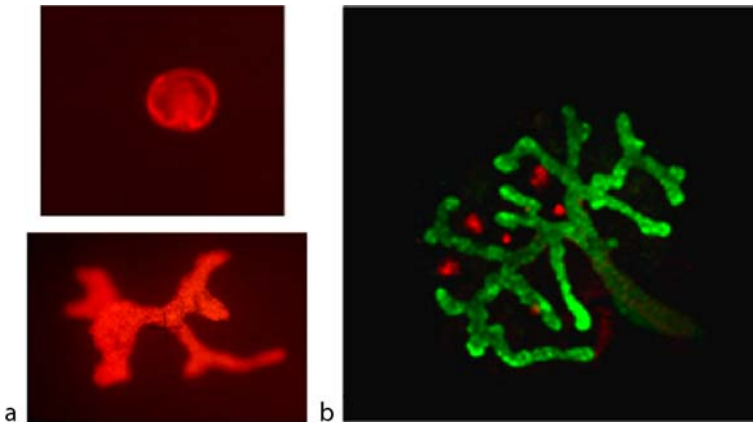


Figure 15-4

(a) Morphogenesis assay: MDCK cells are plated in collagen I gel (top) and treated with HGF (lower) to induce tubulogenesis. Rhodamine phalloidin labeled, confocal microscopy. (b) Organ culture of E11.5 mouse kidney after 3 days in culture, stained with anti-calbindin (green) and anti-WT-1 (red) confocal immunofluorescence microscopy.



injection or incubation of inhibitory dominant negative or shRNA or activating viral constructs (80, 97–100). Although the majority of metanephric organ culture studies are carried out using mouse kidneys, studies of rat and human renal organ culture are also feasible (101, 102).

In Vitro Techniques for the Analysis of the Underlying Mechanisms and Modulation of Renal Cell Injury and Disease

Renal tubule cell injury in vivo can be induced by ischemia, nephrotoxins, genetic abnormalities, or cancer. An understanding of some of the cellular and molecular mechanisms involved is beginning to emerge, in large part because of the use of in vitro techniques, as summarized in *Table 15-6*.

Glomerular Disease

The availability of pure glomerular cell types in vitro has led to their use in elucidating cellular mechanisms of disease. For example, exposure of glomerular podocytes in culture to Heymann nephritis antigen provided experimental evidence for binding in coated pits, patching,

Table 15-6

In vitro techniques for the study of cell injury and diseases

Injury or disease	Cell type	Species	Reference
Ischemia (anoxia)	PT, PCT, PST CTAL, mTALCCT	Human, rat, rabbit	(103)
Gentamicin	LLC-PK1, PTPCT, PST, CCT	Human, dog, rabbit	(104–107)
Cyclosporine	LLC-PK1, PCT, PST, TAL, CCT, mesangial, endothelia, vascular, smooth muscle	Human, dog, rabbit	(108–111)
Nephropathie cystinosis	Proximal tubule	Human	(45)
ADPKD	Primary cystic epithelia	Human	(70)
	Interstitial fibroblasts	Human	(21)
	Conditionally immortalized:		
	Cystic epithelia	Human	(44, 48)
	Interstitial fibroblasts	Human	(112)
ARPKD	Primary cystic epithelia	Human, mouse	(44, 113–115)
	Conditionally immortalized:		
	Cystic epithelia	Human, mouse	(44, 48, 68)
	Organ culture	Mouse	(99)
Nephronophthisis	Primary monolayer	Human	(116)
Wilms' tumor	G401, SK-NEP, WIT 13, GOS-4	Human	(117)
Renal adenocarcinoma	A704, AHCN, RAG	Human, mouse	(118)
Renal carcinoma	CAKI-1, 2: A-498; RC-1; RCC; CCF-RC1, -2, OUR	Human	(119–122)

capping, and shedding from the cell surface (123) while glomerular epithelia from mice with puromycin aminonucleoside nephrosis exhibited loss of negative charge and adhesive properties More recently, the advent of well-differentiated, conditionally immortalized podocyte cell systems from normal mice and their genetically manipulated diseased counterparts has significantly contributed to the understanding of the roles of collagen IVα4 in Alport's syndrome, nephrin in Finnish-type nephropathy, and podocin in immunoglobulin A nephropathies (43, 124). In addition, these cell systems provide new opportunities to study the underlying mechanisms of immune and membranous glomerulonephritides (7) and to provide novel insights into podocyte cell properties (125).

Mesangial cell cultures have also been used as a model system to study diabetes by their isolation from streptozotocin-induced diabetic rat kidneys or by addition of glucose, transforming growth factor (TGF) -β interleukin-lβ or galectin-3 to their tissue culture media in vitro (94, 126, 127). Cell culture studies, utilizing proliferation, contractility, chemotaxis and migration assays are ideal to examine the mechanisms underlying mesangial proliferative disorders, and the effects of ageing (128, 129), although care must be taken to use cells in early passage to prevent loss of phenotypes by long-term adaptation to culture conditions.

Ischemic Injury

Periods of oxygen deprivation lead to ischemic injury and may result in acute renal failure. To fully understand the mechanims underlying hypoxic, anoxic and ischemic renal cell injury, cell culture approaches offer certain advantages and complement the short-term studies carried out in isolated tubule preparation (103, 130). Of the permanent renal cell lines, LLC-PK1 cells have been the most frequently used and have demonstrated apoptotic responses to ischemia, as well as alterations if heat shock proteins leading to necrotic cell death in response to ATP depletion (131–133). Although LLC-PK1 are thought to be of proximal tubule origin, the most susceptible segment to ischemic injury, permanent cell lines have adapted to the relatively hypoxic conditions of tissue culture rendering them abnormally resistant to ischemic damage. For this reason, the cell culture method of choice for studies would be of primary or conditionally immortalized origin. Primary cultures of rat, rabbit, and human

renal proximal tubule epithelial cells have been shown not only to retain their sensitivity to hypoxic, anoxic and ischemic injury and have been used successfully to demonstrate differential tubule cell sensitivity and attenuation of cell death by polyethylene glycol, extracellular calcium restriction, calcium channel blockers, and inhibitors of calmodulin and cysteine protease activities (103, 130). In addition molecular insights have been determined with regard to the integrin-based mechanisms of the loss of cell-matrix adhesion as well as induction of the cell-cell adhesion molecule I-CAM-1 (134, 135). Hypoxia studies have also been carried out on mesangial cells in culture where a role for osteopontin in the resultant proliferation response has been invoked (136).

Nephrotoxic Injury

Renal toxicity is an important topic of study with regard to exposure to environmental toxins, chemotherapeutic, antibiotic and anti-rejection drugs. LLC-PK1 cell lines and primary and conditionally immortalized cultures of rabbit and human proximal tubules have been most widely used to study the mechanisms underlying the toxic effects of a variety of metals (137–139) as well as those of aminoglycoside antibiotics, such as gentamicin where a major role for alterations in proximal tubule phospholipid profiles have been implicated (104–107, 140). Primary and conditionally immortalized renal proximal tubule cells have also been used to study mechanisms of sensitivity to the chemotherapeutic agent cisplatin and to define the relative contribution of epithelial versus microvascular effects of the anti-rejection drugs cyclosporine and FK506 (108–111). Cell culture approaches are highly suited to identification of the intracellular targets of toxicity and the subsequent testing of potential modulators. Primary porcine proximal tubules, LLC-PK1 and MDCK cells, have been used to study mechanisms of transepithelial drug transport (141, 142) and the effects of calcium oxalate crystal formation (143, 144). Interestingly, this was shown to be exacerbated by hypoxia and hypercapnia (145).

Congenital Renal Diseases

Hereditary and non-hereditary renal disease can readily be modeled in vitro by development of renal cell lines from human diseased tissues or from spontaneous or genetically engineered rodent models of disease.

The first cell culture system devised for the study of human autosomal dominant polycystic kidney disease (ADPKD) utilized a primary culture approach in which individual cyst epithelial linings were microdissected and grown in primary culture (65). Together with parallel in vivo studies, these were instrumental in delineating the aberrant cell biological characteristics of ADPKD cystic epithelia including excessive cell proliferation, hypersensitivity to epidermal growth factor (EGF), increased cell-matrix adhesion, alterations in apico-basolateral polarity and fluid secretion defects (146). Subsequently, several primary, immortalized, and conditionally immortalized monolayer systems for human ADPKD, human and mouse autosomal recessive polycystic kidney diseases (ARPKD), and nephronophthisis (44, 49, 68, 113–116) have been established (*Table 15-6*). The derivation of conditionally immortalized cell lines derived from human, rat and mouse models of ADPKD and ARPKD via retroviral delivery to cell cultures of the temperature-sensitive T antigen (*Table 15-4*) (44, 45, 48, 49, 80) or by crossing PKD1, PKD2 mutant mice with the Immortomouse (64) will clearly provide a valuable tool essential tool to analyze the precise function of polycystin-1, and polycystin-2 proteins and to allow development and testing of potential therapeutic interventions.

In addition to genetic cystic diseases, cell culture techniques have also been developed to study X-linked hypophosphatemia (147), nephropathic cystinosis (45) and hydronephrosis (148) and other developmental abnormalities. Although monolayer culture provides several advantages, these studies can be complemented by application of organ cultures of embryonic and postembryonic kidneys (99). Such approaches have been used to define specific defects in ureteric bud morphogenesis and tubule differentiation after inactivation of PKD1 or PKD2 (80). This then provides a 3-dimensional system to test the effects of gene replacement or modulation by delivery via viral transduction technology (100, 149) or of small molecule drugs or antibodies as potential therapeutics agents.

Renal Cancer

Kidney tumors arise predominantly either during early development (Wilms' tumor) or associated with advancing age (renal carcinomas).

Wilms' Tumor

Although it has been surprisingly difficult to culture primary cells from primary Wilms' tumors, some permanently

growing cell lines are available for study, the most widely used of which are G401 and SK-NEP (› *Table 15-6*) (117). The Wilms' tumor suppressor gene encodes a zinc-finger DNA-binding protein, WT-1 that is involved in normal renal development, and its deletion results in tumor formation.

Renal Cell Carcinoma

Numerous permanent cell lines have been established from human renal cell carcinomas including adenocarcinomas, non-metastatic and metastatic clear cell carcinomas with or without additional mutation of associated genes such as Von Hippel Lindau (VHL) (118–122) (› *Table 15-6*). These cell lines are important not only to provide insights into mechanisms of renal carcinogenesis, metastasis and resistance to apoptosis (120, 150, 151) but also to foster the development and testing of potential therapeutic approaches (152, 153).

Renal Fibrotic Disease

Interstitial fibrosis is an important manifestation of renal disease and exacerbates. Renal cell culture techniques employing primary and conditionally immortalized human, rat and mouse renal cortical and medullary fibroblasts have led to important mechanistic insights, including identification of increases in specific integrin (α_5/β_1 and α_v), nonintegrin and discoidin domain matrix receptors (27, 112). Cell culture techniques are also ideally suited to test the potential involvement of growth factors, cytokines and inflammatory mediators such as TGFβ and TNFα and nitric oxide in epithelial to mesenchymal transition (EMT) which is thought to play a role in the induction of fibrosis (27, 154). Co-culture techniques are likely to be particularly instructive with regard to the influence of diseased epithelia on fibroblasts and vice-versa. Mix and match experiments provide the potential to rescue defects and thus could lead to therapeutic insights.

Conclusion and Perspectives

The ability to culture pure populations of renal cells has provided important fundamental tools and has increased understanding of the renal cell biology of normal and diseased kidneys. Together with rapidly increasing molecular understanding of disease processes, due to sequencing of the human, rat and mouse genomes and the identification of many disease genes, it is to be predicted that the future will bring a rapid advancement in our understanding of disease processes. This will be facilitated with the ability to rapidly and efficiently transfect all renal cell types in culture, in a cell specific manner using efficient viral delivery systems. In addition, sophistication of monolayer cell culture techniques and development of clonal, conditionally immortalized cell lines will facilitate development of high-throughput testing of new drugs and potential therapies for a wide range of renal diseases. Finally, with advances in stem cell biology and the ability to derive, modify and deliver renal progenitor cells (155–157), long-term, cell-based regenerative therapeutic approaches to combat renal disease might become a viable option in the foreseeable future.

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16 Animal Models

Jordan Kreidberg

Introduction

The use of animal models has been an essential aspect of nearly all areas of nephrological research since its earliest days. Research on kidney formation and malformation, physiology and pathophysiology, immunological injury, and tolerance or transplant rejection all depend on the use of animal experimentation. This chapter will emphasize genetic approaches that utilize animals, as this area has shown the great progress in the development of novel technologies, that have had great impact in all areas of nephrology.

Institutional Oversight

There is increasing public awareness of the use of animals in research, and with this comes increasing concern about the appropriateness of the use of animals, and whether much of the research that does involve animal models could be accomplished using non-animal models. Therefore it is important to note that all animal research in the United States and presumably in most other countries must be evaluated by institutional committees before any experimentation may commence. Furthermore, the United States Department of Agriculture (USDA) provides constant oversight through the use of frequent and usually unannounced visits to animal facilities with research institutions. These regulatory committees and agencies are charged with evaluating animal protocols to make certain that animals are used in an ethical manner, with proper use of anesthetics or analgesics to minimize or eliminate any source of pain during experimentation. They are also charged with verifying that animals are indeed required for the specific research in question, that large animals are not used when smaller ones would suffice, and that the investigators are trained and knowledgeable about proper use of animals. Despite these several layers of oversight, in the end it is up to the principal investigator to be thoughtful about whether their intended experimental approach will yield sufficiently important and worthwhile results to justify the use of laboratory animals.

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Animal Models of Kidney Disease

The selection of an animal model for some aspect or type of kidney disease takes several factors into consideration. Most importantly, the similarity to human disease that can be observed in a particular model is taken into account. Other important factors include the cost of the animals involved: the cost of maintaining animals larger than rodents increases dramatically with size, and the numbers of animals that can be studied consequently decreases. For this reason, some studies may begin with a rodent model, and then progress to a larger model once the rodent model establishes the feasibility of the hypothesis under study. The size of an animal may be important to the extent that it affects the ability to perform surgical manipulations or physiological measurements. However, since it has become increasingly desirable to obtain physiological measurements on various strains of knockout mice, the equipment available to perform these measurements has improved and become commercially available.

Genetic Models

Animal models of disease that have a genetic basis may either result from spontaneous or induced mutations. Spontaneous mutations or phenotypes are those noticed either by chance or through the directed observation of large numbers of mice, that were not otherwise treated to induce a mutation. In contrast, induced mutations are those resulting from the treatment of mice with irradiation or mutagenic agents known to introduce point mutations or deletions into the genome.

The past 20 years have witnessed an explosion in the use of genetic approaches to understand development and physiology, and thus they will receive appropriate emphasis in this chapter. Several genetic approaches are available for use with animal model systems. A gene of interest may be mutated using gene targeting, or expressed in transgenic mice in such a way to interfere with its normal function. On the other hand, it is possible to start with a phenotype of interest, which could either be obtained as a spontaneous mutation or from mice treated with

a mutagen, and an effort is made to identify the mutated gene responsible for the phenotype. Genetic approaches using gene targeted or transgenic mice are useful for a wide variety of developmental and physiological studies in which there is a need to study the function of a known gene.

Genetic Approaches with known Genes-Genotype to Phenotype

Gene Targeting

Gene targeting was originally used to introduce a deletion or interruption into a gene of interest, using the scheme shown in *Fig. 16-1*, such that it could be determined whether mice would be able to develop in the absence of that gene's function. In cases where a gene was shown not to be essential for development, the homozygous mutant mouse might serve as a useful model in which to study the role of a specific gene in a physiological or disease process. For example, targeted deletions of the Wt1 (1), Pax2 (2), GDNF (3–5), Wnt4 (6), and BMP7 (7, 8), among others, showed these genes to be essential for various aspects of early kidney development. On the other hand, the absence of many immunology-related genes does not result in any developmental impairment, but these mice have served as useful models to study the role of the immune system in transplant rejection.

The advent of gene targeting was made possible through the use of two technologies developed mainly in the 1980s. The first was the development of tissue culture conditions that allowed embryonic stem (ES) cell lines to be grown indefinitely in culture while retaining their totipotency (9). ES cells grown in culture could then be introduced into mouse preimplantation embryos or blastocyts, and become fully integrated into those embryos such that their descendant cells would give rise to all developmental lineages that are found in adult mice (10). The second technology involved the use of homologous recombination to introduce mutations into mammalian genes (11–13). As shown in *Fig. 16-1*, when long stretches of genomic DNA in recombinant DNA constructs are introduced into cells in culture, this DNA will, at variable and often quite low frequency, recombine into the locus from which the genomic DNA was originally derived. Therefore homologous recombination of the correctly designed genomic fragment can be used to introduce a deletion or insertion into a genomic locus, that renders the gene unable to be expressed. This ES cell would in essence be heterozygous for a mutation in the targeted gene, and heterozygous ES cells can be isolated and expanded to provide a population for injection into blastocysts. Therefore, by combining the ES cell technology and homologous recombination, it became possible to target mutations into genes in ES cells, and then introduce ES cells carrying these mutations into blastocysts, finally obtaining a mutant adult mouse.

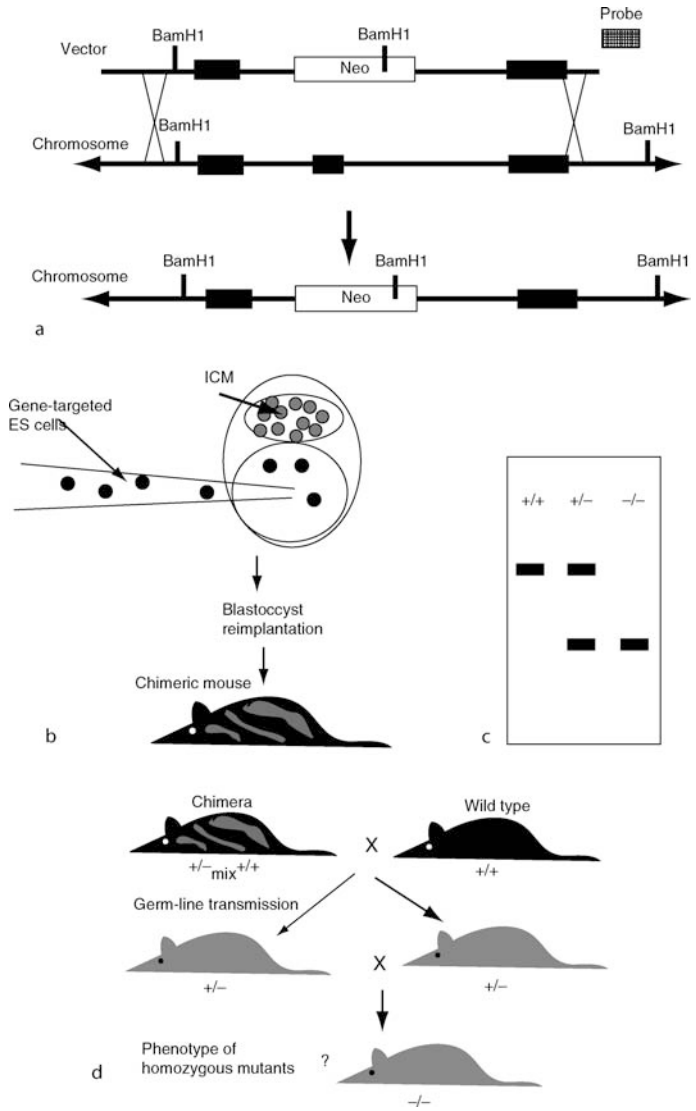
In a typical experiment, gene targeted ES cells would contain one mutated allele and one normal or wild type allele for the gene under study. The targeted ES cells would be injected into preimplantation blastocysts, and groups of these blastocysts would be introduced into female mice that were previously hormonally primed to allow implantation of the injected blastocysts into their uteri, to begin a pregnancy. The resultant mice from these injections are termed "chimeras," because any specific cell is either derived from an ES cells or the original injected embryo, i.e., the chimeric mouse essentially has four parents, the male and female that provided the blastocyst, and the male and female that provided the embryo from which the ES cell line in use was originally derived. In the best cases, a chimera might be nearly entirely derived from the ES cells. Among the tissues that ES cells contribute to are the germ cells: spermatocytes or oocytes. When ES cells heterozygous for a mutation are used to make a chimera, germ cells derived from the ES cells have a 50% chance of carrying the mutant rather than the wild type allele. Therefore, mating a chimeric and wild type mice can result in some of the offspring being true heterozygotes for the mutated gene. After obtaining both male and female heterozygotes, they can be mated to obtain homozygous mutant embryos or mice, depending on whether or not the gene is essential for development.

Conditional Gene Targeting

The process described in the preceding section results in the inactivation of a target gene from the beginning of embryogenesis. In this situation, an embryo will become non-viable at the first point at which expression of the inactivated gene becomes essential for survival. However, it may be highly desirable to study the function of a gene product in many later events during development or adult life. Conditional gene targeting allows the inactivation of a gene in particular tissues or at particular times during development or adult life (14–16). This technology has been developed more recently, and has proved more difficult to employ on a widespread basis thus far, for reasons that will be discussed.

Figure 16-1

Gene targeting in mice. (a) The scheme for targeting a deletion of an exon in embryonic stem cells. Exons are shown as black boxes along a chromosome. Restriction sites for restriction enzyme BamH1A are shown. The replacement vector is constructed such that the neomycin resistance gene (neo) is shown as an open box, in place of one of the exons. An external probe specifically does not overlap with the replacement vector. A double homologous recombination results in then integration of the vector into the chromosome, thus replacing the exon with the Neo gene. The BamH1 site within the neo gene results in a shorter BamH1 restriction fragment detected by probe after homologous recombination. (b) ES cells can be injected through a micropipette into a blastocytst, where they become part of the inner cell mass. The injected blastocyst is introduced into the uterus of a hormonally primed mouse, and gives rise to a chimeric mouse, partially derived from the ES cells, and partially from the original inner cell mass cells. If the ES cells and blastocysts are derived from strains with different coat colors, then the chimeric mouse will have a variegated coat color pattern on its fur, providing an indication of its overall extent of chimerism. In the best cases, the resultant mouse is nearly entirely derived from ES cells. (c) shows a possible pattern obtained in a Southern blot, based on the scheme shown in (a), using the external probe. A wild type mouse shows only the longer band. A heterozygous mouse shows both the wild type and gene targeted band, and the homozygous mutant shows only the shorter band, due to the presence of the BamH1 site in the neo gene. (d) the mating involved in obtaining germ line transmission of the mutation and subsequently obtaining homozygous mutant mice.



The general approach to conditional gene targeting is shown in *Fig. 16-2*. This is a variation on traditional gene targeting, in that it also relies on homologous recombination to introduce a segment of recombinant DNA into the locus of a gene in ES cells. However, whereas traditional gene targeting inactivates the gene, conditional gene targeting must modify the gene such that it can be expressed until such time as its inactivation is desired. The most commonly used approach involved the insertion of LOX sites, which are 34 base pair sites involved in site-specific recombination by Cre recombinase, and enzyme originally derived from a bacteriophage (15). Since LOX sites are rather small, it is usually possible to insert them in introns where they have no effect on gene expression. By placing two LOX sites in a gene to flank an exon, Cre can be used to inactivate a gene by recombining out the DNA segment containing the exon that was situated between the two LOX sites, thus inactivating the gene. There are experimental approaches for expressing Cre in temporally and spatially specific manners or both. Spatial or lineage specific expression of Cre is most often obtained by placing the Cre cDNA downstream of a known tissue specific promoter. Sometimes this is achieved by using homologous recombination to insert the Cre gene into the genomic locus of a gene with known tissue specific expression, such that Cre replaces the first exon of that gene. Temporally specific expression of Cre has proved more difficult to obtain. One approach is to regulate Cre using the tetracycline system for inducible gene expression (17). The other approach makes use of a fusion protein consisting of Cre and a portion of the estrogen receptor that confers steroid mediated nuclear localization (18, 19). The latter is modified to bind tamoxifen or tamoxifen-derivatives instead of estrogen. The Cre-modified estrogen receptor fusion protein will remain in the cytoplasm and therefore not be able to mediate site-specific recombination of LOX sites, until tamoxifen is administered to the mouse to induce nuclear translocation of the Cre fusion protein. This system can be used to induce recombination in embryos, when tamoxifen is administered to pregnant mice. The major obstacle to employing conditional gene targeting on a widespread basis is the availability of promoter/enhancer elements that are able to confer robust tissue or cell-lineage-specific expression of Cre recombinase. However, an increasing number of mouse strains are available that express Cre recombinase in various cell lineages within the developing and adult kidney (20). It is possible to obtain conditional knockouts restricted to nephron progenitor cells (21), podocytes (21, 22–24), proximal tubules (25–27), thick ascending limb (28), ureteric bud (29), juxtaglomerular cells, and collecting ducts (30). It is also possible to obtain vascular knockouts (31–33), though not restricted to the kidney.

As more tissue-promoter elements become available, conditional gene targeting promises to have a large impact on genetic approaches to kidney disease. As noted above, there are many genes expressed both in developing and adult kidney, where the knockout of the gene results in embryonic lethality. This precludes study of how that product of that gene might function in postnatal kidneys, or why a mutation in that gene leads to kidney disease in humans. It also raises the question of why humans carrying such mutations are able survive, albeit with a genetic disease, when mice carrying mutations in the same gene do not survive embryogenesis. Sometimes this is simply because mice and humans differ in their respective requirements for specific genes, but more often, it is because humans with genetic disease often have point mutations that lead to partial loss of function, whereas mouse knockouts often involve complete loss of function mutations. Conditional gene targeting can sometimes offer a solution to this problem, by allowing normal gene expression during embryogenesis, and then inactivating a gene in adult mice. Alternatively, there are variations on the Cre-LOX approach that allow the introduction of point mutations into mice. The introduction of point mutations into mice has been greatly facilitated by recent advancements that facilitate homologous recombination into BACs (Bacterial Artificial Chromosomes) in E. Coli (*Fig. 16-3*) (34–36). BACs are used as they contain large amounts of genomic DNA, and thus are ideal for use as gene targeting vectors. The longer length of BACs compared with shorter genomic clones should improve the frequency of homologous recombination in ES cells.

Animal Models using RNAi Approaches

RNAi technologies have had great impact across the breadth of molecular biology and the study of disease over the past 10 years (37). RNAi is based on a mechanism common to both plant and animals whereby a short strand of RNA, that is associated with a set of proteins known as the RISC complex, hybridizes to a complementary sequence in a mRNA, causes degradation or inhibition of translation of the mRNA (38, 39). In the natural setting, these small RNAs are encoded in the genome as microRNAs. The same molecular machinery used by microRNAs to inhibit expression of mRNAs can be

Figure 16-2

Conditional gene targeting. The targeting vector is different from the previous figure in that LOX sites flank the exon that will eventually be deleted, and the Neo gene is flanked by Frt sites. The vector is incorporated into the chromosome through homologous recombination, and ES cells with this knock-in are used to make chimeric mice, and germline transmission is obtained. Although the LOX sites should not interfere with expression of the gene, the Neo gene is likely to interfere with normal gene expression. However, in most cases mice will tolerate one inactive gene, as long as the other allele is functional. After obtaining heterozygous mice, they are mated with Flp – deleter mice, that express Flp recombinase in germ cells. Flp will recombine the FRT sites and eliminate the neo gene. Mice without the neo gene, but still containing the exon flanked by LOX sites, are mated with mice expressing Cre in a particular tissue or cell type, or expressing an inducible Cre, to obtain the conditional knockout. The breeding scheme shown in the figure is oversimplified. In the actual experiment, a more complicated breeding scheme is required to obtain a mouse that is homozygous for alleles with LOX sites, and that also has the Cre-expressing transgene. An alternative is to breed mice with the conditional allele with mice carrying a traditional knockout. This has the advantage that to obtain the conditional knockout, Cre must only recombine one, and not two, pairs of LOX sites in each cell.

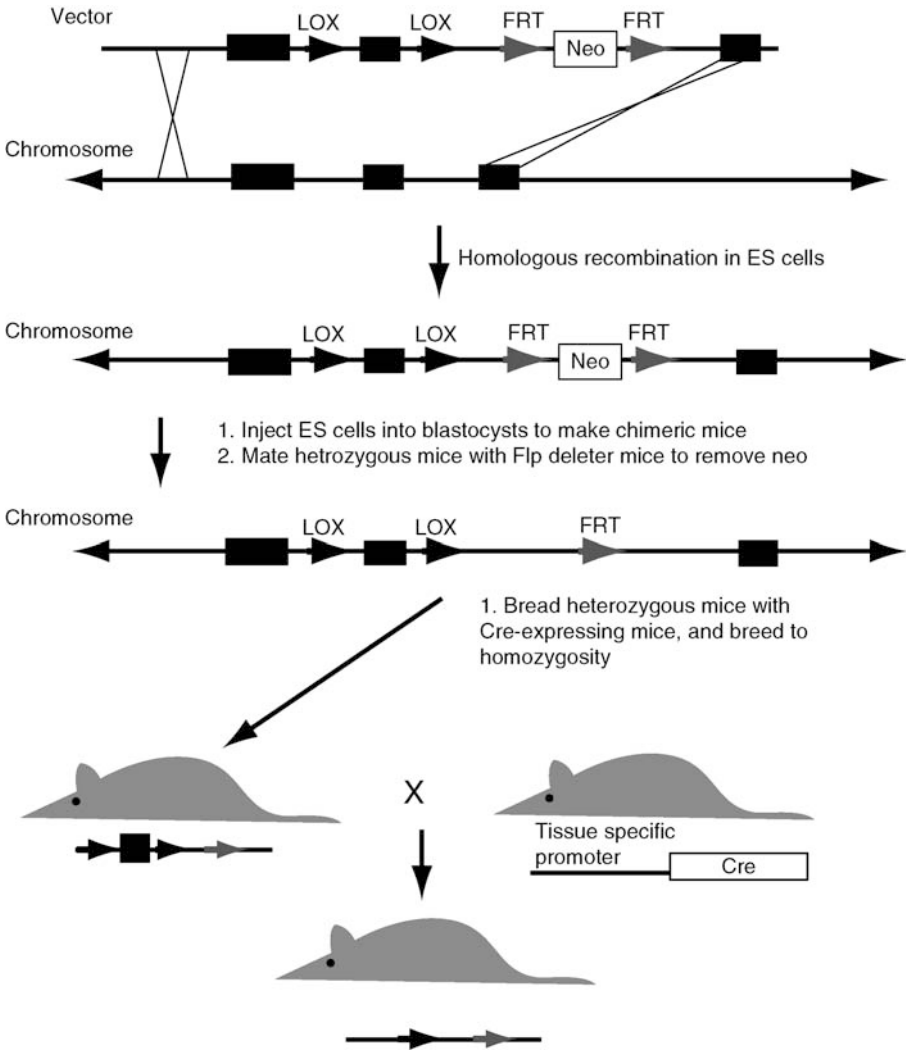
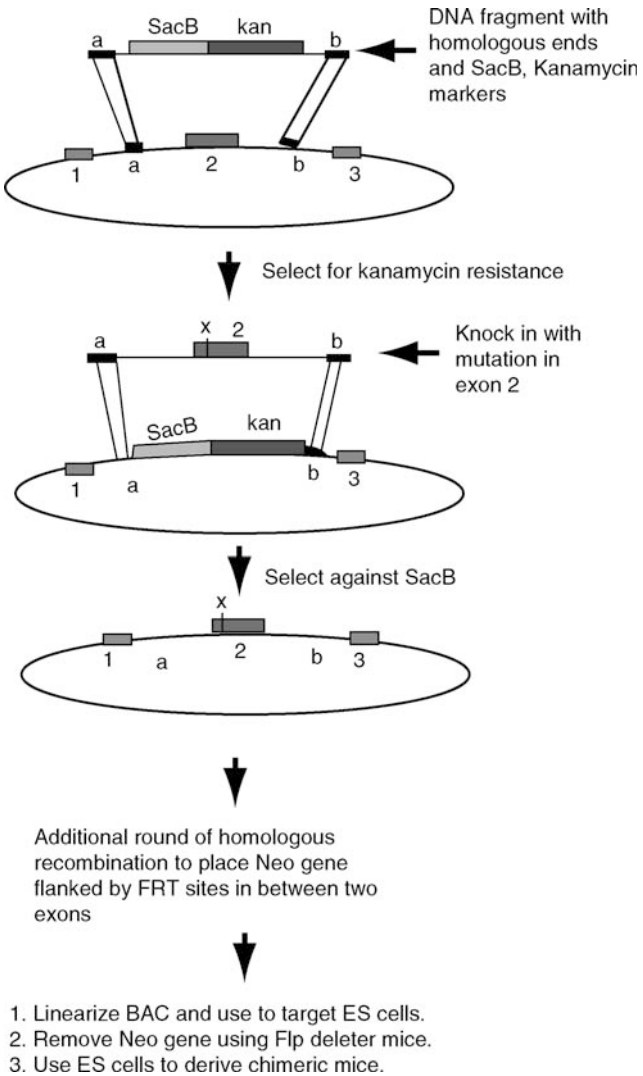


Figure 16-3

Gene targeting using BAC clones. Homologous recombination is done in E. coli instead of in ES cells. In the first step, a DNA fragment is prepared that contains the kanamycin resistance positive selectable marker and the SacB negative selectable marker, and which also contains homologous ends (A and B, each about 50–60 bp) is introduced into E. coli. This fragment can usually be prepared by PCR, using primers that contain the homologies to the genomic region, and also to a vector containing the selectable markers. Usually, a strain of E. coli is used that allows transient activation of the enzymes required for homologous recombination. Selection for kanamycin resistance will obtain BAC clones where the selectable markers have recombined into the BAC. In a second round of homologous recombination, A DNA fragment with the same homologous ends but containing a mutated exon 2, denoted by the "X," is introduced into the E. coli containing the BAC. Selection against SacB will obtain BACs in which the mutated exon 2 has replaced the selectable markers. A third round of homologous recombination is used to insert the neo gene, flanked by FRT sites, so that the BAC can be used for homologous recombination in ES cells. As shown, this scheme is used to introduce point mutations or small deletions into a gene. It can also be used to construct conditional knockout vectors, similar to those shown in *Fig. 16-2*. An additional use is to knock-in a Green Fluorescent Protein (GFP) or β-galactosidase (LacZ) reporter gene into a locus to obtain information about patterns of gene expression.



co-opted for use by siRNA (silencing RNA) or shRNA (short hairpin RNA) that are ectopically expressed in cells. siRNA refers to RNA duplexes that are usually obtained commercially and transfected into cells. shRNA refers to small RNAs that are expressed from plasmids or viral vectors that are introduced into cells. A major addition to the arsenal of approaches involving transgenic mice involves the derivation of transgenic mice that express shRNA molecules capable of reducing levels of mRNA for specific genes in specific tissues or cell lineages (38–41). shRNAs can be expressed either constitutively, or conditionally using the Cre-Lox system (*Fig. 16-4*). An advantage of shRNA technology over gene knockouts is that it is quicker and cheaper, as mice do not need to be bred to homozygosity and also to carry the Cre transgene. Instead, a resulting phenotype can be obtained by mating a Cre-expressing mouse with a mouse carrying a conditional shRNA transgene, and examining the first generation offspring carrying both transgenes. The major disadvantage in comparison with gene knockouts is that expression of the target gene may be variably reduced, and it may be necessary to examine several shRNA transgenic lines in order to obtain ones that demonstrate efficient knockdown of the desired gene.

Transgenic Mice

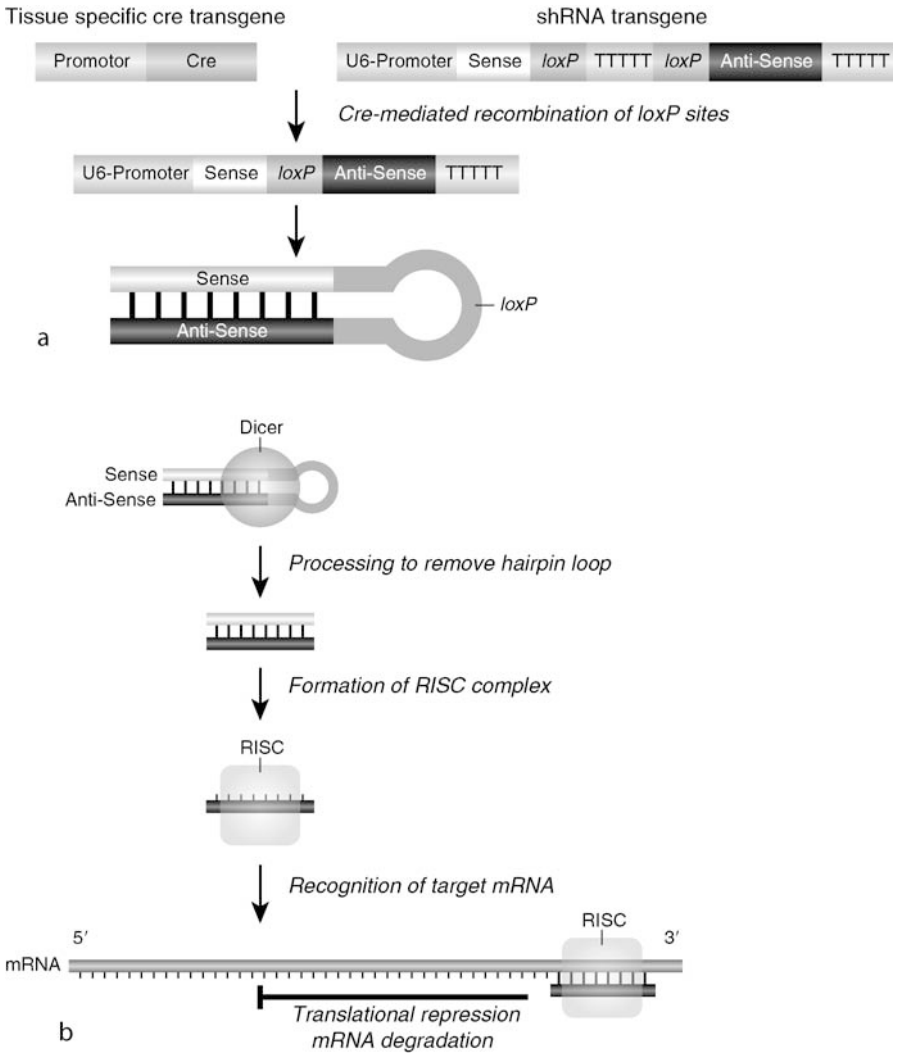
As mentioned above, many mutations that result in human disease are point mutations that result in "hypomorphic," or partial loss of function alleles of a gene. In this case a disease state may result from decreased activity of a gene product. In other cases, a point mutation or deletion mutation may produce a protein that interferes with the function of the normal gene; this is referred to as a dominant negative effect. This could occur in instances where a protein requires homodimerization for activity, and dimerization of a wild type and a mutant form of a protein leads to an inactive complex. Dominant-negative effects can also be found in cases where two proteins heterodimerize, and an inactive mutant protein is able to complex with its partner protein, but as before, the complex is inactive. Dominant – negative effects can be studied in animal models using transgenic mice. Although gene targeted mice discussed in the previous sections can also be considered to be transgenic, because foreign DNA is used to disrupt the endogenous gene, here the term "transgenic" is reserved for those mice in which foreign DNA has been inserted into the murine genome through pronuclear injection (42–45). In contrast to gene targeting schemes in which genes are modified in ES cells, and ES cells are then used to derive chimeric mice, transgenic strategies, DNA is directly microinjected into the pronucleus of a fertilized egg or zygote, and the injected zygotes are then reimplanted into the oviduct of a hormonally-primed female mouse. The injected DNA is able to recombine by non-homologous, or illegitimate recombination into random locations within the genome, and in variable amounts from zygote to zygote. Once mice are derived from the injected zygotes, they are tested to determine if they carry the injected DNA within their genomes as a transgene, and if they do, whether the transgene is expressed. By injecting DNA constructs that contain a tissue-specific promoter and a mutated gene of interest, it is possible to study whether expression of the mutant gene leads to an observable phenotype. In other instances, the gene to be expressed is not mutated, and the experiment is designed to determine if overexpression or de novo expression of the gene results in an observable phenotype or disease model. While the original transgenic studies used relatively short DNA constructs, more recent studies have used BAC (bacterial artificial chromsome) or YAC (yeast artificial chromosome)-based vectors whose much longer stretches of DNA containing promoters and other regulatory regions will hopefully confer more faithful patterns of expression of the transgene directed by those regulatory elements (42, 46). The great majority of transgenic work has been done in murine models, but there have been pioneering efforts in other species such as pigs and rats (47, 48).

Mutagenesis in Zebrafish

Possibly the single most important advancement in the development of novel animal models for kidney disease over the past 10 years is the establishment of zebrafish (*Danio rerio*) as a major model for understanding the genetic and physiological basis for disease. Zebrafish models of glomerular development and disease and polycystic kidney disease have been particularly useful models for study of human disease (49–55). Zebrafish are much less expensive to maintain than mice, and genetic tools to map zebrafish mutations nearly equal or in some cases extend beyond those available for mice (56–58). The zebrafish excretory system involves a pronephric duct and glomus that bears important similarity to mammalian nephrons, and has already been the subject of many research studies (49, 59–65). Two great advantages of Zebrafish are (1) their shorter

Figure 16-4

Conditional expression of shRNA transgenes. (a) Mice containing a Cre recombinase-expressing transgene and a shRNA transgene are crossed to obtain embryos or offspring mice containing both transgenes. The shRNA transgene contains a U6 promoter to direct RNA polymerase III mediated transcription, that is terminated by a poly-T sequence. It also contains a sense and anti-sense sequence, designed to target a mRNA, that will become the double stranded RNA that associates with the RISC complex to mediate the inhibitory effect on mRNA stability or translation. Therefore, transcription of the shRNA transgene is interrupted prior to Cre-mediated recombination of the first poly-T sequence. After removal of the first poly-T sequence by Cre-mediated recombination of the two LoxP sites, the transgene is fully transcribed and terminates at the second poly-T sequence. The resultant shRNA forms a hairpin loop by base pairing of the sense and ant-sense sequences. (b) The shRNA associates with DICER, an RNase III class enzyme that removes the hairpin loop. The double stranded RNA then associates with the RISC complex, the sense strand is removed, and the RISC complex with the anti-sense RNA finds its target mRNA and suppresses translation or decreases mRNA stability.



developmental timing, and (2) that development occurs in nearly transparent embryos that develop outside the mother, allowing for far greater observation and intervention than is possible with rodent embryos. The Zebrafish genome is presently being sequenced at the Sanger Center (66), and a full set of markers exists for gene mapping (57, 67, 68). Importantly, Zebrafish are not inbred like inbred mouse strains and there exists genetic

heterogeneity between isolates used in different laboratories. It is possible to make transgenic Zebrafish, though gene targeting is not yet possible (69–71). However, there have been large scale efforts to saturate the Zebrafish genome through insertional mutagenesis (72–74). An alternative to gene targeting is the use of morpholio oligonucleotides that inhibit expression of specific genes against which the morpholino is targeted; these can be injected into cells of early embryos, and phenotypes can be observed at various developmental stages thereafter (75, 76). In one sense, morpholinos have an advantage over gene knockouts, in that they can be designed to block expression of specific alternative splice forms (75), rather than all forms as is often the case with gene knockouts in mice.

Gene Identification- Phenotype to Genotype

Mutational Screens to Obtain New Phenotypes and Identify Genes

ENU mutagenesis: At present, several major efforts in several countries involve the use of N-ethyl-N-nitrosourea (ENU) to introduce small mutations throughout the mouse genome (77–82). Similar approaches have been used with Zebrafish and many interesting phenotypes have been obtained (83–87). These large genome-scale approaches, which can involve very large mouse or Zebrafish colonies, are justified by the following arguments: (1) Most disease-related human mutations are caused by point mutations, therefore a ENU-mutagenic approach may have a greater chance of producing a phenotype resembling a human disease than will gene-targeted mutations that usually completely inactivate a gene; (2) an ENU-based approach does not rely on previous identification or cloning of the gene, i.e., any gene is a theoretical target, and can be studied, to the extent that some degree of compromise in the gene product's activity will result in an observable phenotype. The obvious disadvantage in comparison with gene targeting is that a large amount of work lies between the observation of a phenotype and the final identification of the mutated gene. (3) Given a large enough effort, it should be possible to eventually "saturate" the genome with mutations, i.e., examination of several hundred thousand mutagen-treated mice or Zebrafish is likely to provide the opportunity to observe the effects of placing a mutation in every gene capable of causing an observable phenotype. However, one important point remains to be mentioned, that dramatically increases the labor and expense of an ENU-based effort. Most observable phenotypes tend to be genetically recessive instead of dominant, meaning that they are not apparent in the first generation offspring of mutagen-treated animals. Instead, it is necessary to breed a second generation and then backcross it to the first generation mice (or Zebrafish), resulting in a third generation (*Fig. 16-5*). Doing this on a large scale will result in many third generation animals that are now homozygous for mutations resulting from the original mutagenic treatment, and some will have observable phenotypes that can be studied for biological interest and to map the responsible gene.

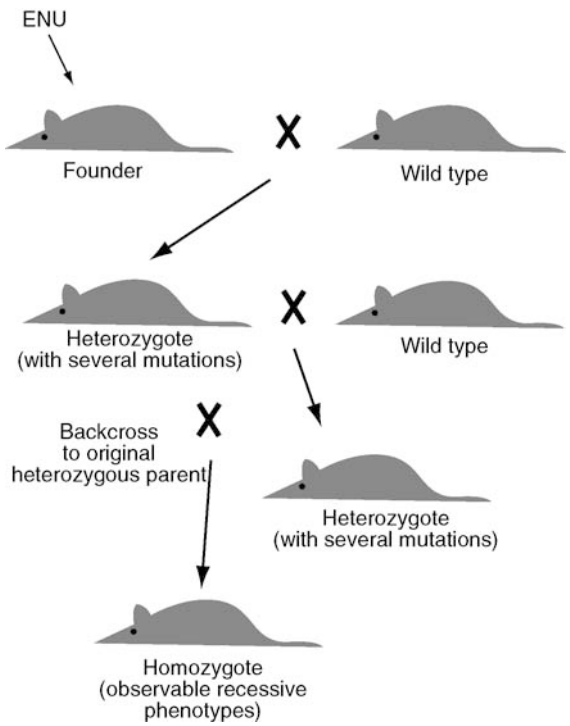
These large scale genetic approaches are not only suited to study developmental anomalies. Some of the large-scale efforts on mouse mutagenesis ongoing around the world will involve performing basic blood work and a urinalysis on each mouse from the group being screened for new phenotypes. Thus this approach has the potential to identify genes involved in disease progression, as well as those responsible for morphogenetic processes.

Gene Identification

Microsatellite repeats: Mapping sites of induced or spontaneous mutations in mice has been greatly aided by the development of sets of microsatellite repeat markers. Microsatellite repeats used in mapping are stretches of CA dinucleotide repeats that are found interspersed throughout mammalian genomes (88, 89). Typically, these CA repeats contain 10–20 CA dinucleotides. These CA repeats are flanked by unique sequences, and thus it is possible to design pairs of PCR primers that correspond to these flanking sequences, that will amplify the $(CA)_n$ sequence between the two primers. Within a genetically inbred strain of mice each individual mouse will contain the same number of CA dinucleotides at each repeat. However, similarly to the variation observed between human individuals, different inbred strains may differ in the number of CA dinucleotides at any particular repeat. Additionally, there are species of mice closely related to *mus musculus*, such as *mus spretus*, that provide even greater differences in the number of CA dinucleotides at many repeats than are found between the inbred strains of mus musculus. As depicted in *Fig. 16-6*, genetic mapping using microsatellite markers takes the following approach: A mouse (or mice) with a phenotype produced by induced or spontaneous mutagenesis is mated with a mouse from a different inbred strain, or from a different species, such as mus spretus to produce F1 mice that are now

Figure 16-5

ENU mutagenesis. A scheme is depicted for finding recessive phenotypes through ENU mutagenesis. Dominant phenotypes require a less complicated approach, as phenotypes will be apparent in the first generation derived from crossing founders with wild type mice. In this scheme, a mutagenized male founder that probably carries many mutations after mutagenesis, is mated with a wild type mouse to produce heterozygote offspring that carry a subset of these mutations. These heterozygotes are mated with wild type mice to produce a second generation, which will carry a smaller subset of the original mutations. These are then mated to the original heterozygous offspring of the founders, and 25% of the offspring of this cross will be homozygous for any particular mutation that was present in the second heterozygous generation.

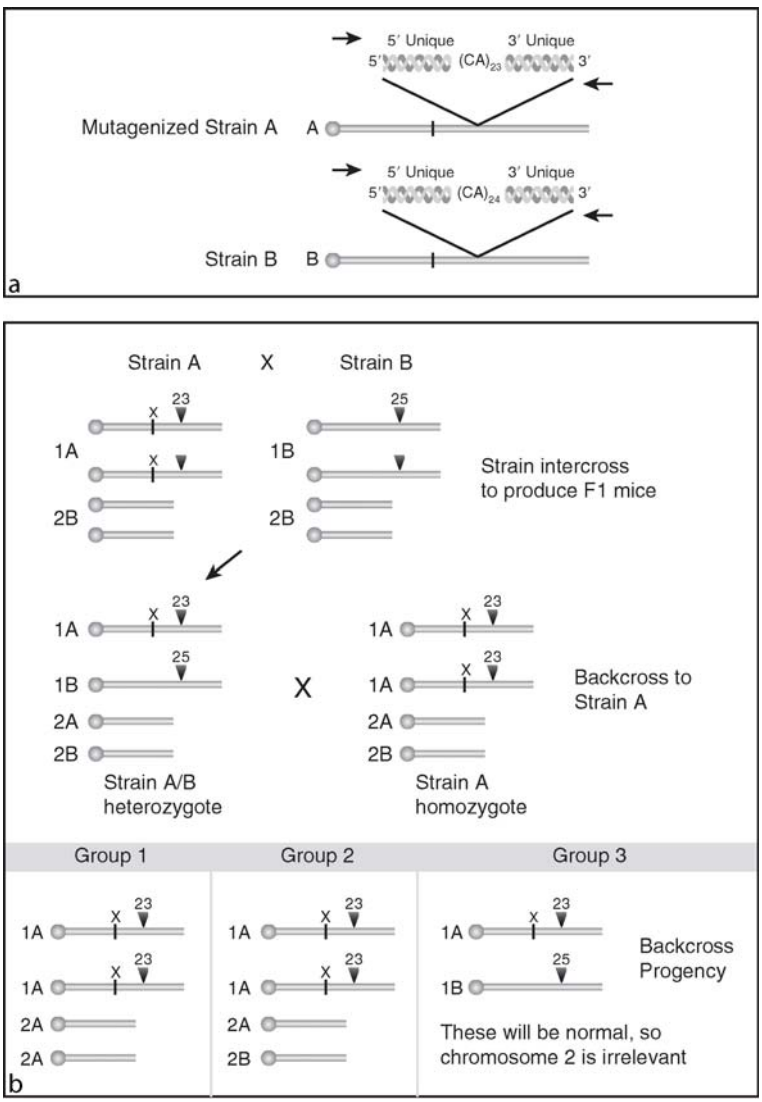


heterozygous at all loci, containing one allele from each of the two parental strains, and one mutant allele. In the case of a recessive phenotype, these F1 mice are now either backcrossed to the original mutant strain, or intercrossed among themselves, to produce approximately 100 progeny mice, about 25% of which can be expected to show the mutant phenotype. For dominant phenotypes, the backcross can be to a wild type mice of either parental strain, and 50% will display the phenotype. Importantly, during this back- or intercross, there is independent segregation of chromosomes, such that each individual of the 100 mice is genetically unique, in that at any given locus, it may be homozygous for alleles from a parental strain, or heterozygous, containing an allele from each strain. A set of PCR primers corresponding to about 40–50 microsatellite repeats (about 2–3 per mouse chromosome) are used in the first round of analysis. These are chosen such that the two parental strains are know to differ in the length of the CA repeat between each primer pair. DNA samples are now obtained from all the progeny, and are tested for the length of the CA sequence at each of the 40–50 microsatellites, and these results are correlated with the observed phenotypes (in practice, a computational result, called a LOD score is produced). Most of the microsatellites will not be genetically linked to the locus containing the mutation, and there will be no observable correlation between the strain genotype at a particular microsatellite and the presence or absence of the phenotype. In contrast, if a microsatellite marker is sufficiently closely linked to the site of a mutation causing a recessive phenotype, both alleles of the microsatellite marker are more likely to be derived from the parental strain originally containing the mutation. Thus, the goal of the first round of screening is to identify at least one marker that is linked to the mutation. Thereafter, subsequent rounds will use sets of markers linked to the original positive marker, with the expectation that it will be possible to identify a marker or pair of markers very closely linked to the mutation that will delimit the region of a single chromosome on which the mutation is located. This can then be used to initiate either a candidate gene approach or a chromosome walking approach to eventually identify the mutated gene.

SNPs and haplotype mapping: Newer more efficient approaches to mapping genetic elements are presently replacing the use of microsatellite repeats described in the previous section. Chief among these are the use of SNP microarrays. SNPs, or single nucleotide polymorphisms, are single base pair differences found between individuals within a species. Among the human population, SNPs tend to be found every 100–300 base pairs within the genome, and can be used as a measure of genetic relatedness among, for example, ethnic groups, or people in different geographic areas (90). SNPs are also found in comparing different inbred strains of mice and between the commonly used laboratory inbred strains that are all derived from *mus musculus* and other mouse strains that are "non-*musculus*" that can be intercrossed with mus musculus strains to aid in genetic mapping, as described above (91). In contrast, within a particular inbred strain of mice, there is by definition genetic homogeneity, and

Figure 16-6

A scheme for mapping a mutation to a genetic locus. (a) depicts the use of microsatellite CA repeats. Strains A and B are two inbred strains of mice, that differ in the length of many CA repeats, including the one shown here. Strain A has 23 CA dinucleotide repeats, whereas strain B has 25. They have the same unique 5′ and 3′ sequences flanking the CA repeats, thus the same PCR primers can be used for both strains, but amplification will yield a longer product from strain B than A. (b) The mating scheme to begin mapping the mutation. Only chromosome 1 and 2 are shown. Strain A has a homozygous mutation on chromosome 1, marked as an ''X,'' that is linked to the CA repeat, here designated by the black inverted triangle. This recessive mutation yields an observable phenotype. Strain A and B are mated to produce an F1 progeny, which will be heterozygous at all loci, including the one mutated in strain A. They will also be heterozygous for all CA repeats, including those on chromosome 2. Thus any CA repeats that differ between the two strains will yield two bands on a PCR reaction using the flanking unique primers for that CA repeat. F1 mice are backcrossed to Strain A homozygotes, and many offspring are examined. 50% of these offspring should be homozygous for X and have the observable phenotype. When these mice are analyzed for the CA repeat close to the locus for X, most mice with the phenotype will show only the strain A amplification product, whereas most of those without the phenotype will show both the strain A and strain B bands. In contrast, amplification of any CA repeats from chromosome 2 or any other chromosome, will not show any correlation of strain A homozyosity with the observed phenotype.



SNPs should not be present between individuals of the same strain. SNPs are also used to map genes in Zebrafish (68). See also *Fig. 16-7*.

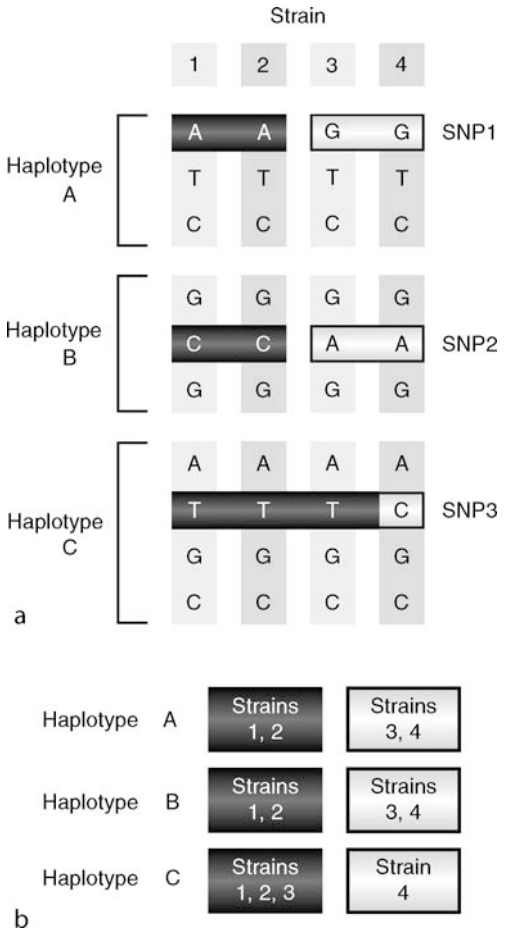
It is now possible to detect SNPs using microarrays (92, 93), such that thousands of oligonucleotides complementary to sequences containing known SNPs can be arrayed on a single microarray chip, and hybridized to an individual human or animal's DNA to determine which of thousands of particular SNPs that individual has in their genome (94). SNPs can be used as genetic markers similarly to the microsatellite repeats described in the previous section. Therefore, a single microarray can provide the same information as hundreds of PCR reactions. To be used in a genetic mapping experiment, the same type of inter-specific cross would be performed as described in the previous section, but instead of using PCR reactions to analyze the segregation of microsatellite repeats, a microarray is used for each individual to analyze the segregation of SNPs, to narrow the interval that contains the gene being mapped.

QTL and Haplotype Analysis

Almost all human disease has a genetic component, whether it is the relative susceptibility to an infectious agent, at one end of a spectrum, or a disease that is primarily due to a genetic mutation in a single gene, at the other end. Between these two extremes lies most human disease, whose etiology derives from a combination of genetic and environmental factors. In many instances, the genetic component is due to the effects of more than one gene. In other instances a single gene might be responsible, but the phenotype is not absolute, but rather of variable penetrance or severity. A genetic elements that contributes to a disease phenotype in a quantitative, as opposed to absolute, manner is referred to as a quantitative trait locus, or QTL (95). It is probably not an overstatement to say that most genetic components of disease occur as QTLs, and indeed, there are over 2,000 known QTLs that have been reported (96). Herein, however, lies the difficulty, as there are not standardized criteria for defining a QTL (95). Moreover, despite this enormous number of reported QTLs, only in the case of around 20–30 QTLs, depending on the criteria used to judge, have the responsible genes been identified and rigorously proven to be responsible for the disease phenotype (96). This is both due to the difficulty in identifying genes responsible for phenotypes in complex genomes, but also because in situations where a phenotype

Figure 16-7

SNPs and haplotypes. (a) A highly simplified of schematic of SNPs and haplotypes is depicted. Four mouse strains, labeled at top are compared. A three nucleotide stretch is shown for haplotypes A and B and four nucleotides for C, though in reality a haplotype may span megabases and be defined by hundreds or more SNPs. The sequences in haplotypes A, B and C are not necessarily contiguous and might even be on different chromosomes. In haplotype A, SNP1 is present at the first nucleotide, in haplotype B, SNP2 is present at the second nucleotide, and in haplotype C, SNP3 is present at the second nucleotide. (b) Haplotype grouping defined by the SNPs. For haplotypes A and B, strains 1 and 2 are the same haplotype, and 3 and 4 define a different haplotype. For haplotype C, strains 1, 2 and 3 are the same haplotype, and strain 4 is a different haplotype, suggesting that strain 3 might be more closely related to strains 1 and 2 than is strain 4. If strains 1 and 2 differ from 3 and 4 for a disease phenotype, it is more likely that the gene is within the area covered by haplotypes A and B, than within haplotype C, that is shared between strains 1, 2 and 3.



may be due to the combined actions of many genes, the contribution of any individual gene may be modest and difficult to prove in an experimental setting.

Nevertheless, many animal models of human disease appear to be caused by one or more QTLs, and the identification of the responsible gene(s) is a pursuit of many research laboratories. Importantly, comparisons of syntenic regions between human and rodent genomes has aided the search for genes at QTLs, as it is often apparent that a QTL identified in humans corresponds to a QTL for a similar phenotype in rodents (97). Comparisons of syntenic regions can also be complicated, as a region harboring a QTL in a rodent genome, if not already delimited to a narrow interval, may be syntenic with several regions of the human genome, that are found on different chromosomes.

The approach to identifying QTLs is similar to that used to detect monogenic phenotypes, that involved the detection of microsatellite repeats or SNPs described above. However, QTL analysis is much more complicated than the analysis of monogenic phenotypes, as one is likely to find multiple regions that appear to be linked to the phenotype, and no single region will stand out as the obvious candidate, until much larger numbers of individual animals or humans are analyzed to obtain statistically significant genetic data for each of several candidate genes.

Haplotype analysis has the potential to make gene identification by SNP mapping more efficient (97–99). Haplotypes are defined as a group of genetic markers that are physically linked on chromosomes and that tend to be inherited together more often than might be predicted if genetic recombination events were evenly distributed along chromosomes. Perhaps the best know examples are the major histocompatibility loci, HLA in humans and H2 in mice. It is now known that haplotype units exist throughout mammalian genomes, and that SNPs can be used as markers to define haplotypes (97–99). It then follows that inbred mouse strains that more closely genetically related are likely to have the same haplotype at a particular genomic location, whereas more distantly related strains would be more likely to have different haplotypes (97, 98). Thus, when SNPs are being analyzed in the progeny of a genetic cross in an attempt to narrow down the location of a candidate gene, haplotype analysis provides an alternative to considering each SNP individually. For example, if strains A and B are being studied with the aim of identifying a disease related QTL only present in strain A and strain B being normal, then genomic areas where strains A and B share the same haplotype are unlikely to harbor the disease locus, whereas areas of the genome where they have differing haplotypes are more likely to have the disease locus. Therefore, consideration of haplotypes, each of which may contain hundreds of SNPs, may allow a means of focusing on candidate genes within differing haplotypes, and ignoring those located within regions where the affected and unaffected strains share the same haplotype, that might otherwise be suggested for further study through a SNP analysis that did not take haplotypes into account. In practice, such an analysis may involve multiple strains of mice and crosses between them in an effort to narrow a genetic interval and physically locate a QTL (100, 101).

Implications of Genome Sequencing

Sequencing of the entire human genome was completed in 2001 (102, 103) and of the mouse genome in 2002 (104). These continue to be refined, with more detailed reports and annotations of the sequence of specific human chromosomes published from 2003 to 2006. As of this writing, the sequences of approximately 180 organisms, including bacteria, plants and animals have been reported. This rapidly expanding database has transformed modern biology and the study of disease, and newer high throughput sequencing technologies promise an even more rapid expansion in of sequence data in the near future (105). For example, in gene mapping studies described above, it is no longer the situation that a disease gene might be mapped to an area within a chromosome that had never been sequenced, and the hunt for the gene becomes a large scale sequencing project. Now, once an area is delimited, the candidate genes in that area are largely known from prior genome sequencing, allowing much more directed sequencing to be done in attempts to find disease-causing mutations. The genome sequences for most, if not all, animals used as disease models is also known, greatly accelerating studies such as those involving disease-related gene identification in animal models. A major frontier in genome sequencing related to disease models is not so much the sequencing of additional species as it is the sequencing of multiple genomes within a species, so that we can increase our understanding of intra-species genetic variation, and how it relates to disease susceptibility (105–107).

Other Animals in Nephrology Research

The emphasis on mice and Zebrafish thus far in this chapter is not meant to overlook the enormous benefit that has derived from the use of other species. Despite

some pioneering efforts, gene targeting technology has not been developed in rats. However, ENU mutagenesis has been used to obtain rat mutant models of disease and gene mapping of disease phenotypes in rats has yielded important insights (108–112). Rats have also been widely used in studies of nutrition as it related to kidney development and disease (113). Furthermore, their larger size makes rats more amenable to studies that require precise physiological monitoring or imaging, though the technology to perform such studies in murine models, to take advantage of knockout mouse models, has also improved dramatically (114–121).

Large animal research have also had an important legacy in nephrology that continues to this day. Historically, several animal models have been used to study renal physiology, including swine, sheep, guinea pigs, rabbits, rats and mice. Fetal lambs have been a particularly important model in which developmental aspects of physiology have been studied, particularly relating to obstructive uropathy (122–134). Studies in large animals are also vital in efforts to use tissue engineering to develop artificial tissues or in models of tissue regeneration (135–137). As in other situations, there is a constant need to balance the advantages of a large animal model with the lower costs of smaller animals models.

Models of Renal Failure

Approaches to the study of renal failure include acute and chronic models. Acute renal failure has been induced using pharmacological agents, antisera against kidney tissue or other antigens in which immune complex formation leads to glomerular disease (138–144). Ischemia-reperfusion models of acute renal failure are achieved by temporarily ligating a renal artery allowing study of the pathological processes involved in tubular damage, as well as the effect of various pharmacological treatments on the pathologic process (e.g., (145–165)). An alternate form of tubular injury involves temporary obstruction of a ureter (166–168). Combining these injury models with genetic models is an emerging frontier in nephrology research.

Models of Immunological Injury

There are many models of autoimmune injury to the kidney. A traditional model for a lupus-like autoimmune disease is the NZB mouse, that has been studied for many years (169–173). These mice develop autoantibodies similar to those observed in humans with systemic lupus erythematosis and other related autoimmune disorders. More recently, many strains of mice carrying mutations in genes involved in the regulation of the immune response have been used to increase our understanding of the role the immune system plays in the onset and progression of kidney disease (174–184). These knockout strains have allowed investigators to begin a genetic dissection of genes involved in autoimmune and other disorders. Transgenic technologies have also had an important impact in the development of new immunological models. One particular contribution is the use of live-cell imaging that exploits fluorescent transgenic reporter genes which mimic the expression of genes expressed in specific immunological cell types (185, 186). These can be used either to trace the location of these cells in animals, or to show evidence of gene expression in an in vivo setting.

Transplant Models

Animal models have been used extensively to study transplant rejection, and in efforts to understand how tolerance to transplanted tissue may be improved. Over the past 20 years there has been an extraordinary advancement in our mechanistic understanding of the immune function, and this has been brought to bear on the study of transplant rejection and tolerance (187–193). Important models under study include skin and heart transplants in mice, and kidney transplants in rats (194–201). Additionally, it is possible to produce "humanized" mice by transplanting human tissue into immunodeficient or irradiated mice whose immune system has been reconstituted with human lymphocytes, thus allowing the study of human immune function in an animal model (202–205). One area of research that remains controversial is that involving xenografts (206, 207). Since the supply of human kidneys and other organs for transplants continues to fall far short of the demand, there is a desirability of determining whether non-human animals provide an alternative source of organs for transplantation. The major concerns here include the strong immunological rejection to a xenograft that must be overcome, and the danger that xenografts might serve as vectors for the introduction of novel infectious agents into the human population.

Summary

Animal models are of increasing importance in the study of kidney disease. An important shift over the past 10 or

more years is the use of rodent models, and the use of genetic models. Large animal use remains an important aspect of these studies. The use of large versus small animals must take into account cost and ethical considerations.

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17 Clinical Investigation

Susan L. Furth · Jeffrey J. Fadrowski

Clinical Research Question

How can we best evaluate, treat, and assess long-term risks for children with kidney disease? Who is at risk of developing end-stage renal disease (ESRD) in childhood or young adulthood? Clinicians are often faced with questions such as these with uncertain answers in the practice of pediatric nephrology. Parents ask, "Why did my child get this disease?" "What is the most effective method to treat this condition?" "What's the prognosis of this condition in my child?" Frequently, these answers are not known, and these questions are the inspiration for high-quality clinical research. The first step in developing a valuable clinical study is determining whether the initial query can be translated into a good research question.

Hallmarks of a Good Research Question

A good research question gives *useful* information, is interesting to the researcher, builds on what is known, and can be answered with available resources. Research is a labor of love, demanding attention to detail, perseverance, honesty, and imagination. Developing a good research question is an iterative process. One needs input from knowledgeable colleagues and collaborators. The researcher must become thoroughly familiar with what is already known about the topic by reviewing the literature and consulting with experts in the area. Investigating what is already known has several benefits. First, it can reveal that the candidate research question has already been answered adequately. Second, learning what is already known provides insight into potentially useful methods for addressing a research question. For example, previous studies may demonstrate good ways to measure a variable of interest or provide background information for determining sample size. Third, a literature review may suggest ways to frame the research question at hand. For example, a literature review may reveal that particular risk factors are consistently associated with a disease process, and an intervention to modify these risk factors may form a sound basis for a clinical trial.

Finally, a good research question needs to be answerable with available resources. These include subjects available for study, technical expertise of the research staff, and the time and money that can be devoted to the project. Once a question is framed, the researcher needs to outline the study protocol or methods, which include specifying the recruitment method, number of subjects and how they will be recruited, how each variable will be defined, and the plan for data analysis. A poorly designed study is worse than no study at all because, like imprecise measurements and an improper analytic plan, it can also lead to false conclusions.

Steps in Refining a Good Research Question

A good research question usually begins with a broadly stated concept. The initial question is then made more specific by identifying independent and dependent variables. Often, research questions are concerned with causal relationships. *Independent variables* are those conceptualized to be causes; *dependent variables* are those conceptualized to be effects. The research question can be modified to ask about the role of multiple potential causes in leading to the specific outcome. A simple research question asks whether x (independent variable) causes y (dependent variable). More complex research questions could assess the relative importance of x and other variables (e.g., a and b) as causes of y. A different research question might ask how strongly x predicts y in one population versus another.

The next step is to translate a research question into a hypothesis. In our simple example, the researcher may hypothesize that x causes y. In the actual research project, the information collected is examined to determine whether it is reasonable to conclude that x does cause y. In examining the data, the investigator tests the *null hypothesis* that x does not cause y versus the *alternative hypothesis* that it does.

Scientific Method

A study's potential value is determined by the relevance of the research question. Its ultimate worth is determined by

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the study methods. Methodologic issues concern the study design, subject selection, data collection techniques, and the analytic plan. Subsequent sections in this chapter discuss each of these aspects. As a foundation, this section describes the concepts of inference, generalizability, and validity.

Inference

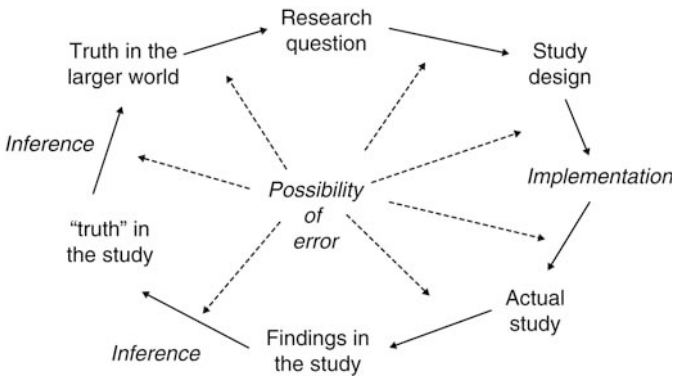
As *Fig. 17-1* shows, scientific research begins with the research question. It then moves (clockwise in the figure) to the controlled arena of the study design and then through the implementation of the actual study and findings. Inferences from the findings in the study approximate the "truth in the study." From these "truths" we attempt to infer the applicability of the findings to general clinical practice. Researchers describe and explain reality by sampling a portion of it, measuring characteristics of the sample, analyzing the measurements, and interpreting the results. Errors in the design or implementation of the study can lead to false conclusions. The strength of inference depends largely on the research methods used in the recruitment of study subjects (sampling) and in the choice and integrity of the study design. At any stage in study design and implementation, bias can occur. Bias is the result of any process that tends to produce results that depart systematically from the truth. Three broad categories of bias include selection bias, measurement bias, and confounding bias. Selection bias arises if the manner in which subjects with the outcome of interest and the comparison group were selected yields an apparent association when, in reality, exposure and disease are not associated. Measurement bias occurs when the methods of measurement systematically differ between groups. Confounding occurs when a factor is associated both with the exposure and the outcome and the effect of the main exposure under study is confused or distorted by the confounder.

Statistical inference depends on the methods used to define and sample the population. The researcher uses inferential statistics to *extrapolate* the sample findings to the larger population of children from which the sample was drawn. Inferential statistics assume that the studied sample is drawn by probability methods and can be used to make inferences about the larger population. The size of a probability sample determines the certainty of inferences from it. All other things being equal, the larger the sample size, the greater the certainty of inferences to the population.

The researcher's ability to make a *causal inference* from study results depends largely on issues of study design. Study designs are *observational* when the investigator does not manipulate the risk factor but merely selects children with and without disease and compares them in terms of the risk factor(s). Study designs are *experimental* when the investigator not only observes but actually manipulates the *relationship* between two variables. Observational designs provide somewhat weaker evidence of causation, because they fail to rule out explanations other than association between the variables studied. Experimental designs can provide much stronger evidence for causation. In an experimental study, the investigator controls the independent variable, which is the factor hypothesized to produce change in a dependent variable. In an experimental study, subjects are randomized to receive or not receive the independent variable. The goal of the process of randomization is to produce

Figure 17-1

The role of inference in drawing conclusions from clinical research studies.



study groups that are "balanced" in terms of other factors that could influence the dependent variable. Unfortunately, experimental designs often are not feasible, ethical, or desirable. Epidemiologic studies of disease preclude manipulation of risk factors in humans. Health services researchers studying the public health impact of changes in health policy rarely can control these changes.

Within the broad categories of observational and experimental designs, there are many variations. These variations, distinguishing characteristics, primary uses, strengths, and weaknesses are discussed in the section Study Design. Study design also influences the validity of study results.

Validity

Validity is the extent to which study findings correctly reflect and explain reality (Fig. 17-2). Validity can also be thought of as accuracy. Systematic bias undermines validity. As a research question's relevance increases, so does the need for validity. The clarity and rigor of study design as well as the careful implementation of the research plan increase the likelihood that inferences from the study are valid.

Cook and Campbell (1) define several *aspects* of validity. *Statistical validity* is the correctness of study conclusions regarding the existence of a relationship between two variables. A study lacks statistical validity when it concludes there is no relationship between variables when in fact there is one or when it concludes there is a relationship when in fact there is none. Statistical validity is jeopardized most often by inadequate sample size and by improper use of statistical tests.

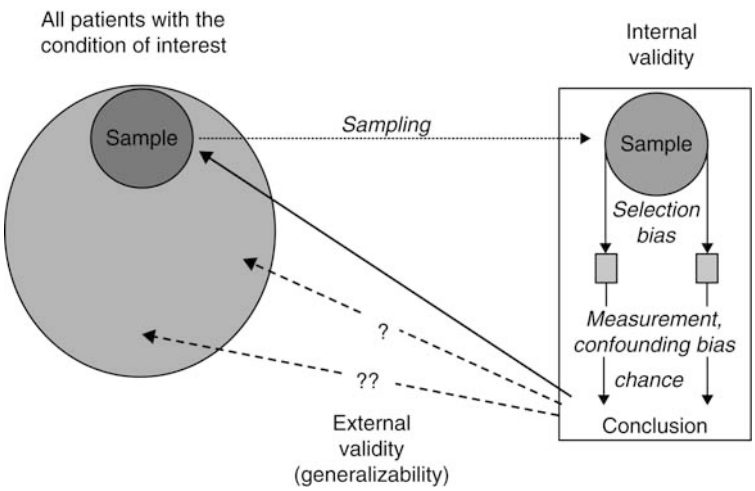
If there is a relationship between two variables, *internal validity* is the correctness of conclusions about whether the relationship between the independent and dependent variables is causal. Internal validity is jeopardized when a study design fails to control for factors that could confound the hypothesized causal relationship.

Finally, given a causal relationship between the independent and dependent variables, *external validity* is the correctness of generalizing to other persons, settings, and times. Poor choice of a study population and inadequate research procedures are common challenges to external validity.

Reliability. While validity is the degree to which data measure what they were intended to measure, reliability is the extent to which repeated measurements at different times and places, or by different people, are reproducible. An instrument measuring an assay may be accurate if, on average, the measures vary around the true value, but may not be reliable, because the measures are widely scattered around the true value. Reliability and precision are related concepts. A precise measure is one that has nearly the same value each time it is measured. It is very reliable. Precision can be described statistically using the standard deviation of repeated measures. The standard deviation

Figure 17-2

External and internal validity in experimental designs (from Fletcher RH, Fletcher SW, Wagner EH. Clinical Epidemiology: The Essentials, 3rd edn. Baltimore, Williams & Wilkins, 1996:12, with permission).



divided by the mean is the coefficient of variation. Small coefficients of variation imply precise measurements.

In summary, the scientific method involves extrapolating inferences from a study situation to the larger world. The value of research depends on the validity of such inferences, which in turn is determined by the researcher's choice of reliable methods. The following sections explain the strengths and weaknesses of the study designs available.

Study Design

Observational Studies

There are four major types of observational studies: case series, cross-sectional, case-control, and cohort. Observational designs are weaker than experimental designs in establishing causation, but they are useful when it is not feasible to manipulate the independent variable. Studies of disease etiology usually are observational. In these types of observational studies, risk factors or exposures are the independent variables, and disease is the dependent variable.

Case Series

In a *case series* study, a sample of cases is chosen, and the presence of the risk factor is measured. A case series study is easy to conduct and is useful as a preliminary study to reinforce anecdotal evidence, to generate hypotheses, or to establish variable distributions in planning future research. Case series can sometimes identify previously unrecognized constellations of symptoms or morbidities attributed to exposures to drugs or toxins. An example of a case series is the report by Furth et al. on diabetes associated with the use of tacrolimus in pediatric renal transplant recipients (2). The authors identified a number of pediatric transplant recipients treated with tacrolimus who developed diabetes. The authors summarized the case histories and reviewed existing literature regarding diabetes associated with immunosuppressive therapy in adult transplant recipients. A case series such as this can provide useful information for the clinician. However, as a method to determine the risk associated with a particular factor, this design is extremely weak because there is no means of comparison. Even if a risk factor is highly prevalent among the cases (in this example, all the cases of diabetes posttransplant had been treated with tacrolimus), there is no way of knowing whether the risk of the disease is greater with exposure to tacrolimus than with exposure to other immunosuppressive medications – for example, cyclosporine or steroids. The case series design cannot provide an estimate of risk.

Cross-Sectional Design

A cross-sectional study is one in which the disease and risk factors are measured at the same time in a sample of subjects. Subjects can be categorized as either having or not having the risk factor. Within each group, the presence of the disease can be determined. Analytically, the association between a particular risk factor and the disease is measured as the relative prevalence of the disease among those with, versus those without, the risk factor. The cross-sectional study design is superior to the case series in that it provides a means for comparison. Cross-sectional studies are relatively economic, easy to conduct, and allow simultaneous examination of multiple risk factors.

An example of a cross-sectional design is an analysis of hemoglobin levels among the first 340 children enrolled in the Chronic Kidney Disease in Children Prospective Cohort Study (CKiD) (3). Although the CKiD Study is a "cohort study," because hemoglobin in the present analysis was described in each subject at *only one time point*, the first study visit, it would be considered a cross-sectional design. As the CKiD Study progresses, serial hemoglobin measurements among the CKiD participants will be available, allowing for cohort study designs (see "*Cohort Design*"). *Table 17-1* from this cross-sectional study describes the prevalence of anemia. The prevalence of anemia increased as the severity of chronic kidney disease increased.

Cross-sectional studies have a number of limitations. Studying prevalent patients runs the risk of missing those patients who were "cured" or who died soon after

Table 17-1

Prevalence ratio of anemia by CKID stage in cross-sectional study of children with chronic kidney disease

KDDQI CKD stage[a]	*n*	% Anemic	Prevalence ratio	95% CI
2	39	21	1.0	–
3	217	39	1.9	1.0–3.6
4	82	73	3.6	1.9–6.7

[a]Based on GFR measured by plasma disappearance of iohexol (3)

developing the disease. Also, because in cross-sectional analyses the presence or absence of two factors is assessed at the same time, it is not possible to attribute causality.

Case-Control Studies

A case-control study starts with the identification of persons with the disease or other outcome variables of interest in the population at risk (*Fig. 17-3*). A suitable control group of persons without the disease or outcome is also selected from the population at risk. This is pictured on the right side of *Fig. 17-3*. To examine the possible relation of one or more exposures to the given disease or outcome, the researcher then looks back in time to compare the proportions of the cases and controls exposed and not exposed to the risk factor in question.

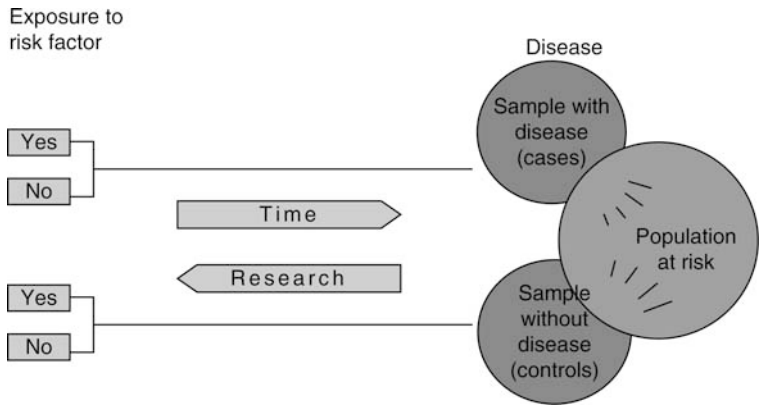
The case-control design has several advantages. It provides stronger evidence of causation than the cross-sectional design. In a cross-sectional study, outcomes and exposures are assessed simultaneously, and the investigator must infer cause and effect relationships because the temporal sequence cannot be established. In a case-control design, an attempt to establish a temporal relationship between the outcome and exposure is made by starting with a population of persons with and without the outcome and then working backwards to examine suspected exposures. Thus, compared to the cross-sectional design, the investigator is more confident that the exposure of interest came before the outcome, not as a result of the outcome. As compared to other study designs, case-control studies are efficient in the study of rare diseases or those with long latent periods between exposure and outcome. Whereas cross-sectional or cohort designs would require a large number of subjects and time to identify risk factors for a rare disease, a well-designed case-control study can identify similar risk factors with comparatively fewer subjects and much less time and expense. Also adding to the efficiency of the case-control design, several potential risk factors for a disease or outcome can be examined simultaneously.

A case-control study by Fored et al. confirmed the association of acetaminophen and aspirin with chronic renal failure (4). Adult Swedish patients with early-stage chronic renal failure were identified as cases ($N = 918$) from monthly reports of serum creatinine measurements from medical laboratories. Controls were randomly selected throughout the ascertainment period from the Swedish Population Register ($N = 980$). Aspirin and acetaminophen were used regularly by 37 and 25%, respectively, of the patients with renal failure and by 19 and 12%, respectively, of the controls. Regular use of either drug in the absence of the other was associated with an increase by a factor of 2.5 (odds ratio via logistic regression) in the risk of chronic renal failure from any cause.

Case-control studies yield an odds ratio as an estimate of relative risk. This measure is calculated by dividing the odds that a patient was exposed to a given risk factor by the odds that a control was exposed to the risk factor. It can also be obtained from logistic regression analysis. Logistic regression allows the investigator to obtain the odds ratio for a given risk factor independent of other potential risk factors or confounders using the technique of adjustment. Odds ratios are generally a good approximation of relative risk if the outcome is rare.

Figure 17-3

Design of a case-control study (from Fletcher RH, Fletcher SW, Wagner EH. Clinical Epidemiology: The Essentials, 3rd edn. Baltimore, Williams & Wilkins, 1996:213, with permission).



As with any study design, the case-control method has limitations. Case-control studies allow for the study of only one disease at a time, as opposed to cross-sectional or cohort studies. This design does not allow for the measurement of incidence, prevalence, or excess risk. Case-control studies are also subject to error, or bias, which can threaten the validity of the study. Selection and information biases, the two major categories of bias, are possible in the case-control design. Selection bias arises if the manner in which cases and controls were selected yields an apparent association when, in reality, exposure and disease are not associated. For example, cases, by definition, include only individuals who have been identified as having the disease and who are available for study. Those who have not been diagnosed, have been misdiagnosed, or have died are excluded. If diagnosis or availability is related to the exposure being studied, the sample of cases will be biased.

Avoiding selection bias can be even more challenging in the selection of controls. The control group must be comparable to the cases. They should not be chosen in such a way that important differences between cases and controls exist that might influence exposure history and thus limit the inferences derived from the study. A number of strategies exist to select a control group that is at risk for the disease and otherwise representative of the same population as the cases. These include sampling cases and controls in the same way (e.g., from the same clinical setting), matching controls to cases on key variables related to the disease (e.g., age), using multiple control groups, and using population-based samples of both cases and controls (e.g., using disease registries).

Information bias occurs when the case and control groups differ in terms of the quality of the data collected to measure risk factors. The retrospective approach to measuring an exposure in the case-control design introduces the possibility of differential recall between the cases and controls. Cases may have been asked more often about the presence of a given exposure and/or may be more circumspect in their recall of such exposures. This introduces *recall bias*, a form of information bias because of better, and sometimes exaggerated, recollection of exposures by cases as compared to controls.

It can be difficult for an investigator to remain objective in collecting exposure information. In interviewing subjects and in reviewing records, there may be a tendency to look more carefully or evaluate evidence differently for cases than for controls. Strategies for dealing with this problem include the use of objective measures and to ensure that the individuals collecting data are unaware of the subject's group status (*blinding/masking*). The more subjective the method for measuring the exposure, the more important it is to mask the observer. Blinding as to the specific exposure being studied or study hypothesis is useful and also can be used to attempt to control recall bias.

Nested case-control studies and nested case-cohort studies are alternative case-based hybrid designs that have many advantages. A nested case-control study involves selecting all cases and control subjects from a known cohort. In this design, the controls are free of the outcome or disease. Nested case-control studies eliminate the problem of recall bias, because the exposure information is obtained before the outcome has developed (cohort design). Also, the temporal sequence between exposure and outcome is defined. This design is also much more economical and efficient; the entire cohort need not be analyzed for a given exposure (e.g., via a laboratory specimen). Nested case-cohort studies also use the selection of cases and controls from a known cohort. However, in this design, controls are randomly selected from the initial cohort irrespective of outcome. This design permits the delineation of *relative risk* for an exposure.

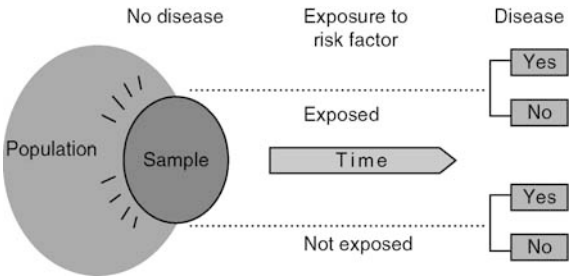
Cohort Design

Various names, including prospective, follow-up, and longitudinal, have been used to label cohort studies in the past, reflecting the temporal sequence of exposure and disease in this category of observational studies. The word *cohort* originated from the Latin word *cohors*, describing a group of warriors that marched together. Clinical investigators have adapted this term to a specific type of research study: a group of individuals free of the disease(s) of interest is assembled, their risk status is determined, and the group is followed over time to measure the incidence of disease. Comparison of the incidence of disease (or rate of death from disease) between those with and without the exposure of interest permits measurement of the association between the risk factor and the disease (*Fig. 17-4*).

The significance of the cohort design has been emphasized by the wealth of scientific data obtained from famous cohorts such as the Framingham Study or the Physicians Health Study. In the field of pediatric nephrology, the Chronic Kidney Disease in Children Prospective Cohort Study (CKiD) is ongoing and designed to improve the understanding of the occurrence and progression of chronic kidney disease and its multiple complications (5). Pediatric nephrology has previously benefited from studies

Figure 17-4

Design of a cohort study (from Fletcher RH, Fletcher SW, Wagner EH. Clinical Epidemiology: The Essentials, 3rd edn. Baltimore, Williams & Wilkins, 1996:102, with permission).



using the cohort design. For example, Wong et al. used the cohort design to demonstrate a 17-fold increase in the risk of hemolytic uremic syndrome (HUS) associated with antibiotic use in children with *Escherichia coli* 0157:H7 diarrhea infections (6). In this study, children with *E. coli* 0157:H7 were followed to assess risk factors for the development of HUS.

The cohort design has an obvious niche in clinical research. Ethical and practical considerations often do not allow for randomization of individuals to an exposure of interest. Cohort designs allow for the examination of exposure and disease associations under such circumstances.

Cohort studies can be classified as concurrent or nonconcurrent. In a concurrent cohort study (also referred to as a prospective or longitudinal study), the clinical investigator identifies the population and collects extant exposure information and then follows the cohort to a designated point in the future. Nonconcurrent cohort studies (i.e., retrospective, historical, and nonconcurrent prospective) require the investigator to identify a cohort that has been delineated in the past, along with information regarding the exposure(s) of interest. This population can then be followed for the development of a given disease in the more recent past, the present, or into the future.

Traditionally, the outcome of interest in cohort studies is the ratio of the incidence of disease in those with the exposure divided by the incidence of disease in those without the exposure. This can be interpreted as the *relative risk* for disease in many cases. When calculating and interpreting risks in the cohort design, the absence of randomization must be taken into account. Because the investigator is merely observing the exposure and not controlling for it via randomization, subjects with and without the risk factor might differ in terms of other characteristics that are related to the disease. If the characteristic is related to both the exposure being evaluated and the disease, it can lead to a misleading association between the exposure and the disease. Such a characteristic would be a *confounder*.

To avoid misinterpreting such an association, the investigator must measure potential confounders and adjust for them in the analysis. Multivariable analyses are examples of statistical tools used to adjust for confounders. However, unsuspected confounders might still jeopardize the validity of conclusions.

For example, Wong et al. (6) used a multivariate logistic-regression analysis to account for potential confounders in the association of antibiotic use for *E. coli* 0157:H7 associated diarrhea and HUS. Adjustments were made for the initial white blood cell count and the day of illness on which the initial stool culture was obtained for analysis. These factors had been previously associated with increased risk of HUS. A higher initial white blood cell count could be a potential confounder, for example, because it is associated with an increased risk of HUS (the outcome) and might make the physician more likely to prescribe antibiotics (the exposure), thus potentially falsely linking antibiotic usage with HUS. After adjustment for these factors, the multivariate analysis revealed a persistent association, reassuring the discerning reader.

Cohort studies have several advantages. Because risk factors are measured before disease, the temporal sequence of risk and disease is established, and the potential for biased risk measurement is avoided. Several diseases or outcomes can be measured, and disease occurrence can be measured in terms of incidence, not just prevalence. Cohort studies often require large sample sizes and are unsuitable for studying rare diseases. Large sample size and long follow-up periods can make cohort studies costly. A nonconcurrent cohort design can reduce cost, but it decreases the investigator's control over subject selection and risk factor measurement.

Experimental Studies

In an experimental study, the investigator controls the independent variable, also known as the intervention or treatment, and then observes the effect on an outcome or series of outcomes. The classic form of experimental design is the clinical trial.

Randomized Controlled Clinical Trials

Most investigators, clinicians, and patients are familiar with the "gold-standard" of experimental designs, the

randomized blinded controlled clinical trial. Such trials are considered "gold-standard" because the rigid design helps minimize the influence of confounding variables and bias, allowing the true effect of the intervention to be elucidated.

The IgA Nephropathy Study is an example of a randomized controlled clinical trial. In this study, eligible patients younger than 40 years with immunoglobulin A (IgA) nephropathy on kidney biopsy, estimated glomerular filtration rate $\geq$50 mL/min/1.73 m^2, and evidence of moderate to severe proteinuria are randomly assigned to receive alternate day prednisone, fish oil, or placebo (7). The goal of this study is to determine the relative benefits of fish oil or alternate day prednisone on the progression of IgA nephropathy. In this and other controlled clinical trials, randomization is the key feature of its experimental nature. Participants are *randomly* assigned to the group that will receive the intervention (the intervention, treatment, study or experimental group) or the group that will not receive the intervention (the control or placebo group) (*Fig. 17-5*). Through randomization, all potential confounders, both those recognized by the investigator and those that are not suspected, are likely to be balanced between the study groups. In other words, the three groups in this study are considered to be the same except for the treatment they receive. Differences in rates of progression of kidney disease in the three groups are attributable to the intervention, because the effect of confounders has been ruled out by the balance achieved by randomization. Therefore, experimental designs offer stronger evidence of causality than do observational designs.

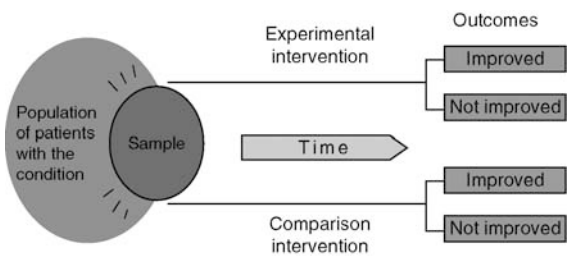
In an experimental study, it is important to ensure that subject assignment is truly random. This can be achieved by using random numbers, either through computer-assisted assignment or manually, with a table of random numbers. Sometimes *blocking* is used in conjunction with random assignment. A block of subjects is simply a set number of consecutive study enrollees. Within each block, a predetermined number of subjects are randomly assigned to each study group. For example, if the block size is set at six and two study groups of equal size are desired, then three subjects in each block of six are randomly assigned to one group and three to the other. Blocking is useful when study enrollment is expected to be prolonged. Over extended periods, both study procedures and outside conditions can change. Blocking ensures that the study groups will be balanced with regard to such changes. For example, given the prolonged enrollment period due to the relative rarity of IgA nephropathy in children and young adults, block randomization was used in the IgA Nephropathy Study.

Experimental studies, like observational studies, are subject to measurement bias. Research staff should be masked or *blinded* to the subject's group assignment during data collection, especially if any outcome measures are not strictly objective. In the IgA study, for example, both the research staff and the study subjects are unaware of which treatment they receive. In one arm of the study, placebo capsules are made to look exactly like fish oil capsules. In another arm, placebo tablets are distributed that are identical to prednisone. If outcomes are measured, without subject or staff knowledge of the subject's group status, they are less likely to be influenced by expectations about potential differences between treatment and control group outcomes. Studies in which both the participant and the staff are unaware of the treatment group status are known as "double-blinded." There may be situations in which blinding the clinical staff and patients is difficult or impossible. Clinical trials that are not blinded are known as open-label trials. Such designs are common in cancer clinical trials, as treatments being compared are often complex, with different side effect profiles and delivery protocols, and thus masking is not feasible.

Clinical trials typically provide the necessary medical evidence to bring a new treatment into use, and for drugs, such trials are referred to as "phases." A *phase I* trial is most often the first stage in testing a new drug in humans, and may include healthy participants and/or patients. In these trials, information on the distribution, metabolism, excretion and side effects of the drug is investigated. The optimal dose of the drug to deliver is also investigated. Such studies are typically not randomized or blinded.

Figure 17-5

Design of a randomized trial (from Fletcher RH, Fletcher SW, Wagner EH. Clinical Epidemiology: The Essentials, 3rd edn. Baltimore, Williams & Wilkins, 1996:139, with permission).



Phase II clinical trials are designed to evaluate the effectiveness and short-term safety and side effects associated with the drug. These trials are generally carried out in persons having the disease of interest. *Phase III* trials are expanded trials to prove the efficacy of a drug and provide the information necessary for physician labeling of the drug. These studies are most often randomized and blinded. Once completed, the pharmaceutical manufacturer may apply to market the drug for the indication(s) studied in the phase III trial. *Phase IV* clinical trials are postmarketing studies (post licensure) designed to delineate more information about the drug's risks, benefits, and optimal use in "real-world" conditions.

Alternative Designs

It is not always feasible to conduct a traditional randomized controlled clinical trial for every treatment. In such scenarios, multiple alternative experimental study designs are available and may provide useful information regarding the treatment under consideration. A few examples of such alternative designs follow. *Cross-over trials* randomly assign half of the study population to start with the control period and then subsequently switch to active treatment; the other half are on the opposite schedule. Such trials allow each participant to serve as their own control, allowing for increased statistical power and thus fewer participants. However, these studies generally take a longer period of time. The analysis and interpretation of the results may also be complicated if the treatment effects are thought to persist for a period of time even after the intervention has been ceased. A before-after trial compares the outcomes of different types of treatments in a group or groups of interest by taking advantage of calendar time. In such a study, outcomes in individuals receiving one type of treatment during a given period are compared with individuals at a subsequent time, who have received a different treatment. Although economical to perform, the results of such designs may be more prone to error as it may be difficult to know and/or control factors independent of the treatment that may have also influenced the outcome of interest.

Issues with Analysis in Experimental Designs

It is not uncommon in clinical trials to have patients who were assigned to one group switch to a different group. For example, a patient assigned to receive the active treatment under study may discontinue the treatment. Alternatively, a patient assigned to the control group may end up receiving active treatment. To avoid introducing bias in the results, it is common practice in clinical trials to analyze the results by "*intention-to-treat*." In such conservative analyses, every patient is grouped according to his/her original randomization assignment when analyzing the results, regardless of whether the patient actually received the assigned treatment or not. Intention-to-treat analyses may dilute the effect of the treatment of interest, but more importantly, likely minimize the introduction of bias into the study which may lead to erroneous conclusions.

To maximize the yield of arduous and costly clinical trials, investigators may perform *subgroup analyses*, defined as an evaluation for treatment effects within a subset of patients. For example, in the IgA Nephropathy Study, baseline proteinuria was found to be associated with "time to failure," defined as a decline in estimated GFR to $\leq$60% of baseline. The authors performed a subgroup analysis comparing the effects of the treatments and placebo among those with more severe proteinuria, defined as a first morning baseline urine protein/creatinine ratio between 1 and 3; no significant difference between the treatment groups or the placebo group in the time to failure was observed (7). Related to subgroup analyses, *post hoc analysis* refers to examining the data after the study has concluded for associations that were not specified a priori. Although subgroup analyses and post hoc analyses may provide additional useful information, due to analytic challenges, particularly in the area of sample size, results may be misleading. Given these statistical limitations, it is recommended that all hypotheses and intended analyses be stated prior to the initiation of the study, which may help in planning the design of the study, including the sample size. In the event that post hoc analyses are performed, they should be clearly labeled as such so that the reader is able to identify the potential limitations to any conclusions derived from such analyses. A recent report in the New England Journal of Medicine reviews the challenges of subgroup analyses, and provides guidelines for their use within the Journal (8).

In summary, many research designs are available to the investigator. No single design is best for all research questions. Although experimental designs are superior to observational designs in addressing threats to internal validity, they are not always feasible or ethical. The most appropriate design for a given research question is the design that maximizes internal validity within the constraints of the research environment.

Important Issues in Carrying out a Research Plan

Selection of Subjects

In any research study, one would like to extrapolate the findings to all patients with the condition of interest. The study population is the group that is meant to represent the target population from which a sample is drawn. Sampling decisions involve defining the study population and sample.

Defining the Target Population

Although there is no one single ideal target population, the investigator needs to consider the ramifications of one definition versus another. If the investigator were interested in studying risk factors for a specific disease, the target population could be defined as all children with this disease or a subset of them (e.g., children of a certain age). The broader the target population, the greater the generalizability of the study findings. On the other hand, the increased heterogeneity of a broadly defined target population could introduce variability among subgroups in terms of the importance of risk factors. For example, a particular characteristic could be a major risk factor in some population subgroups but not in others. Assessing the importance of risk factors within subgroups requires a larger study sample and perhaps a more complex sampling design.

Defining the Study Population

A practical consideration in defining the target population is availability of the population for study. The investigator could have all children available seen in a particular clinical setting. Insofar as children seen in this setting are representative of the target population, the clinical site would be a good choice for study; the experience of its enrollees could be considered *generalizable* to the target population. If children enrolled in the clinical setting differ systematically from the target population, *sampling bias* is introduced. For example, tertiary care pediatric nephrology centers might be more likely to serve children with advanced *stages* of the kidney disease or more severe or complicated cases. Studying only these cases may introduce bias toward only studying the most complex forms of a particular disease. Sampling bias impairs the generalizability of study findings. Representativeness, therefore, is a prime consideration in defining a study population. Investigators should evaluate the representativeness of candidate settings and the likely implications of potential biases. One possible approach to this in studies of patients with kidney disease is to compare the characteristics of participants in a study to known characteristics of the larger population to whom one would like to generalize the results.

Defining the Sampling Scheme

Just as we generalize from the study population to the target population, we generalize from the study sample to the study population. *Sample statistics* are measures that pertain to the samples that are studied. A sample mean, for example, is the sample's average score on a particular measure, and a sample standard deviation expresses the variability of the sample scores. The sample statistics are the investigators best estimates of the *population parameters*. The sample mean is the best estimate of the population mean; the sample standard deviation is the best estimate of the population standard deviation.

Extending beyond inference to hypothesis testing, sample statistics of the association between variables are the best estimates of these associations in the target population. The association between a hypothesized risk factor and the occurrence of disease in the study sample (perhaps measured by odds ratios or relative risks) is the investigator's best estimate of the association between the risk factor and disease in the target population.

Probability theory is the rationale for extrapolating inferences from a study sample to the reference population. A *probability sample* is one in which every subject, or element, in the study population has a known probability of being selected. A *nonprobability sample* is one in which the probabilities of selection are unknown. It is legitimate to extrapolate from a sample to its population only if probability sampling has been used.

There are several types of probability sampling. In *simple random sampling*, each element has an equal chance of being selected. In *systematic sampling*, each element in the population is assigned a consecutive number, and every *nth* element is sampled. Systematic sampling is easy to use, but it will generate a biased sample if the sampling fraction (e.g., every tenth case) is the same as some periodicity in the ordering of cases in the population. For example, if every tenth patient is sampled in a clinic where ten patients are seen each session and the most complex cases are scheduled first, then the sample will contain either all complex cases or no complex cases, depending on the first element drawn. *Stratified random*

sampling is useful when one believes that population subgroups differ in important ways. The population is divided into the subgroups, or *strata*, of interest. Simple or systematic random samples are then drawn from each stratum. *Cluster sampling* is useful when it is difficult or costly to sample elements in a population individually. Instead of elements, groups of elements are sampled. For example, in a study of school children, the investigator could take a probability sample of classrooms and then study all the students within each selected classroom. The selected classrooms, in combination, must be representative of the overall population. As with stratified sampling, formulas for calculating variance must be modified, and consultation with a statistician is recommended.

Nonprobability sampling techniques include *convenience sampling*, *quota sampling*, and *purposive sampling*. A convenience sample is one that is most readily obtained without the use of random sampling. A quota sample is a convenience sample drawn to assure specified numbers of subjects in specified strata, without the use of random sampling. A purposive sample is one in which subjects are selected because they are judged to be representative of the population of interest.

Probability sampling is preferred but not always possible. In clinical research, the investigator is often limited to a particular clinic population. If a clinic population is believed to be representative and if it is larger than the number of subjects needed for study, the investigator should use a probability sampling technique to draw the study sample.

An example of probability sampling using stratified sampling techniques can be seen in a recent survey study of adult and pediatric nephrologists (9). The authors created a survey containing ten case vignettes to assess whether increased experience with pediatric patients influenced nephrologists' recommendations for peritoneal or hemodialysis in otherwise identical patients with ESRD described in the vignettes. Because the authors wanted the survey respondents to represent the population of U.S. adult and pediatric nephrologists, they randomly selected a representative sample of nephrologists in five geographic regions of the United States. Each randomly selected nephrologist was mailed a survey containing ten case vignettes to assess what factors affected the nephrologists' dialysis recommendations.

Determining Sample Size

In any study, several factors determine the required sample size. This section describes those that come into play in several common types of investigations. Detailed sample-size formulas and tables are beyond the scope of this chapter, but several excellent references are listed in Suggested Reading. Briefly, to estimate sample size, the researcher needs to set the acceptable level of α (probability of type I error), β (probability of type II error), and determine the effect size that one is likely to see. In determining sample size, the probability of making a type I error, α, is usually set at 0.05. This is the probability of concluding that an association between two variables exists when it does not. β error is the probability of concluding that no association exists, when in fact it does. The reader will be more familiar with β error in terms of its relationship to "power." The power of a study is equal to 1-β. In many studies, β is customarily set at 0.2 for a power of 80%. If β is set to 0.1, the power of the study is 90%.

In addition to specifying α and β, the researcher must also determine an estimate of the response to treatment in one of the groups (in a clinical trial) or the rate of occurrence of disease (in a cohort study). The effect size is an estimate of how much better than the comparison group you expect a treatment group to be in a clinical trial or how increased the risk of a particular disease is in the setting of a particular risk factor (in a cohort study). An illustration of estimated sample sizes for given α, β, and effect sizes is shown in *Table 17-2* for a study comparing differences in proportions in two groups (10).

Table 17-2 illustrates how varying the acceptable levels of α, β, and effect size influences sample size. For example, if we designed a study to determine whether a new drug could "cure" 40% of patients compared to an old drug that "cured" 10% of patients, we would have 90% power to see such an effect with 95% confidence in a total sample of 96 patients (see row 2 in *Table 17-2*). In contrast, to obtain a significant result documenting a smaller effect size from the old drug cure rate of 10% to a new drug cure rate of 20% with the same $a = 0.05$ and 90% power, we would need to study 572 patients.

Attrition of Study Subjects

Sample size calculations determine the number of subjects needed at the study's conclusion. In determining the number of subjects to enroll, the investigator must estimate attrition rates and enroll a sufficiently large sample to compensate for study dropout.

Even if probability sampling is used to define the study, subject attrition could produce a biased sample at the study's conclusion. An attrition rate of more than 25% is cause for concern. In data analysis, subjects completing the study should be compared with those who drop out to

Table 17-2

Example illustrating how α, β, and effect size affect sample size

Confidence 1-α (%)	Power 1-β (%)	Effect size (%)	Total sample No.
95	80	10→40	76
95	90	10→40	96
95	90	10→20	572
95	99	10→40	156

Adapted from sample size calculator (Statcalc) in Epi-info Stat Calc (10)

determine whether the two groups differ in a clinically significant way. Such differences must be considered in interpreting the study findings.

Data Collection: Measurement

Decisions on what data to collect and how to do so begin with specifying the variables that need to be measured and operationally defining each. The investigator will need to evaluate the suitability of existing measures and determine whether to use an existing measure or develop a new one. The data sources for each variable must also be identified. Finally, the investigator should specify the level of measurement of each variable. An efficient way to document the data collection plan is to make a table with columns listing the variables to be measured, their definitions, the data source(s) for each variable, and level of measurement for each. This section describes issues pertaining to each of these tasks.

Identifying the Variables to Be Measured

Researchers are often tempted to collect as much information as possible. This can be costly, in terms of time, money, and data quality. The investigator should be able to justify each variable to be measured. Most important are the hypothesized independent and dependent variables. In addition, identified potential confounders should be mea\sured. Finally, data characterizing the study population and sample will be needed to describe the study's generalizability.

Types of Variables

Variables can be continuous or categorical. Age, height, and weight are examples of continuous variables. Categorical variables can be binary, with two possible outcomes such as male or female gender; nominal, i.e., nonordered categories such as race: White, African American, Asian, Native American or Pacific Islander; or ordinal, i.e., ordinal categories such as a pain rating scale: none, mild, moderate, severe.

Sources of Data

Study data can be collected from existing sources or can be generated specifically for the specific research hypothesis being tested, using surveys, interviews, or observations. Most studies combine both strategies.

An enormous variety of existing data sources is available, including medical records, vital records, national and local health surveys, and census data. Health programs often keep records of services provided, and billing records can be especially helpful. Examples of existing data sources in pediatric kidney disease include the United States Renal Data System (USRDS) (11) and data collected by the North American Pediatric Renal Trials and Collaborative Studies (NAPRTCS) (12). Existing sources can provide data for time periods and individuals otherwise unavailable to the investigator. The number of studies published by the NAPRTCS and their tremendous contribution to our understanding of clinical outcomes in pediatric kidney transplantation and dialysis illustrate this point. The chief disadvantage of existing sources gleaned from registry data is that the data are often not collected as systematically as in a prospective research study. Important data elements are sometimes missing. Incomplete data and inaccuracies are also possible with registry data.

Primary data collection is expensive and is limited to subjects available to the investigator. On the other hand, the investigator's control over data collection makes data quality more certain. Many primary data collection strategies are available, including mail surveys, mass-administered questionnaires, telephone and in-person structured and unstructured interviews, direct observation, and videotaping and audiotaping. Choosing a strategy should be based on the research question, the sensitivity of the data to be collected, the literacy of the population to be studied, and the resources available for the study. The key factor should be data quality – that is, *which* method will provide the most complete and accurate information within budgetary constraints.

Assessing Data Quality

One strategy to enhance data quality is to train research staff thoroughly. Often, data collection staff works

independently. To ensure that they follow study procedures, the protocol for data collection should be detailed in a study manual. Training sessions should be held to explain the study's aim to staff, as well as how each one's role fits into the big picture. Staff should be given ample time to practice their data collection skills. Once the fieldwork of the main study begins, staff should be encouraged to bring problems to the attention of supervisory staff. Such problems should be resolved in a timely fashion, and the resolution should be documented and added to the study manual. In this way, research staff are kept apprised of changes in the study protocol and are impressed with the importance of adhering to it.

After the instrument pretesting and staff training, the investigator should pilot test the data collection activities. A pilot test is a dress rehearsal of the activities for selecting study subjects, contacting them, securing informed consent, and collecting and processing data. Activities that do not work as planned should be modified and the pilot testing continued until the fieldwork procedures run smoothly.

Data quality should be monitored during the main study. Interviews and questionnaires should be reviewed as they are completed to allow recontacting subjects to correct errors. The reliability of subjective measures and those requiring special technical skill should be assessed. For studies with unsupervised interviewing of subjects, it is wise to validate a portion of the completed interviews handed in by fieldworkers. This can be done by recontacting a random sample of subjects and then asking them to verify their responses to a subsample of the interview questions.

Analytic Plan

Data Analysis

Investigators often defer considering data analysis until the data have been collected. This is a serious mistake. Study planning should include an analytic plan of the steps needed to answer the *study* questions once the fieldwork is completed.

Use of Statistical Tests

As noted earlier, statistical validity is the correctness of study conclusions regarding group differences and variable relationships. A key threat to statistical validity is the use of inappropriate statistical tests. Variable types and variable distribution, as well as the hypothesis being tested, determine the correct type of statistical test. The important properties of a variable's distribution are its location, spread and shape. Measures of location include the *median* (middle observation), the *mean* (arithmetic mean or average) and the *mode* (most frequent value). Measures of spread of a distribution include the *range*, which equals the maximum minus the minimum value, the *interquartile range*, which equals the 75th minus the 25th percentile, the *variance*, which equals the average squared deviation from the mean, and the *standard deviation*. The shape of a distribution can be described by its skewness i.e., symmetry, and its kurtosis i.e., "peakedness." *Parametric statistical tests* are based on assumptions about parameters of the population and are the most powerful tests available in situations in which these assumptions are met. *Nonparametric statistical tests* are based on fewer assumptions about the population, so they are appropriate in situations in which the assumptions underlying parametric statistics are not met.

Assumptions vary by statistical test. If a test is used in a situation that violates its assumptions, it will be inaccurate, leading to a misleading measure of statistical significance. This, in turn, will lead to an incorrect estimate of the likelihood of a Type *I* error.

In developing the analytic plan, the investigator should consider the assumptions of candidate tests in determining which ones to use. A discussion of specific statistical tests is beyond the scope of this chapter, but a framework for deciding which tests to use can be given. In this framework, three factors determine the type of test to use: the major analytic question to be answered, the levels of measurement used, and the number and independence of comparison groups.

In preparing the analytic plan, the investigator needs to translate the research question into analytic terms. Three major analytic approaches are to describe characteristics of the sampled population, to compare groups of subjects, and to measure associations among variables.

Where group comparisons are to be made, the appropriate statistical test is also determined by the number of groups to be compared (two vs. three or more) and by whether comparison groups are independent or matched. Thus, in a study of cases matched with sibling controls, it would be inappropriate to use a statistical test for independent groups.

As decisions about level of measurement and the selection of study groups are part of study planning, it is easy to see how these decisions are better informed if their ramifications for data analysts are considered. Level of measurement, study group formation, and data analysis

Table 17-3
Bivariates Statistical Tests

Level of measurement	Two groups		Three or more groups	
	Independent (unpaired groups)	Paired groups	Independent (unmatched groups)	Matched groups
Nominal dichotomy	Chi-square of Fisher's exact test	McNemar's test	Chi-square	Cochran's test
More than two categories	Chi-square	McNemar's test	Chi-square	Cochran's test
Ordinal	Mann-Whitney test	Sign test	Kruskal-Wallis one-way analysis of variance ANOVA	Friedman two-way ANOVA
		Wilcoxon matched-pairs signed-ranks test		
Interval	*t* Test for groups	*t* Test for pairs	One-way ANOVA	ANOVA for repeated measures

are all interrelated, and should all be considered part of study planning.

When the study's purpose is to assess the relationship between two continuous variables, the degree of concordance can be expressed as a simple *correlation coefficient*. A related approach is the use of a *kappa statistic* which expresses the degree of concordance beyond that due to chance. *Chronbach's alpha* expresses internal consistency among three or more variables that measure the same general characteristic.

When the study's purpose is to describe a population, the investigator makes inferences from sample statistics to population parameters. Sample proportions and measures of central tendency (mean, median, and mode) and dispersion (standard deviation, range) are used to estimate these parameters in the population. *Confidence intervals* can be constructed around proportions and means to express the certainty of the sample-based population estimates. When the study's objective is to compare two or more groups, sample group differences in proportions and means are used to estimate such differences in the population. Statistical tests of significance can be used to assess the certainty of sample-based inferences about group differences in the population. The appropriate statistical test depends on the number of groups compared, whether subjects in the groups are matched, and the level of measurement of the variable on which the groups are being compared. *Table 17-4* displays bivariate statistical tests commonly used in assessing the significance of group differences. For a variable with a normal distribution, a *t-test* compares two means, while *ANOVA* can compare means in three or more groups. *Chi-square* compares proportions.

When normality assumptions cannot be made, the *sign test* can be used for a single sample or paired sample to assess whether the medians of the sample and a reference, or two samples being compared differ. The *Wilcoxan signed rank test* can be used when the data are on an interval scale, and makes use of the magnitudes of the differences between measurements and a hypothesized location parameter. If the variable of interest is measured on an ordinal scale, the *Mann-Whitney test* can be used to assess whether the two populations have different median values.

When the research aim is to measure the association between variables, sample statistics again are used to estimate population parameters. The variables' levels of measurement determine the appropriate statistical measure of the strength of their association, the appropriate test of the statistical significance of the association, and the certainty of estimates of its strength. For continuous data, Pearson's correlation coefficient is used to measure association. For dichotomous variables, the *odds ratio* and *relative risk* (RR) are measures of the degree of association between two factors. Analytic studies in the medical literature often are designed to determine whether there is an association between exposure to a factor and development of disease. If there is an association, the question is how strong the association is. To assess the strength of the association, we measure the ratio of the incidence of disease in exposed individuals to the incidence of disease in nonexposed individuals. This ratio is called the *relative risk*. If the risk in exposed individuals is equal to the risk in unexposed, the relative risk is 1.0 and there is no association. If the risk in exposed individuals is greater than in unexposed (RR $>$ 1.0), then there is an association that may suggest that the exposure

Table 17-4
Sensitivity and specificity of urinalysis components (13)

Test	Sensitivity % (range)	Specificity % (range)
Microscopy: WBCs	73 (32–100)	81 (45–98)
Microscopy: bacteria	81 (16–99)	83 (11–100)
Leukocyte esterase	83 (67–94)	78 (64–92)
Nitrite	53 (15–82)	98 (90–100)
Leukocyte esterase or nitrite positive	93 (90–100)	72 (58–91)
Leukocyte esterase or nitrite or microscopy positive	99.9 (99–100)	70 (60–92)

confers risk. If the risk in exposed is less than in unexposed (RR < 1.0), the exposure may be protective against risk. The relative risk can only be calculated in a prospective study, as it requires incidence of disease. In a case-control study, since we do not know incidence, we cannot calculate the RR directly. Instead of the proportion of the exposed population who develop disease compared to the proportion of the unexposed population who develop disease, in case-control studies, we have the proportion of the cases who were exposed and the proportion of the controls who were exposed. In case-control studies we utilize the concept of odds to define the odds ratio, which approximates the relative risk if the incidence of disease is low. We compare the odds of a case having been exposed to a particular factor to the odds of a control being exposed to that factor. As in the case of relative risk, if the exposure is not related to the disease, the odds ratio will equal 1.0. If the exposure is positively related to the disease, the odds ratio will be greater than one.

Studies of the combined and relative impacts of multiple independent variables, or the effect of an independent variable after controlling for other factors, will require multivariable analytic tests. The appropriateness of a multivariable technique is determined by the levels of measurement of the independent and dependent variables. Multiple linear regression analysis can be used to assess association between a putative exposure and a continuous outcome while adjusting for other possibly confounding factors. Multiple logistic regression analysis can be used to assess the association between a putative risk factor and a binary outcome measure, while adjusting for other potential confounding factors. These measures can be used to calculate an adjusted relative risk, or an adjusted odds ratio. The references cited at the end of this chapter describe the applicability and interpretation of the most commonly used multivariable statistical tests.

In addition to describing association between two variables, and assessing the risk associated with an exposure and an outcome, another common research question in the medical literature includes assessing the time from a particular exposure until an outcome such as death, or hospitalization or transplantation. A commonly used statistical tool to assess the time to an event is survival analysis, or *Kaplan Meier analysis*. Kaplan Meier analyses can be used to compare survival between two treatment modalities. When adjusting for other potential confounding variables in a survival or time to event analysis, Cox *proportional hazards methods* can be used, yielding a hazard ratio which can be thought of as comparable to a relative risk.

Statistical Significance and Confidence Intervals

Most readers of the medical literature will be familiar with the term *statistical significance*, which is most often referred to in clinical reports as a "p value $<.05$." This highly sought after result of a statistical test refers to the probability of α, or a type I error. A p value of .05 in a study means that there is a 5% chance that the results seen in the study could have occurred by chance. However, the authors have concluded that this probability is low enough for them to accept the alternative hypothesis (that there is a real difference between groups) and to reject the null hypothesis (there is no difference between groups). It is important to note that the p value in a study result depends on the size of the observed difference between the groups in question and the size of the sample of patients studied. Standing alone, the p value does not convey any sense of the magnitude of the treatment effect seen in the study or the precision of the estimate of the size of the treatment effect. *Confidence intervals*, in contrast to p values, can convey this information in a more meaningful way.

For any estimated value, it is useful to have an idea of the uncertainty of the estimate in relation to the true

value it is trying to approximate. For example, if we designed a study to estimate the beneficial effect of a new lipid-lowering medication in chronic kidney disease in adolescents, we would try to recruit a large representative sample of hyperlipidemic adolescents and randomize them to treatment with a new lipid-lowering medication. From our study, we might want to estimate the magnitude of lipid level reduction associated with the new medicine. We might also want to use this estimate as an approximation for the "true" reduction in lipid levels that would be seen in the "universe" of pediatric patients with hyperlipidemia and chronic kidney disease. To estimate the "true" reduction in lipid levels (which can never be directly measured), we can generate a confidence interval around our estimate.

In any study, construction of a *confidence interval* around the point estimate gives us a range of values in which we can be confident that the true value resides. A confidence interval gives a sense of the estimates precision; it extends evenly on either side of the estimate by a multiple of the standard error (SE) of the estimate. In our example, our study might yield an estimate of the drop in serum low-density lipoprotein cholesterol levels of the treatment group of 30% with a standard deviation of that estimate of 20%. One could then use this estimate to generate a confidence interval around this estimate. In the medical literature, one will most often see references to 95% confidence intervals. The general equation for a 95% confidence interval is equal to the estimate ± 1.96 times the SE of the estimate. The factor 1.96 comes from the standard normal distribution, in which 95% of estimates would fall within ± 1.96 SEs of the mean. If one wanted to increase the probability of including the true estimate in the confidence interval, one could generate a 99% confidence interval, which would equal the estimate ± 2.56 times the SE of the estimate. Because the SE of an estimate is equal to the standard deviation of the population divided by the square root of the sample size, n, one can see that a larger sample size is needed in a study to generate a precise estimate of a treatment's effect. Given the same standard deviation, the SE in our study would be smaller if we studied 100 children compared to 20 children. The larger study would generate a narrower 95% confidence interval. The strict interpretation of a 95% confidence interval is that this is the range of values for the true population estimate that is consistent with the data observed in the study. In our hypothetical example, the smaller study might give us the opportunity to conclude that the new lipid lowering is associated with a 30% reduction in low-density lipoprotein cholesterol with a relatively broad 95% confidence interval of 21–39%, whereas the larger study yields a more precise estimate. The 95% confidence interval around the same point estimate of a 30% reduction is 26–34% in the study with the larger sample size.

Topics Related to Clinical Decision Making

Clinicians are routinely faced with patients with unknown diagnoses, and patients expect that the clinician will know how to efficiently and accurately diagnose the problem with which they are presenting. Diagnostic acumen relies in large part in understanding the "epidemiology" of the possible diagnoses, as well as the strengths and limitations of the various tests that might be performed to diagnose such diseases. The following section provides an introduction to concepts and terms that are related to the science of clinical decision making.

The Disease

When describing the burden of a disease in a population, the terms incidence and prevalence are often used. *Incidence* is defined as the number of new cases of a disease that occur in a population at risk for developing the disease during a specified period of time. *Prevalence* is defined as the proportion of persons present in the population currently affected by the disease at a specified point in time. Distinguishing between these terms is important when considering the burden of given disease. When prevalence is reported, people affected by the disease for varying amounts of time are included; these are not necessarily all new cases. Those with severe forms of the disease may have died and thus depending on the time specified in the definition, may not be included in the prevalence. The incidence and prevalence of end-stage renal disease (ESRD) are routinely reported by the United States Renal Data System (USRDS). In 2005, the incidence was 347 (new) cases of ESRD per million population per year. This was only 1% higher than the incidence in 2001. The prevalence of ESRD in 2005 (also referred to as point prevalence) was 1,569 cases per million population (11). Based on these definitions, we know that 347 (22%) of the 1,569 prevalent cases of ESRD per million population in 2005 were new cases.

The Diagnostic Test

When utilizing a diagnostic or screening test to aid in the care of a patient, a clinician must know "how good" the test is. Terms used to describe "how good" a test is

include sensitivity and specificity. The *sensitivity* of a test describes the ability of the test to correctly identify those that have the disease. A sensitive test has a low *false-negative rate*, meaning the test result will not frequently be negative in those who have the disease. The *specificity* of a test describes the ability of the test to correctly identify those who do *not* have the disease. A specific test has a low *false-positive rate*, meaning the test result will not frequently be positive in those who do *not* have the disease.

Table 17-4 from the American Academy of Pediatrics' Practice Parameter for the diagnosis of initial urinary tract infections in children examines the sensitivity and specificity of various components of the urinalysis (13). For example, the sensitivity of nitrite is reported as 53%. Thus, among children with a urinary tract infection, this test will be positive approximately 53% of the time. Knowing the relatively low sensitivity of the nitrite test, a clinician would not be reassured that a urinary tract infection does not exist if the result is negative. The specificity of the nitrite test is 98%. Thus, among children *without* a urinary tract infection, this test is negative approximately 98% of the time. The high specificity of the nitrite test informs the clinician that a low false-positive rate exists. As can be observed in the figure, and as often happens in clinical practice, using a combination of diagnostic tests can significantly increase the overall sensitivity and specificity, and thus the accuracy of the diagnosis.

Often in clinical medicine, clinicians are faced with a positive test result, and the next question asked is, "among patients with a positive test result, what proportion will actually have the disease?" This is known as the *positive predictive value* of the test. The *negative predictive value* of a test relays the probability that if the test is negative, the patient does not have the disease. It is important to remember that the predictive value of a test is affected by both the prevalence of the disease in the population being considered, and, if the disease in uncommon, the specificity of the test being used. Higher disease prevalence generally leads to an increase in the positive predictive value of a test. However, as most diseases are rare, tests with higher specificity likely have a greater impact on increasing the positive predictive value for a given test. These values can be easily calculated as demonstrated in *Fig. 17-6*.

Likelihood ratios (LR) are another measure of test performance that helps the clinician utilize the results of a given test diagnostically. When presented with a patient with particular signs and symptoms, the clinician has an initial assessment of the probability, (pretest probability) of a particular disease. A diagnostic test ordered to help determine the presence of a disease may be positive or negative. LRs help inform the clinician as to how much the test result should shift his/her initial assessment of the probability (pretest probability) of the disease being present or absent to the posttest probability. Strong, conclusive tests yield very big or very small LR's. Weak, inconclusive tests yield modest LR's, close to 1.0. For example, following the results of a positive test result, if the positive LR is found to equal 1, then there is no change in the likelihood of the disease. If the LR was 10 after the positive test result, then the posttest odds of the disease is equal to the likelihood ratio multiplied by the pretest odds. The odds of disease can be calculated from the probability as probability/1- probability. The higher the LR, the better the test is for ruling in a diagnosis. These ratios depend upon the validity of the test being ordered in distinguishing who has

Figure 17-6

Calculation of sensitivity, specificity, positive and negative predictive values.

	Population	
Test results	**With disease**	**Without disease**
Positive	True positive (TP) = have disease and test result positive	False positive (FP) = no disease and test result positive
Negative	False negative (FN) = have disease and test result negative	True negative (TN) = no disease and test result negatve

$$\text{Sensitivity} = \frac{TP}{TP + FN} \qquad \text{Specificity} = \frac{TN}{TN + FP}$$

$$\text{Positive predictive value} = \frac{TP}{TP + FP} \qquad \text{Negative predictive value} = \frac{TN}{FN + TN}$$

the disease in question from who does not; hence these ratios may be derived from sensitivity and specificity of the test (see below). LR's may be more useful than sensitivity and specificity in certain situations. LR's can be calculated for tests without dichotomous results such as those that are resulted as "positive, intermediate, or negative." The results of several diagnostic tests may be combined to provide a single LR. Finally, with some relatively simple calculations, the posttest probability of a disease can be calculated using the LR and the pretest odds of the disease. Formulas for calculating LRs follow.

$$\text{Positive LR}: \frac{\text{probability of an individual with the disease having a positive test}}{\text{probability of an individual without the disease having a positive test}}$$

$$\text{Negative LR}: \frac{\text{probability of an individual with the disease having a negative test}}{\text{probability of an individual without the disease having a negative test}}$$

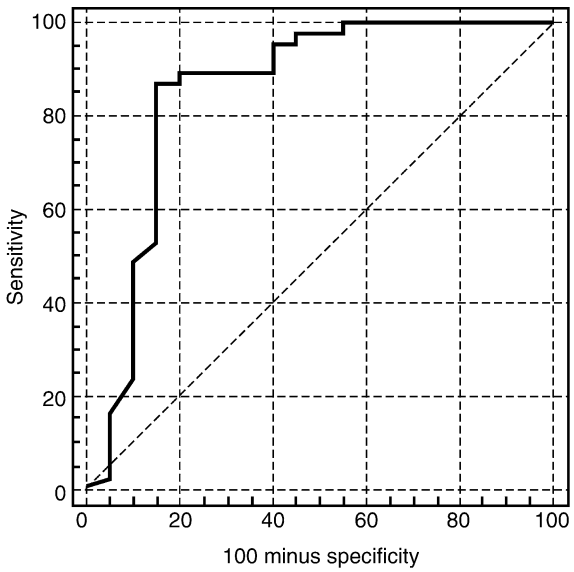
For dichotomous tests:
Positive LR: sensitivity/(1-specificity)
Negative LR: (1-sensitivity)/specificity

Receiver operator characteristic (ROC) curves, originally developed in the field of electronics, allow for a graphical display of the trade-off between sensitivity and specificity for diagnostic tests with ordinal or continuous results, in which several values of sensitivity and specificity are possible. Several cutoff points are determined and the sensitivity and specificity are determined at each point. The sensitivity (or true-positive rate) is graphed on the Y axis as a function of 1-specificity (the false-positive rate) on the X axis. Tests with values falling in the upper left corner of the graph are considered ideal (100% true positives and no false positives). If a test followed the diagonal line from the lower left corner to the upper right corner, it would be considered useless–on this diagonal line the true-positive rate equals the false-positive rate. The area under the ROC curve can range from 0.5 for a worthless test to 1.0 for a perfect test.

Filler et al. made use of both an ROC curve and LRs in a study examining nonminimal change nephrotic syndrome in children referred to a tertiary care medical center in Canada, as can be seen in the *Fig. 17-7* included from this study (14). The authors noted an increase in the incidence in focal segmental glomerulosclerosis (FSGS) over a 17 year study period. Based on the International Study of Kidney Disease in Children (ISKDC), the approach to a child with new-onset nephrotic syndrome has been to perform a kidney biopsy if the disease was unresponsive to a standard dose of corticosteroid therapy of at least 28 days in duration (15). Given the increased incidence of FSGS observed in their study, Filler et al. considered that kidney biopsies to distinguish FSGS from minimal change disease may need to be performed sooner after presentation with the nephrotic syndrome, and based on their data, investigated the ideal time to perform a kidney biopsy for the detection of nonminimal change nephrotic syndrome (i.e., FSGS). The clinical feature (as opposed to a "diagnostic" test) represented in the ROC curve is "time to remission after starting corticosteroid therapy." By plotting and comparing the various sensitivities and specificities of values for "time to remission," the authors concluded that the cutoff of 28 days was statistically the best point when a biopsy should be considered. At this cutoff point, the detection of "true positives" (nonminimal change nephrotic syndrome) was maximized, and the detection of "false-positives" (minimal change nephrotic syndrome) was minimized. The LRs for "time to remission" are also listed in the manuscript. The longer a patient took to enter remission, the

Figure 17-7

ROC curve for the detection of non-MCNS in relation to remission time (14). The graph plots the true-positive rate expressed as sensitivity (%) as a function of the false-positive rate (*100-specificity* [%]) at different cutoff points. Area under the ROC CURVE = 0.859; SE = 0.057; 95% confidence interval, 0.793 to 0.911.



higher the positive LR for the diagnosis of a nonminimal change pathology underlying the nephrotic syndrome.

Screening Programs

Screening programs are designed to detect or diagnose a disease as early as possible, in hopes of improving the prognosis. A common screening program is the use of mammography for the earlier detection of breast cancer. Detecting breast cancer early, before it is "symptomatic" or advanced, has been proven to improve outcomes and survival in older women. A sampling of the many factors that must be considered when evaluating the feasibility and effectiveness of a screening program follows.

Disease

Is there a preclinical phase of the disease – a time when the disease is present but clinical symptoms have not yet manifested? Does intervening earlier in the natural history of the disease make treatment easier and/or improve morbidity or mortality? Is the disease prevalence high enough to make a screening program cost-effective?

Test

Does a screening test exist with acceptable sensitivity and specificity for a screening program – are the false-positive and false-negative rates acceptable? Is the test acceptable to the population – will they consent to the test? Do the benefits gained from early diagnosis of the disease outweigh the cost of the test?

Person/Population

Does screening/early diagnosis improve outcomes for an individual and the population? Do those with earlier diagnosis of the disease comply with treatment recommendations and regimens – how many of those who screen positive receive a final diagnosis and treatment? Is there an improvement in the quality of life in those screened?

Ethics in Research

The foundation of medical research is to help improve the lives of patients. In keeping with this, it is important to be familiar with the various terms and concepts related to the responsible and ethical conduct of medical research.

Conflicts of interest may occur between a researcher's interest in advancing medical knowledge versus his/her self-interest in fame, prestige, academic or financial advancement. Transparency of contractual obligations and relationships is mandatory, and in some circumstances, these must be dissolved, or the researcher should not participate in a related project. For example, a conflict of interest would exist if a physician was paid by a pharmaceutical company as a medical consultant, and this physician also served as a primary investigator in a clinical trial of a medication produced by the same drug company. Conflicts of interest may also occur in the clinician-investigator role when a researcher is also the clinician for the research subject. In such situations, what may be best for the research project may not be best for the individual patient. In such conflicts, the physician is expected to do what is best for the patient.

Several types of scientific *misconduct* have been described. Scientists have a responsibility to report misconduct, and institutions have the responsibility to investigate the misconduct, and protect the person alleging the misconduct. *Fabrication/forgery* is the invention and reporting of data that does not exist from an experiment that was not performed. *Falsification/fraud* is the manipulation of research data such that what is reported misrepresents the actual findings. *Plagiarism* is the presentation of another person's words or ideas as one's own or without giving appropriate credit to the original author(s).

Research on *human subjects* requires special ethical considerations and protections. Respect for research participants requires investigators to obtain informed consent or assent, maintain privacy and confidentiality, and protect the vulnerable. *Informed consent* involves relaying an unambiguous description of the research project and allowing the subject to make an informed decision regarding participation. It must be clearly stated that the patient will be involved in a research project and participation is entirely voluntary. A clear description of the potential risks and benefits, and any compensation, should be provided. For pediatric patients participating in research, *assent* is also required. Children cannot legally give permission to participate in a research study, nor can they give "consent" as consent implies

full understanding. However, ethicists and medical and legal professionals have agreed that children should be routinely asked if they agree (assent) to participate in a research project, and their wishes should be respected. Other *vulnerable* populations that require special attention to ensure their safety include prisoners, pregnant women and their fetuses and embryos, and people with impaired capacity to make decisions, such as the mentally ill. During the consent process, *confidentiality and privacy* procedures utilized by the study should be outlined. The extent of confidentiality should be disclosed – the subject should understand who will and who will not have access to the data.

Evaluating the Literature: Rating the Strength of Scientific Evidence

Health care decisions should be based on research-based evidence. Whether the individual nephrologist is making a clinical decision or a national organization is developing clinical practice guidelines, efforts should be made to systematically assess the strengths of scientific evidence related to a particular clinical diagnostic or treatment plan. Guidelines first developed more than 20 years ago at the Department of Clinical Epidemiology and Biostatistics at McMaster University first introduced tools to allow clinicians to critically review original articles on etiology, diagnosis, prognosis, and therapy (16). In the following decade, the series was widely read and cited, was modified for use by the general public, and was published in clinical epidemiology texts (17). At the same time, clinicians at McMaster University and across North America continued to expand and improve the guidelines. Their focus has expanded to include clinicians' ability to access, summarize, and apply information from the literature to everyday clinical problems, transforming the Readers' Guides to Users' Guides (18–41).

Such systematic approaches have also been adapted to assess entire bodies of research on particular subjects. In 1999, the U.S. Congress directed the Agency for Health Care Policy Research and Quality to identify methods to assess health care research results. The results of that effort were published in a report entitled "Systems to rate the strength of scientific evidence." The goals of this project were to describe systems to rate the strength of scientific evidence, including evaluating the quality of individual articles that make up a body of medical evidence related to a particular disease, allowing for the most informed medical assessments and decision making. The report provides framework for the clinician regarding the evaluation of the various types of study design as described in this chapter (42).

In summary, the busy clinician can afford to be selective in reviewing the literature. In rating the strength of scientific evidence in evaluating a specific clinical problem or treatment, one needs to be selective. The simplest criteria for choosing which studies to read in detail or which to weigh heavily in evidence are clinical relevance and methodologic soundness. This chapter has introduced a simple framework for evaluating such features in the context of sound clinical research methodology and has outlined the most recent guidelines for assessing the strength of scientific evidence for making decisions in clinical care. These tools for systematic assessment of existing research can also guide the clinical investigator toward areas that require further study in which current evidence for treatment or outcomes is scant.

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18 Genomic Methods in the Diagnosis and Treatment of Pediatric Kidney Disease

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The completion of the Human Genome Project (HGP) in 2003 has laid the foundation and driven the technological advancements necessary for the study of the genetics of complex, multi-factorial diseases, such as those affecting the kidney. The International HapMap Project has built upon the HGP through the systematic identification and cataloguing of genetic variation across human populations. Translating the mass of data generated by these studies into useful clinical knowledge is now a major undertaking in nearly all areas of medicine, including the field of Pediatric Nephrology. Much of this work will revolve around linking particular patient phenotypes to genomic and proteomic data, such as genotype, expression profile, and protein biomarkers. As evidenced by the etiological advances made in various kidney disorders resulting from the application of genome-wide linkage analyses in the 1990's, there are a number of unique aspects of Pediatric Nephrology that make it an area particularly suitable to genomic exploration with such novel technologies as genome-wide association and expression analyses. These aspects relate both to the clinical characteristics, as well as to public health and epidemiological concerns, of pediatric kidney diseases.

A number of pediatric kidney diseases are considered to have a multi-factorial pathogenesis, often reflected in their variable clinical presentations. Such diseases are often difficult to diagnosis; and invasive biopsies are often required. Genotype and/or expression information obtained from large-scale association or profiling studies may refine the diagnosis of a number of kidney diseases and even allow for individualized disease management and therapy. Additionally, there remains an urgent need for clinically useful biomarkers which can assist in the prevention and early diagnosis of both acute and chronic kidney diseases in children. Novel serum and/or urine biomarkers identified through the application of high-throughput proteomics offers another possibility for enhanced diagnosis and treatment. Moreover, from a public health standpoint, proper management of renal disease is expensive, particularly renal replacement and dialysis. The treatment of kidney patients based on genomic and proteomic data may help lessen this burden by enhancing disease risk prediction and creating disease subsets with associated prognostic and therapeutic implications. While these issues are relevant to all of nephrology in general, as well as to many other areas of medicine, they are particularly germane to the study of childhood kidney diseases where there can be high mortality, the need for expensive lifetime treatment, as well as serious growth and nutrition implications. The proper application of genomics in Pediatric Nephrology is well-suited to address the unique challenges posed by this field.

This chapter aims to provide an overview of the application of genomic technologies to the study of pediatric kidney diseases. We will begin with a discussion of the polygenic nature of complex diseases and the recent genomic advances enabling their study. This will include outcomes of the HGP and International HapMap Project. We will then discuss the application of various genomic methods to the more common pediatric kidney diseases, covering the range of both chronic and acute, with a focus on the DNA level. We will end with a discussion of the future prospects and recommendations for applying genomics to pediatric nephrology.

The Genomics Revolution

In 2003, the completion of the Human Genome Project brought the scientific community one step closer to identifying the genes underlying common, polygenic diseases. Prior to this achievement, the goal of identifying the genetic factors responsible for diseases presenting substantial public health burdens was elusive. Research efforts focused on monogenic disorders following Mendelian patterns of inheritance. Linkage analysis was the main tool for the identification of genes behind these monogenic diseases. Although linkage analysis proved to be an effective method for the mapping of numerous genes in monogenic disorders during the 1980's and 90's, including for familiar forms of various kidney diseases (1–5), it's subsequent application to complex diseases did not meet

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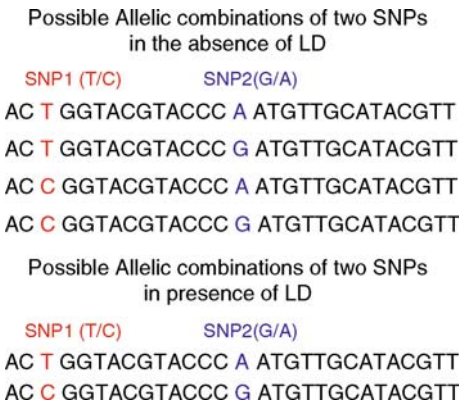
with the same success. As linkage analysis is more suitable for the identification of genes with relatively large effect sizes, its application to polygenic diseases, where the contribution of each locus or allele is believed to be relatively small, resulted in less than significant findings and results that could not be replicated. Only a handful of genes contributing to common diseases have been identified to date through linkage and subsequent positional cloning methods (6–9).

In 1996, Risch and Merikangas demonstrated the power of genome-wide association analysis (GWA) over linkage methods for the identification of the genes behind complex diseases (10). Association analysis relied upon the availability of a standardized dense set of markers covering the genome that could be easily and inexpensively typed, as well as sample sizes of thousands of cases and controls. Until the technology existed to identify, accurately map and then genotype such large numbers of markers in such great numbers of individuals, linkage analysis followed by fine-mapping remained the best option at that time for uncovering complex disease genes. However, the work of the HGP began to drive the technological breakthroughs needed to map polygenic diseases.

Analysis of the human genome sequence has demonstrated the abundance and uniformity of single nucleotide polymorphisms (SNPs), single base changes in DNA that occur in at least 1% of the population (This is in contrast to a mutation, which occurs in < 1% of the population). It is some of these common DNA variants that are believed to be responsible for at least some of the phenotypic variation observed within and between populations, including various diseases. The HGP contributed to developing technologies for the rapid, large-scale identification and scoring of SNPs and creating the intellectual resources needed to study sequence variation. The International HapMap Project built upon these discovery efforts by characterizing the patterns of linkage disequilibrium (LD) and haplotype structure across the human genome. LD occurs when two or more markers segregate together with significantly different frequencies than would be expected if they segregate independently from each other. LD is generally greater for SNPs in close physical proximity. As LD tends to reduce the number of possible haplotypes, or set of alleles, present in a population, it is useful for association mapping, in that knowing the genotype of one marker can predict the genotype of another marker in LD with it (*Fig. 18-1*). The work of the HapMap Project, along with others, has resulted in the identification, mapping, characterization, and the public availability of over four million validated SNPs to date (www.hapmap.org). The identification, mapping and cataloging of these SNPs in various human populations has helped make association studies a reality. In conjuction with the advent of mass throughput genotyping platforms, such data has potential to contribute extensively to our basic understanding of human biology and disease.

Figure 18-1

The Prinicipal of Linkage Disequilibrium. A Set of DNA sequences illustrating two SNPs. In the top half, the SNPs are not in LD with each other and segregate independently. With two SNPs in linkage equilibrium, four possible allelic combinations are possible. In the bottom half, two SNPs in the presence of LD are illustrated. Notice that LD reduces the number of possible allelic combinations, as the SNPs no longer segregate independently. Here, the 'T' allele of SNP1 segregates only with the 'A' allele of SNP2, while the 'C' allele of SNP1 segregates only with the 'G' allele of SNP2. In such a case, only one of these two SNPs would need to be genotyped, as the genotype at one SNP will provide the genotype information for the other.



The two types of association studies that are commonly used are the candidate-gene approach and the genome-wide approach (*Table 18-1*, *Fig. 18-2*). Candidate-gene association studies rely upon a specific hypothesis and are often limited by our current knowledge of biological and/or disease mechanisms. The strategy has been a frequently employed method in the study of numerous traits and diseases, including many related to nephrology. Nevertheless, replicable results have been difficult to achieve for various reasons, including the small, underpowered sample sizes characteristic of many of these types of studies (11).

In the second type of association study, the genome-wide strategy is hypothesis-free and does not make any assumptions regarding the mechanisms behind the phenotype being studied. Genome-wide association studies are ideally suited for common complex diseases, where

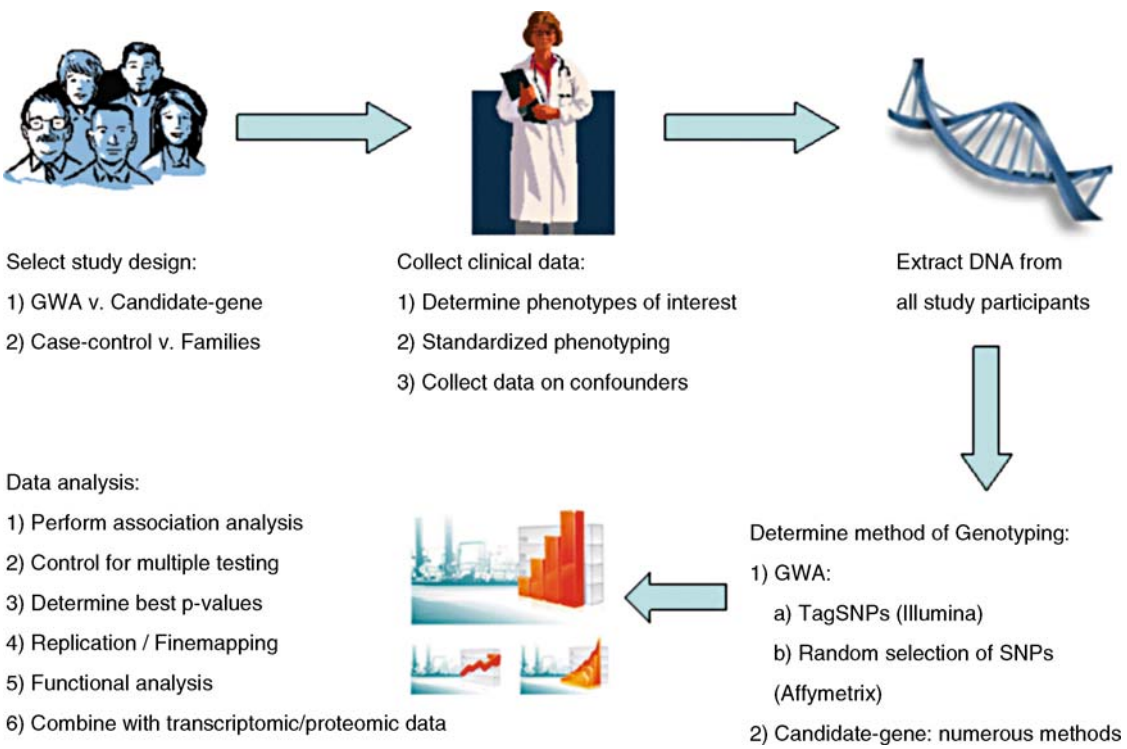
Table 18-1

Comparison of Genome-wide (GWA) and Candidate-gene Association Studies

Study design	Advantages	Disadvantages
GWA	Hypothesis-free	Genotyping more technically demanding
	May be more economical and efficient when large numbers of loci need to be worked-up	May be costly and time-consuming
	Ideal in cases where little is known regarding disease mechanisms	
	Often allow for collection of data relating to copy number polymorphisms (CNPs) in addition to SNPs – two genetic variants for the price of one.	Requires sophisticated bioinformatic approaches to data analysis and storage
Candidate-gene	Less costly and time-consuming if large numbers of loci do not need to be analyzed	Candidate-gene selection constrained by known disease biology
	Ideal in situations where evidence implicates particular disease mechanisms and related genes; or when following-up a QTL containing likely functional candidates	Less economical and efficient when testing great numbers of loci
	Straightforward data-handling and analytical approaches	
	Various methods of genotyping available; less technically demanding	

Figure 18-2

Workflow of a Genetic Association Study.



the physiological mechanisms and genetic factors that contribute to them are often not well understood. GWA studies are based on hundreds of thousands to millions of SNPs spread across the genome, each one tested for association with a particular outcome. There are currently two main approaches to this type of study. One type employs an LD-based selection strategy, as exemplified by the Illumina HumanHap-550 and Human1M sets of variants. The LD-based strategy exploits patterns of genomic LD to select a minimum number of SNPs (tagSNPs) to be assayed which captures the maximum amount of information across the genome. SNPs on these arrays serve as proxies for those not assayed. The second approach ignores patterns of LD and relies on the random selection of markers. This approach is the basis for the Affymetrix 500 K and 1 million SNP array products. A number of GWA reports have recently been published identifying novel genes for various diseases in adult populations, including many outcomes relevant to kidney disease, such as obesity and Type II Diabetes (12–14), as well as quantitative traits like urinary albumin excretion (15).

Within the field of Pediatric Nephrology, many of the known disease genes were initially identified through the study of familiar forms of a disease using linkage analysis followed by positional cloning methods (16–19). These genes have become obvious candidates to examine in the context of seemingly sporadic occurrence, where a patient has no prior family history of the disease, and which constitutes the majority of many childhood-onset kidney diseases. This is often done through mutation scanning in case series. While linkage analysis proved to be a useful tool for identifying the first disease genes in pediatric kidney disease, allowing for some of the first insights into the molecular pathogenesis of many of these diseases, these genes generally only account for a minority of cases. New methods which result in the identification of novel genes can assist in making further advances. While GWA studies are still relatively new, candidate gene studies have been an actively pursued approach in nephrology for novel gene identification. The popularity of this approach is illustrated by a review of the 2004 and 2005 abstracts of the American Society of Nephrology (ASN) and the European Renal Association/European Dialysis and Transplantation Association (ERA/EDTA), where over 180 studies encompassing 205 polymorphisms in 92 different genes were submitted (20). The genes most frequently studied were those of the renin-angiotensin system (RAS) and those related to inflammation (20). (The best evidence to date indicates minor statistical associations and a negligible clinical relevance of the most frequently studied RAS polymorphism, the insertion/deletion (I/D) variant of the angiotensin I-converting enzyme (ACE) gene (21–23). Despite their frequent study, a genetic association has yet to be convincingly demonstrated for any of the RAS genes. These genes will not be further discussed in the chapter. Of particular note, the authors highlight the extremely small sample sizes of the vast majority of these studies. In 2004, the average study size was 185 (cases and controls combined), with a slight increase in 2005 to 276. In addition, the authors found that while only 16% of these studies met the newly-adopted criteria for publishing candidate gene association studies in the Journal of the American Society of Nephrology (JASN) in 2004, this number jumped to nearly half in 2005 (20). These data highlight two important points. First, as it is unlikely that the often thousands of subjects needed for adequately-powered studies can be collected at a single site, there is a need for large-scale, multi-site collaborative studies. Large-scale candidate-gene association studies of novel genes in adult or pediatric populations are currently lacking. Second, by adopting strict publishing guidelines, journals can help assure the proper design and analysis of association studies. To address the first point, European investigators interested in nephrology and genetics established the Renal Genome Network (ReGeNet; www.regenet.eu) in 2003 to encourage large, collaborative clinical studies focused on the genetic epidemiology of renal disease (24). To date, a comparable formal renal network of investigators is lacking in the States. Such a network dedicated to studying the genetic basis of pediatric renal disease would greatly assist in establishing the infrastructure needed to design and implement adequately-powered association studies, whether those studies are candidate-gene-based or genome-wide.

When many genes are to be tested, or when one desires a hypothesis-free approach, it may be more efficient and economical to perform a GWA study. While these studies may assist in the identification of novel genes in acute kidney disorders, where currently half or less of all cases are explained by known mutations, GWA approaches in pediatric nephrology are likely to be most beneficial when applied to chronic kidney disease (CKD) and end-stage renal disease (ESRD) resulting from the increasing rate of hypertension, diabetes and obesity in children. The mechanisms leading to CKD and ESRD in these settings are likely to be polygenic in nature and the current lack of understanding regarding all disease mechanisms leading to these outcomes precludes a selection of appropriate candidate genes. In addition, the mechanisms of these disorders and their precipitating causes may be different from those occurring in adults. While some of the GWA findings from adult populations

may be relevant to pediatric populations as well, more work will be required to determine this. A more in-depth discussion on the application of candidate gene association and GWA approaches to the more common pediatric kidney diseases will be presented in the following section.

While much of this discussion has focused exclusively on the role of DNA sequence variation in disease outcomes, there are also extensive research efforts dedicated to the role of mRNA and proteins in the prevention, diagnosis and treatment of kidney disease. Expression profiling, as with high-throughput SNP genotyping, has benefited substantially from recent advances in microarray technology, which are allowing for the measurement of genome-wide expression levels, also called 'transcriptomics,' and their correlation with various disease states. As with cancer, the iden- tification of certain common expression 'signatures' through the simultaneous profiling of thousands of genes from kidney biopsy samples may help determine treatment course and patient prognosis in various renal diseases. The genome-wide study of proteins, or proteomics, has also benefited from recent technological breakthroughs in mass spectrometry, allowing for a greater number of proteins to be simultaneously analyzed. There is currently an urgent need in pediatric nephrology for clinically-relevant biomarkers which can be obtained non-invasively and which will eventually allow for earlier disease detection (25, 26). Because these two fields are closely aligned with the study of DNA sequence variation, in that changes affecting gene sequence can ultimately affect gene expression and protein production, we focus in the following section on the application of the study of DNA sequence variation to the various forms of pediatric renal disease. The application of all three fields to the study of pediatric nephrology can ultimately lead to enhanced prevention efforts, diagnoses and treatment options through the identification of novel genes and genetic markers, gene expression signatures and biomarkers associated with various renal disease states and outcomes in pediatric populations.

The Application of Genomic Methods to Pediatric Kidney Diseases

Acute Diseases

Table 18-2 provides a summary of the genes and loci implicated in the following conditions, as well as information on known genotype-phenotype correlations.

Nephrotic Syndrome (NS) NS as a heterogeneous collection of disorders represents an excellent example of where the application of genetic and genomic methods can assist in a greater understanding of disease pathogenesis and treatment.

In children, minimal change nephropathy (MCN) is the overwhelming cause of NS, such that renal biopsies are often not performed before beginning treatment with potentially harmful immunosuppressive therapies (27). Corticosteroids are often prescribed first. The majority of children respond well to this treatment and they are said to have 'steroid-sensitive nephrotic syndrome' (SSNS). However, there is a subset of children who do not respond to this therapy and they are said to have 'steroid-resistant nephrotic syndrome' (SRNS). In such cases, a renal biopsy may be indicated and the underlying cause may be focal segmental glomerularsclerosis (FSGS). Current treatment strategies for MCN and FSGS are similar, however, they are generally less effective for FSGS; and children with the latter may go on to develop ESRD. Despite the existence of these therapeutic strategies, the underlying pathological mechanisms of these two histological classifications are poorly understood. It is a subject of debate in the literature as to whether or not these classifications represent distinct diseases or subtypes of a single condition (27). In addition to a need for a greater understanding of the disease processes involved in NS, less toxic therapies are also needed for treatment in children. In the last decade, much progress has been made in the area of NS genetics and more can be expected in the coming years with novel genomic technologies. These advances should help to clarify NS-related pathologies and offer a pharmacogenetic approach to the treatment of NS.

In the late 90's, genome-wide linkage analysis followed by positional cloning was a particularly successful method for the identification of the genes behind the familial form of NS, although sporadic cases, those without any previous family history, account for the majority of pediatric NS cases. In 1998, Kestila et al. identified the first NS-related gene, NPHS1 (nephrin), to be the cause of an autosomal-recessive form of congenital NS in the Finnish population (16, 17). This was a particularly severe form of NS, characterized by heavy proteinuria in utero, lesions of the glomerular filtration barrier, and often death within the first two years of life (17). These findings were quickly followed by the identification of the NPHS2 (podocin) gene and its mutations as the cause of autosomal recessive steroid-resistant NS in older children from families of Northern European and North African descent (18, 19). This form presented between three months and five years of age and was characterized by minimal glomerular changes on early biopsy samples, FSGS present at later stages and rapid progression to ESRD. Both nephrin and

Table 18-2

Genes and genotype-phenotype correlations in Selected Pediatric Renal Diseases

Disease	Gene(s)/Loci	Genotype-Phenotype Correlations	Comments
Nephrotic Syndrome (steroid-resistant)	NPHS1, NPHS2, LAMB2, WT1, PLCE1	NPHS1,NPHS2,LAMB2,WT1 commonly mutated in congenital onset & resistant to steroid treatment; Type & number of NPHS2 mutations correlates with age of onset.	Additional susceptibility genes and modifier genes likely. GWA studies may assist with their discovery.
Atypical Hemolytic Uremic Syndrome	CFH, CFI, CFB, MCP (complement regulatory genes)	CFH -severe clinical course, rapid progression to ESRD, earlier age at onset, disease recurrence after transplant; MCP -most favorable course, no recurrence post transplant; CFI – intermediate course.	Known complement genes account for ~50% of cases. Additional susceptibility genes likely. Modifier genes likely due to high incomplete penetrance. SNPs may also play a role along with known mutations.
Autosomal Dominant Polycystic Kidney Disease	PKD1, PKD2	PKD1 – more severe course & earlier onset; 5′ mutations may progress to ESRD earlier & be more susceptible to ICAs.	PKD1 accounts for ~85% of families. Most mutations in these genes are unique to single family. Genetic background may account for variability w/in a family. Medical re-sequencing can assist in increasing the numbers of particular mutational profiles. GWA studies may help to uncover modifier genes.
Autosomal Recessive Polycystic Kidney Disease	PKHD1	Presence of two truncating mutations results in neonatal death, regardless of position. Missense mutations associated with milder phenotype.	~33% of mutations are unique to a single family. Medical re-sequencing can assist in increasing the numbers of particular mutational profiles.
IgA Nephropathy	6q22–23; 4q26–31; 17q12–22; 2q36; megsin gene, uteroglobin, C1GALT1, E & L selectins, PIGR, IGHMBP2, TNFa, TGFb, IL-4, IFg, and HLA-DRA	Gene verification and discovery and genotype-phenotype correlations still needed.	No gene(s) yet identified in families. Many results from case-control studies need verification. GWA studies needed.
End-Stage Renal Disease	10p; 18q; See Iyengar, et al. for additional loci; D10S558, D10S1435, D6S281, D4S2937, D2S291, D17S515, CNDP1, ELMO1, PVT1	Gene verification and discovery and genotype-phenotype correlations in pediatric cohorts still needed.	Genes identified in adult populations and many in setting of DN. Verification in adults and children required. GWA studies needed.

podocin localize to the slit diaphragm and are key glomerular filtration barrier proteins.

Since the identification of these genes, numerous studies have investigated their role in all forms of NS. It is now well-established that 10–30% of sporadic NS cases with FSGS are due to mutations within NPHS2 (28). Mutations within the laminin-β-2 (LAMB2), Wilms' tumour 1 (WT1) and phospolipase C-ε (PLCE1) genes have also been reported in pediatric NS, although they are far less frequent (29–31). Hinkes et al. reported that in 89 patients from 80 families manifesting NS within the first year of life, mutations in one of four genes (NPHS1, NPHS2, WT1, and LAMB2) were identified in 66% of the cases, with nearly 38% and 23% due to NPHS2 and

NPHS1 mutations, respectively (32). When examined by age of onset, the proportion of congenital cases explained by mutations in one of the four genes was nearly twice that of infantile-onset cases. Also of note, the study reported that children with mutations in any one of the four genes did not respond to steroid treatment. The authors concluded that children with onset of NS during the first year of life should be screened for mutations within these four genes in order to guide therapeutic course; and that there are likely additional unknown genes playing a role in early-onset NS.

Previous studies have suggested both allelic and locus heterogeneity of NS. While nephrin mutations have been found only in patients with congenital onset, podocin mutations have been found in cases presenting at varying ages (32, 33). The reasons for this phenotypic variability are not yet completely understood, however, there is data supporting that the specific type or number of NPHS2 mutations may play a role (34, 35).

Perhaps the largest study to date on podocin mutations in sporadic cases best demonstrates the significant locus and allelic heterogeneity of NS. Using direct sequencing in 430 patients from 404 families from around the world, Hinkes, et al. was the first to document that patients with a specific type and number of mutations presented with symptoms at a significantly earlier age than patients without such mutations (33). Nevertheless, the authors did observe that identical mutations did result in different ages of onset within their study. Consequently, Hinkes, et al. concluded that there are likely to be additional modifying factors affecting the phenotype. It must also be noted that nearly 300 individuals in this study did not carry any mutation within the podocin gene, highlighting the likelihood of as yet undiscovered genes. This is the largest report to date, and the first to establish statistical significance of an important genotype-phenotype correlation. It serves as an example of the advantages of multi-national collaborations and demonstrates that further such studies are required in order to identify additional correlations with therapeutic and prognostic implications in the treatment of NS.

Currently, genetic screening is recommended for those children presenting with NS during their first year of life, as this could allow carriers of mutations in the NPHS1, NPHS2, LAMB2, and WT1 genes to be spared the potentially harmful treatments to which they have been shown not to respond (32, 36). Genetic testing may eventually become more widespread in children presenting with NS, regardless of age, as further studies establish links between currently known mutations and clinically-relevant phenotypic outcomes, as well as with the discovery of novel genes and variants. It should be noted that a different set of genetic variants may predispose to the steroid-sensitive form of NS (37), however, it has not been as intensively studied as SRNS due to its relatively benign clinical course. While there is currently much research focused on the genes and proteins of the slit diaphragm complex, further in-roads may be made in both SRNS and SSNS with the application of genome-wide association and expression studies to uncover novel susceptibility and modifying genetic variants. Such studies will require large-scale cohorts resulting from multi-institution and/or multi-national collaborations in order to further elucidate the pathogenetic mechanisms behind this rare and heterogenous syndrome.

Hemolytic Uremic Syndrome (HUS)

Hemolytic Uremic Syndrome (HUS) is similar to NS in that there are different manifestations of the disease and it appears to be genetically heterogenous. HUS occurs in its typical form following an infection associated with diarrhea, often from the E. Coli serotype 0157:H7. This form is relatively benign, and once resolved, complete renal function is generally restored. However, approximately 5–10% of HUS patients present with no previous infection. This atypical form, aHUS, can occur at any age, can be familiar or sporadic in nature and is associated with a poor clinical outcome, including a 25–30% mortality rate (38). While a number of environmental factors triggering aHUS have been reported (39–45), a genetic basis for this form has been suggested by familial cases (46–48). In 1998, the first aHUS-associated gene, complement factor H (CFH), was localized (49). Since then, aHUS has become largely recognized as a disease of complement alternative pathway dysregulation.

As with NS, some of the initial advances in understanding the etiology of aHUS were the result of genetic linkage and subsequent association studies. Using a candidate-gene linkage approach in three families, Warwicker et al. first demonstrated linkage of aHUS to a region on chr. 1 containing a number of genes involved in complement activation regulation (49). Subsequent mutational screening of the CFH gene identified two different mutations in one of the three families and in a sporadic aHUS patient. Since this first identification, numerous studies have reported various mutations within CFH associated with aHUS (49–54). Most mutations are missense mutations occurring in the C-terminal region of the protein; and functional analysis of CFH mutations show that they result in a reduced ability of CFH to protect cells from

complement lysis (55). Additional mutations within the complement regulatory genes of membrane co-factor protein (MCP) (56–58) and factor I (CFI) (59, 60), as well as within complement activating components factor B (CFB) (61) and C3 genes (62), have also been associated with aHUS. Together, these genes are estimated to account for approximately 50% of aHUS cases (39, 63–65). There is now an on-line database, www.fh-hus.org, which catalogues the various complement activation gene mutations associated with aHUS and provides a host of information regarding this disease and its genetic risk factors.

In order for complement gene mutation status to be translated into clinically useful information, a few reports have examined genotype-phenotype correlations in aHUS. Although each report is based on a small number of children, the results are consistent across studies. Carriers of CFH mutations have the most severe clinical course, demonstrating earlier age of onset, rapid progression to ESRD, and disease recurrence after kidney transplantation (63, 66). Those with MCP mutations show the most favorable prognosis, with no disease recurrence post-transplantation (39, 63, 66). CFI mutations carriers have an intermediate course, with 50% quickly progressing to ESRD, 50% recovering and a majority with disease recurrence post-transplantation (63, 66). These data demonstrate that aHUS presentation, response to treatment and long-term outcome are closely linked to genotype status. This should allow for more tailored management of children with aHUS and underscores the urgent need for new and improved therapies for those with CFH and IFH mutations.

An interesting feature of aHUS is its high rate of incomplete penetrance, approaching 50%, where disease-causing mutations are seen in those who do not present with the disease. It has been speculated that additional genetic factors may be required in addition to the known causative mutations in order for the disease to manifest itself. In addition, approximately half of aHUS patients do not carry a known causative mutation, suggesting as yet unidentified genetic variants. In this regard, a handful of polymorphisms, or SNPs, have also been associated with aHUS (50, 56, 65, 67,68). Caprioli et al. documented association of three SNPs in CFH with aHUS, concluding that they may predispose to the disease in those without CFH mutations, as well as contributing to the full manifestation of aHUS in CFH mutation carriers (50). Findings from Fremeaux-Bacchi, et al. support these data (56). A report by Esparza-Gordillo identified a SNP haplotype block spanning the complement activation gene cluster or chr.1q32 which was more frequent in aHUS patients, particularly those with CFH, MCP or F1 mutations, and which associated strongly with the severity of the disease (68). This same group later reported on a pedigree where three independent aHUS genetic risk factors were segregating, but only those members who inherited all three manifested aHUS (69). The authors hypothesize that multiple "hits" are required in complement activation genes in order to induce the aHUS phenotype. These data demonstrate the allelic heterogeneity and polygenic nature of aHUS.

Clearly, more work remains to be done, as only 50% of cases are explained by currently known mutations. It is likely that more genes and mutations await discovery. Moreover, the role of SNPs and their functional impact along side the currently known mutations needs to be better elucidated. As with NS, large-scale genomic association and expression studies may be helpful in this regard.

Chronic Kidney Diseases and End-Stage Renal Failure

As with the acute disorders, *Table 18-2* provides a summary of the genes, loci and genotype-phenotype correlations relevant to the below conditions.

Polycystic Kidney Disease (PKD) PKD represents a diverse collection of disorders of the tubules of the kidney, where healthy renal tissue is progressively replaced by fluid-filled cysts. Extra-renal manifestations are also seen in PKD, including hypertension, biliary ectasia and fibrosis leading to portal hypertension, hepatic cysts, abnormal heart valves, and intra-cranial aneurysms. Autosomal dominant PKD (ADPKD) is the most common form, with symptomatic onset usually during the third and fourth decades of life, although childhood onset has been documented (70, 71). Autosomal recessive PKD (ARPKD) is rarer, with symptoms often beginning *in utero* or during the neonatal period. Aside from palliative care, there are currently no known specific therapies for PKD. While the genetics of both ADPKD and ARPKD are well-established, additional advancements await through medical re-sequencing and the identification of genetic disease modifiers.

As with the aforementioned acute conditions, the genes for ADPKD, polycystin-1 (PKD1) and polycystin-2 (PKD2)/transient receptor potential channel (TRPP2), and the gene for ARPKD, fibrocystin (PKHD1), were identified through linkage analyses and subsequent positional cloning efforts (1–5). In ADPKD, the PKD1 gene accounts for approximately 85% of all families (72–74) and is associated with both a more severe clinical course and earlier onset (74–77). There is substantial allelic

heterogeneity of both PKD1 and PKD2, with most mutations unique to a family. The ADPKD database shows 314 and 91 likely pathogenic germ-line mutations within PKD1 and PKD2, respectively (http://pkdb.mayo.edu/; accessed 6/6/08).

As most mutations are private, genotype-phenotype studies must generally classify mutations in some way, usually based on type (missense v. truncating) and position within the protein. However, no strong correlations have yet been established between either type or position and phenotypic outcome for either gene. There is some evidence to suggest that patients with mutations in the 5' region of PKD1 progress to ESRD earlier and may be more susceptible to intra-cranial aneurysms (78, 79). While these two genes account for nearly all cases, the disease often presents with significant variability within families. While some of this variability is likely due to environmental factors, additional genetic factors are also suspected in modifying disease outcomes. Studies have estimated that 18–78% of the phenotypic variance in PKD1 and PKD2 populations may be attributable to genetic background (80–82). Many candidate gene studies have been performed in an attempt to identify some of these modifying factors; however, the results to date have been inconsistent (23, 83, 84). Because of the diversity of disease mutations and a lack of phenotypic correlations, the usefulness of genetic testing in ADPKD is limited, although it may be helpful in childhood cases with no previous family history. This may change as genotype-phenotype correlations and new therapies are eventually established.

In ARPKD, allelic effects play a greater role than in ADPKD. There are currently just over 300 mutations in the PKHD1 gene, according to the ARPKD database (http://www.humgen.rwth-aachen.de/; accessed 6/6/08). About 33% of mutations in this gene are unique to a single family (74). Although genotype-phenotype studies are limited by the substantial amount of allelic heterogeneity present within PKHD1, some conclusions can be drawn from these reports. In all studies to date, the presence of two truncating mutations has been shown to result in neonatal death, regardless of their position (85–87). All ARPKD patients which survive the neonatal period have been shown to carry at least one amino-acid substitution, as opposed to all chain-terminating mutations (85). While truncating mutations in general are associated with more severe disease, missense mutations have been shown to result in a milder phenotype (85). Larger cohorts of patients will be required to establish consistent and more detailed genotype-phenotype correlations that can be used to improve management of the disease, and in particular, to assist in the development of novel therapeutic approaches. As ARPKD is quite rare, and thus the numbers small, no candidate gene studies have been carried out to identify genetic modifiers, although some have been mapped in mouse models (74, 88).

In both ADPKD and ARPKD, the availability of fast, high-throughput medical re-sequencing, where patients are re-sequenced in-depth over long genomic regions in a matter of hours or days, offers a new opportunity for mutation detection, with a consequent increase in numbers of patients with specific mutational profiles. This should eventually aid in establishing clinically useful genotype-phenotype correlations. Moreover, the use of GWA studies in ADPKD, where the numbers are larger, may allow for the identification of novel genetic modifiers.

Primary IgA Nephropathy (IgAN) Primary IgAN is the most common form of glomerulonephritis in children worldwide, although its prevalence varies according to country (89–91). Some of this variance is attributable to geographic differences in the policies affecting renal biopsy in the young (92), while some may also be due to geographic differences in genetic and environmental factors (93, 94). Its incidence has been estimated as high as 25–30% in some Asian countries where children routinely undergo annual urinary screening, however, lower rates have been reported in Europe and the United States (95–98). IgAN is characterized by glomerular mesangial deposits of IgA and a widely-varying presentation (99). IgAN often presents with gross hematuria attendant to an upper respiratory tract infection (95, 98, 100, 101). However, it is estimated that in as many as half of all cases, there is only microscopic hematuria, which can continue for years before the development of proteinuria (95, 98, 101, 102). Because symptoms can be intermittent, and because in most countries there are no routine urinary screening programs, the diagnosis of IgAN can be missed.

IgAN was previously considered a benign disease, however, this outlook is changing based on the recognition of increased morbidity and mortality after long-term follow-up (95, 100, 103, 104), including the finding that approximately 10% and 20% of children progress to ESRD from ten and twenty years postdiagnosis, respectively (95, 100, 105–108). The disease progresses slowly over decades and with clinical symptoms often not presenting until years after onset, it is possible that many adult IgAN cases actually had a pediatric onset. Consequently, childhood IgAN may be considered an early stage of adult IgAN with life-long follow-up and evaluation required to detect the signs of disease progression (95). Enhanced detection of the disease in childhood may allow

for its earlier treatment and more aggressive management, offering a reduced risk of chronic kidney disease and ESRD at its later stages.

A complete understanding of the pathogenesis of IgAN is still lacking, although abnormally low glycosylation of IgAI molecules is a consistent finding in patients (109–111). Treatment of the disease is based upon symptoms, but ACE inhibitors are commonly prescribed due to their reno-protective effects. Some new drugs are currently under investigation, but more specific novel treatments are needed which can be used safely over the long-term in children. Obtaining a better understanding of this disease from a genetic standpoint should allow for insights into IgAN pathogenesis, as well as offering options for its improved diagnosis and treatment.

As with the diseases previously discussed, IgAN is genetically heterogenous, with familial aggregation identified in a subset of cases, while the bulk of cases present with seemingly sporadic onset. Currently, it is estimated that 15% of cases are "familial," however, it may actually be higher due to the difficulty in diagnosing the disease.

Within families, IgAN appears to follow an autosomal dominant inheritance pattern with incomplete penetrance. The most likely genetic model for IgAN resembles that of NS or aHUS, where there are a handful of primary susceptibility genes with additional modifying genetic and environmental factors also required. Yet the identification of genes for IgAN lags behind that of the acute kidney disorders, with no gene yet identified in familial cases. However, three genome-wide linkage studies have established a number of chromosomal regions linked to the disease. Gharavi, et al. identified a locus, termed *IGAN1*, on chromosome 6q22–23 as being linked to IgAN in 60% of the families from a Caucasian cohort (112). Using 22 informative Italian families from the IgAN biobank (113), Bisceglia, et al. reported linkage to *IGAN1* and also identified two suggestive loci on chromosomes 4q26–31 and 17q12–22(114). The most recent genome-wide linkage scan identified a locus on chromosome 2q36 in a Canadian family of Austrian-German descent with 14 affected subjects (115). These studies support the likely genetic heterogeneity of the disease. Review of the genes within these loci do not reveal likely candidates, however, this is not unexpected given the lack of understanding surrounding IgAN pathological mechanisms (116).

Not unlike the previous diseases, numerous smaller-scale case-control and family-based association studies have been undertaken for IgAN. Some of the genes studied to date, and for which significant associations have been reported, include the megsin gene, found in glomerular mesangium and upregulated in IgAN (117, 118); the uteroglobin gene, a multifunctional anti-inflammatory protein (119–121); and the C1GALT1 gene, an enzyme involved in the O-glycosylation process (122); and the E- and L- selectin genes, involved in endothelial and leukocyte cell-cell interactions, respectively (123). Associations have also been reported for a number of inflammatory candidates as well, including TNFα, TGFα, IL-4, IFγ, and HLA-DRA (124–127). In a partial genome-wide screen, a Japanese group has reported significant associations of the polymeric immunoglobulin receptor (PIGR) gene (128) and the immunoglobulin mu-binding protein 2 gene (129) using over 80,000 gene-based SNPs in a stepwise association design. Finally, while not associated with IgAN onset, a 32 bp deletion in the chemokine receptor 5 (CCR5) gene has been associated with increased renal survival in IgAN patients (130, 131). Results for many of the above genes require confirmation in larger, independent cohorts.

High-throughput genotyping of dense sets of SNPs within the currently known linkage peaks, now made possible through the HapMap Project and the commercial availability of mass-throughput SNP genotyping platforms, may assist in the identification of the genes responsible for the signals. GWA studies may also be useful and more economical in this regard. Once susceptibility genes are firmly established, the IgAN field can progress to identifying genotype-phenotype correlations to aid in diagnosis and treatment.

End-stage Renal Disease (ESRD) While many chronic kidney diseases can lead to ESRD, the causes of it differ between children and adults. Although hypertension and diabetes are the primary causes of ESRD in adult populations, FSGS and congenital structural kidney abnormalities are the primary causes in children. However, the well-documented worldwide increase in rates of childhood obesity, and its related complications of diabetes and hypertension, has the potential to significantly impact the rate of pediatric renal failure (132–137). The prevalence of ESRD in pediatric cohorts is generally less than that in adults, nevertheless, these children experience significant morbidity and mortality (138–140). In addition, ESRD disproportionately affects minorities, with African-American children having nearly double to triple the rates of Caucasian children within the same age categories (140).

Due to well-established statistics indicating a potentially catastrophic increasing public health burden of CKD and ESRD overall, the identification of genes related to these two outcomes represents a substantial opportunity for improvement in understanding the complex pathological mechanisms behind CKD progression.

A vast majority of the work in this area has been conducted in adult populations due to the higher prevalence of these outcomes, as well as to the availability of large cohorts of adults well phenotyped for the more common complex diseases which result in CKD and ESRD within this population. We will briefly review findings from the adult studies here, however, as the precipitating causes differ between adults and children, disease mechanisms leading to the end-point of ESRD may also differ. Therefore, genes identified in adults need to be tested for relevance in pediatric patients. Large, multi-institutional/multi-national cohorts of CKD and ESRD pediatric patients phenotyped in a standardized manner will be required to dissect the genetics of these complex and critical outcomes of various pediatric kidney diseases.

A number of groups have performed linkage analyses for ESRD in the context of one of its major precipitating causes in adults, diabetic nephropathy (DN) (141–153). Results from these linkage studies have identified numerous distinct loci, however, the best evidence supports DN susceptibility loci on chromosomes 10p and 18q (154). A susceptibility gene, carnosinase-1 (CNDP1), has been identified from the 18q locus. Two independent groups have demonstrated that diabetic patients harboring a particular leucine repeat polymorphism of CNDP1 are at a significantly reduced risk of developing nephropathy and ESRD (155, 156).

Although numerous association studies examining single genes have been published for ESRD, as in other cases, the small sample sizes and limited coverage of the candidate genes make results difficult to interpret (154). However, some larger-scale and more in-depth association scans, including a GWA study, have been published in this area and warrant discussion (15, 157, 158–160). In the first comprehensive, family-based screen of candidate genes for ESRD, Ewens identified twenty nominally significant genes, including twelve novel ones, in 72 type I diabetic trios (160). McKnight et al., used 6,000 microsatellites in 400 Irish patients with and without type I DN to identify two significant makers on chromosome 10, and four additional markers worthy of investigation on chromosomes 2, 4, 6, and 17 (159). A Japanese scan using over 80,000 SNPs in 87 type II diabetics with nephropathy and 92 diabetic controls without nephropathy identified the engulfment and cell motility 1 (ELMO1) gene as a DN susceptibility gene (158). In a recent GWA SNP association study, Hanson et al. found a significant association with the plasmacytoma variant translocation (PVT1) gene with ESRD in 207 type II diabetics with and without DN (157). Unfortunately, many of the above results still await verification (154).

It is noteworthy that the above studies all examined a qualitative outcome and were conducted in the setting of DN. However, there have been a number of both large-scale linkage and association studies published examining quantitative kidney function traits, such as glomerular filtration rate and urinary albumin excretion, in various populations (15, 141, 161, 162). Results from most of these studies require verification. A number of advantages have been suggested for the use of quantitative traits in the genetic dissection of complex diseases (163–165). The continued use of large and comprehensive linkage and association scans should assist in the further identification and replication of ESRD susceptibility loci. However, their relevance to children will have to be tested. The establishment of cohorts of children with ESRD is challenging given the small numbers, nevertheless, such cohorts can be collected through long-term collaborative efforts. Such cohorts are required to map genetic variation which may ultimately allow for more aggressive and tailored prevention and treatment efforts in this life-threatening disorder.

The Past, Present and Future Application of Genomic Methods in Pediatric Nephrology

With the application of genome-wide linkage analyses as one of the first genomic technologies, pediatric nephrology witnessed some of the first fundamental advances in the understanding of many chronic and acute kidney diseases. With the completion of the HGP and the availability of genome-wide SNP markers that were easily and inexpensively typed, the field witnessed the candidate-gene era, where one or a few genes were genotyped for a handful of SNPs in usually small to moderate size samples with inadequate statistical power for detecting true disease associations. The popularity of this method was due in large part to its ease of application; however, the current and widespread use of candidate gene studies has limited ability to result in the novel breakthroughs required to enable paradigm changes in the prevention, diagnosis and treatment of these often life-threatening conditions. In order for the field to take the next step in the understanding of such diseases, the application of the next generation of genomic technologies to pediatric renal diseases in required, including GWA studies with hundreds of thousands to millions of SNPs. In order for these studies to become a reality within the field, large, well-phenotyped cohorts of children are necessary. The sample sizes needed for GWA studies to be successful, those samples approaching

1,000 or more, cannot be collected at a single center due to the often modest number of cases of any particular kidney disorder. Consequently, it becomes apparent that investigators need to form inter-institutional and even international collaborations with standardized methods of phenotyping. This is being pursued to some extent with adult renal patients and it is just beginning in the pediatric case. More collaborations can be expected as the importance of genomic technologies in pediatric nephrology is increasingly recognized, as such technologies become more readily available to investigators and as the infrastructure required for such studies becomes more firmly established. GWA studies offer a hypothesis-free design to gene discovery and are therefore ideal for many complex kidney diseases where there is limited knowledge of disease mechanisms. Their analysis, in conjunction with the other genome-wide technologies of transcriptomics and proteomics, offers the best hope for future fundamental advances in the field of pediatric nephrology.

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19 Tissue Engineering

Anthony Atala

Patients suffering from diseased or injured genitourinary organs are often treated with reconstructive surgery or transplants, but there is a severe shortage of donor tissue and organs. This shortage worsens yearly as modern medicine increases the human lifespan. The aging population grows, and the need for organs grows with it. Physicians and scientists have begun to look to the fields of regenerative medicine and tissue engineering to provide new options for these patients. These fields apply the principles of cell transplantation, material science, and bioengineering to construct biological substitutes that can significantly improve the quality of life of the urologic patient by eliminating the need for intensive grafting procedures or transplant surgery.

Tissue engineering, one of the major components of regenerative medicine, follows the principles of cell transplantation, materials science, and engineering to develop biological substitutes that can restore and maintain normal organ function. Tissue engineering strategies generally fall into two categories: the use of acellular matrices designed to direct the body's natural ability to use its own cells to regenerate damaged tissue, and the use of matrices seeded with cells in the laboratory to produce novel tissues and organs. Acellular tissue matrices are usually prepared by manufacturing artificial scaffolds, or by removing cellular components from donor tissues via mechanical and chemical manipulation to produce collagen-rich matrices (1–4). These matrices slowly degrade after implantation and are replaced by the extracellular matrix (ECM) proteins secreted by the in growing cells. Cells themselves can also be used for therapy via injection, either with carriers such as hydrogels, or alone.

The most common way to use cells in tissue engineering is to obtain a small piece of donor tissue and dissociate it into individual cells in the laboratory. These cells are either implanted directly into the host, or are expanded in culture and attached to a support matrix. The cell/matrix construct is then reimplanted into the host. The source of the donor tissue can be heterologous (such as bovine), allogeneic (same species, different individual), or autologous. Ideally, autologous cells are used, because in this case both structural and functional tissue replacement will usually occur with minimal complications. To accomplish this, a biopsy of tissue is obtained from a host, the cells are dissociated and expanded in culture, and the expanded cells are implanted back into the same host (2, 5–12). The use of autologous cells, although it may cause an inflammatory response, avoids rejection and thus, the deleterious side effects of lifelong immunosuppression can be avoided.

However, for many patients with extensive end-stage organ failure, a tissue biopsy may not yield enough normal cells for expansion and transplantation. In other instances, primary autologous cells cannot be expanded from a particular organ, such as the pancreas. In these situations, stem cells are envisioned as an alternative source of cells from which the desired tissue can be derived. Stem cells can be derived from discarded human embryos (human embryonic stem cells), from fetal tissue, or from adult sources (bone marrow, fat, skin). However, there are ethical issues involved in the use of embryonic stem cells. Most human applications are currently banned in the United States, a policy that may be dramatically changed with the inauguration of a new President in January 2009. Despite this, the field of stem cell biology is advancing rapidly, and cutting-edge techniques such as therapeutic cloning and somatic cell reprogramming circumvent some of the ethical questions and offer potentially limitless sources of these cells for tissue engineering applications.

This chapter will review the major components of most tissue engineering techniques, and will describe how these techniques are being applied to the reconstruction and regeneration of the genitourinary system.

The Basic Components of Tissue Engineering

Cells

Native Cells

In the past, one of the limitations of applying cell-based regenerative medicine techniques to organ replacement was the inherent difficulty of growing certain cell types in

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large quantities. Even when some organs, such as the liver, have a high regenerative capacity in vivo, cell growth and expansion in vitro can be difficult. By studying the privileged sites for committed precursor cells in these organs, as well as by exploring the conditions that promote differentiation and/or self-renewal of these cells, it has been possible to overcome some of the obstacles that limit cell expansion in vitro. One example is the urothelial cell. Urothelial cells could be grown in the laboratory setting in the past, but only with limited success. Several protocols were developed over the past two decades that identify the undifferentiated cells in a mixed culture of cells isolated from the urinary tract, and keep them undifferentiated during their growth phase (11, 13–16). Using these methods of cell culture, it is now possible to expand a urothelial culture that initially covered a surface area of 1 cm^2 to one covering a surface area of 4,202 m^2 (the equivalent of one football field) within 8 weeks (11). These studies indicated that it should be possible to collect autologous bladder cells from human patients, expand them in culture, and return them to the donor in sufficient quantities for reconstructive purposes (11, 14–19). Major advances in cell culture techniques have been made within the past decade, and these techniques make the use of autologous cells possible for clinical application.

Embryonic Stem Cells

In 1981, pluripotent cells were found in the inner cell mass of the human embryo, and the term "human embryonic stem cell" was coined (20). These cells are able to differentiate into all cells of the human body, excluding placental cells (only cells from the morula are totipotent; that is, able to develop into all cells of the human body). These cells have great therapeutic potential, but their use is limited by both biological and ethical factors.

The political controversy surrounding stem cells began in 1998 with the creation of human embryonic stem (hES) cells derived from discarded embryos. hES were isolated from the inner cell mass of a blastocyst (an embryo 5 days post-fertilization) using an immunosurgical technique. Given that some cells cannot be expanded ex vivo, ES cells could be the ideal resource for tissue engineering because of their fundamental properties: the ability to self-renew indefinitely and the ability to differentiate into cells from all three embryonic germ layers. Skin and neurons have been formed, indicating ectodermal differentiation (21–23) (24). Blood, cardiac cells, cartilage, endothelial cells, and muscle have been formed, indicating mesodermal differentiation (25–27). Pancreatic cells have been formed, indicating endodermal differentiation (28). In addition, as further evidence of their pluripotency, embryonic stem cells can form embryoid bodies, which are cell aggregations that contain all three embryonic germ layers while in culture, and can form teratomas in vivo (29). These cells have demonstrated longevity in culture and can maintain their undifferentiated state for at least 80 passages when grown using currently published protocols (30, 31).

However, in addition to the ethical issues surrounding the use of embryonic stem cells, their clinical application is limited because they represent an allogenic resource and thus have the potential to evoke an immune response. New stem cell technologies (such as somatic cell nuclear transfer and reprogramming) promise to overcome this limitation.

Therapeutic Cloning (Somatic Cell Nuclear Transfer)

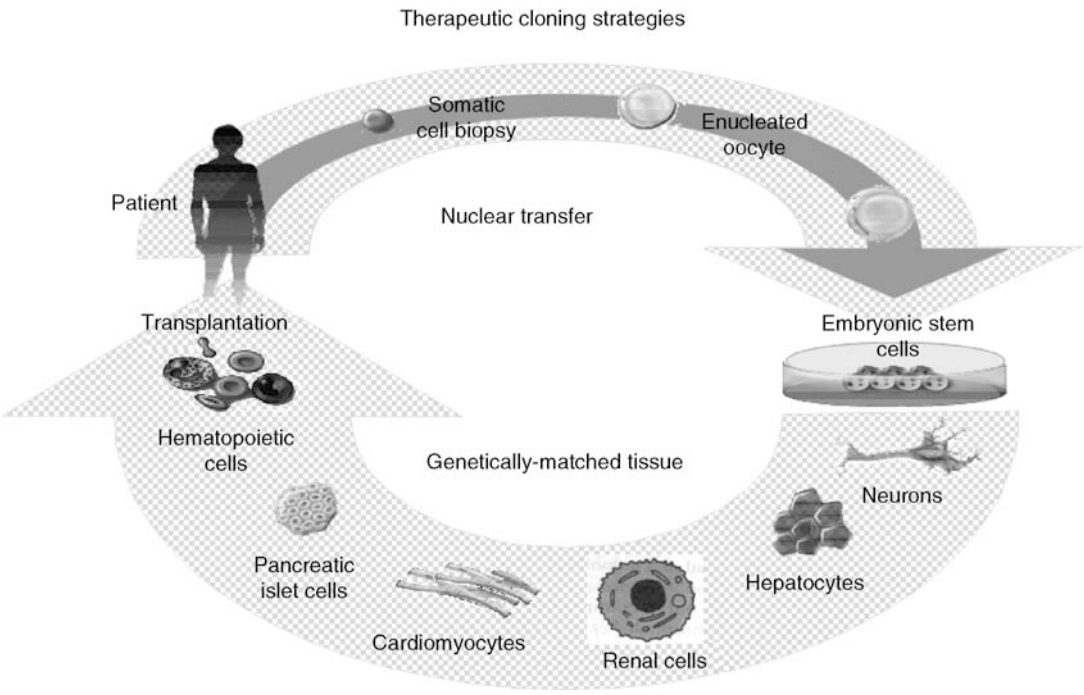
Somatic cell nuclear transfer (SCNT), or therapeutic cloning, entails the removal of an oocyte nucleus in culture, followed by its replacement with a nucleus derived from a somatic cell obtained from a patient. Activation with chemicals or electricity stimulates cell division up to the blastocyst stage.

At this point, it is extremely important to differentiate between the two types of cloning that exist – reproductive cloning and therapeutic cloning. Both involve the insertion of donor DNA into an enucleated oocyte to generate an embryo that has identical genetic material to its DNA source. However, the similarities end there. In reproductive cloning, the embryo is then implanted into the uterus of a pseudopregnant female to produce an infant that is a clone of the donor. A world-famous example of this type of cloning resulted in the birth of a sheep named Dolly in 1997 (32). However, there are many ethical concerns surrounding such practices, and as a result, reproductive cloning has been banned in most countries.

While therapeutic cloning also produces an embryo that is genetically identical to the donor, this process is used to generate blastocysts that are explanted and grown in culture, rather than in utero. Embryonic stem cell lines can then be derived from these blastocysts, which are only allowed to grow up to a 100-cell stage. At this time the inner cell mass is isolated and cultured, resulting in ES cells that are genetically identical to the patient. This process is detailed in *Fig. 19-1*. It has been shown that nuclear transferred ES cells derived from fibroblasts, lymphocytes, and olfactory neurons are pluripotetent and can generate live pups after injection into blastocysts.

Figure 19-1

Strategies for therapeutic cloning in regenerative medicine.



This shows that cells generated by SCNT have the same developmental potential as blastocysts that are fertilized and produced naturally (33–36). In addition, the ES cells generated by SCNT are perfectly matched to the patient's immune system and no immunosuppressive medications would be required to prevent rejection should these cells be used in tissue engineering applications.

Although ES cells derived from SCNT contain the nuclear genome of the donor cells, mitochondrial DNA (mtDNA) contained in the oocyte could lead to immunogenicity after transplantation. To assess the histocompatibilty of tissue generated using SCNT, Lanza et al. microinjected the nucleus of a bovine skin fibroblast into an enucleated oocyte (37). Although the blastocyst was implanted (reproductive cloning), the purpose was to generate renal, cardiac and skeletal muscle cells, which were then harvested, expanded in vitro, and seeded onto biodegradable scaffolds. These scaffolds were then implanted into the donor steer from whom the cells were cloned to determine if cells were histocompatible. Analysis revealed that cloned renal cells showed no evidence of T-cell response, suggesting that rejection will not necessarily occur in the presence of oocyte-derived mtDNA. This finding represents an important step in overcoming histocompatibility problems of stem cell therapy.

Although promising, SCNT has certain technical limitations that must be overcome prior to clinical application. There are also obvious etical concerns which must be resolved re: the potential abuses of therapeutic cloning. In addition, this technique has not yet been successful in humans. The initial failures and fraudulent reports of nuclear transfer in humans have reduced enthusiasm for human applications (38–40). However, it was recently reported that non-human primate ES cell lines were generated by SCNT of nuclei from adult skin fibroblasts (41, 42).

Before SCNT-derived ES cells can be used as clinical therapy, careful assessment of quality of the lines must be determined. For example, some cell lines generated by SCNT have contained chromosomal translocations and it is not known whether these abnormalities originated from aneuploid embryos or if they occurred during ES cell isolation and culture. In addition, the low efficiency of SNCT (0.7%) and the inadequate supply of human oocytes further hinder the therapeutic potential of this technique. Still, these studies provide promise that ES cell lines could one day be generated from human cells to produce patient-specific stem cells. Such cells would have the potential to cure many human diseases that are currently untreatable.

Reprogrammed Somatic Cells

Recently, exciting reports of the successful transformation of adult cells into pluripotent stem cells through specific genetic "reprogramming" has been published. Reprogramming is a technique that involves de-differentiation of adult somatic cells to produce patient-specific pluripotent stem cells, eliminating the need to create embryos. Cells generated by reprogramming would be genetically identical to the somatic cells (and thus, the patient who donated these cells) and would not be rejected. Yamanaka was the first to discover that mouse embryonic fibroblasts (MEFs) and adult mouse fibroblasts could be reprogrammed into an "induced pluripotent state (iPS)" (43). These iPS cells possessed the immortal growth characteristics of self-renewing ES cells, expressed genes specific for ES cells, and generated embryoid bodies in vitro and teratomas in vivo. When iPS cells were injected into mouse blastocysts, they contributed to a variety of cell types. However, although iPS cells selected in this way were pluripotent, they were not identical to ES cells. Unlike ES cells, chimeras made from iPS cells did not result in full-term pregnancies. Gene expression profiles of the iPS cells showed that they possessed a distinct gene expression signature that was different from that of ES cells. In addition, the epigenetic state of the iPS cells was intermediate between that found in somatic cells and that found in ES cells, suggesting that the reprogramming was incomplete.

These results were improved significantly by Wernig and Jaenisch in July 2007 (44). In their study, DNA methylation, gene expression profiles, and the chromatin state of the reprogrammed cells were similar to those of ES cells. Teratomas induced by these cells contained differentiated cell types representing all three embryonic germ layers. Most importantly, the reprogrammed cells from this experiment were able to form viable chimeras and contribute to the germ line like ES cells, suggesting that these iPS cells were completely reprogrammed.

It has recently been shown that reprogramming of human cells is possible (45, 46). Yamanaka generated human iPS cells that are similar to hES cells in terms of morphology, proliferation, gene expression, surface markers, and teratoma formation. Thompson's group showed that retroviral transduction of the stem cell markers *OCT4, SOX2, NANOG, and LIN28* could generate pluripotent stem cells. However, in both studies, the human iPS cells were similar but not identical to hES cells. Although reprogramming is an exciting phenomenon, our limited understanding of the mechanism currently limits the clinical applicability of the technique. The future potential of reprogramming is quite exciting.

Placental and Amniotic Fluid Stem Cells

Recently, it has been shown that pluripotent cells may be derived from the amniotic fluid and placenta. Both amniotic fluid and placenta are known to contain multiple partially differentiated cell types derived from the developing fetus. Stem cell populations have been isolated from these sources. Called amniotic fluid and placental stem cells (AFPSC), they express embryonic and adult stem cell markers (47). The undifferentiated stem cells expand extensively without a feeder cell layer and double every 36 h. Unlike human embryonic stem cells, the AFPSC do not form tumors in vivo. Lines maintained for over 250 doublings retained long telomeres and a normal complement of chromosomes. AFPSC are broadly multipotent, and human lines can be induced to differentiate into cell types representing each embryonic germ layer, including cells of adipogenic, osteogenic, myogenic, endothelial, neuronal and hepatic lineages. Examples of differentiated cells derived from AFPSC and displaying specialized functions include neuronal lineage secreting the neurotransmitter L-glutamate or expressing G-protein-gated inwardly rectifying potassium (GIRK) channels, hepatic lineage cells producing urea, and osteogenic lineage cells forming tissue engineered bone. In this respect, they meet a commonly accepted criterion for pluripotent stem cells, without implying that they can generate every adult tissue. The cells could be obtained either from amniocentesis or chorionic villous sampling in the developing fetus, or from the placenta at the time of birth. They could be preserved for self use, and used without rejection, or they could be banked. A bank of 100,000 specimens could potentially supply 99% of the US population with a perfect genetic match for transplantation. Such a bank may be easier to create than with other cell sources, since there are approximately 4.5 million births per year in the USA (47).

Biomaterials

In the most common tissue engineering procedures, isolated cells are seeded onto a scaffold composed of an appropriate biomaterial. These biomaterials replicate the biologic and mechanical function of the native extracellular matrix (ECM) found in tissues in the body by serving as an artificial ECM. Biomaterials provide a three-dimensional space for the cells to develop into new tissues with appropriate structure and function. They can also allow delivery of appropriate bioactive factors (e.g., cell adhesion peptides, growth factors) to the developing tissue (48) to help regulate cellular function. As the majority of mammalian

cell types are anchorage-dependent and will die if no cell adhesion substrate is available, biomaterials provide this substrate that can deliver cells to specific sites in the body with high loading efficiency. Biomaterials can also provide mechanical support against in vivo forces so that the predefined three-dimensional structure of the engineered implant is maintained during tissue development.

The ideal biomaterial should be biodegradable, bioresorbable, and support the replacement of normal tissue without inducing inflammation. Incompatible materials are destined for an inflammatory or foreign-body response that eventually leads to rejection and/or necrosis. Degradation products, if produced, should be removed from the body via metabolic pathways at an adequate rate so that the concentration of these degradation products in the tissues remains at a tolerable level (49). The biomaterial should also provide an environment in which appropriate regulation of cell behavior (adhesion, proliferation, migration, and differentiation) can occur. Cell behavior in the newly formed tissue has been shown to be regulated by multiple interactions of the cells with their microenvironment, including interactions with cell-adhesion ligands (50) and with soluble growth factors. Since biomaterials provide temporary mechanical support while the cells undergo spatial reorganization into tissue, the properly chosen biomaterial should allow the engineered tissue to maintain sufficient mechanical integrity to support itself in early development, while in late development, it should begin to degrade so that it does not hinder further tissue growth (48).

Generally, three classes of biomaterials have been utilized for engineering tissues: naturally derived materials (e.g., collagen and alginate), acellular tissue matrices (e.g., bladder submucosa and small intestinal submucosa), and synthetic polymers such as polyglycolic acid (PGA), polylactic acid (PLA), and poly(lactic-co-glycolic acid) (PLGA). These classes of biomaterials have been tested for biocompatibility (51, 52). Naturally derived materials and acellular tissue matrices have the potential advantage of biological recognition. However, synthetic polymers can be produced reliably on a large scale with controlled properties such as strength, degradation rate, and microstructure, which would aid in the preparation of easily used, "off-the-shelf" scaffold material.

Naturally Derived Materials

Collagen is the most abundant and ubiquitous structural protein in the body, and may be readily purified from both animal and human tissues with an enzyme treatment and salt/acid extraction (53). Collagen implants, under normal conditions, are degraded through a process involving phagocytosis of collagen fibrils by fibroblasts (54). This is followed by sequential attack by lysosomal enzymes including cathepsins B1 and D. Under inflammatory conditions, the implants can be rapidly degraded largely by matrix metalloproteins (MMPs) and collagenases (54). However, the in vivo resorption rate of a collagen implant can be regulated by controlling the density of the implant and the extent of intermolecular cross-linking – the lower the density, the greater the space between collagen fibers and the larger the pores for cell infiltration, leading to a higher rate of implant degradation. Collagen contains cell adhesion domain sequences (e.g., RGD) that may help to retain the phenotype and activity of many types of cells, including fibroblasts (55)and chondrocytes (56).

Alginate, a polysaccharide isolated from seaweed, has been used as an injectable cell delivery vehicle (57) and a cell immobilization matrix (58) owing to its gentle gelling properties in the presence of divalent ions such as calcium. Alginate is relatively biocompatible and is approved by the Food and Drug Administration (FDA) for human use as wound dressing material. Alginate is a family of copolymers of D-mannuronate and L-guluronate. The physical and mechanical properties of alginate gel are strongly correlated with the proportion and length of polygluronic block in the alginate chains (57).

Acellular Tissue Matrices

Acellular tissue matrices are collagen-rich matrices prepared by removing cellular components from tissues. The matrices are often prepared by mechanical and chemical manipulation of a segment of tissue (1–4). These matrices slowly degrade upon implantation, and are replaced and remodeled by ECM proteins synthesized and secreted by transplanted or in growing cells.

Synthetic Polymers

Polyesters of naturally occurring *a*-hydroxy acids, including PGA, PLA, and PLGA, are widely used in tissue engineering. These polymers are FDA-approved for a variety of applications, including sutures (59). The ester bonds in these polymers are hydrolytically labile, and they degrade by nonenzymatic hydrolysis. The degradation products of PGA, PLA, and PLGA are nontoxic natural metabolites and are eventually eliminated from the body in the form of carbon dioxide and water (59). The degradation rate of

these polymers can be tailored to theapplication by altering crystallinity, initial molecular weight, and the copolymer ratio of lactic to glycolic acid. Generally, the optimal degradation time ranges from several weeks to several years. Since these polymers are thermoplastics, they can be easily formed into a three dimensional scaffold with a desired microstructure, gross shape, and dimension by various techniques, including molding, extrusion, solvent casting (60), phase separation techniques, and gas foaming techniques (61). Many applications intissue engineering often require a scaffold with high porosity and ratio of surface area to volume. Other biodegradable synthetic polymers, including poly(anhydrides) and poly(orthoesters), can also be used to fabricate scaffolds for tissue engineering with controlled properties (62).

Engineering Specific Tissues and Organs

Investigators around the world, including our laboratory, have been working towards the development of several cell types, tissues, and organs for clinical application. The following sections will describe this research in detail.

Urethra

Various biomaterials without cells, such as PGA and acellular collagen-based matrices derived from decellularized small intestine and bladder, have been used in animal models for the regeneration of urethral tissue (4, 63–67). Some of these biomaterials, like acellular collagen matrices derived from bladder submucosa, have also been seeded with autologous cells for urethral reconstruction. Our laboratory has been able to replace tubularized urethral segments with cell-seeded collagen matrices (68, 69).

Acellular collagen matrices derived from bladder submucosa by our laboratory have been used experimentally and clinically. In animal studies, segments of the urethra were resected and replaced with acellular matrix grafts. Histological examination showed complete epithelialization and progressive vessel and muscle infiltration, and the animals were able to void through the neo-urethras (4). These results were confirmed in a clinical study of patients with hypospadias and urethral stricture disease (70) (*Fig. 19-2*). Decellularized cadaveric bladder submucosa was used as an underlying matrix for urethral repair in patients with stricture disease and hypospadias. Patent, functional neo-urethras were noted in these patients with up to a 7-year follow-up. The use of a readily available, "off-the-shelf" matrix appears to be beneficial for patients with abnormal urethral conditions and obviates the need for obtaining autologous grafts, thus decreasing operative time and eliminating donor site morbidity.

Unfortunately, the above techniques are not applicable for tubularized urethral repairs. The collagen matrices are able to replace urethral segments only when used in specific physical contact with existing tissue. However, if a tubularized repair is needed, the collagen matrices should be seeded with autologous cells to avoid the risk of stricture formation and poor tissue development (68). Therefore, tubularized collagen matrices seeded with autologous cells can be used successfully for total penile urethra replacement.

Bladder

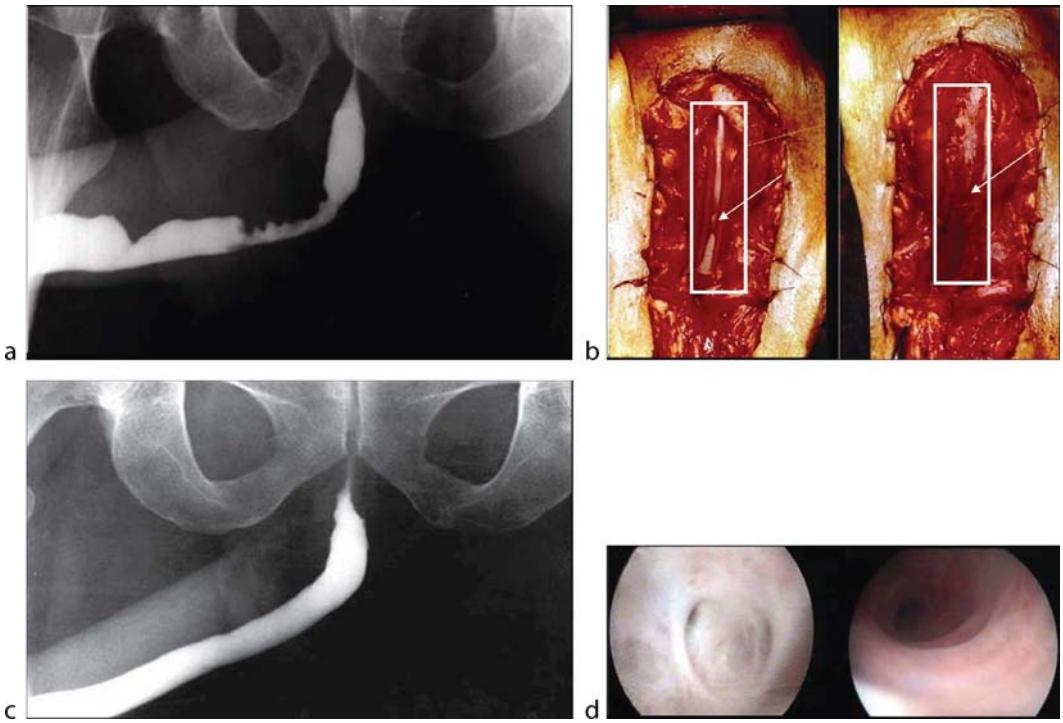
Currently, gastrointestinal segments are commonly used as tissues for bladder replacement or repair. However, gastrointestinal tissues are designed to absorb specific solutes, whereas bladder tissue is designed for the excretion of solutes. Due to the problems encountered with the use of gastrointestinal segments, numerous investigators have attempted alternative materials and tissues for bladder replacement or repair.

The success of cell transplantation strategies for bladder reconstruction depends on the ability to use donor tissue efficiently and to provide the right conditions for long term survival, differentiation, and growth. Urothelial and muscle cells can be expanded in vitro, seeded onto polymer scaffolds, and allowed to attach and form sheets of cells (71). These principles were applied in the creation of tissue engineered bladders in an animal model that required a subtotal cystectomy with subsequent replacement with a tissue engineered organ in beagle dogs (12). Urothelial and muscle cells were separately expanded from an autologous bladder biopsy, and seeded onto a bladder-shaped biodegradable polymer scaffold. The results from this study showed that it is possible to tissue engineer bladders that are anatomically and functionally normal. Clinical trials for the application of this technology are currently being conducted.

A clinical experience involving engineered bladder tissue for cystoplasty reconstruction was conducted starting in 1999. A small pilot study of seven patients was reported, using a collagen scaffold seeded with cells either with or without omentum coverage, or a combined PGA-collagen scaffold seeded with cells and omental coverage (*Fig. 19-3*). The patients reconstructed with the engineered bladder tissue created with the PGA-collagen cell-seeded scaffolds showed increased compliance, decreased end-filling pressures, increased capacities and

Figure 19-2

Urethral repair using a collagen matrix. (a): Representative case of a patient with a bulbar stricture. (b): During surgery, strictured tissue is excised, preserving the urethral plate on the left side, and the matrix is anastamosed to the urethral plate in an onlay fashion on the right. The boxes in both photos indicate the area of interest, including the urethra, which appears white in the left photograph. In the left photograph, the arrow indicates the area of stricture in the urethra. On the right, the arrow indicates the repaired stricture. Note that the engineered tissue now obscures the native white urethral tissue in an onlay fashion in the right photograph. (c): Urethrogram 6 months after repair. (d): Cystoscopic view of urethra before surgery on the left side, and 4 months after repair on the right side. (See color plate 5)



longer dry periods (72) (*Fig. 19-4*). Although the experience is promising in terms of showing that engineered tissues can be implanted safely, it is just a start in terms of accomplishing the goal of engineering fully functional bladders. Further experimental and clinical work is being conducted.

Kidney

Renal tissue is arguably one of the most difficult tissues to replicate in the laboratory. The kidney is a complex organ and its unique structural and cellular heterogeneity creates many challenges. The system of nephrons and collecting ducts within the kidney is composed of multiple functionally and morphologically distinct segments, arranged in an elaborate architectonic pattern. For this reason, appropriate conditions must be provided to ensure the long-term survival, differentiation and growth of many types of cells. Recent efforts in kidney tissue regeneration have focused on the development of a reliable cell source (73, 74) (75, 76) (77, 78). Moreover, optimal growth conditions have been extensively investigated to provide adequate enrichment to achieve stable renal cell expansion systems (79, 80) (81–83).

Isolation of particular cell types that produce specific factors may be a good approach for selective cell therapies. For example, cells that produce erythropoietin could be isolated in culture, and these cells could eventually be used to treat anemia that results from end stage renal failure. However, total renal function would not be achieved using this approach. To create kidney tissue that would deliver full renal function, a culture containing all of the cell types comprising the functional nephron units should be used. Optimal culture conditions to nurture renal cells have been extensively studied and

Figure 19-3

Construction of engineered bladder. (a): Scaffold material seeded with cells for use in bladder repair. (b): The seeded scaffold is anastamosed to native bladder with running 4–0 polyglycolic sutures. (c): Implant covered with fibrin glue and omentum. (See color plate 6)

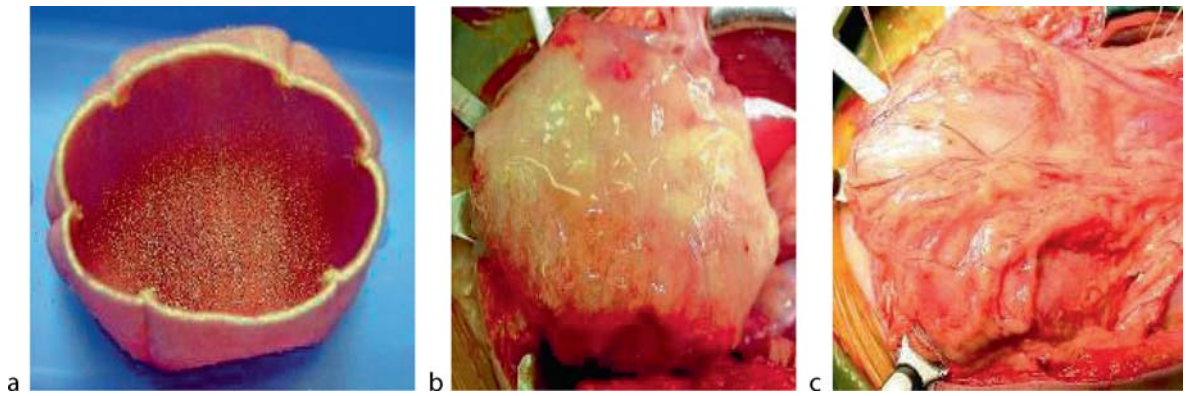
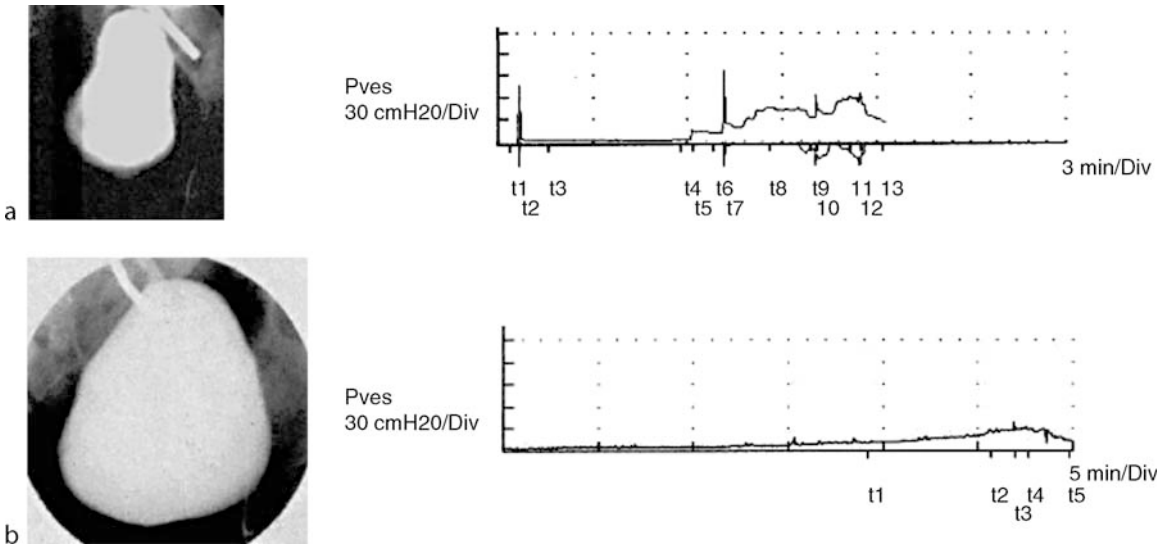


Figure 19-4

Cystograms and urodynamic studies of a patient before and after implantation of the tissue engineered bladder. (a): Preoperative results indicate an irregular bladder in the cystogram and abnormal bladder pressures as the bladder is filled via urodynamic study. (b): Postoperatively, findings are significantly improved.



cells grown under these conditions have been reported to maintain their cellular characteristics (84). Moreover, renal cells placed in a three-dimensional culture environment are able to reconstitute into renal structures.

Recent investigative efforts in the search for a reliable cell source have been expanded to stem and progenitor cells. Use of these cells for tissue regeneration is attractive due to their ability to differentiate and mature into the many specific cell types needed. This is particularly useful in instances where primary renal cells are unavailable due to extensive tissue damage. Bone marrow-derived human mesenchymal stem cells have been shown to be a potential source due to their ability to differentiate into several cell lineages (73, 74, 77). These cells have been shown to participate in the kidney development when they are placed in a rat embryonic niche that allows for continued exposure to a repertoire of nephrogenic signals (78). These cells, however, were found to contribute mainly to

regeneration of damaged glomerular endothelial cells after injury. In addition, the major cell source of kidney regeneration was found to originate from intrarenal cells in an ischemic renal injury model (73, 76). Another potential cell source for kidney regeneration is circulating stem cells, which have been shown to transform into tubular and glomerular epithelial cells, podocytes, mesangial cells, and interstitial cells after renal injury (74, 85–87) (75, 88, 89). These observations suggest that controlling stem and progenitor cell differentiation may lead to successful regeneration of kidney tissues.

Although isolated renal cells are able to retain their phenotypic and functional characteristics in culture, transplantation of these cells in vivo may not result in structural remodeling. In addition, cell or tissue components cannot be implanted in large volumes due to limited diffusion of oxygen and nutrients (90). Thus, a cell-support matrix, preferably one that encourages angiogenesis, is necessary to allow diffusion across the entire implant. A variety of synthetic and naturally derived materials has been examined in order to determine the ideal support structures for the regeneration (70, 72, 91–93). Biodegradable synthetic materials, such as poly-lactic and poly-glycolic acid polymers, have been used to provide structural support for cells. Synthetic materials can be easily fabricated and configured in a controlled manner, which make them attractive options for tissue engineering. However, naturally derived materials, such as collagen, laminin and fibronectin, are much more biocompatible and provide a similar extracellular matrix environment to normal tissue. For this reason, collagen based scaffolds have been used increasingly in many applications (94–97).

Developmental Approaches to Kidney Regeneration

Transplantation of a kidney precursor, such as the metanephros, into a diseased kidney has been proposed as a possible method for functional restoration. In an animal study, human embryonic metanephroi, transplanted into the kidneys of an immune deficient mouse model, has developed into mature kidneys (98). The transplanted metanephroi produced urine-like fluid, however, failed to develop ureters. This study suggests that development of an in vitro system in which metanephroi could be grown may lead to transplant techniques that could produce a small replacement kidney within the host. In another study, the metanephros was divided into mesenchymal tissue and ureteral buds, and each of the tissue segments was cultured in vitro (99). After eight days in culture, each portion of the mesenchymal tissues had grown to the original size. A similar method was used for ureteral buds, which also propagated. These studies indicate that if the mesenchyme and ureteral buds were placed together and cultured in vitro, a metanephros-like structure would develop and suggest that the metanephros could be propagated under optimal conditions.

In another study, transplantation of metanephroi into a non-immunosuppressed rat omentum showed that the implanted metanephroi are able to undergo differentiation and growth that is not confined by a tight organ capsule (100). When the metanephroi with an intact ureteric bud were implanted, the metanephroi are able to enlarge and become kidney-shaped tissue within 3 weeks. The metanephroi transplanted into the omentum were able to develop into kidney tissue structure with a well-defined cortex and medulla. Mature nephrons and collecting system structures are shown to be indistinguishable from those of normal kidneys by light or electron microscopy (101, 102). Moreover, these structures become vascularized via arteries that originate at the superior mesenteric artery of the host (101, 102). It has been demonstrated that the metanephroi transplanted into the omentum survive for up to 32 weeks post-implantation (103). These studies show that developmental approach may be a viable option for regenerating renal tissue for functional restoration.

Tissue Engineering Approaches to Kidney Regeneration

The ability to grow and expand renal cells is one of the essential requirements in engineering tissues. The feasibility of achieving renal cell growth, expansion and in vivo reconstitution using tissue engineering techniques was investigated (91). Donor rabbit kidneys were removed and perfused with a non-oxide solution which promoted iron particle entrapment in the glomeruli. Homogenization of the renal cortex and fractionation in 83 and 210 micron sieves with subsequent magnetic extraction yielded three separate purified suspensions of distal tubules, glomeruli, and proximal tubules. The cells were plated separately in vitro and after expansion, were seeded onto biodegradable polyglycolic acid scaffolds and implanted subcutaneously into host athymic mice. This included implants of proximal tubular cells, glomeruli, distal tubular cells, and a mixture of all three cell types. Animals were sacrificed at one week, two weeks, and one month after implantation and the retrieved implants were analyzed. An acute inflammatory phase and a chronic foreign body

reaction were seen, accompanied by vascular in growth by 7 days after implantation. Histologic examination demonstrated progressive formation and organization of the nephron segments within the polymer fibers with time. Renal cell proliferation in the cell-polymer scaffolds was detected by in vivo labeling of replicating cells with the thymidine analog bromodeoxyuridine (76). BrdU incorporation into renal cell DNA was confirmed using monoclonal anti-BrdU antibodies. These results demonstrated that renal specific cells can be successfully harvested and cultured, and can subsequently attach to artificial biodegradable polymers. The renal cell-polymer scaffolds can be implanted into host animals where the cells replicate and organize into nephron segments, as the polymer, which serves as a cell delivery vehicle, undergoes biodegradation.

Initial experiments showed that implanted cell-polymer scaffolds gave rise to renal tubular structures. However, it was unclear whether the tubular structures reconstituted de novo from dispersed renal elements, or if they merely represented fragments of donor tubules which survived the original dissociation and culture processes intact. Further investigation was conducted in order to examine the process (104). Mouse renal cells were harvested and expanded in culture. Subsequently, single isolated cells were seeded on biodegradable polymers and implanted into immune competent syngeneic hosts. Renal epithelial cells were observed to reconstitute into tubular structures in vivo. Sequential analyses of the retrieved implants over time demonstrated that renal epithelial cells first organized into a cord-like structure with a solid center. Subsequent canalization into a hollow tube could be seen by two weeks. Histologic examination with nephron segment specific lactins showed successful reconstitution of proximal tubules, distal tubules, loop of Henle, collecting tubules and collecting ducts. These results showed that single suspended cells are capable of reconstituting into tubular structures, with homogeneous cell types within each tubule.

Although these studies demonstrated that renal cells seeded on biodegradable polymer scaffolds are able to form some renal structures in vivo, complete renal function could not be achieved in these studies. In a subsequent study we sought to create a functional artificial renal unit which could produce urine (105). Mouse renal cells were harvested, expanded in culture, and seeded onto a tubular device constructed from polycarbonate (97). The tubular device was connected at one end to a silastic catheter which terminated into a reservoir. The device was implanted subcutaneously in athymic mice. The implanted devices were retrieved and examined histologically and immunocytochemically at 1, 2, 3, 4 and 8 weeks after implantation. Fluid was collected from inside the implant, and uric acid and creatinine levels were determined.

Histological examination of the implanted device demonstrated extensive vascularization as well as for mation of glomeruli and highly organized tubule-like structures. Immunocytochemical staining with anti-osteopontin antibody, which is secreted by proximal and distal tubular cells and the cells of the thin ascending loop of Henle, stained the tubular sections. Immunohistochemical staining for alkaline phosphatase stained proximal tubule-like structures. Uniform staining for fibronectin in the extracellular matrix of newly formed tubes was observed. The fluid collected from the reservoir was yellow and contained 66 mg/dl uric acid (as compared to 2 mg/dl in plasma) suggesting that these tubules are capable of unidirectional secretion and concentration of uric acid. The creatinine assay performed on the collected fluid showed an 8.2 fold increase in concentration, as compared to serum. These results demonstrated that single cells form multicellular structures can become organized into functional renal units that are able to excrete high levels of solutes through a urine-like fluid (105).

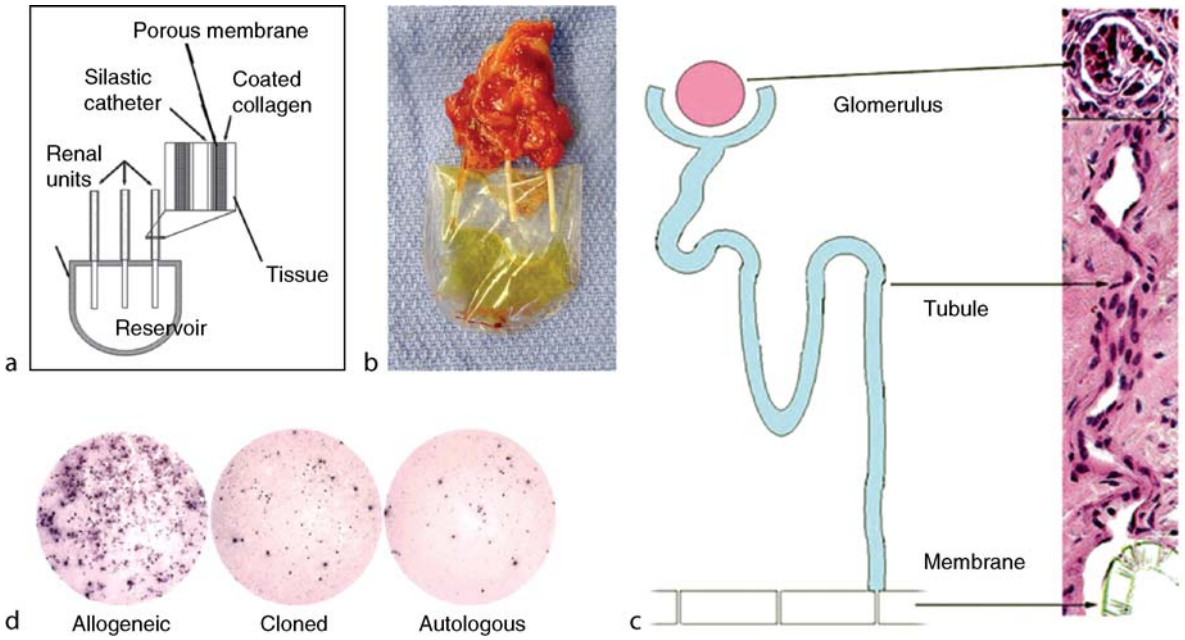
To determine whether renal tissue could be formed using an alternative cell source, nuclear transplantation (therapeutic cloning) was performed to generate histocompatible tissues, and the feasibility of engineering syngeneic renal tissues in vivo using these cloned cells was investigated (84). Nuclear material from bovine dermal fibroblasts was transferred into unfertilized enucleated donor bovine eggs. Renal cells from the cloned embryos were harvested, expanded in vitro, and seeded onto three-dimensional renal devices (*Fig. 19-5a*). The devices were implanted into the back of the same steer from which the cells were cloned, and were retrieved 12 weeks later.

This process produced functioning renal units. Urine production and viability were demonstrated after transplantation back into the nuclear donor animal (*Fig. 19-5b*). Chemical analysis suggested unidirectional secretion and concentration of urea nitrogen and creatinine. Microscopic analysis revealed formation of organized glomeruli and tubular structures (*Fig. 19-5c*). Immunohistochemical and RT-PCR analysis confirmed the expression of renal mRNA and proteins.

Since previous studies have shown that bovine clones harbor the oocyte mitochondrial DNA (106–108) the donor egg's mitochondrial DNA (mtDNA) was thought to be a potential source of immunologic incompatibility. Differences in mtDNA-encoded proteins expressed by cloned cells could stimulate a T-cell response specific for

Figure 19-5

Combining therapeutic cloning and tissue engineering to produce kidney tissue. (a): Illustration of the tissue-engineered renal unit. (b): Renal unit seeded with cloned cells, three months after implantation, showing the accumulation of urine-like fluid. (c): Clear unidirectional continuity between the mature glomeruli, their tubules, and silastic catheter. (d): Elispot analyses of the frequencies of T cells that secrete IFNγ after stimulation with allogeneic renal cells, cloned renal cells, or nuclear donor fibroblasts. Cloned renal cells produce fewer IFNγ spots than the allogeneic cells, indicating that the rejection response to cloned cells is diminished. The presented wells are single representatives of duplicate wells. (See color plate 7)



mtDNA-encoded minor histocompatibility antigens when the cloned cells are implanted back into the original nuclear donor (109). Maternally transmitted minor histocompatibility antigens in mice have been shown to stimulate both skin allograft rejection in vivo and cytotoxic T lymphocytes expansion in vitro (109) that could prevent the use of these cloned constructs in patients with chronic rejection of major histocompatibility matched human renal transplants (110, 111). We tested for a possible T-cell response to the cloned renal devices using delayed-type hypersensitivity testing in vivo and EliSPOT analysis of interferon-γ secreting T-cells in vitro. Both analyses revealed that the cloned renal cells showed no evidence of a T-cell response, suggesting that rejection will not necessarily occur in the presence of oocyte-derived mtDNA (*Fig. 19-5d*). This finding may represent a step forward in overcoming the histocompatibility problem of stem cell therapy (111).

These studies demonstrated that cells derived from nuclear transfer can be successfully harvested, expanded in culture, and transplanted in vivo with the use of biodegradable scaffolds on which the single suspended cells can organize into tissue structures that are genetically identical to that of the host. These studies were the first demonstration of the use of therapeutic cloning for regeneration of tissues in vivo.

However, a naturally derived tissue matrix with existing three-dimensional kidney architecture would be preferable to the artificial matrix used in these experiments, because it would allow for transplantation of a larger number of cells, resulting in greater renal tissue volumes. Thus, we developed an acellular collagen-based kidney matrix, which is identical to the native renal architecture. In a subsequent study we investigated whether these collagen-based matrices could accommodate large volumes of renal cells and form kidney structures in vivo (112).

Acellular collagen matrices, derived from porcine kidneys, were obtained through a multi-step decellularization process. During this process, serial evaluation of the matrix for cellular remnants was performed using histochemistry, scanning electron microscopy (SEM) and RT-PCR. Mouse renal cells were harvested, grown,

and seeded on 80 of the decellularized collagen matrices at a concentration of 30×10^6 cells/ml. Forty cell-matrix constructs grown in vitro were analyzed 3 days, 1, 2, 4 and 6 weeks after seeding. The remaining 40 cell-containing matrices were implanted in the subcutaneous space of 20 athymic mice. The animals were sacrificed 3 days, 1, 2, 4, 8 and 24 weeks after implantation for analyses. Gross, SEM, histochemical, immunocytochemical and biochemical analyses were performed.

Scanning electron microscopy and histologic examination confirmed the acellularity of the processed matrix. RT-PCR performed on the kidney matrices demonstrated the absence of any RNA residues. Renal cells seeded on the matrix adhered to the inner surface and proliferated to confluency 7 days after seeding, as demonstrated by SEM. Histochemical and immunocytochemical analyses performed using H & E, periodic acid schiff, alkaline phosphatase, anti-osteopontin and anti-CD-31 identified stromal, endothelial and tubular epithelial cell phenotypes within the matrix. Renal tubular and glomerulus-like structures were observed 8 weeks after implantation. MTT proliferation and titrated thymidine incorporation assays performed 6 weeks after cell seeding demonstrated a population increase of 116% and 92%, respectively, as compared to the 2 week time points. This study demonstrates that renal cells are able to adhere to and proliferate on the collagen-based kidney matrices. The renal cells reconstitute renal tubular and glomeruli-like structures in the kidney-shaped matrix. The collagen based kidney matrix system seeded with renal cells may be useful in the future for augmenting renal function.

We also investigated the feasibility of creating three-dimensional renal structures for in situ implantation within the native kidney tissue. Primary renal cells from 4 week old mice were grown and expanded in culture. These renal cells were labeled with fluorescent markers and injected into mouse kidneys in a collagen gel for in vivo formation of renal tissues. Collagen injection without cells and sham operated animals served as controls. In vitro reconstituted renal structures and in vivo implanted cells were retrieved and analyzed.

The implanted renal cells formed tubular and glomerular structures within the kidney tissue, as confirmed by the fluorescent markers. There was no evidence of renal tissue formation in the control and the sham operated groups. These results demonstrate that single renal cells are able to reconstitute kidney structures when placed in a collagen-based scaffolding system. The implanted renal cells are able to self assemble into tubular and glomerular structures within the kidney tissue. These findings suggest that this system may be the preferred approach to engineer functional kidney tissues for the treatment of end stage renal disease.

Genital Tissues

Reconstructive surgery is required for a wide variety of pathologic penile conditions, such as penile carcinoma, trauma, severe erectile dysfunction, and congenital conditions such as ambiguous genitalia, hypospadias, and epispadias. One of the major limitations of phallic reconstructive surgery is the scarcity of sufficient autologous tissue.

The major components of the phallus are corporal smooth muscle and endothelial cells. The creation of autologous functional and structural corporal tissue de novo would be beneficial. Autologous cavernosal smooth muscle and endothelial cells were harvested, expanded, and seeded on acellular collagen matrices and implanted in a rabbit model (113, 114). Histologic examination confirmed the appropriate organization of penile tissue phenotypes, and structural and functional studies, including cavernosography, cavernosometry, and mating studies, demonstrated that it is possible to engineer autologous functional penile tissue. Our laboratory is currently working on increasing the size of the engineered constructs.

Congenital malformations of the uterus may have profound implications clinically. Patients with cloacal exstrophy and intersex disorders may not have sufficient uterine tissue present for future reproduction. We investigated the possibility of engineering functional uterine tissue using autologous cells (115). Autologous rabbit uterine smooth muscle and epithelial cells were harvested, then grown and expanded in culture. These cells were seeded onto preconfigured uterine-shaped biodegradable polymer scaffolds, which were then used for subtotal uterine tissue replacement in the corresponding autologous animals. Upon retrieval 6 months after implantation, histological, immunocytochemical, and Western blot analyses confirmed the presence of normal uterine tissue components. Biomechanical analyses and organ bath studies showed that the functional characteristics of these tissues were similar to those of normal uterine tissue. Breeding studies using these engineered uteri are currently being performed.

Similarly, several pathologic conditions, including congenital malformations and malignancy, can adversely affect normal vaginal development or anatomy. Vaginal reconstruction has traditionally been challenging due to the paucity of available native tissue. The feasibility of

engineering vaginal tissue in vivo was investigated (116). Vaginal epithelial and smooth muscle cells of female rabbits were harvested, grown, and expanded in culture. These cells were seeded onto biodegradable polymer scaffolds, and the cell-seeded constructs were then implanted into nude mice for up to 6 weeks. Immunocytochemical, histological, and Western blot analyses confirmed the presence of vaginal tissue phenotypes. Electrical field stimulation studies in the tissue-engineered constructs showed similar functional properties to those of normal vaginal tissue. When these constructs were used for autologous total vaginal replacement, patent vaginal structures were noted in the tissue-engineered specimens, while the non-cell-seeded structures were noted to be stenotic (116).

Other Emerging Technologies

Injectable Therapies

Both urinary incontinence and vesicoureteral reflux are common conditions affecting the genitourinary system. Both conditions are usually the result of damage to or malformation of a specific sphincter muscle. Currently, injection of bulking agents around the defective sphincter can be used clinically for these conditions, but biocompatibility of current synthetic bulking agents is a concern. The ideal substance for endoscopic treatment of reflux and incontinence should be injectable, nonantigenic, nonmigratory, volume stable, and safe for human use. Animal studies have shown that chondrocytes (cartilage cells) can be easily harvested and combined with alginate in vitro and the resulting suspension can be easily injected cystoscopically. A similar technique using muscle and muscle precursor cells with the hope of repairing the defective sphincter muscle has been studied. These technologies have been applied in humans for the correction of vesicoureteral reflux in children and for urinary incontinence in adults (117, 118).

Autologous Chondrocytes as Bulking Agents

Injectable bulking agents can be endoscopically used in the treatment of both urinary incontinence and vesicoureteral reflux. The advantages in treating urinary incontinence and vesicoureteral reflux with this minimally invasive approach include the simplicity of this quick outpatient procedure and the low morbidity associated with it. Several investigators are seeking alternative implant materials that would be safe for human use (119).

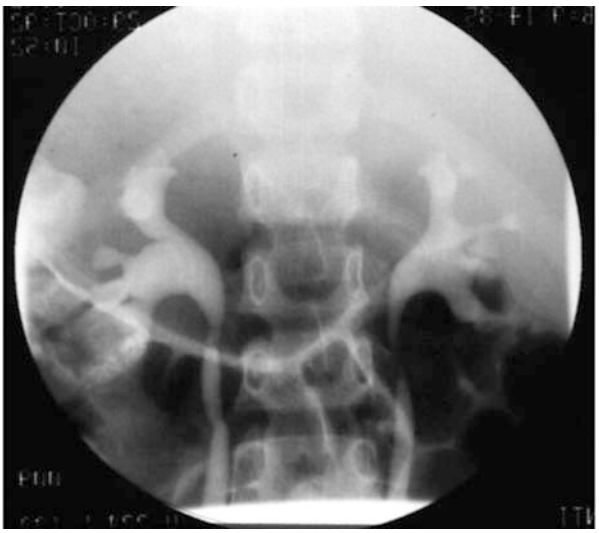
The ideal substance for the endoscopic treatment of reflux and incontinence should be injectable, nonantigenic, nonmigratory, volume stable, and safe for human use. Toward this goal long term studies were conducted to determine the effect of injectable chondrocytes in vivo (120). It was initially determined that alginate, a liquid solution of gluronic and mannuronic acid, embedded with chondrocytes, could serve as a synthetic substrate for the injectable delivery and maintenance of cartilage architecture in vivo. Alginate undergoes hydrolytic biodegradation and its degradation time can be varied depending on the concentration of each of the polysaccharides. The use of autologous cartilage for the treatment of vesicoureteral reflux in humans would satisfy all the requirements for an ideal injectable substance.

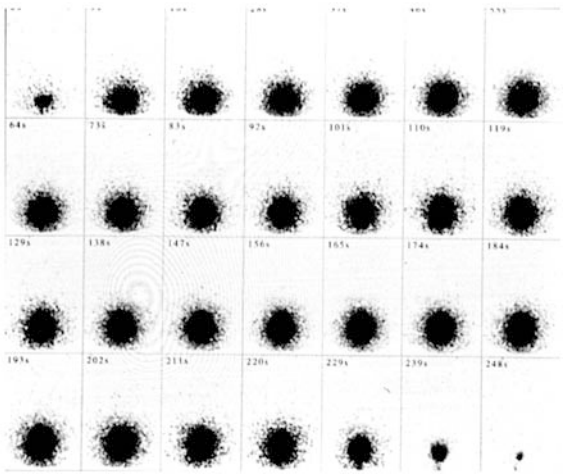
Chondrocytes derived from an ear biopsy can be readily grown and expanded in culture. Neocartilage formation can be achieved in vitro and in vivo using chondrocytes cultured on synthetic biodegradable polymers. In these experiments, the cartilage matrix replaced the alginate as the polysaccharide polymer underwent biodegradation. This system was adapted for the treatment of vesicoureteral reflux in a porcine model (121). These studies showed that chondrocytes can be easily harvested and combined with alginate in vitro, the suspension can be easily injected cystoscopically, and the elastic cartilage tissue formed is able to correct vesicoureteral reflux without any evidence of obstruction.

Two multicenter clinical trials were conducted using this engineered chondrocyte technology. Patients with vesicoureteral reflux were treated at ten centers throughout the US. The patients had a similar success rate as with other injectable substances in terms of cure (*Fig. 19-6*). Chondrocyte formation was not noted in patients who had treatment failure. It is supposed that the patients who were cured have a biocompatible region of engineered autologous tissue present, rather than a foreign material (117). Patients with urinary incontinence were also treated endoscopically with injected chondrocytes at three different medical centers. Phase 1 trials showed an approximate success rate of 80% at follow-up 3 and 12 months postoperatively (118). Several of the clinical trials involving bioengineered products have been placed on hold because of the costs involved with the specific technology. With a bioengineered product, costs are usually high because of the biological nature of the therapies involved. As with any therapy, the cost that the medical health care system can allow for a specific technology is limited. Therefore, the costs of bioengineered products have to be lowered for them to have an impact clinically. This is currently being addressed for multiple tissue-engineered technologies.

Figure 19-6

Autologous chondrocytes for the treatment of vesicoureteral reflux. (a): Preoperative voiding cystourethrogram of a patient with bilateral reflux. A catheter was inserted into the bladder via the urethra, and contrast material was instilled intravesically. Here, contrast material can be seen within both ureters and within the kidneys, indicating reflux is present. (b): Postoperative radionuclide cystogram of the same patient 6 months after injection of autologous chondrocytes. A catheter was inserted into the bladder via the urethra, and a radioactive solution was inserted into the bladder. The bladder was scanned during filling and emptying phases. This panel includes sequential images of the bladder as it was filled and emptied. This shows a normal, round bladder that fills and empties properly. If reflux had been present, the ureters would have been visible in the scan above the round bladder.





As the technologies advance over time, and the volume of the application is considered, costs will naturally decrease.

Injectable Muscle Cells

The potential use of injectable cultured myoblasts for the treatment of stress urinary incontinence has been investigated (122, 123). Myoblasts were labeled with fluorescent latex microspheres (FLM) in order to track them after injection. Labeled myoblasts were directly injected into the proximal urethra and lateral bladder walls of nude mice with a micro-syringe in an open surgical procedure. Tissue harvested up to 35 days post-injection contained the labeled myoblasts, as well as evidence of differentiation of the labeled myoblasts into regenerative myofibers. The authors reported that a significant portion of the injected myoblast population persisted in vivo. Similar techniques of sphincteric derived muscle cells have been used for the treatment of urinary incontinence in a pig model (124). The fact that myoblasts can be labeled and survive after injection and begin the process of myogenic differentiation further supports the feasibility of using cultured cells of muscular origin as an injectable bioimplant.

The use of injectable muscle precursor cells has also been investigated for use in the treatment of urinary incontinence due to irreversible urethral sphincter injury or maldevelopment. Muscle precursor cells are the quiescent satellite cells found in each myofiber that proliferate to form myoblasts and eventually myotubes and new muscle tissue. Intrinsic muscle precursor cells have previously been shown to play an active role in the regeneration of injured striated urethral sphincter (125). In a subsequent study, autologous muscle precursor cells were injected into a rat model of urethral sphincter injury, and both replacement of mature myotubes as well as restoration of functional motor units was noted in the regenerating sphincteric muscle tissue (126). This is the first demonstration of the replacement of both sphincter muscle tissue and its innervation by the injection of muscle precursor cells. As a result, muscle precursor cells may be a minimally invasive solution for urinary incontinence in patients with irreversible urinary sphincter muscle insufficiency.

Fetal Tissue Engineering

The prenatal diagnosis of fetal abnormalities is now more common and more accurate. Improvements in prenatal diagnosis have led to demand for novel interventions designed to reverse potentially life-threatening processes before birth. Having a ready supply of urologic-associated tissue for immediate surgical reconstruction of congenital malformations at birth may be advantageous. Theoretically, once the diagnosis of the pathologic condition is confirmed prenatally, a small tissue biopsy could then be obtained under ultrasound guidance. These biopsy materials could then be processed expanded in vitro. Using tissue engineering techniques, in vitro-reconstituted structures could then be readily available at the time of birth for reconstruction.

Summary and Conclusions

Regenerative medicine efforts are currently underway experimentally for virtually every type of tissue and organ within the human body. As regenerative medicine incorporates the fields of tissue engineering, cell biology, nuclear transfer, and materials science, personnel who have mastered the techniques of cell harvest, culture, expansion, transplantation, as well as polymer design and biomedical engineering are essential for the successful application of these technologies for patients. Various tissues are at different stages of development, with some already being used clinically, a few in preclinical trials, and some in the discovery stage. Recent progress suggests that engineered tissues may have an expanded clinical applicability in the future and may represent a viable therapeutic option for those who would benefit from the life-extending benefits of tissue replacement or repair.

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Clinical Methods

20 Clinical Evaluation

Mohan Shenoy · Nicholas J. A. Webb

Presentation of Renal Disease in Children

Introduction

Renal disease in children may present with overt abnormalities clearly associated with the urinary tract, such as the development of macroscopic haematuria or profound oliguria. However, in many instances symptoms may be very non specific or seemingly mild. Children with chronic renal failure present with a wide variety of symptoms including enuresis, failure to thrive, short stature, lethargy and pallor. The onset may be silent and the progress insidious, with symptoms only developing late in its course. Urinary tract infection in infants and small children may, in contrast to older children, present with non-specific manifestations including poor feeding, vomiting, irritability, abdominal pain, failure to thrive, lethargy and restlessness. The possibility of renal disease should therefore be considered in the differential diagnosis of any child presenting to hospital with acute or chronic symptoms.

Antenatal Imaging

Since the first report of renal abnormalities being detected by antenatal ultrasonography in the 1970s (1), there have been major developments in the antenatal assessment of the urinary tract (2–4). In most countries, pregnant women undergo routine ultrasound assessment of the fetus at various stages throughout pregnancy, including a detailed scan performed at around 20 weeks gestation. Ultrasound is particularly informative with regard to renal abnormalities, which account for around 20% of all significant fetal abnormalities detected during gestation (5). Recent developments including 3D ultrasound and magnetic resonance imaging of the fetus will allow these anomalies to be studied in further detail. This has resulted in many significant congenital and inherited abnormalities of the urinary tract being detected antenatally, notably posterior urethral valves, the multicystic dysplastic kidney (MCDK), pelvi-ureteric junction obstruction and polycystic kidney diseases. In the case of posterior urethral valves, this has resulted in the possibility of antenatal therapy, currently the subject of an ongoing randomized controlled trial investigating vesico-amniotic shunting (www.pluto.bham.ac.uk). Large kidneys on antenatal imaging may suggest hydronephrosis, polycystic kidney disease (PKD), MCDK, congenital nephrotic syndrome or rarely a renal tumor whereas small kidneys may point to the presence of renal dysplasia or hypoplasia. PKD, cystic dysplasia and glomerulocystic disease are common causes of echobright kidneys. TCF2 gene mutations are found in almost a third of the children with antenatally diagnosed bilateral echogenic kidneys (6). The presence of renal macrocysts in the antenatal period should alert the physician towards a diagnosis of autosomal dominant PKD, PKD associated with tuberous sclerosis, MCDK or cystic dysplasia.

The sensitivity of ultrasound imaging does, however, result in the detection of many minor abnormalities, particularly unilateral renal pelvic dilatation, which appear to resolve spontaneously in later pregnancy or after birth and are of no long term significance. Their detection may be a source of significant maternal anxiety and multiple invasive investigations where appropriate protocols are not in operation.

Abnormalities of Appearance of Urine

Red or Dark Urine

The presence of blood in the urine causes it to develop a pink to red color. Only a relatively small amount of blood is necessary to produce discoloration. Prolonged contact between blood in the urinary tract and acidic urine causes the haem pigment to become oxidized to a methaem derivative, giving the urine a brown color. In general, the longer the contact and the more acidic the urine, the darker brown the urine becomes.

Contamination with blood from menstruation in older girls needs ruling out. There are a number of causes of false positive haematuria, where alternative substances produce red discoloration (*Table 20-1*). One of the most common of these is the pink discoloration seen in nappies caused by

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Table 20-1

False positive haematuria; alternative causes of red urine

Foods e.g., beetroot, berries containing anthocyanins and food dyes
Haemoglobinuria e.g., in intravascular haemolysis
Myoglobinuria e.g., in rhabdomyolysis
Urate crystals (a cause of pink discoloration of nappies)
Drugs e.g., rifampicin, phenothiazines, desferroximine, phenindione
Inborn errors of metabolism e.g., porphyria and alkaptonuria

the precipitation of urate crystals. Urine microscopy is therefore mandatory following the detection of red or dark urine. It is important that this is performed on a fresh sample as red cell lysis occurs where samples are allowed to stand for prolonged periods prior to examination.

The passage of fresh red blood in the urine, with or without clots, is most likely to originate from the lower urinary tract, particularly where this is most marked at the beginning or the end of the urinary stream. This highlights the importance of obtaining a comprehensive clinical history in such children. Causes include urethritis, trauma, bladder calculus and schistosomiasis. Urological assessment and cystoscopy in indicated in most of these cases. More uncommon causes of bleeding from the upper tracts include trauma, arteriovenous malformations, tumors and angiomyolipoma associated with tuberous sclerosis.

Fabricated and induced illness by proxy, previously termed Munchausen syndrome by proxy is a very rare disorder, though falsification of urine samples by contamination with maternal or other blood is one of the more common modes of presentation (7). A high index of suspicion needs to be maintained to detect such cases. Absolute confirmation of contamination requires analysis of urinary red cells for differences in blood group type, or if required, DNA profiling.

Dark urine associated with jaundice in the neonate or older child should alert the physician to the presence of conjugated hyperbilirubinaemia, indicative of significant liver or biliary tract disease. Bilirubin and urobilinogen will be detected on dipstick testing the urine in such cases.

Cloudy Urine

The urine may become cloudy secondary to the presence of white blood cells (pyuria) associated with urinary infection, calcium phosphate crystals or a combination of calcium salts, uric acid, oxalate, cystine or struvite. These substances are normally present in the urine, though may be present in excess in various disease states. The precipitation of phosphates and urates is enhanced by refrigeration of the urine sample.

Frothy Urine

The presence of significant quantities of protein in the urine will result in it becoming frothy. Presentation with frothy urine should therefore prompt the physician to perform dipstick analysis followed by formal quantification of protein content.

Offensive or Unusual Smelling Urine

A number of factors may result in an alteration in the smell of urine. Infection with urea splitting organisms including *Ureaplasma urealyticum, Proteus, Staphylococcus, Klebsiella, Providentia* and *Pseudomonas* species may generate an ammonia-like smell, though the positive predictive value of offensive urine for bone fide urinary infection is very low. A number of the inborn errors of metabolism, including maple syrup urine disease, phenylketonuria and isovaleric acidaemia also produce characteristic odors. The change in urine odor following the ingestion of asparagus was first reported in the eighteenth century by a physician to the French royal family (8). This is thought to be due to the presence of S-methylthioacrylate and S-methyl-3-(methylthio)thiopropionate (9), though a combination of methyl mercaptan, dimethyl sulfide, and small amounts of sulfur-oxidized compounds could also be responsible (10).

Passage of Gravel or Stones

The passage of gravel or stones in the urine is an unusual, though recognized mode of presentation of urinary tract calculi. Stones are either metabolic or infective in origin (Chapter 58).

Abnormalities of Volume of Urine

Oliguria

The otherwise healthy neonate is oliguric for the first 2–3 days of life until the onset of the postnatal diuresis.

Ninety-two percent of neonates will pass urine within the first 24 h of life and almost all newborns will do so in the first 48 h (11). Beyond the immediate neonatal period, oliguria is defined a urine output of less than 500 ml/24 h/1.73 m^2; that which is sufficient to maintain homeostasis. The most common cause of oliguria in children is intravascular volume depletion. Where this has been excluded or adequately treated and volume depletion persists, a search for intrinsic or obstructive renal disease should be commenced.

Polyuria and Polydipsia

Of those children presenting to medical practitioners with symptoms of polyuria, only a very small proportion will have a significant renal or other pathology. A strict definition of polyuria does not exist, though a daily urine output exceeding 2 l in school-aged children is unusual. It is important to distinguish polyuria from frequency of micturition where small volumes of urine are passed frequently though the 24 h urine output is within normal limits.

Significant pathologies which result in polyuria are shown in *Table 20-2* and include excessive fluid intake, increased osmotic load, failure to produce or release antidiuretic hormone (ADH) and resistance to the actions of ADH in the kidney. The distinction between primary polydipsia and primary polyuria may be inferred from the relative osmolality of plasma and urine and the response to controlled water deprivation. The distinction between pituitary and renal causes of polyuria may be made by the plasma ADH concentration and the urinary response to D-amino-arginine vasopressine.

Table 20-2
Causes of polyuria in children

Increased fluid intake
Psychogenic/behavioral polydipsia
Hypothalamic polydipsia
Hyperreninaemia including Wilm's tumor
Increased osmotic load
Diabetes mellitus and other causes of hyperglycaemia
Chronic renal failure (urea)
Following mannitol infusion
Failure of ADH production
Cranial diabetes insipidus
Basal skull fracture
Cranial tumors
Post hypophysectomy
Infection; encephalitis, meningitis, tuberculosis
Vascular aneurysm or thrombosis
Failure of renal response to ADH
Nephrogenic diabetes insipidus (X-linked)
Acquired unresponsiveness to ADH
Obstructive uropathy
Hypokalaemia
Hypercalcaemia
Sickle cell disease
Chronic renal failure
Drugs e.g., amphotericin, methoxyflurane, tetracycline

Wetting and Abnormalities of the Passage of Urine

Wetting is the most common urinary tract disorder of childhood, though in most cases there is no organic pathology. The majority of children will achieve daytime bladder control between 3 and 4 years of age (12). The prevalence of nocturnal enuresis is 15–20% at age 5 years and up to 3% in young adults (13). Nocturnal enuresis has a strong genetic predisposition and on its own, is a benign self-limiting condition. Daytime wetting, which can occur as a result of a number of disorders including detrusor overactivity, dysfunctional voiding and, rarely, giggle incontinence which is more common in girls and usually resolves by the age of 9 years. Secondary enuresis, both diurnal and nocturnal raises the possibility of an organic cause (e.g., neuropathic bladder) especially in boys; however it is commonly associated with psychosocial disturbances such as parental separation, the birth of a new child or a death in the family. Diabetes mellitus should always be excluded. A careful voiding and wetting history along with a focused physical examination will help to identify any organic pathology. The presence of dysuria and frequency might suggest a diagnosis of urinary tract infection whereas poor urinary stream and an enlarged bladder would point to a diagnosis of a neuropathic bladder or an obstructive pathology such as posterior urethral valves. Examination of the urine for infection is essential in all children with wetting. In a child with persistent daytime wetting a pre and post-void ultrasound of the renal tract is useful to screen for urinary tract anomalies and to assess bladder emptying. Urodynamic assessment to identify abnormalities of bladder and sphincter function is indicated in children with a neuropathic bladder and in some children with

persistent and troublesome wetting who do not demonstrate a good response to conservative management.

Pollakiuria, a benign, self-limiting condition is characterized by the very frequent (every 5–20 min) passage of urine during the daytime hours. It most commonly presents in pre-school age children and is not associated with wetting.

Edema

Children with renal disease may present with edema, a major clinical manifestation of ECF volume expansion. Edema may occur in the acute nephritic syndrome and other causes of acute renal impairment as a result of failure of salt and water excretion. Here, peripheral edema is accompanied by intravascular volume expansion with hypertension and pulmonary edema. In the nephrotic syndrome, loss of plasma oncotic pressure secondary to hypoproteinaemia and increased vascular permeability result in the loss of fluid from the intravascular into the extravascular space. Here, edema is initially first evident as swelling of the periorbital region, which is often most marked in the morning. With progressive fluid retention, more generalized edema develops in a gravity dependent distribution, the ankle and sacral areas being the most severely affected. This may be associated with abdominal distension secondary to ascites.

Asymptomatic Presentation Following Screening or Routine Assessment

Whilst the universal routine testing of children's urine by dipstick examination is not performed in the large majority of countries, this has been undertaken in Japan, having been introduced by the Ministry of Education in 1973 with the aim of the early detection of asymptomatic renal disease (14). Outside of universal screening programs, children may have their urine tested at the time of attendance at hospital Emergency Departments and out-patient clinics or as part of a routine medical assessment for insurance or immigration purposes. Such testing may detect abnormalities in the urine (commonly microscopic haematuria) which results in referral for further assessment.

Children may have hypertension detected following a routine medical examination performed at school or for participation in particular sporting activities etc. In such instances it is firstly essential to rule out erroneously high blood pressure which has occurred as a result of the use of incorrect measuring equipment or white coat hypertension. Whilst renal disease is widely reported to be the commonest cause of hypertension in children, the rising tide of childhood obesity may soon see this become the predominant cause (15).

The screening of asymptomatic siblings of index cases of renal disease is a difficult area. Clearly where the detection of significant pathology necessitating the commencement of specific therapy is possible e.g., cystinosis, then appropriate testing should be performed. Siblings and close contacts of children presenting with diarrhea associated haemolytic uraemic syndrome are not infrequently found to have evidence of varying degrees of renal impairment and anemia in the absence of any significant symptoms and such detection will allow the commencement of appropriate therapy and follow-up. In contrast, in the asymptomatic siblings of children with autosomal dominant PKD, many authorities would recommend that ultrasound or genetic screening does not take place. Potentially affected individuals should undergo an annual check of blood pressure and urinalysis until they are able to make a fully informed decision about the relative merits of presymptomatic detection, bearing in mind the implications that this may have. There are a number of situations where medical opinion is divided, for instance whether newborn siblings of children with vesicoureteric reflux should undergo radiological assessment; definitive answers to these question are only likely to be obtained through the enrolment of such children in prospective randomized controlled trials.

Urinary Tract Infection

Urinary tract infection is common. Swedish data report a cumulative incidence by 2 years of age of 2.2% in boys and 2.1% in girls (16). In the northern United Kingdom, cumulative referral rates by 7 years of age are as high as 2.8% for boys and 8.2% for girls, rising to 3.6 and 11.3% respectively by 16 years of age (17). Whilst older children may present with classical symptoms of either lower urinary tract infection (dysuria, frequency, wetting) or upper urinary tract infection (systemic upset, fever, loin pain), these features are much less pronounced in the younger child; here nonspecific manifestations may include poor feeding, vomiting, irritability, failure to thrive, lethargy and abdominal pain.

Diagnosis of urinary tract infection is important, as aside from the acute morbidity associated with infection, the development of infection is an important marker of underlying congenital abnormalities of the urinary tract, in particular vesico-ureteric reflux. Furthermore, infection itself is an important cause of renal cortical scarring in those predisposed to this. Therefore a diagnosis of urinary tract infection should always result in

consideration being given to radiological assessment of the urinary tract. Previous guidelines recommended that all children be investigated after a single infection, though more recent guidelines, for instance those produced by the UK National Institute for Health and Clinical Excellence, have recommended radiological investigations are only performed on the very young and those with recurrent or difficult to treat infections (http://www.nice.org.uk/guidance/index.jsp?action=byID&o=11819).

Clinical History

Antenatal History

A record should be made of the results of antenatal imaging studies, particularly the detailed scan performed at around 20 weeks gestation. Abnormalities of liquor volume should be recorded. In the absence of rupture of the amniotic membranes, oligohydramnios or anhydramnios after 16 weeks' gestation represents failure of urine excretion due to obstruction to urine flow or inability of the kidneys to produce urine. Polyuric states including neonatal Bartter's syndrome and congenital nephrotic syndrome are associated with polyhydramnios; it should be remembered, however, that only a small proportion of cases of polyhydramnios are caused by renal disease.

A raised maternal serum alpha-fetoprotein level between 15 and 20 weeks gestation is associated with spinal cord defects and the congenital nephrotic syndromes and is an indication for a detailed antenatal ultrasound scan and amniocentesis.

Close attention should be paid to drug history. Maternal intake of medications such as COX-2 inhibitors, angiotensin converting enzyme inhibitors (ACEi) or angiotensin receptor blockers in the second and third trimester is associated with renal failure in the neonatal period. First trimester exposure to ACEi can lead to major cardiovascular, central nervous system and renal malformations (18).

Other aspects of maternal medical history should be noted. Previous recurrent miscarriages should alert the physician to the possibility of a chromosomal abnormality. There is an increased risk of MCDK among infants of mothers with gestational diabetes. Maternal obesity is associated with increased risk of neural tube defects (19).

Birth History

An enlarged placenta (greater than 25% of the child's birth weight) is a feature of congenital nephrotic syndrome. The presence of hematuria following perinatal asphyxia may be due to renal venous thrombosis. Umbilical arterial catheterization is associated with vascular damage and is the commonest cause of hypertension in the neonatal period (20). The presence of a single umbilical artery in an infant is associated with an increased risk of a variety of renal anomalies including vesicoureteric reflux, MCDK and renal aplasia and dysplasia (21). It is debatable as to whether these infants merit further imaging unless the antenatal scan has demonstrated any abnormality; in such cases an ultrasound scan of the renal tract will provide useful initial information and guide further evaluation.

Family History

A detailed family history forms an essential component of the clinical evaluation and may often provide important clues to the diagnosis. It is considered good practice to document the family tree in the case records. A variety of renal diseases including PKD, familial glomerulonephritis and tubular disorders e.g., Bartter's and Gitelmann's syndromes are inherited. An increasing number of hereditary renal disorders can now be diagnosed by DNA analysis and it is therefore essential that the family is provided with detailed counseling with regard to the potential future implications.

Parental consanguinity is common in a number of societies including the Muslim Middle Eastern countries and the UK Pakistani community, where up to 55% are married to a first cousin (22). This should raise the possibility of conditions which are transmitted in an autosomal recessive manner such as autosomal recessive PKD, juvenile nephronophthisis and cystinosis. End stage renal failure of all causes is also more prevalent in this population (23).

Deafness and renal failure in a male relative may suggest a diagnosis of Alport's syndrome which is most commonly inherited in an X-linked manner. The inheritance of isolated vesicoureteric reflux is felt to be consistent with multifactorial or autosomal dominant with reduced penetrance and has a sibling recurrence rate of 30–50% (24).

General Medical History

Renal disease may be feature of a number of childhood disorders. A Pediatric Nephrologist working in a tertiary centre will often be asked to see children under the care of other specialists and must therefore be familiar with the

renal manifestations of systemic disorders and iatrogenic problems. Renal involvement may be a part of a multisystem disorder such as Henoch Schönlein purpura or systemic lupus erythematosus. Hyponatraemia due to cerebral salt wasting or syndrome of inappropriate ADH secretion is a common problem on the neurosurgical ward. A diagnosis of retinitis pigmentosa should alert to the possibility of juvenile nephronophthisis. Tubulopathy is often seen in children on the pediatric oncology ward following chemotherapy with agents such as adriamycin or ifosfamide. Asymptomatic renal calculi are known to occur after a short course of ceftriaxone and nephrocalcinosis is associated with prolonged use of furosemide.

Urine and Micturition

The clinical history should record information regarding daytime and night-time wetting (see above) and abnormalities of micturition, including the nature of the urinary stream (e.g., hesitancy, staccato micturition, terminal dribbling, the need to stand to void etc). Poor urinary stream should raise the possibility of posterior urethral valves in a male infant, though following the introduction of routine ultrasound scanning, the majority of such cases would be detected in the antenatal period. By 3 years of age most children have some conscious control over micturition and achieve daytime control, but enuresis and daytime accidents can continue to occur. In an older child, the voiding frequency and an estimate of the urinary volume should be documented (25).

Dietary History

The fluid and dietary restrictions in renal disease depend on the type and stage of disease. For instance, while salt and fluid restriction is not necessary in polyuric states such as proximal renal tubular disorders and Bartter's syndrome, anephric children on dialysis will require tight control of their salt and fluid intake. Most children with advanced renal failure will be on a restricted potassium, phosphorus and protein diet. Anorexia associated with renal failure means that these children are often malnourished and input from an experienced pediatric dietician is mandatory.

One of the consequences of the obesity epidemic has been the increasing numbers of overweight children with hypertension, disturbed glucose metabolism and hyperlipidaemia (26). Excessive salt intake may be associated with hypercalciuria.

Psychosocial History

The impact of the disease on the child, for example the amount of school missed, body image, limitations on lifestyle, self-esteem and peer reactions should be assessed. There is a wealth of evidence supporting the view that chronic physical illness and disability are risk factors for mental health problems in children. Social disadvantage, poverty, poor housing, educational failure and family instability are other risk factors. Caring for a chronically ill child is a huge challenge for any family and parents often have to give up employment in order to achieve this. Impact on siblings should be given consideration. A skilled social worker and child psychiatrist or psychologist are therefore essential members of a multiprofessional pediatric nephrology team. The impact of the disease on the child's intellectual, emotional and social progress must be assessed. An awareness of the risk factors will help the physician identify those children who are at particularly high risk of developing mental health problems. Adherence with prescribed medications is a particular problem during adolescence and is the commonest cause of rejection and graft loss in this age group (27).

Clinical Examination

General Assessment (› *Fig. 20-1*)

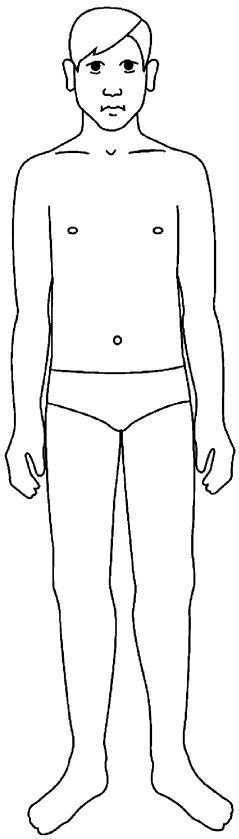
An initial rapid assessment of the level of consciousness, airway, breathing and circulation should be performed to determine how ill the child is. Any obvious dysmorphic features should be recorded. Pain can be a manifestation of renal disease and is often confused with back pain and other causes of abdominal pain in childhood. The state of the child (clothing and hygiene) should be noted. A record of the mood and demeanor of the child together with the family interaction is informative.

Growth

Growth is an invaluable measure of health during childhood and accurate measurement of height and weight along with the head circumference in younger children is therefore essential. Most growth charts express anthropometric measures in terms of percentiles, however not infrequently children with renal disease are significantly below the lowest percentile lines and therefore expressing height, weight and body mass index in terms of standard deviation score (SDS or Z score) is sometimes more

Figure 20-1

Assessment of the child with renal disease



1. **Airway**
   Breathing
   Circulation
   Ill or well child
2. **General assessment**
   Hydration
   Growth - centile charts
   Nutrition
   Dysmorphic features
   Tanner staging
3. **Skin**
   Sallow
   Pallor
   Jaundice
   Oedema
   Cushingoid features
   Scratch marks
   Rash
   Central venous/ haemodialysis catheter

   Eyes
   Face and oral cavity
   Ear deformity
4. **Cardiovascular system**
   Pulse including femorals
   BP
   JVP
   AV fistula
   Cardiomegaly
   Murmur
   Bruits
5. **Chest**
   Kussmaul breathing
   Rib rosary
   Harrison's sulcus
   Pleural effusion/pulmonary oedema
6. **Abdomen**
   Scars, PD catheter
   Distension, peritonitis
   Renal mass
   Bowel sounds
   Transplant kidney- tenderness/bruit
   Genitalia, urethral opening
   Anus
7. **Central nervous system**
   Mental status
   Cranial nerves
   Tone, power, reflexes
   Gait, coordination
8. **Musculoskeletal system**
   Muscle wasting, weakness
   Hemihypertrophy
   Joint swelling
   Spine, sacrum
   Linb deformity
   Wrist swelling
9. **Urine analysis**
   Microscopy
   Dipstick for blood, protein, nitrite and glucose

informative. Pubertal development (Tanner staging) should also be formally assessed.

Nutrition

Assessment of nutrition in children with renal disease can be a challenge; fluid overload and abnormalities in the distribution of fat and lean body tissue can lead to misinterpretation of the nutritional anthropometric measures (28). The commonly used nutritional markers are dietary assessment, height, estimated dry weight, body mass index, serum albumin and skin fold thickness. Tools for assessing body composition such as dual energy x-ray absorptiometry and bioelectrical impedance analysis can sometimes provide useful information.

Hydration/Volume Status

Assessment of the state of hydration and circulatory status is an important skill for all physicians dealing with children with kidney disease. An understanding of total body water and its distribution between the intravascular (plasma) and extracellular fluid (interstitial) compartments is essential. A child with nephrotic syndrome usually presents with a recent increase in weight and peripheral edema, reflecting an increase in ECF volume, though at the same time may exhibit signs of intravascular volume depletion such as reduced capillary refill time, cold extremities, tachycardia and oliguria. Occasionally, clinical signs may be subtle and measurement of urinary sodium, fractional excretion of sodium (FeNa) and urine osmolality

may provide additional information. In contrast, expansion of the intravascular volume, such as in nephritic states results in hypertension, raised jugular venous pulse, hepatomegaly and the development of pulmonary edema.

Cardiovascular System

Pulse

The rate, rhythm and volume of the pulse should be recorded. Tachycardia with a low volume pulse is a feature of intravascular volume depletion. The presence of a gallop rhythm suggests congestive cardiac failure. The femoral pulses should always be examined; coarctation of the aorta may present with hypertension and cardiac failure. The presence of bruits on auscultation over major arteries such as the carotid or renal arteries suggests extensive vascular disease or renal artery stenosis.

Blood Pressure

Measurement of the blood pressure is an integral part of the assessment of the child with renal disease. It is important to ensure that the right equipment and standards are used to ensure that hypertension is correctly diagnosed; a wrong diagnosis due to incorrect measurement can lead to a battery of unnecessary invasive tests. There is increasing use of ambulatory blood pressure monitoring in children, especially to diagnose essential and white coat hypertension (29). The investigation of hypertension is discussed in Chapter 62.

Precordium

Fluid overload, long standing hypertension and cardiomyopathy will give rise to cardiomegaly. Pericarditis with a rub may be a feature of severe uremia. A heart murmur may suggest a structural anomaly but can also be a feature of infective endocarditis or a hyperdynamic state as occurs in anemia.

Respiratory System

Hyperventilation with Kussmaul respiration suggests the presence of metabolic acidosis. Rachitic deformity of the ribs can be seen in longstanding chronic renal failure. Dullness on percussion over the lung fields might suggest pleural effusion in a child with nephrotic syndrome or a peritino-pleural leak in a patient on peritoneal dialysis. Fine crepitations over the lung bases may indicate the presence of pulmonary edema.

Abdomen

Abdominal Distension

Abdominal distension in renal disease may be due to ascites, bladder enlargement due to urinary retention and mass (see below) and the presence of peritoneal dialysis fluid. In children who have undergone previous abdominal surgery, for example urinary diversion procedures, one should be aware of the possibility of adhesions and obstruction presenting as an acute surgical abdomen. Tenderness with guarding and rigidity is highly suggestive of peritonitis and in these circumstances the opinion of a pediatric surgeon should be sought.

Abdominal Mass

Prior to the onset of routine antenatal imaging, the detection of an abdominal mass was a reasonably frequent mode of presentation of a variety of renal and urological disorders, including the MCDK, pelvi-ureteric junction obstruction and other causes of hydronephrosis. In the neonatal period, 55% of abdominal masses are renal in origin (hydronephrosis 25% and multicystic dysplastic kidney 15%). In later infancy and childhood, the proportion of abdominal masses which are renal in origin increases, largely due to the increased rate of malignant tumors in the age group (30).

Renal causes of an abdominal mass are shown in *Table 20-3*.

Features in the clinical history may help in ascertaining the likely cause of the mass. In the newborn period, the large majority of these lesions will have been identified antenatally, though additional clinical information may inform subsequent investigation and likely diagnosis i.e., the presence of oligohydramnios or polyhydramnios. Beyond the newborn period, a family history of severe hypertension may point to a diagnosis of PKD. Presentation with symptoms and signs of urinary tract infection may point to many of the anomalies associated with urinary obstruction or stasis.

Table 20-3

Causes of an abdominal mass of renal origin

Abnormality	Causes if unilateral	Causes if bilateral
Hydronephrosis (obstructive and non-obstructive)	PUJ obstruction	Causes of bladder outlet obstruction including posterior urethral valves
	VUJ obstruction	Eagle Barrett syndrome
	Primary megaureter	
Cystic mass	Multicystic dysplastic kidney	PKD (dominant and recessive)
	Simple cyst	Cystic disease associated with syndromal diagnosis
	Cystic dysplasia	Cystic dysplasia
Infection	Acute pyelonephritis	
	Xantogranulomatous pyelonephrtis	
	Perinephric abscess	
Vascular	Renal venous thrombosis	
	Arterial thrombosis	
	Acute cortical necrosis	
Tumors	Wilm's tumor	
	Mesoblastic nephroma	
	Leukaemic infiltrate	
Miscellaneous	Compensatory hypertrophy	Beckwith Weidemann syndrome
	Duplex kidney	Acute renal failure
	Fused crossed ectopia	Storage disorders
		Congenital nephrotic syndrome

Hepatosplenomegaly

Liver enlargement in association with renal disease can be seen in a number of conditions including glycogen storage disease type 1, tyrosinaemia and Fanconi-Bickel syndrome. Splenomegaly in a child with haematuria might suggest infective endocarditis. Autosomal recessive PKD may cause hypersplenism as a consequence of hepatic fibrosis. Hepatosplenomegaly in association with renal disease reflects a multi-system disorder which can be inflammatory such as systemic lupus erythematosus or a neoplastic process, for instance lymphoma presenting with acute renal impairment secondary to uric acid nephropathy.

Urinary Bladder

An enlarged urinary bladder can be detected by palpation and percussion and is characteristically associated with suprapubic pain. Gross constipation and local genital inflammation are the common causes of acute urinary retention in children and rarely require urethral catheterization. Lower urinary tract obstruction due to urological causes such as posterior urethral valves, pelvic tumors and the neuropathic bladder should be considered an emergency, immediate decompression of the urinary tract being indicated.

Genitalia

Clinical evaluation of the renal patient is incomplete without examination of the genitalia. The presence of vulvovaginitis, a common gynaecological problem in pre pubertal girls causing dysuria should be noted. In male children the state of the foreskin and the position of the urethral meatus should be recorded. Hypospadias is a common problem with an incidence of up to 1:130 live births; parents should be made aware that circumcision is contraindicated where this is present. Eagle Barratt syndrome is associated with bilateral cryptorchidism. The presence of male pseudohermaphroditism in association with proteinuria and renal impairment in an infant is suggestive of Denys Drash syndrome.

Anus

The presence of an imperforate anus in a neonate should alert the physician towards other associated anomalies such as sacral dysplasia, spinal dysraphism and vesicoureteric reflux.

Nervous System

Whilst a detailed neurological examination is often not required, it is important to record the level of consciousness,

mental status and examination of the cranial nerves, muscle tone, power and reflexes along with the gait and co-ordination. Hypertension can present with papilloedema and is the commonest cause of bilateral Bell's palsy. Seizures are associated with hyponatraemia, uremia, hypocalcaemia, hypertensive encephalopathy, vasculitis and haemolytic uremic syndrome. Blindness can result from rapid lowering of blood pressure in severe hypertension and is also known to occur in association with the use of immunosuppressive agents including methylprednisolone (31) and tacrolimus following renal transplantation (32). In a child with continence issues, careful examination of the peripheral sensory and motor functions together with the assessment of the anal tone is essential.

Skin

SLE can present with the characteristic fixed erythematous malar rash. The presence of a palpable purpura over the extensor surfaces and buttocks suggests Henoch Schönlein purpura. Multiple café au lait spots are seen in neurofibromatosis, which can give rise to hypertension secondary to renal artery stenosis. Tuberous sclerosis is characterized by typical dermatological lesions including facial adenofibromas (adenoma sebaceum), shagreen patches over the lower back and hypopigmented ash leaf macules which may be present anywhere in the body. Icthyosis is a recognized feature of the arthrogryposis, renal Fanconi syndrome and cholestasis (ARC) syndrome. Dystrophic nails should raise the possibility of nail patella syndrome.

Face

Examination of the face can provide valuable clues to the underlying clinical problem. Any obvious dysmorphic features such as Down's or William's syndrome should be noted. Long-term ciclosporin use is associated with hypertrichosis, particularly in the Asian population. Hirsutism is a well recognized feature of steroid toxicity and Cushing's syndrome. Nephrotic syndrome often presents with early morning periorbital swelling and is frequently misdiagnosed as allergic rhinitis. Examination of the oral cavity is important and may reveal gingival overgrowth as a consequence of ciclosporin therapy. Asking the child to smile may reveal a facial expression resembling crying in the child with Ochoa syndrome, which is associated with the presence of a neuropathic bladder.

Eyes

The eyes are affected in a number of renal diseases and therefore input from an experienced pediatric ophthalmologist is invaluable. Coloboma of the eyelid is seen in Goldenhar syndrome. Uncontrolled renal osteodystrophy can lead to scleral calcification. The presence of hypercalcaemia may cause redness of the eyes secondary to the metastatic crystallization of calcium, phosphate and hydroxyapatite in the conjunctiva (33). Slit lamp examination may reveal characteristic crystal deposits in the cornea in cystinosis. In a child with unexplained acute renal failure, examination of the eyes may clinch the diagnosis; for example in tubulointerstitial nephritis and uveitis (TINU) syndrome. Uveitis is also a feature of systemic diseases such as juvenile rheumatoid arthritis. Aniridia is associated with an increased risk of Wilm's tumor. Presence of anterior lenticonus, which is a conical protrusion in the anterior aspect of the lens (seen almost exclusively in males) along with macular changes, is a characteristic feature of Alport's syndrome. Cataracts are seen in Lowe's syndrome and galactosaemia, but can also be a consequence of steroid therapy.

Not infrequently, a child with severe hypertension is referred by an optician with papilloedema and retinal hemorrhages and exudates. Diabetic retinopathy, which parallels nephropathy is rare in childhood and correlates with the duration and control of disease. Nephronophthisis is associated with several characteristic eye lesions; retinitis pigmentosa in Senior Loken syndrome, tapetoretinal degeneration in juvenile nephronophthisis and oculomotor apraxia in children with Cogan syndrome. Retinitis pigmentosa is a feature of Bardet-Biedl syndrome.

Ears

Minor external ear malformations such as preauricular skin tags and pits are found in 0.5–1% of newborns and do not warrant renal evaluation unless accompanied by other systemic malformations (34). Branchial fistulae are a feature of branchio-oto-renal syndrome. Alport's syndrome is characterized by the insidious onset of high tone sensorineural deafness. Sensorineural deafness is also a feature of type 4 Bartter's syndrome which is the most severe phenotype presenting in the neonatal period with life-threatening volume depletion and chronic renal failure. Deafness can be a consequence of aminoglycoside toxicity and is also seen following rapid administration of a large dose of furosemide.

Musculoskeletal System

Muscles

Muscle wasting is a feature of chronic uremia. Myopathy can also be secondary to rickets and renal osteodystrophy which may improve following vitamin D therapy. One of the recognized side-effects of steroid therapy is proximal myopathy, demonstrated by eliciting Gower's maneuver. Up to half of children with mitochondrial disorders will have renal involvement with tubular dysfunction being the commonest pathology.

Skeletal System

Florid skeletal deformities of the weight bearing limbs, such as genu varum or valgum can be seen in infants with rickets. Rachitic rosary and swelling of the wrists and ankles due to epiphyseal swelling are the other well recognized features of rickets. Slipped femoral capital epiphysis is a feature of renal osteodystrophy, and a vascular necrosis of the head of the femur may complicate corticosteroid therapy, particularly following renal transplantation.

Whilst polydactyly is a recognized feature of syndromes such as Bardet Biedl and Meckel Gruber syndrome, isolated polydactyly is usually associated with a favorable outcome. Hemihypertrophy and Beckwith Wiedeman syndrome are associated with an increased risk of Wilm's tumor. Spinal dysraphism should be suspected in infants with a lower midline back lesion such as a subcutaneous mass, dermal vascular malformation, hypertrichosis, a midline dimple or sinus tract, a skin tag or an asymmetric gluteal cleft. Sacral agenesis is seen in infants of insulin dependent diabetic mothers but may also be part of the familial Currarino triad syndrome (presacral mass, sacral agenesis and anorectal malformation) (35).

Joints

Arthropathy is a characteristic feature of Henoch Schönlein purpura and SLE. It is also seen in systemic onset juvenile idiopathic arthritis, which if poorly controlled can lead to renal amyloidosis. Arthralgia and arthritis affect almost half of children with sarcoidosis. Metabolic disorders such as Lowe's syndrome and Lesch-Nyhan syndrome may present with arthropathy. Although hyperuricemia is common in pediatric transplant recipients, gouty arthritis is rare.

Examination of the Urine

Macroscopic Examination

The usual yellow color or urine is due to the presence of a number of pigments, some of which are derived from food (i.e., riboflavine) and others produced endogenously. The intensity of the coloration depends upon the urinary flow rate; where large quantities of urine are produced e.g., following high fluid intake, the urine is paler in color though darker in color at times of reduced urine production.

Where the urine is abnormally red or dark in color, dipstick testing and microscopy examination of the sample should be performed.

Dipstick Examination of Urine

Dipsticks

It is important that urine dipsticks are kept dry in their container with the lid on and that the manufacturer's expiry date is adhered to. A number of automated devices are available. These ensure that the stick is read at the correct time, thus reducing interobserver variability. Most will produce a printout which can be attached to the patient's medical record.

Specific Gravity

Most modern urine dipsticks measure urinary specific gravity, though this information is rarely utilized in clinical practice. Specific gravity is a measure of the mass of individual solutes present per unit volume of urine, as opposed to osmolality, which is the measure of the total particle concentrations, irrespective of the mass of the individual particles. Low values of specific gravity (less than 1.010) are found at times of maximal water excretion and high values (greater than 1.025) at time of maximal urinary concentration.

pH

The pH of healthy urine varies between 4.5 and 8.0. Fasting produces low values and the highest pH measurements are seen following meals. pH values are low where acidaemia is present, except for where this is secondary to renal tubular acidosis.

Blood

Urine dipsticks detect the presence of hemoglobin in the urine through its ability to catalyze a reaction between hydrogen peroxide and o-tolidine. Spotted positivity indicates intact red cells, whereas uniform positivity indicates free hemoglobin (e.g., in intravascular haemolysis or red cell lysis in the urinary tract).

There are a number of causes of false positivity, including the presence of myoglobinuria, oxidizing agents in the urine and heavy bacterial contamination. The presence of reducing agents, such as ascorbic acid in the urine may cause a false negative result. These highlight the importance of confirmation of the presence of red blood cells by microscopy of a fresh sample of urine.

Protein

Urine dipsticks undergo color change from yellow to green following binding with proteins. Albumin is better detected than other urinary proteins including globulins, tubular proteins etc. There are a number of causes of false negative and false positive results (*Table 20-4*). Dipstick analysis is not a good quantitative test because of the effect of urinary concentration; more concentrated urine will show higher protein content, and where proteinuria is detected, formal quantification with a urinary protein/creatinine or albumin/creatinine ratio is indicated.

It is well recognized that urinary protein excretion increases throughout the daytime with prolonged duration in the upright position, and first morning samples should be assessed to rule out any element of orthostatic proteinuria. Transient proteinuria may occur following exertion, fever or acute illness and is of no significance with regard to long term renal outcome.

Table 20-4
Causes of false positive and false negative proteinuria on dipstick testing

False positive proteinuria	False negative proteinuria
Concentrated urine	Dilute urine
Alkaline urine	Acidic urine
Gross haematuria, pyuria, bacteriuria	
Dipstick left in urine too long or delay in reading	
Contamination and drugs	
Antiseptics: chlorhexidine, benzalkonium	

Glucose

Glucose is a small molecule which is freely filtered in the glomerulus. In health, the proximal tubule reabsorbs all of the filtered glucose by an active process., which has an upper rate limit or transport maximum (T_M). Certain normal individuals have a lower T_M for glucose and will have glycosuria at normal or only slightly increased plasma glucose concentrations (so called renal glycosuria). Glycosuria also occurs where overt hyperglycaemia is present, e.g., in diabetes mellitus and in a number of tubular abnormalities, including Fanconi syndrome. The lower limit of detection for glucose in the urine is 0.5 mmol/l.

Leucocytes

A number of routinely available urine testing sticks will detect the presence of leucocyte esterase, indicating the presence of pyuria. Where testing is positive, urine microscopy should be used to confirm this finding.

Nitrites

A number of routinely available urine testing sticks will also detect the presence of nitrites. These are produced by the majority of pathogenic bacteria and their detection provides further screening data to assist in the diagnosis of urinary tract infection. The test has a high specificity but a low sensitivity for the diagnosis of UTI. As such, as a standalone test, it is of limited usefulness. Where UTI is suspected or needs to be excluded, a urine culture is necessary to determine the bacteriological cause and antibiotic sensitivities or to confidently rule out UTI.

Microscopy of Urine

There are many who advocate the routine bedside microscopy of urine in all children who present with suspected renal disease or urinary tract infection. This is not, however, widely performed for a variety of reasons including unfamiliarity with the technique and availability of appropriate microscopes in clinical areas.

Casts

Casts are produced by the aggregation of Tamm-Horsfall protein with cells or cellular debris in the renal tubule. They are best seen in unspun urine as centrifugation may damage casts. Where urine has centrifuged for other reasons, casts are most frequently seen at the edge of the coverslip.

Hyaline casts are present in proteinuric states, though may be found in concentrated specimens of urine from normal individuals. These may appear waxy if lipid droplets are present.

Cellular casts contain cellular material, the source of which may provide some clues regarding the underlying pathology. Red blood cell casts are always pathological and indicate glomerular bleeding. White blood cell casts indicate renal inflammation secondary to pyelonephritis or immunologically mediated disease. Epithelial cell casts (often present with red and white blood cell casts) are produced from shed tubular epithelial cells and may be seen during recovery from acute tubular necrosis.

Red Blood Cells

The excretion of a small number of red cells is a normal phenomenon, which increases with increasing age and following vigorous exercise. Children with febrile illness may develop a transient increase in red cell excretion. However, the persistent presence of greater than 5 red cells/mm^3 in uncentrifuged urine is abnormal. Urine microscopy can distinguish anatomically normal RBCs of lower urinary tract origin from dysmorphic RBCs of glomerular origin which have been distorted during their passage through the filtration barrier. This is best performed using phase contract microscopy, though possible with ordinary light microscopy. In its purest form the distinction is useful, though often a mixture of abnormal and normal cells is present and interpretation is difficult. Acanthocytes are red blood cells with thorn-like projections of varying lengths distributed over the surface due to an increase in membrane cholesterol:lethicin ratio. Where acanthocytes constitute greater than 5% of the urinary red cell population, this may indicate the presence of a glomerulonephritis.

White Blood Cells

The presence of greater than 10 white cells/mm^3 in a midstream sample of urine is considered abnormal. Neutrophils are detected in urinary tract infection, but are also seen in contaminated urine samples, proliferative glomerulonephritis and interstitial nephritis. The presence of osinophils in the urine is a sensitive and specific sign of acute interstitial nephritis.

Bacteria and Other Organisms

Urinary bacteria may be clearly visible without Gram staining. Their detection may be enhanced by the use of phase contrast microscopy. Fungi e.g., *Candida* and *Schistosoma* species (a rare cause of haematuria) may also be detected.

Epithelial Cells

The presence of epithelial cells in the urine may represent desquamation from the urinary tract. Tubular cells may be seen following tubular injury (acute tubular necrosis, acute transplant rejection). Squamous cells are commonly exfoliated from urethra and are a normal finding.

Crystals

Normal urine contains crystals of calcium phosphate and oxalate. Other crystals may be cystine, uric acid or dihydroxyadenine.

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21 Laboratory Assessment and Investigation of Renal Function

Aaron Friedman

Introduction

The kidney can be injured by a variety of different mechanisms. Investigating the type of injury and assessing the degree of injury and its progression involves laboratory assessment and often tissue sampling. This chapter will discuss laboratory assessment and investigation with emphasis on the use of blood and urine samples to investigate renal function.

There are a number of terms frequently used in laboratory assessment that are now more precisely defined and help us think about how we use laboratory assessment. In general, laboratory studies are used to help with diagnosis, prognosis, follow up of approach or effect of therapy. Usually the specific laboratory study performed fits the definition of a biomarker – a characteristic that is measured and evaluated objectively as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention (1). This chapter will discuss those measurements used primarily in patient care.

Glomerular Function and Injury

Glomerular filtration rate (GFR) is the most widely used indicator of kidney function. The measurement of GFR is based on clearance. Clearance is the removal ("clearance") of a given substance from a fixed volume of plasma in a fixed period of time. This is expressed by the formula: Clearance = $U_x \times V$ where U_x is the urine $\bar{P}_x$ concentration of the substance X, V is volume over time or flow rate and P_x is the plasma concentration of the substance X.

For a clearance measurement to be equal to the GFR, a number of principles must be met. The substance to be measured must be freely filterable through the glomerulus. The substance cannot be reabsorbed or secreted by tubules. Thus, the substance is neither added to urine nor removed from urine during its path through the tubule. The substance must be inert, cannot be toxic to the patient or the kidney, or alter function.

The materials used to measure clearance fall into two basic categories – exogenous and endogenous. Exogenous materials must be infused to provide a constant serum concentration. Endogenous substrate is produced by the body often at a relatively constant rate and in this way usually results in consistent plasma concentration.

Methods of Assessment

1. *Urinary clearance*: In this method plasma and urine are measured. The urine measurement is over a fixed period of time (a few hours to 24 h) with volume and concentration measured. A plasma measurement during or immediately coincident with the urine collections are routinely used. This provides the UV/P. The classic exogenous material used – the gold standard – is inulin. This is true because inulin meets all the criteria listed above for the ideal substance for glomerular filtration rate measurement. The classic endogenous substance is creatinine. When using creatinine the GFR is adjusted to a standard of 1.73 m^2 ($UV/P \times 1/1.73\ m^2$) because the amount of creatinine produced daily does depend on muscle mass. Infants have lower plasma creatinines in part because their endogenous production is relatively low compared with adults (males > females) even if the filtration rate of creatinine is similar (*Table 21-1*). Finally, clearances are by convention expressed as mL/min and in the case of creatinine, mL/min/1.73 m^2.
2. *Constant infusions without urine collection*: This method is based on the simple notion that when an infused substance (which meets the criteria above for a clearance substance) achieves a constant plasma concentration, the rate of infusion (IR) equals the rate of excretion – $C_x = IR_x/P_x$ where x is the substance, C the clearance, IR the infusion rate and P the plasma concentration (2). This methodology is not frequently used. It requires an infusion. GFR may be overestimated if the plasma concentration is measured too early and a constant plasma concentration is not

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Table 21-1

Plasma creatinine in children

Age (yr)	Plasma creatinine		Creatininuria	
	µmol/L	mg/dL	µmol/kg/d	mg/kg/d
<2	35–40	0.4–0.5	62–88	7.1–9.9
2–8	40–60	0.5–0.7	108–188	12.2–21.2
9–18	50–80	0.6–0.9	132–212	14.9–23.9

Adapted from García-Nieto V, Santos F. Pruebas funcionales renales. In: Garcia-Nieto V, Santos F, eds. *Nefrología pediátrica*. Madrid: Aula Médica, 2000; and García-Nieto V, Santos F, eds. *Grupo aula medica*. Madrid: Aula Médica, 2000;15–26

reached. The methodology also assumes that the substrate infused will reach a constant plasma concentration in a similar period of time. This means that the area of distribution is similar from individual to individual. This assumption is not true for newborns who have a higher than normal extracellular volume (3).

3. *Single injection–plasma disappearance curves* (4): The concept behind this form of measurement is: following an injection, a substance will leave a space (intravascular space) by urinary excretion and further distribution into another space such as the extracellular space. The rate of decline from the intravascular will be rapid early and will slow as distribution progresses. Once fully redistributed, the rate of decline from the intravascular space will reflect the excretion rate. If using a substance that is excreted by filtration alone, the GFR can be measured. This technique requires frequent blood sampling. Some models have been designed that are based on single compartment mathematics (usually waiting to sample after the typical distribution time); this reduces the number of blood samples needed but also reduces accuracy. Also, patients with edema and patients with cardiovascular insufficiency will not fit typical models.
4. *Plasma concentration*: Using an endogenous substrate, the concept of just measuring the plasma concentration is based on the principle that as the GFR goes down, the substrate concentration in plasma must go up. While true, this relationship is not linear. A number of formulas have been used. The most common used in children is $GRF = K \times ht/P_{creat}$ where K is a constant which takes into account age and muscle mass and is different for neonates, boys and girls (see below); ht is height in centimeters, and P_{creat} is plasma concentration of creatinine (3). The two most commonly used endogenous substances are creatinine and cystatin C.

Use of Endogenous and Exogenous Substrates in Clinical Medicine and in Studies for Measure of CFR

Endogenous Substrates

Because creatinine and cystatin C are endogenously produced they are most commonly used for standard clearance methods or plasma concentration methods.

Creatinine

Creatinine is produced from creatine, an intracellular component of skeletal muscle. The circulating concentration of creatinine is dependent on muscle mass and GFR. Creatinine, in fact, is not a perfect substrate for measuring clearance. It is primarily filtered at the glomerulus but is also secreted into urine by tubular epithelial cells (5). Evidence for reabsorption of creatinine has also been reported (6). Because of the combination effects of muscle mass and filtration rate the plasma concentration of creatinine in prematures, neonates, children and adults undergoes change with time. *Tables 21-1* and *21-2* demonstrate plasma concentrations for prematures and newborns *Table 21-2* and children across three age ranges (*Table 21-1*). As the GRF falls, plasma creatinine increasingly overestimates GRF (7,8). This is, in part, because the tubular secretion of creatinine contributes an increasing fraction of total creatinine excreted, leading to a clearance which is increasingly greater than actual GFR.

Creatinine was measured by a "classic" technique using a Jaffe reaction – the production of an orange-red color when creatinine was mixed with alkaline sodium picrate. Recently, modern autoanalyzers are using creatinase and relying on this enzymatic reaction to measure creatinine (3).

The use of creatinine urinary clearances continues. However, the potential errors introduced when obtaining

Table 21-2

Mean values of plasma creatinine during the first weeks of life of term and very low birth weight neonates

Birth weight (g)	Plasma creatinine (μmol/L)[a]			
	Postnatal period (d)			
	1–2	8–9	15–16	22–23
1001–1500	95 ± 5	64 ± 5	49 ± 4	35 ± 3
1501–2000	90 ± 5	58 ± 7	50 ± 8	30 ± 2
2001–2500	83 ± 5	47 ± 8	38 ± 8	30 ± 10
Term	66 ± 3	40 ± 4	30 ± 8	27 ± 7

[a]Mean values ± SEM

Table 21-3

Normal values of the glomerular filtration rate (gfr) as measured by creatinine clearance and renal plasma flow (rpf) as measured by *p*-aminohippuric acid and reference values of maximal urine osmolality

	Neonate	1–2 wk	6–12 mo	1–3 yr	Adult
GFR[a] mL/min × 1.73 m^2	26 ± 2	54 ± 8	77 ± 14	96 ± 22	118 ± 18
RBF[a] mL/min × 1.73 m^2	88 ± 4	154 ± 34	352 ± 73	537 ± 122	612 ± 92
Maximal urine osmolality[a] mOsm/kg H_2O	543 ± 50	619 ± 81	864 ± 148	750 ± 1330	825 ± 1285

[a]Mean values ± SEM

Adapted from García-Nieto V, Santos F. Pruebas funcionales renales. In: Garcia-Nieto V, Santos F, eds. *Nefrología pediátrica*. Madrid: Aula Médica, 2000; and García-Nieto V, Santos F, eds. *Grupo aula medica*. Madrid: Aula Médica, 2000;15–26

a creatinine clearance from plasma plus urine should be understood. First, certain foods can change the plasma creatinine. A cooked meat meal especially in large volumes can influence plasma creatinine by introducing a "nonendongenous" source of creatinine (9). Certain medications and substances can interfere with the tubular secretion of creatinine (cimetidine, trimethoprium, urate) (10). Urine collections are timed. Inaccurate collection times can affect the results. A 24 h collection (1440 min) will report too low a GFR if the collection is actually under 24 h or too high a GFR if the collection is actually >24 h when the time used for calculation is precisely 24 h. Normal values are found in *Table 21-3*.

In aggregate, the use of endogenous substrates are preferred for GFR estimations in clinical situations. The commonly used estimations of GFR are: in children the Schwartz formula (11,12)

$$\text{eGFR} = \frac{\text{KL}}{\text{Scr}}$$

eGFR – estimated GFR in mL/min/1.73 m^2
K – empirically derived constant
L – length in centimeters
Scr – serum creatinine in mg/dL

K is 0.45 in infants, 0.55 in children and adolescent girls and.0.7 in adolescent males.

in adults the Cockraft–Gault equation (13)

$$\text{eGFR} = \frac{[140 - \text{age(y)}][\text{bodyweight(kg)}]}{(72 \times \text{Scr})}$$

eGFR – estimated GFR
140 and 72 are empiric numbers allowing a close correlation between plasma (serum) creatinine and GFR

In adult females the eGFR is corrected by 0.85 because of smaller muscle mass.

More recently, cystatin C has been used as an endogenous marker of renal function and to estimate GFR. Cystatin C is a nonglycosylated protein produced in all cells with relative constancy. Cystatin C is not influenced by age, gender, body habitus or composition (14). Renal handling of cystatin C is filtration with nearly complete catabolism and reabsorption of catabolized products by the proximal tubule. Little or no cystatin C appears in the urine (15,16). Cystatin C circulating level is approximately 1 mg/L in healthy humans.

Is cystatin C a better indicator of GFR than serum creatinine? Because cystatin does not appear in urine it

cannot be used as direct measure of GFR like creatinine or inulin. Studies suggest that serum cystatin C is better correlated with *measured* GFR and that smaller changes in GFR are more readily measured by serum cystatin C than by serum creatinine (14,17). Formulas that have been tested for the use of cystatin C to estimate GFR in children have been reported by Filler (18) and Zappitelli (19).

$$\log_{10}(\mathrm{GF}) = 1.962 + \left[1.123 \times \log_{10}\left(\frac{1}{\mathrm{cys}^{\mathrm{C}}}\right)\right]$$

Estimated GFR using cystatin C($\mathrm{cys}^{\mathrm{C}}$) according to Filler.

There continues to be debate as to whether cystatin C as a measure of estimated GFR is clearly better than serum creatinine (3). *Figure 21-1* shows creatinine and cystatin C levels from prematures to adults.

Exogenous Markers of Clearance

Inulin

This is the classic marker for measuring GFR, since it meets all the criteria for accurate measure of GFR (3). Inulin is a fructose polysaccharide, which is inert. Inulin urinary clearance, constant infusion techniques and even plasma disappearance have provided considerable evidence as to normal GFR for neonates, children and adults (*Figs. 21-2* and *21-3*) (20). Inulin is the gold standard, but because it requires infusion and is most accurate when used as an infusion plus urine collection technique, inulin is rarely used clinically.

Markers using radiolabelled isotopes (radionuclides): In clinical medicine radionuclides are often used to estimate total GFR or to measure difference in clearance between one kidney and the other in the same patient. The fundamentals of this approach rely primarily on single injection, plasma disappearance curves to "measure" GFR. The three commonly used radionuclides for GFR measurements are diethylene triamine penta-acetic acid (DTPA), ethylene diamine tetra-acetic acid (EDTA) and iothalamate. All three require an injection and then measuring the signal from the radiolabelled form to obtain measurement (technetium 99 for DTPA, chromium 51 for EDTA and iodine 125 for iothalamate). Briefly stated, DTPA correlation to 24 h inulin clearance is very good (21), EDTA correlation to inulin clearance is good (21,22), and iothalamate correlation to inulin clearance is only fair (23). In general, DTPA and EDTA do not perform well in neonates. EDTA appears to have an extrarenal source of elimination. The Tc radiolabel may dissociate from DTPA during clearance studies and iothalamate appears to undergo both tubular secretion and reabsorption (20).

Finally, iohexol has been touted as an alternative to inulin. Iohexol is a nonionic, non-radioactive, X-ray contrast medium of low molecular weight which is eliminated from plasma solely by glomerular filtration (24). It is easily measured by HPLC using very small volume increasing the usefulness in children (25). Iohexol GFR measurements correlate well with inulin measurements (26). Iohexol requires an infusion, and the plasma disappearance adheres to a two compartment model (fast peak in 5 min corresponding to peak plasma concentration, slow curve at approximately 90 min corresponding to renal excretion) (27). Iohexol may prove useful as a study tool, but probably not useful as a regular clinical measure of GFR.

Proteinuria

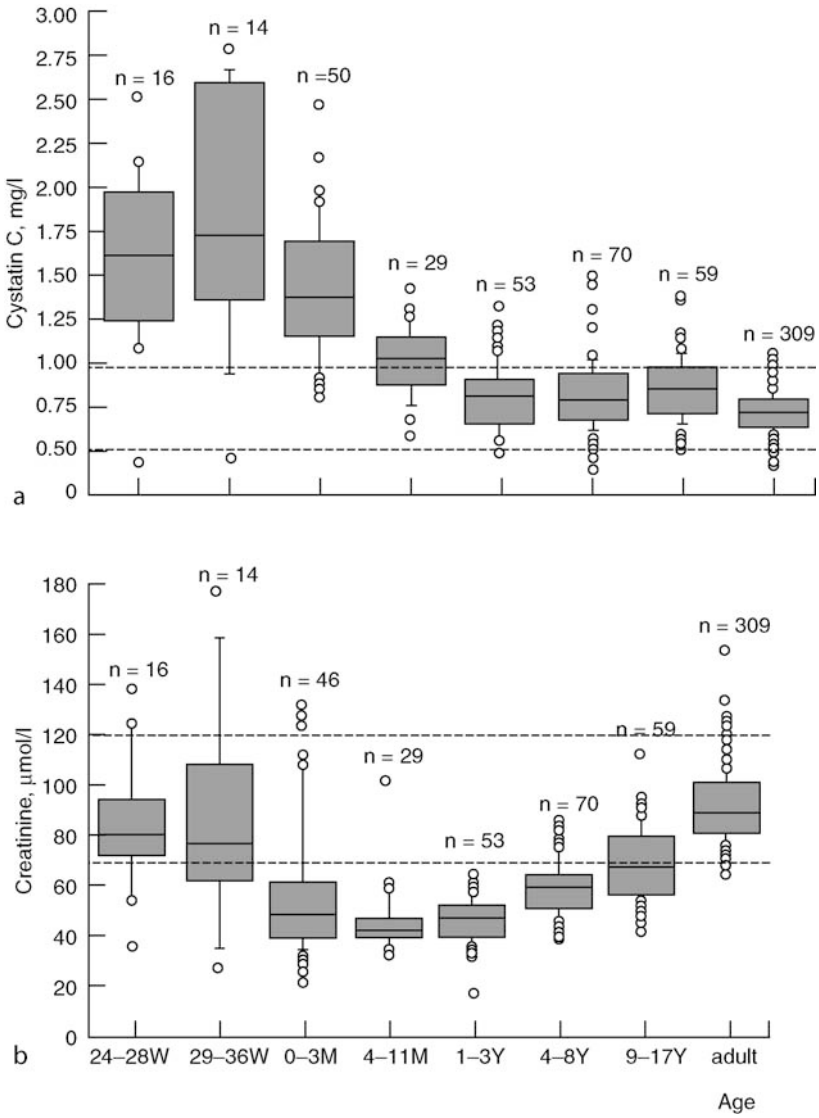
The glomerulus prevents the passage of proteins from plasma to the Bowman's space. This restriction is mediated by charge- repelling primarily negatively charged proteins such as albumin and by size. This combination explains why so little protein appears in Bowman's space as part of ultrafiltrate (Bowman's space albumin concentration is 210 mg/L). Any protein filtered under normal conditions is reabsorbed by the proximal tubule through endocytosis at the luminal membrane. Situations of glomerular injury, where the glomerular basement membrane charge and/or "pore" size is changed, will result in large amounts of protein (including albumin) being filtered. This will readily overwhelm proximal tubule endocytosis and proteinuria will ensue.

Under normal conditions some protein will appear in the final urine. As noted above essentially no albumin will appear in urine, but some *tubular* proteins are routinely measured in normal urine. Most commonly Tamm–Horsfall protein, a glycoprotein secreted by the ascending limb of the loop of Henle can be measured. Generally, the finding of Tamm–Horsfall protein in urine is of no clinical significance.

Proteinuria as a measure of renal injury is used ubiquitously by nephrologists. Protein that appears in urine as a result of the loss of glomerular barrier (termed altered permselectivity) usually results from physical damage to the glomerulus, loss of anionic charge along the glomerular membrane, and hemodynamically mediated protein loss. Loss of anionic charge alone results in increased albumin excretion (selective proteinuria), but as injury worsens and especially if physical injury to the glomerular barrier

Figure 21-1

Box plot distributions showing (a) cystatin C and (b) creatinine values (tenth, twenty-fifth, fiftieth, and ninetieth percentiles) across the age groups. The categories of 24 to 36 and 29 to 36 weeks refer to gestational ages of preterm babies. Dotted lines indicate 95% confidence interval of adult range. Preterm babies born between 24 to 36 weeks' gestation were 1 day old. (Adapted from Finney H, Newman DJ, Thakkar H, et al. Reference ranges for plasma cystatin C and creatinine measurements in premature infants, neonates and older children. *Arch Dis Child* 2000;82:71–75).



results, protein loss becomes non-selective. This degree of selectivity is determined by measuring albumin and another protein usually of higher molecular weight such as immunoglobulin G (IgG) or transferrin. Highly selective proteinuria will have an IgG:albuimin ratio of <0.1.

Proteinuria is used routinely in diagnosis, prognosis, and response to therapy. Often the first clue to renal injury is the finding of proteinuria on a urinalysis. Prognostically, persistent proteinuria or failure of protein excretion to respond to treatment is viewed as a bad prognostic sign. When protein excretion does diminish, apparently in response to therapy, this is viewed not only as a good sign, prognostically, but as a predictor of prolongation of renal function and prolongation of time to severe chronic kidney disease (CKD) or end stage renal disease (ESRD). In this way, the response of

Figure 21-2

Inulin clearance as a function of age X±SD.

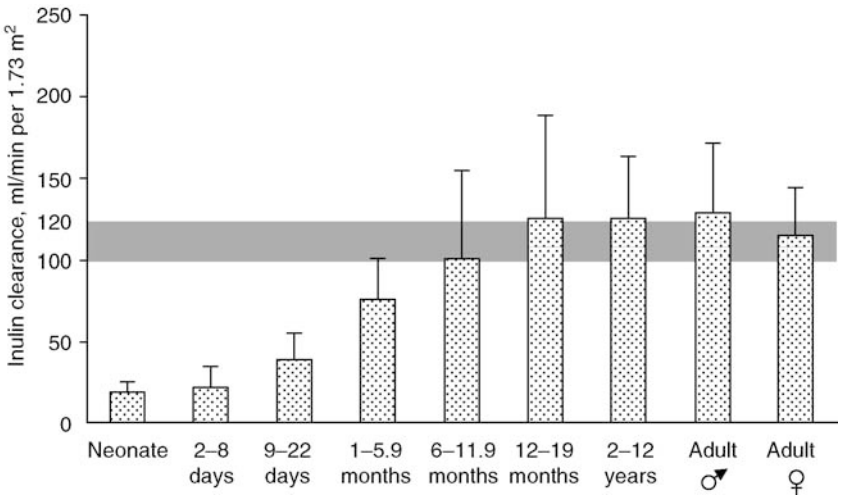
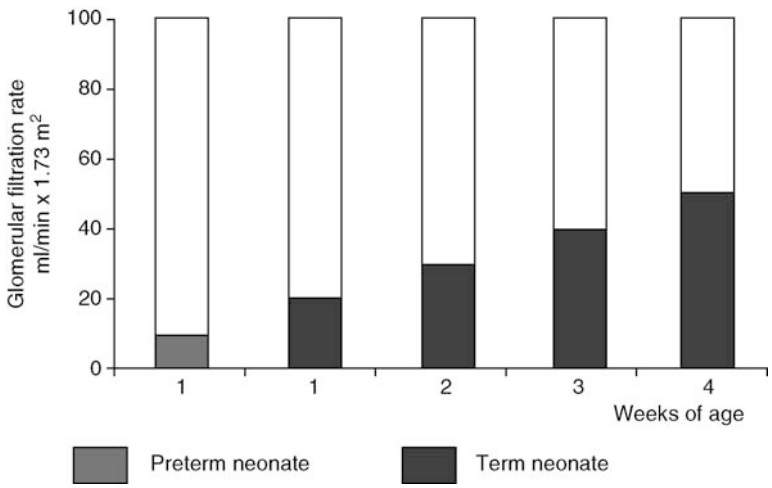


Figure 21-3

Postnatal increase in glomerular filtration rate in term and preterm infants. Open part in columns represents mature levels of glomerular filtration rates. (Adapted from Guignard JP, Torrado A, Da Cunha O, et al. Glomerular filtration rate in the first three weeks of life. *J Pediatr* 1975;87:268–272.)



protein excretion to treatment is used as a surrogate to predict later changes in glomerular filtration rate. The evidence for proteinuria as a surrogate to predict later change or time to change in renal function is somewhat limited (1).

Another commonly used marker for diagnosis of renal injury is microalbuminuria (preferable terminology – albuminuria). Very small amounts of albumin are excreted in normal individuals when this amount is between 30 mg/g creatinine and 300 mg/g creatinine (or 0.7 mg/m^2/24 h to 7 mg/m^2/24 h). The finding suggests subtle glomerular injury. Albuminuria has been used extensively to diagnose and follow early diabetic nephropathy (28).

Some low molecular weight (LMW) proteins are filtered, but in normal situations these proteins are metabolized and reabsorbed by the proximal tubule. With

impaired proximal tubule function (e.g., Fanconi syndrome, drug injury) these proteins will appear in urine. Commonly measured "tubular" proteins include β_2-microglobulin (more stable but more variable because of higher MW), ∂_1-microglobulin (not protein bound, but unstable in acid urine), retinol-binding protein (strongly bound to plasma proteins).

Other tubular proteins – of tubular origin – have been suggested as markers of tubular injury and especially of tubular necrosis or transplant rejection. Markers of tubular necrosis have included *N*-acetyl glucosaminidase and alanine-aminopeptidase. Both are brush border enzymes released with injury (29). Another promising marker is NGAL (neutrophil gelatinase-associated lipocalin), a tubular protein whose urinary levels increase shortly after tubular injury. Suggestive data from patients' status post cardiac surgery, critically ill patients, contrast induced nephropathy and conditions such as lupus, nephritis and hemolytic uremic syndrome are promising (30). Confirmatory studies are still needed.

Protein Measurement

Dipsticks use a colorimetric measurement of urine protein. It is *not* quantitative, but qualitative, and measures protein: absence or presence in small or large quantities.

A somewhat more quantitative measure is the protein/creatinine ratio measured in a random urine sample. The correlation to 24 h urine protein is reasonably good (31). The sample most frequently used is a first or second morning void sample. First morning void samples should be used when considering orthostatic proteinuria (see below), otherwise, use second morning void samples. *Table 21-4* describes the upper limit of normal for protein/creatinine in the pediatric age range. Nephrotic range protein is >400 mg/mol creatinine or >2 g/g creatinine (in adults a standard definition is >2 g of protein excreted per 24 h).

A 24 h urine collection is the gold standard for defining proteinuria. Such collections are cumbersome, especially in young children, and subject to error (undercollection of fewer than 24 h or overcollection of >24 h).

Table 21-4

Normal values of protein excretion as a function of age

Age (yr)	Proteinuria	
	g/mol creatinine	g/g creatinine
0.1–0.5	80	0.70
0.5–1.0	60	0.55
1–2	45	0.40
2–3	30	0.30
3–5	20	0.20
5–7	19	0.15
7–17	18	0.15

Hematuria

Red blood cells in urine (hematuria) is caused by renal trauma, glomerular injury leading to red cells passing through the glomerular barrier, interstitial or tubular injury, stone disease, cystic disease, malignancy or injury to the urinary tract. Red cells can appear in urine even though they derive from outside the urinary tract. Two examples include menses or excoriation of the epidermis at the urethral meatus. Normal urine has few, if any, intact red cells, generally <2 red blood cells (RBC)/HPF (high powered field) on a urine specimen, which has been centrifuged. This small number will not result in a positive result on urinalysis. At the other extreme, urine will appear red or burgundy with red cells in the urine or with hemoglobin in urine. A urine hematocrit of ≤1 will still turn urine red. Because hematuria is a nonspecific finding, the finding usually indicates some injury but usually does not provide more information. There are two exceptions. First, dysmorphic red cells seen using phase contrast microscopy on microscopic examination of the urine may help determine the site of injury along the urinary tract. If 75% or greater of red cells seen are dysmorphic, the kidney and most likely the glomerulus is the site of injury. If <25% of RBC are dysmorphic (32), the injury site is the urinary tract – renal pelvis or below. Second, if a urinalysis is positive for RBC's but no RBC's are noted, then hemoglobinuria (or myoglobinuria) should be suspected and direct measurement of hemoglobin or myoglobin should be undertaken.

Urinalysis

Likely the most commonly used laboratory test for examining renal function or injury to the kidney or urinary, the urinalysis is easy to perform and is used as a screening test, a diagnostic test and, at times, a follow up examination to test the efficacy of therapy. Its utility is at times overestimated and the risk of either false positives or false negatives should be understood (33).

Urinalysis usually characterized three aspects of urine: (a) physical character: color, clarity, specific gravity and/or osmolality; (b) chemical character: pH, presence of glucose, protein, blood, ketones, bile pigments; (c) microscopic character: red cells (RBC), white cells (WBC), organism casts and crystals. Urine should be clear. Cloudy urine may represent crystals (e.g., calcium phosphate in alkaline urine), cells – WBC, RBC or both. Proteinuria, hematuria and glucose in the urine are often diagnosed first by using urinalysis as the screening test.

The most commonly used method for some physical, chemical and even cellular elements analysis of urine is a dipstick test which employs a colorimetric change to identify abnormalities. These impregnated sticks should be used appropriately. Certain rules pertain:

- Urine tested should be around body or even room temperature. Refrigeration will affect the test results.
- The observations of change should be made after 15 s and up to 1 min after dipping the reagent stick in urine. Too rapid or too late an observation of color change can give a false reading.
- Moisture, sunlight, cold, heat or time (strips have expiration dates) will result in false readings.
- Certain conditions or substances will cause false reading. For example, urinary ascorbic acid will result in false positive proteins and false negative glucose readings. Very alkaline urine will yield a false positive protein reading.

Leukocyte esterase and nitrite measurements are commonly found on urinalysis dipsticks. When WBC, especially neutrophils, are present leukocyte esterase is positive. Nitrite is found in urine as a result of metabolic release by bacteria. Unfortunately, neither is sensitive or specific enough to be able to definitively diagnose urinary tract infections even when used together (34). Routine urinalysis as a screening in the normal population remains controversial (35).

Renal Blood Flow (RBF) (*Table 21-4*)

The measurement of renal blood flow has been of interest to renal physiologists and nephrologists for decades. Normally, blood flow to the kidneys is approximately 1200 mL/min/1.73 m^2. The filtration fraction, that portion of renal blood flow that is filtered through glomeruli, normally is approximately 20% of RBF. The classic measurement method for RBF is the clearance of para-aminohippurate (PAH). To measure RBF, the substance used must be fully extracted from blood after a single pass through the kidney. PAH comes close, as it is both filtered by the glomerulus and secreted by the proximal tubule. It appears that approximately 90% of PAH is extracted in a single pass through the kidney. The classic measurement requires constant infusion and blood and urine measurements to examine clearance. This technique is now used almost exclusively (and not frequently) in the laboratory (20).

Other techniques are used more frequently to measure renal blood flow. These include iodine labeled hippuron (orthoiodo-hippurate), which is handled in a manner similar to PAH, but extraction is less than PAH (36). Another technique is the use of technetium labeled mercaptoacetyl-triglycerine (MAG-3). This technique is not very accurate for the measurement of renal blood flow, but has been used to evaluate urinary tract infection (see chapter on urinary tract infection). Other methods used clinically include contrast enhanced ultrasound (37), color Doppler ultrasound (38), magnetic resonance imaging (39). These may be used to measure renal blood or to compare flow between kidneys; however, renal blood flow measurements are not that accurate, but do provide estimates of blood flow (40).

Tubular Function

The composition of final urine is dependent on the composition of filtrate and then the contribution made by tubular function (reabsorption and/or secretion). The concentration of solute in glomerular filtrate is dependent on diet, water intake, intestinal absorption, non renal losses and age. Thus, normal values for the excretion of solutes are really no more than a typical range for excretion assuming a normal GFR (*Table 21-5*).

Sodium

Under normal conditions, sodium excretion approaches sodium intake. During growth children are in positive balance with respect to most solutes. Day to day, this positive balance amount for sodium or other solutes is small. On a Western diet, sodium excretion in children is approximately 3–4 mmol/kg/24 h (*Table 21-5*). However, body fluid composition, especially extracellular fluid volume, is regulated in part by the regulation of sodium excretion (hormonal influence by aldosterone and atrial natriuretic peptide). The plasma sodium concentration typically does not predict the urinary sodium concentration.

Table 21-5

Urinary solute/creatinine (creat) ratios (fifth and ninety-fifth percentiles) as a function of age

Solute/creat	Age (yr)															
	1/12–1		1–2		2–3		3–5		5–7		7–10		10–14		14–17	
mol/mol	P5	P95	P5	P95	P5	P95	P5	P95	P5	P95	P5	P95	P5	P95	P5	P95
Sodium/creat	2.5	54	4.8	58	5.9	56	6.6	57	7.5	51	7.5	42	6	34	–	28
Potassium/creat	11	74	9	68	8	63	6.8	48	5.4	33	4.5	22	3.4	15	–	13
Calcium/creat	0.09	2.2	0.07	1.5	0.06	1.4	0.05	1.1	0.04	0.8	0.04	0.7	0.04	0.7	0.04	0.7
Magnesium/creat	0.4	2.2	0.4	1.7	0.3	1.6	0.3	1.3	0.3	1	0.3	0.9	0.2	0.7	0.2	0.6
Phosphate/creat	1.2	19	1.2	14	1.2	12	1.2	18	1.2	5	1.2	3.6	0.8	3.2	0.8	2.7
Oxalate/creat	0.06	0.17	0.05	0.13	0.04	0.1	0.03	0.08	0.03	0.07	0.02	0.06	0.02	0.06	0.02	0.06
Urate/creat	0.7	1.5	0.5	1.4	0.47	1.3	0.4	1.1	0.3	0.8	0.26	0.56	0.2	0.44	0.2	0.4

Adapted from Matos V, Melle G van, Boulat O, et al. Urinary phosphate/creatinine, calcium/creatinine, and magnesium/creatinine ratios in a healthy pediatric population. *J Pediatr* 1997;131:252–257; and Matos V, Melle G van, Werner D, et al. Urinary oxalate and urate to creatinine ratios in a healthy pediatric population. *Am J Kidney Dis* 1999;34:1–6

Because sodium reabsorption consumes a substantial amount of energy utilized by the kidney and because sodium reabsorption especially during extracellular volume contraction is essential to restoring normal body physiology, the fractional excretion of (FE) of sodium is often used during episodes of oliguria to differentiate volume contraction from acute renal injury. Fractional excretion is the fraction of solute filtered, which appears in urine.

$$FE = \frac{Us}{Ps} \times \frac{Pcr}{Ucr} \times 100$$

Us – urine solute concentration
Ps – plasma solute concentration
Pcr – plasma creatinine concentration
Ucr – urine creatinine concentration

By concentration FE is reported in percentage, thus multiplication by 100.

If one substitutes sodium in the equation above the FE of sodium can be determined. In other than neonates, during episodes of low urine output a normally functioning kidney will maximally reabsorb sodium and the FE_{NA} will be <1%.

Potassium

On a normal diet potassium excretion in children averages 1–2 mmol/kg/24 h (*Table 21-5*). Potassium excretion in urine under normal circumstances depends on the secretion rate of potassium by the distal tubule. This secretion is regulated by distal tubule sodium delivery and aldosterone. To estimate the regulation by the distal tubule of potassium excretion, the preferred method is the transtubular potassium (concentration) gradient (TTKG). This measure evaluates the gradient between luminal peritubular potassium concentrations and the interstitial potassium concentration in the late distal tubule or collecting duct. The following assumptions make up the foundation of the use of TTKG. The potassium concentration of the distal collecting duct is the same as the systemic potassium concentration. The luminal potassium concentration at the distal tubule is deduced by adjusting the urinary potassium concentration to medullary water reabsorption by the distal tubule and collecting duct. This is done by dividing the final urine potassium concentration by the urine to plasma osmolality ratio. All these also require that there is sufficient sodium in the distal tubule for exchange of sodium and potassium and the urine/plasma osmolality is >1 (41). The calculation is:

$$TTKG = \frac{U_k \times P_{osm}}{Pk \times U_{osm}}$$

TTKG – transtubular potassium gradient
U_k – urine potassium concentration
P_{osm} – plasma osmolality
Pk – plasma potassium concentration
U_{osm} – urine osmolality

In adults a TTKG >5 means aldosterone is active; <3 suggests limited or no mineralocorticoid activity. In children with hyperkalemia a TTKG <4.1 (or 4.9 in infants) suggests decreased mineralocorticoid activity. With hypokalemia a TTKG >2 is indicative of ongoing aldosterone secretion.

Chloride

Urinary chloride excretion is tied to urinary sodium excretion. Urinary chloride depends on diet and extracellular volume status. With extracellular volume contraction, enhanced sodium reabsorption results in enhanced chloride reabsorption. During metabolic alkalosis the urinary chloride concentration can be useful diagnostically. A urinary chloride concentration >10 mmol/L is consistent with diuretic induced renal loss, whereas urine chloride concentration under 10 mmol/L is consistent with gastric loss and volume contraction.

Calcium

Hypercalciuria is defined as calcium excretions >4 mg/kg/24 h or >0.1–0.125 mmol/kg/24 h. A commonly used technique to screen for hypercalciuria is the calcium/creatinine ratio with a value of >0.2 mg/mg (>0.6 mmol/mmol) (42). The value for neonates and infants is higher (*Table 21-5*).

Phosphate

Urinary phosphate excretion is primarily dependent on dietary phosphate intake. Normal urinary phosphate excretion is 10–15 mg/kg/24 h (0.3–0.5 mmol/kg/24 h) in adults and 20–25 mg/kg/24 h (0.6–0.8 mmol/kg/24 h) in children (*Table 21-5*).

Phosphate is handled by reabsorption at the level of proximal tubule. For solutes handled by reabsorption 100-FE represents the tubular reabsorption (percentage of filtered load reabsorbed). For phosphate the TRP (tubular reabsorption of phosphate) represents the percent of phosphate reabsorbed by the proximal tubule. Generally the TRP on a Western diet (high in protein and phosphate) in a child with normal renal function will actually be the maximum that can be absorbed. The TMP (tubular maximum phosphate) reabsorption is reflected in this equation.

$$TMP = GFR \times Sp - Up \times V$$

Sp – serum phosphate concentration
Up – urine phosphate concentration
V – volume

This equation represents the best indicator of renal tubular handling of phosphate (43). With the above assumptions and rearrangement of the equation the commonly used TMP/GFR measurement can be obtained and reflects the serum phosphate concentration at the tubular threshold – the serum phosphate concentration at which phosphate concentration is at its maximum and phosphate begins to appear in urine:

$$\frac{TMP}{GFR} = TRP \times Sp$$

$$\frac{TMP}{GFR} = \left[1 - \left(Up \times \frac{Scr}{Sp \times Ucr}\right)\right] Sp$$

Scr – serum creatinine concentration
Ucr – urine creatinine concentration
TMP/GRF = Sp−(Up × Scr/Ucr) – this is most commonly used

In children >age 2 years, the normal TMP/GFR is 4–6 mg/dL. This falls to 2.8–4.2 mg/dL by age 20 (44).

Glucose

Glucose is filtered and reabsorbed by the proximal tubule. Like phosphate if the filtered load exceeds the maximal capacity of the proximal tubule to reabsorb glucose, the threshold for glucose excretion is reached. The normal plasma threshold for plasma glucose is approximately 180 mg/dL (9.9 mmol/L). Glucose filtration testing where glucose in increasing concentrations is infused (changed every 30 min) until the plasma glucose concentration reaches 350–400 mg/DL. Urine samples are collected at the last 15 min of each 30 min infusion period. This test was used to determine the glucose threshold especially in situations of renal glycosuria. Under normal conditions urinalysis test for glucose will be negative.

Magnesium

Magnesium is filtered and reabsorbed primarily at the proximal tubule and the ascending limb of the loop of Henle. In determining if renal handling of magnesium is normal the FE of magnesium FE_{Mg} is the typical measurement performed. Normally, approximately 5% of filtered magnesium is excreted. With hypomagnesemia the FE_{Mg} can fall to 1%. Under hypomagnesemic conditions the FE_{Mg} of ≥5% suggests renal wasting of magnesium.

Uric Acid, Oxalate, Citrate

Uric acid handling by the kidney is complicated. Uric acid is filtered, reabsorbed and secreted. In children (>age 2 year) and adolescents uric acid excretion is *not* age dependent and is approximately 520 ± 145 mg/1.73 m^2/24 h (3093 ± 875 μmol/1.73 m^2/24 h) (45). A screening test for hyperuricosuria was devised correcting urine uric acid concentration to GFR.

$$\text{Urine uric acid concentration} \times \frac{\text{Plasma creatinine concentration}}{\text{Urine creatinine concentration}}$$

A value >53 mg/dL or 32 μmol/L on first morning void urine in children >2 year of age is considered abnormal (46).

Oxalate forms highly insoluble crystals in urine. Oxalate excretion is in the range of 20–50 mg/1.73 m^2/24 h (0.3–0.55 mmol/1.73 m^2/24 h). In infants the value may be slightly higher (47). Above this range is suspicious for hyperoxaluria.

Citrate excretion in urine is important in reducing the risk of stone formation especially calcium containing stones. Hypocitraturia contributes to stone formation. Citrate excretion below 300 mg/g creatinine (0.176 mol/mol) in girls and 125 mg/g/ creatinine (0.074 mol/mol) in boys suggests hypocitraturia (48).

Acid Base

Anion Gap

The anion gap is a readily available measure to determine if a metabolic acidosis (acidemia) is associated with an endogenous or exogenous unmeasured serum anion leading to an *elevated* anion gap versus the loss of bicarbonate and concomitant increase in chloride leading to a normal serum anion gap. The anion gap represents the fact that routinely measured serum cations, specifically sodium and potassium, outnumber routinely measured anion chloride and bicarbonate. Because electroneutrality must be maintained there must be unmeasured anions that are routinely present in serum. In fact, there are both unmeasured cations and anions. The unmeasured cations add little to the electrical charge – Ca, Mg. Unmeasured anions include: protein such as albumin, phosphate, sulfate and others (e.g., urate). The two most commonly used ways to measure *anion gap* in serum are:

$$\text{Anion Gap (AG)} =$$
$$\text{Na} - (\text{Cl} + \text{HCO}_3) = 10 \pm 2$$
$$(\text{Na} + \text{K}) - (\text{Cl} + \text{HCO}_3) = 12 \pm 4$$

High serum anion gaps reflect endogenously produced anions such as lactate or keto acids, or exogenous anions such as salicylate. A low anion gap can be seen with hypoalbuminuria, or rarely in children, with abnormal cationic proteins.

A related concept is the urinary anion gap. The most easily measured ions in urine are Na, K and Cl. The urinary gap is calculated as:

$$\text{Urinary anion gap} = (\text{Na} + \text{K}) - \text{Cl}$$

Under metabolic acidosis conditions in patients with normal renal function, two important ions are not routinely measured in urine, NH_4 and HCO_3. Ammonium is positively charged, HCO_3 is negatively charged. When urine is acidic especially under a pH of 6 there is little or no HCO_3 in urine. However, with metabolic acidosis NH_4 production is stimulated. NH_4 will be excreted with Cl and therefore, the urinary anion gap will measure more Cl than Na + K. The urinary anion gap will be negative. Therefore, the urinary anion gap is a rough estimate of ammonium excretion. In acidemic patients with impaired NH_4 production (distal renal tubular acidosis, Type IV renal tubular acidosis or chronic kidney disease with impaired NH_4 production) the urinary anion gap remains positive. In patients receiving bicarbonate it is more difficult to use the urinary anion gap especially if the urine pH is high as bicarbonate may make up an important unmeasured anion (49,50). Urinary anion gap has been used in the neonate, but is more difficult to interpret especially in the premature (51).

Another method used to estimate ammonium excretion is the modified urine osmolar gap. Like the urinary anion gap this method recognizes that urinary osmolality is dependent on the concentration of ions in the urine. In particular, this method is useful when the urine pH is high. The equation is (52):

$$\text{Estimated } NH_4 \text{ excretion (mmol/L)} =$$
$$1/2\left[U_{osm} - 2(U_{Na} + U_K) + \frac{U_{urea}}{2.8} + \frac{U_{gluc}}{18}\right]$$

U_{osm} – urine milliosmoles
U_{Na} – urine sodium concentration (mmol/dL)
U_K – urine potassium concentration (mmol/dL)
U_{gluc} – urine glucose concentration (mg/dL)
U_{urea} – urine urea concentration (mg/dL)

Urinary Acidification Test

The assessment of the ability of the kidney to handle an acid load- urinary acidification testing is an exam that may be needed to test for renal tubular acidosis (RTA) and to distinguish type I (distal) RTA from type 2 (proximal) RTA (53–56). Distal RTA fundamentality is an inability to secrete sufficient H+ into the distal tubule lumen to acidify urine. At least three types of studies have been employed: (a) give acid to lower serum pH; (b)furosemide test to deliver large amounts of sodium and chloride to distal tubule and thereby, encourage H+ ion secretion; (c)acetazolamide (or bicarbonate), which delivers large amounts of bicarbonate to the distal tubule. Normal H+ ion secretion will result in HCO_3 and H combining to form water and carbon dioxide. Thus, the pCO_2 of final urine will be considerably higher than blood pCO_2. If a first morning urine pH is <5.5, no additional testing is needed. This is a normal distal acidification.

(a) *Acid test*: Either during spontaneous metabolic acidosis or with the delivery of ammonium chloride (75–100 mmol/m^2 intravenously over 4–6 h, orally with enteric coated capsules over 1 h or orally at 2 mmol/kg/24 h over 3–5 days) such that the blood pH drops to 7.33 or less and the serum bicarbonate falls to below 18 mmol/L. At this point the urine pH should be below 5.5. A concomitant urine measurement of sodium, potassium, chloride and osmolality will assure that insufficient sodium at the distal tubule is not a cause for reduced acidification or that very high ammonia production (urinary anion gap or osmolar gap measurement) has resulted in a urine pH which does not fall below 5.5. Finally, very dilute urine (urine osmolality) will mask good distal H+ secretion. Patients with type II RTA will respond normally once the serum bicarbonate falls below threshold (typically, serum bicarbonate <14 mmol/L).

(b) *Furosemide Test*: 3–4 h following a furosemide dose of 1 mg/kg orally or intravenously, urine pH should fall below 5.5. Patients with type I RTA will fail to reduce urine pH sufficiently; however, patients with type II RTA will respond normally. This test is not predictive of type IV RTA. Measurement of urinary electrolytes (as noted above) will also demonstrate high potassium excretion expected as a result of furosemide.

(c) *Acetazolamide or bicarbonate test*: With this test oral acetazolamide (0.5–1 g/1.73 m^2 or 15–20 mg/kg) or oral bicarbonate (2.5 mmol/kg) should result in bicarbonaturia. A normal response is a urine pCO_2 of >70 mmHg or difference between urine pCO_2 and blood pCO_2 of >20 mmHg (urine – blood pCO_2). This exam requires that urine be collected and stored under oil and in glass to prevent the loss of CO_2 into the air. Patients with type I (distal RTA) will not show the normal pCO_2 response. Patients with type II TA will.

Urinary Concentrating and Diluting (56,57)

Concentrating Ability

Patients may have reduced ability to concentrate urine as a result of a primary or secondary renal defect; as a result of vasopressin deficiency or due to large volumes of water intake (primary polydipsia). Two tests performed separately or sequentially can help delineate causes for failure to concentrate urine. In children or adults urine can be concentrated to 1200 mOsm/kgH_2O). In neonates maximal concentration is rarely above 700 mOsm/kgH_2O.
Tests:

- *Fluid restriction*: A first morning void after 12 h overnight fluid restriction with an osmolality >725 mOsm/kgH_2O, means normal concentrating ability. No further testing is needed. This test cannot be used in children under 2 years of age (see exam below). In children with large volumes of fluid intake for weeks or month (primary polydipsia), the urinary concentrating ability is reduced. Progressive reductions in fluid intake over days or weeks may be necessary in order to perform a thirst test. A thirst test still requires 12–16 h of fluid deprivation or loss of at least 2% of body weight. A loss of 2% of body weight may occur in children with vasopressin deficiency or nephrogenic diabetes insipidus and can occur well short of 12 h of fluid deprivation. When these diagnoses are being considered fluid deprivation should be performed in a controlled setting.
- *Vasopressin administration*: In patients following fluid deprivation where the urine osmolality does not rise above 600 mOsm/kgH_2O or in patients where vasopressin deficiency or nephrogenic diabetes insipidus is suspected and no fluid restriction is performed, vasopressin administration may help make the diagnosis. With a kidney capable of responding to vasopressin, the administration of DDAVP (1-desa-

mino-8-1) arginine vasopressin intranasally at a dose of 10 micrograms for infants and 20 μg in children or adolescents should result in urine osmolality increasing to above 750 mOsm within 6–8 h after administration of vasopressin. This test is best performed under some fluid restriction. In infants a restriction to 50–75% of usual fluid intake is preferred (57,58). In nephrogenic diabetes insipidus, no response to DDAVP will be noted.

Dilution Ability

Rarely is there a need to determine the maximal ability to dilute urine. It can be helpful when trying to determine *excessive* electrolyte loss (sodium, potassium, chloride) in patients with tubular disorders such as Bartter's syndrome. Urine and blood measurements of creatinine, sodium, potassium, chloride, osmolality and urine volume are measured prior to and then after oral water intake of 20 mL/kg over 1–2 h followed by an IV infusion 1–2 L of 2.5% dextrose plus 0.45 saline over 2–3 h. Urine is collected from the start of the study and on hourly intervals. The study results in a determination of the osmolar fractional clearance and the volume of urine produced per 100 mL GFR. The equation that defines the findings is as follows:

$$\mathrm{V} = \text{Volume of urine}/100\text{mlGFR} - \mathrm{Pcr}/\mathrm{Ucr}) \times 100$$

Pcr – plasma creatinine concentration
Ucr – urine creatinine concentration

$$C_{osm} = \text{Osmolar fractional clearance} = \frac{\text{Urine osmolality}}{\text{Plasma osmolality}} \times \mathrm{V}$$

This approach was described by Rodriguez-Soriano and normal values are reported (59). The normal C_{osm} in infants is 4.3 ± 1.3 mL/dL GF and in children the normal is 3.2 ± 0.7 mL/dL GF.

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22 Evaluation of Growth and Development

Sandra Amaral · Alicia Neu

Normal Growth

Growth is an invaluable biological indicator of overall health status in children. For this reason, careful assessment of growth should be an integral component in the routine care of any child with kidney disease. To optimize growth and development in children with kidney disease, it is vital to understand both the normal physiology of growth and the impact of kidney disease on growth. It is also imperative to be familiar with current methods of growth assessment and their limitations.

Measurements of Growth

Linear Growth

Pediatric growth charts have been used since the 1970s to assess how a child's growth compares with the growth of age- and gender-matched peers. The most commonly used growth charts were originally developed by the National Center for Health Statistics in 1977 to describe the distribution of height and weight of children in the United States (1). The 1977 growth charts were adopted by the World Health Organization (WHO) for international use. In 2000, the growth charts were updated based on data from the United States National Health and Nutrition Examination Survey (NHANES) from 1971 to 1994. NHANES has collected data on the health and nutrition of Americans since the 1960s (*Figs. 22-1*–*22-4*). In 2006, WHO launched new Child Growth Standards (2). These new standards resulted from a WHO study initiated in 1997 to develop an international standard for assessing physical growth, nutritional status and motor development in children from birth to 5 years of age. The study was a community based, multi-country project conducted in Brazil, Ghana, India, Norway and the United States. Over 8,440 children were included in the study (2). In 2007, WHO constructed growth references for children and adolescents 5 years through 19 years of age to be in accord with the WHO Child Growth Standards for preschool children and the body mass index (BMI) cut-offs for adults (3) (*Figs. 22-5* and *22-6*).

To identify problems with growth, it is necessary to examine growth changes over time. Growth must be assessed accurately and frequently. In the first year of life, linear growth should be assessed at birth, within 1 week after delivery, and then at 1, 2, 4, 6, 9, and 12 months. From 1 to 3 years of age, linear growth should be assessed every 6 months, at 18, 24, 30 and 36 months. Thereafter, linear growth should be measured once yearly.

To ensure accurate assessment of height, both measurement equipment and technique must be precise. Inaccurate height measurements on either an infant measuring board or stadiometer may lead to gross misinterpretation of growth patterns. Length boards and stadiometers should be zeroed on a daily basis and checked with standard length rods at least monthly. *Figure 22-7* demonstrates proper positioning of an infant and older child for height measurement. Special attention should be directed to positioning of the feet and hands. With an infant measuring board, feet must be flat and positioned against the board in line with the back of the head. The toes should point up. The body must be straight. The best way to ensure the head and feet are aligned and against the board is to have one person hold the head and another person hold the feet. In infants, it is often necessary to hold the knees down. Buttocks and shoulders should also touch the back board. The head should be in the Frankfurt plane, with the outer canthus of the eye and the external auditory canal forming a line perpendicular to the back board. For standing measurements, the feet must again be flat. The child should be measured after the removal of socks and shoes so that proper foot positioning can be ensured. Any hair accessories that may impact measurement of the top of the head should be removed if possible. If hair arrangements cannot be removed but may affect the accuracy of the measurement, this should be noted on the growth chart. Have the child stand with his/her back to the height rule. Heels should be slightly apart. The back of the head,

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Figure 22-1

Height and weight percentiles in boys from birth to 36 months.

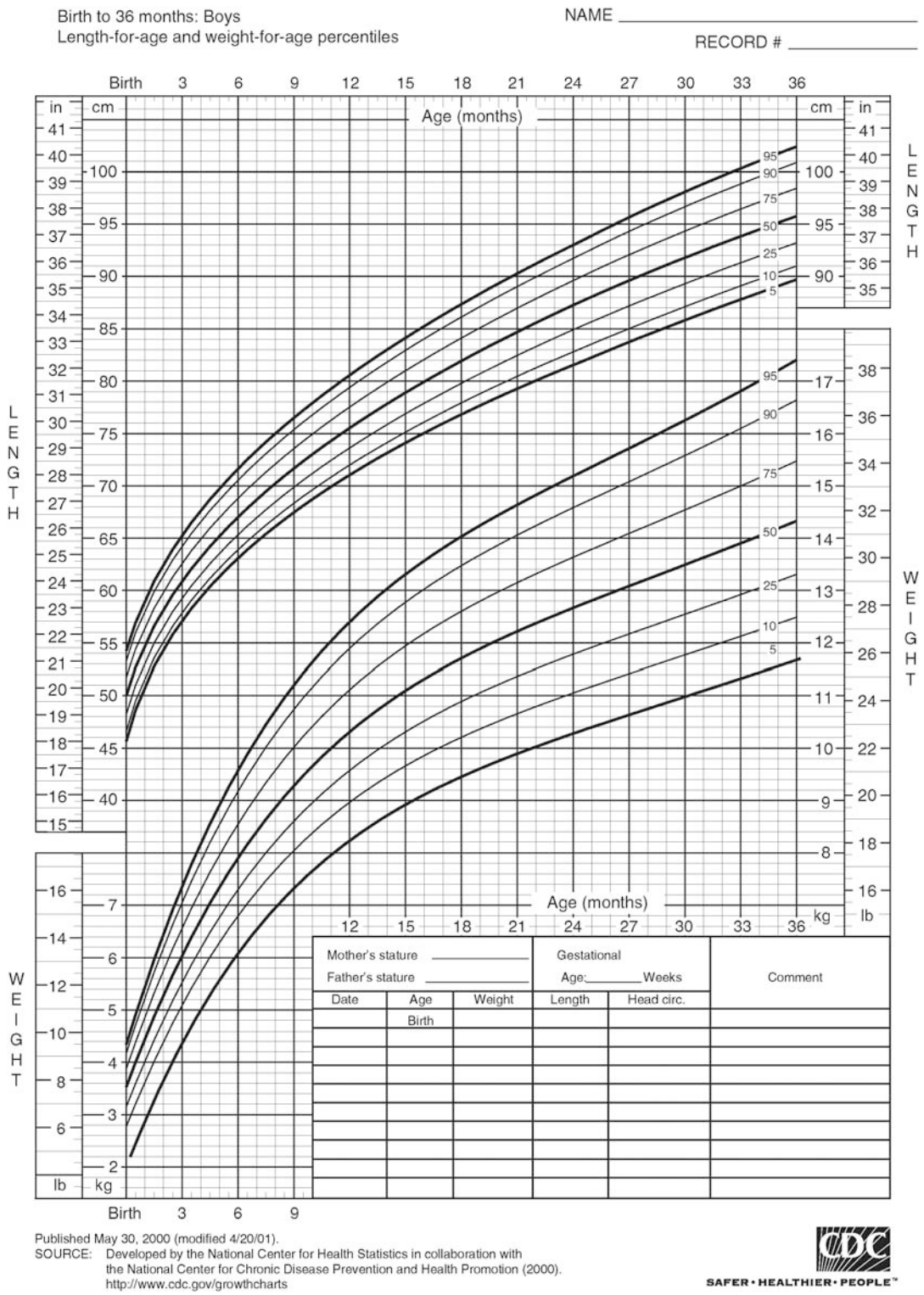


Figure 22-2

Height and weight percentiles in girls from birth to 36 months.

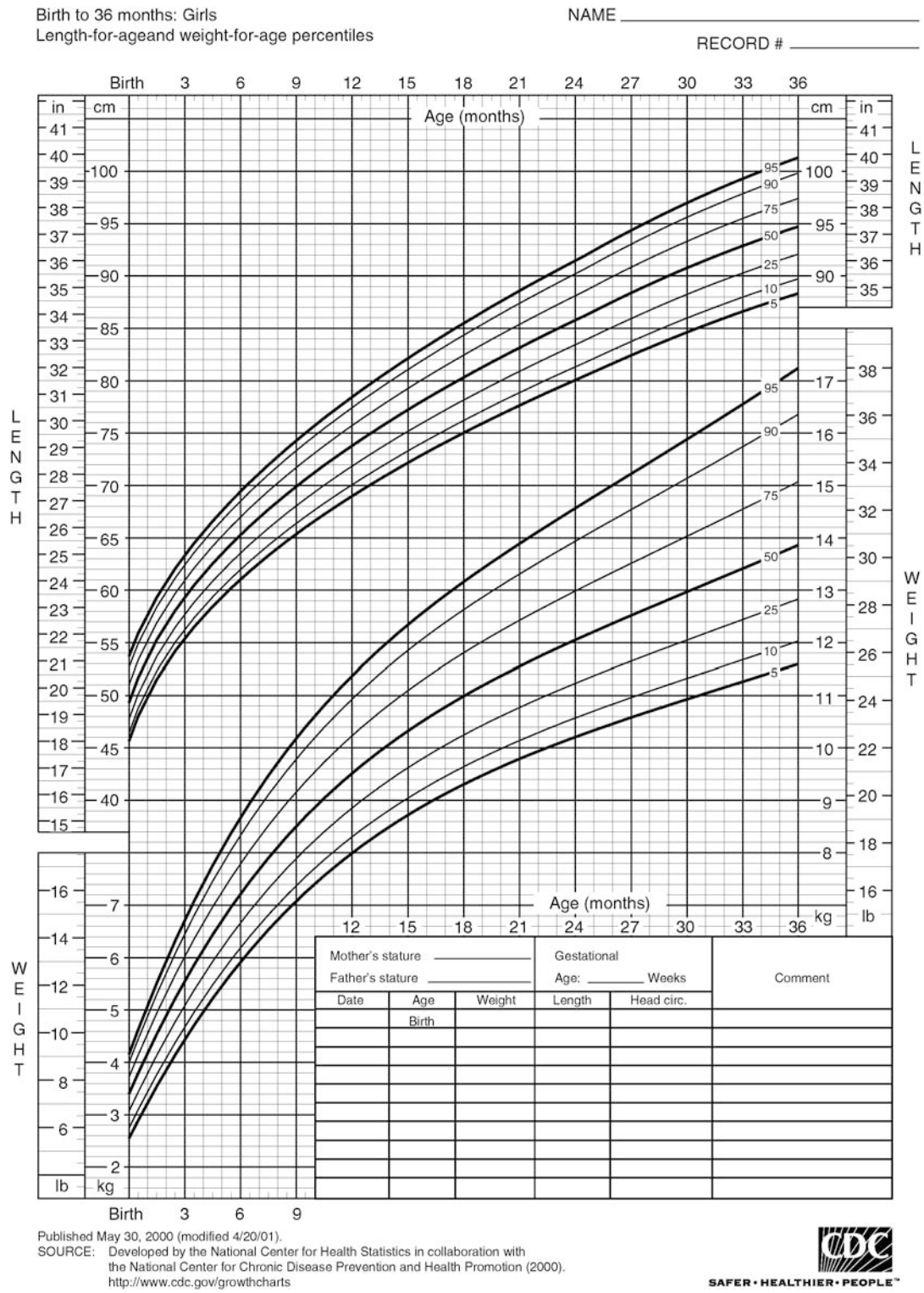


Figure 22-3

Height and weight percentiles in boys 2 to 20 years of age.

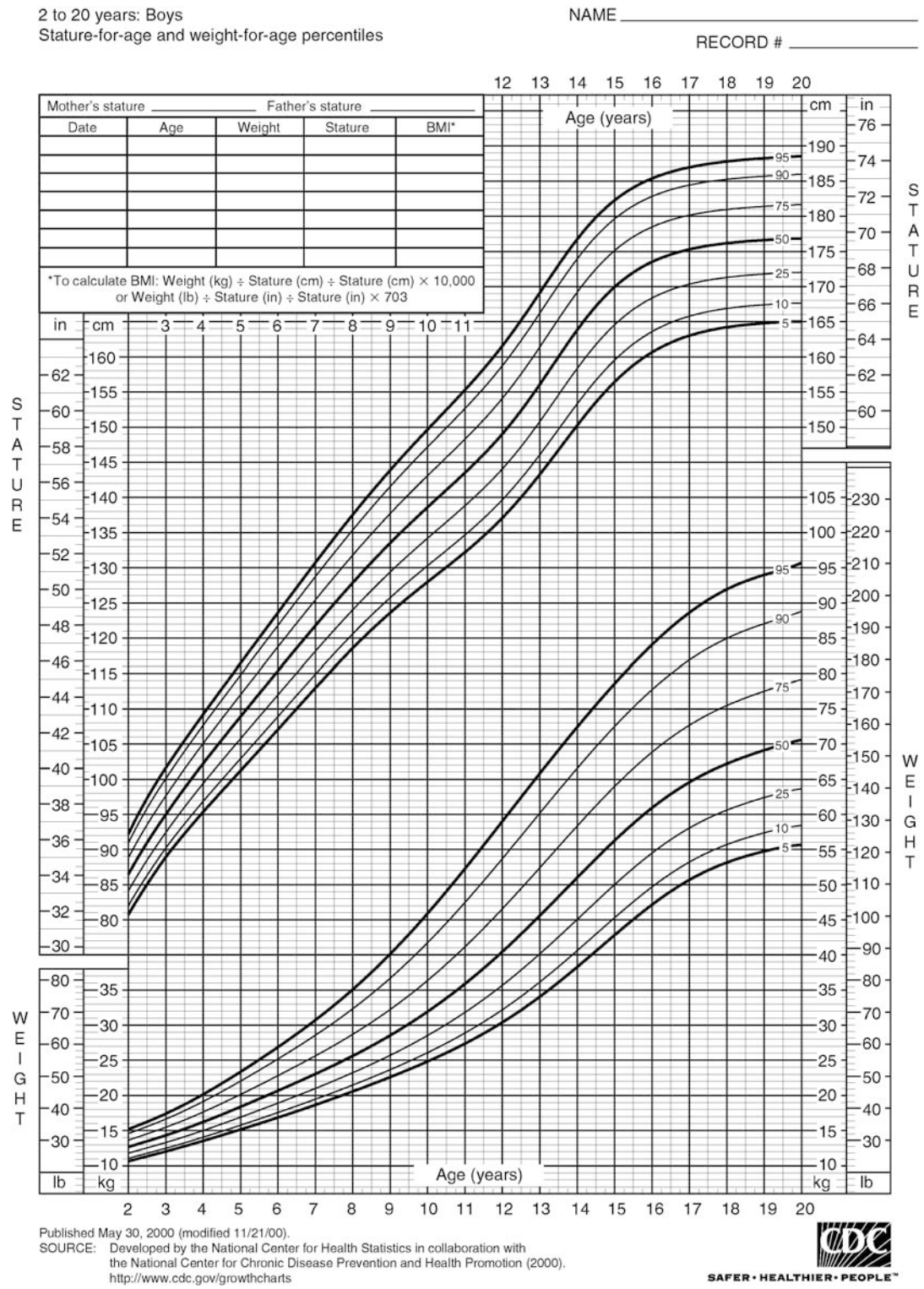


Figure 22-4

Height and weight percentiles in girls 2 to 20 years of age.

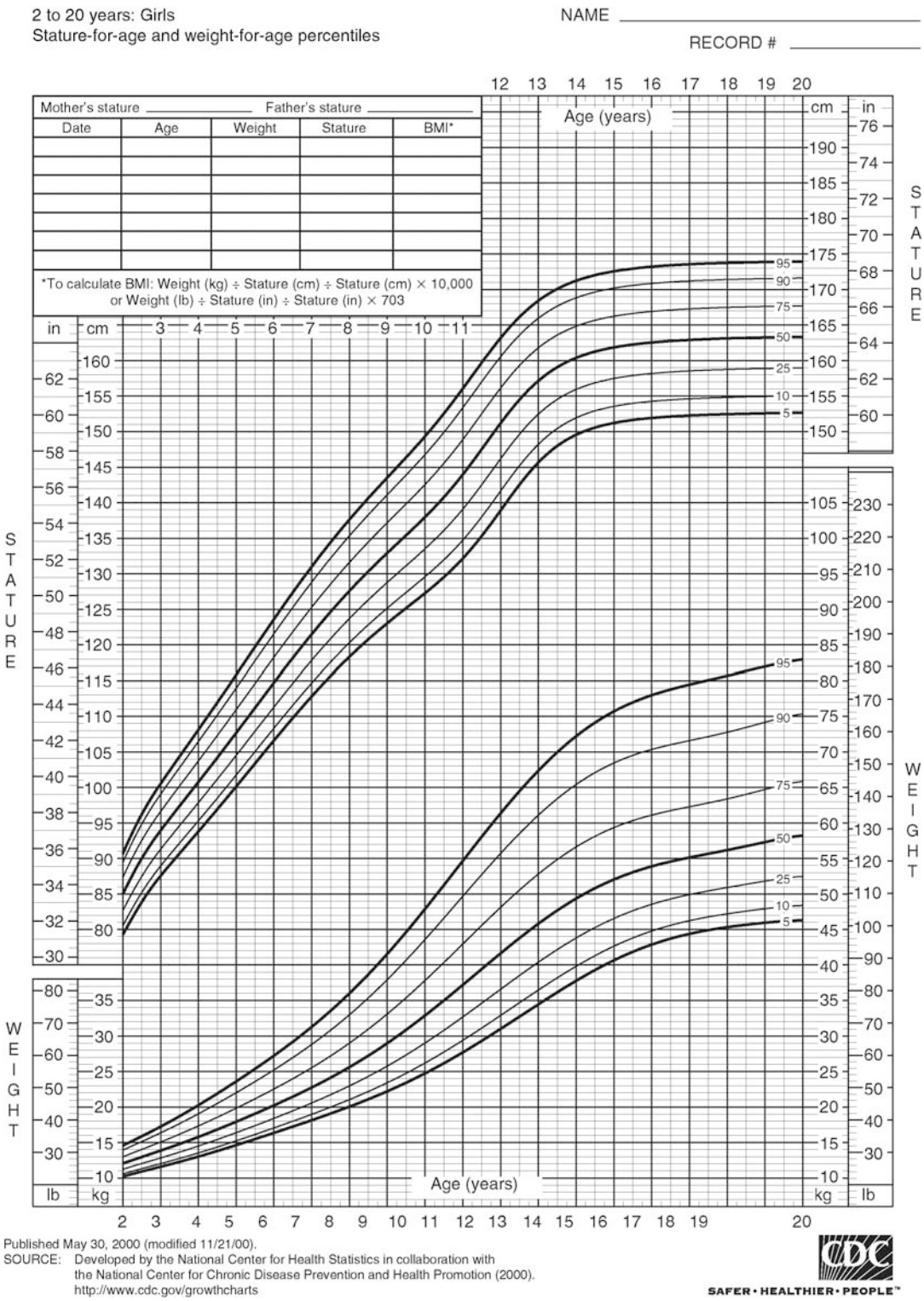
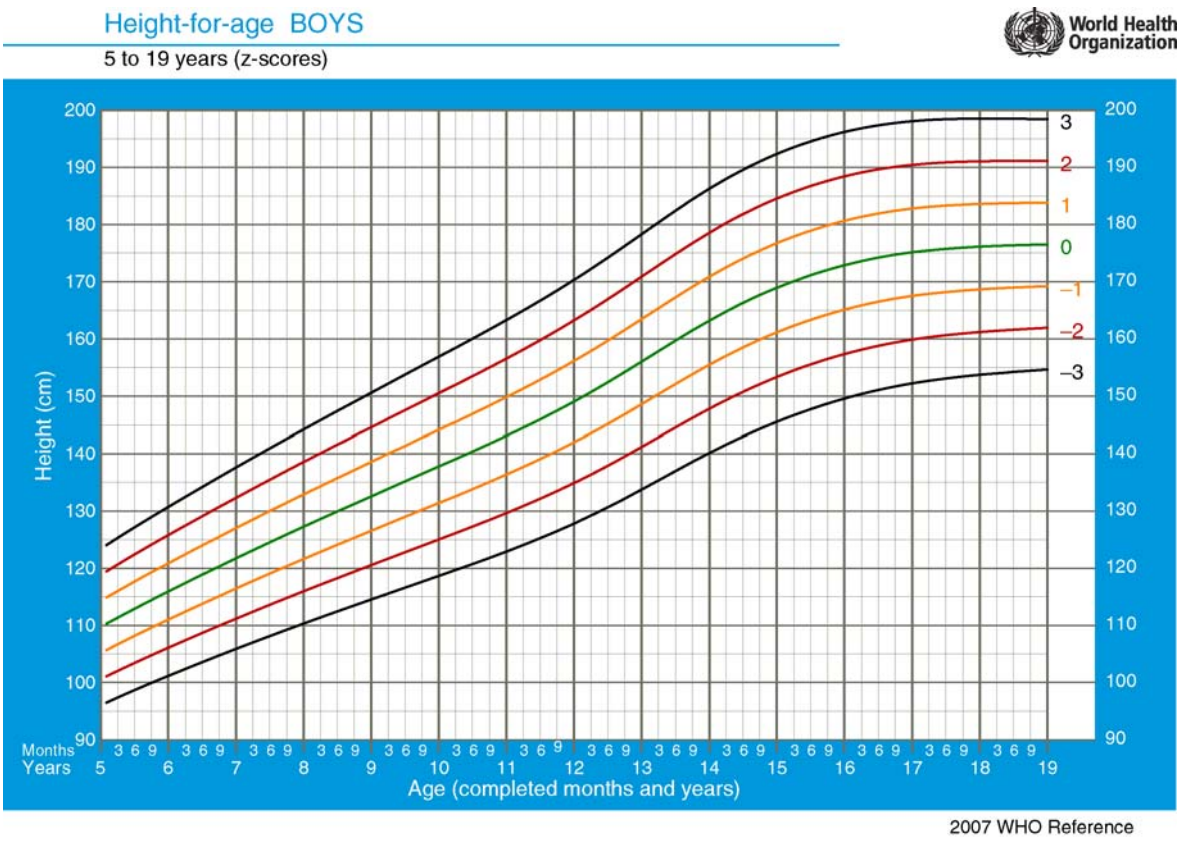


Figure 22-5

World Health Organization growth reference curves, providing z-scores for height in boys 5–19 years of age (http://www.who.int).



shoulder blades, buttocks, calves and heels should be touching the height rule. The knees should not be bent. Eyes should be forward, arms at sides and shoulders relaxed. The top of the external auditory meatus should be level with the inferior margin of the bony orbit. The head piece of the stadiometer is lowered until it is touching the top of the head and is level. Height is recorded to the resolution of the height rule (i.e., nearest millimeter/half a centimeter). If the participant is taller than the measurer, the measurer should stand on a platform so that he/she can properly read the height rule.

There are several common errors that may occur when obtaining height measurement. A period of increased risk for misinterpretation of height measurement occurs when an infant progresses from sitting to standing. Standard growth charts for infants from 0 to 36 months are based on recumbent length measured on infant measuring boards. Growth charts from 2 to 20 years are based on standing heights only. It is crucial to use the growth chart that correlates with the measurement technique between the ages of 2 and 3 years. Using the incorrect growth chart may lead to gross misinterpretation of a height pattern during the toddler years. Another potential error may occur when plotting the height measurement on the growth chart; height measurement should be recorded on the growth chart as close to the fractional age as possible rather than plotting on the nearest year or half-year. Inaccurate plotting of age may lead to significant discrepancies in height trends.

Most growth charts express height as a percentile for a reference population. The updated 2000 CDC growth charts can be used to obtain both percentiles and z-scores. A z-score is a statistical measure that quantifies the number of standard deviations (SD) a data point is from the mean of the data set. A z-score of zero lies at the mean. The WHO growth standard and reference charts make z-score calculation readily accessible (*Figs. 22-5* and *22-6*). Height percentiles are informative when a child's height measurement lies within them, however z-scores are more helpful for children who are significantly shorter

Figure 22-6

World Health Organization growth reference curves, providing z-scores for height in girls 5–19 years of age (http://www.who.int).

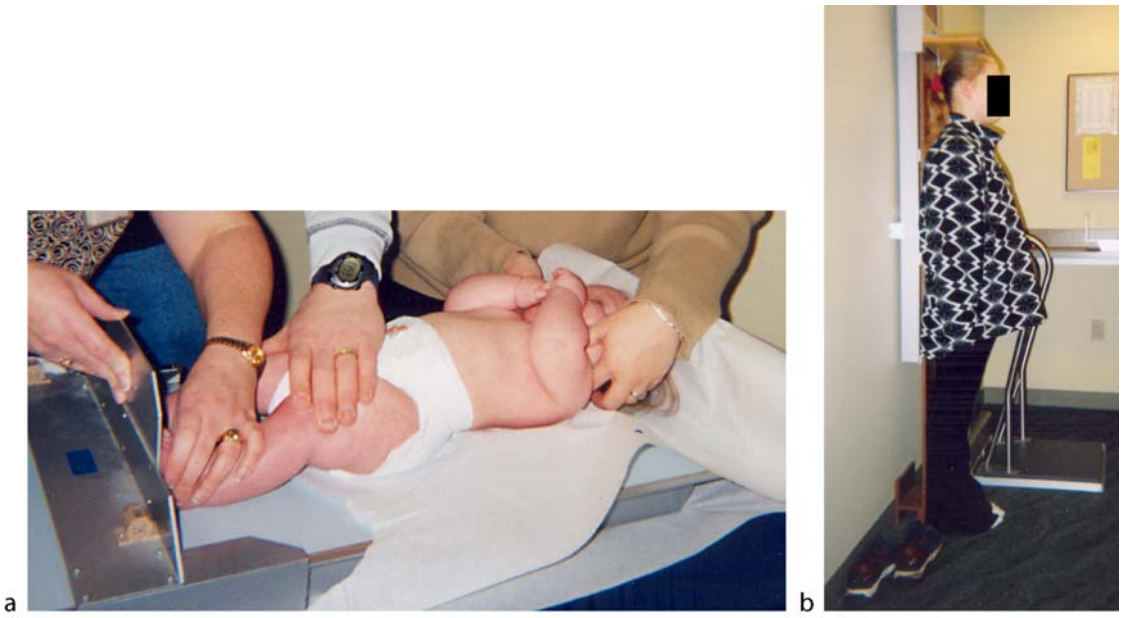
or taller than the standard percentiles. Z-scores (or SDS) are also used for other growth parameters, such as weight and body mass index. Besides the WHO z-score references, z-scores may be calculated using several computer programs that are widely available. Z-scores should be calculated regularly for any child with kidney disease. For children with incident end-stage renal disease (ESRD), height less than 1% for age (z-score < -2.5) at dialysis initiation has been shown to be associated with a more complicated medical course, included increased risk of mortality and prolonged hospitalization (4). For prevalent ESRD pediatric patients, decrease by 1 SDS in height has been shown to be associated with 12% increase in risk of death (5).

Special Considerations in Linear Growth

Several pediatric populations require special consideration when interpreting the standard CDC growth charts. The updated 2000 growth charts represent a more varied pediatric sample than the previous growth charts from the 1970s, however several groups are still underrepresented. Very low birth weight infants ($< 1{,}500$ g) are not included in the standard CDC growth charts. There are separate growth charts available for infants 1,500 g or less and infants 1,501–2,500 g. These charts are based on a large longitudinal cohort of premature infants from the Infant Health and Development Program (IHDP) (6, 7). For both low birth weight and very low birth weight infants, length, weight and head measurements should be recorded under the patient's corrected (or gestation-adjusted) age. The corrected age is calculated by subtracting the number of weeks of prematurity from the infant's chronological age. For most premature infants, length, weight, and head circumference should be plotted under the child's corrected age until the child reaches 2 years. For the extremely low birth weight infant weighing less than 1,000 g at birth, the corrected age is often used until

Figure 22-7

Proper positioning of an infant (a) and child (b) for accurate measurement.



3 years of age. If the child's growth normalizes before 24–36 months of age, chronologic age may be used instead of corrected age.

The standard CDC growth charts also may not adequately represent normal growth patterns for children with special healthcare needs. Several diagnosis-specific growth charts have been developed for conditions in which growth patterns may be impaired, such as Trisomy 21, Turner's syndrome and cerebral palsy (8). Although many of these diagnosis-specific growth charts are based on relatively small sample sizes, they may be useful in understanding expected growth trends in these special populations.

Although the updated CDC growth charts include a more diverse ethnic sampling, racial and ethnic differences in growth remain incompletely understood. Currently, one set of growth charts is promoted for all racial and ethnic groups because studies have suggested that growth differences among racial and ethnic groups are generally the result of environmental rather than genetic influences, and these data are supported by the WHO Growth Reference Study Group (2, 9). There is evidence to suggest that blacks may have an earlier acceleration in growth compared with whites (10). There have also been several studies noting decreased height SDS in Hispanics, especially Mexican Americans(11, 12). Since Hispanics represent up to 30% of pediatric ESRD, it is particularly disconcerting that standard growth charts include a random population comprised of less than 10% Hispanic children (13). Thus, although the racial and ethnic differences in growth trends demonstrated in children with ESRD may not represent genetic influences, it is important to understand that the differences exist and may be in part related to factors other than those associated with kidney failure.

Sitting Height

Measurement of sitting height may be a more useful measure of stature than standing height for children who are wheelchair bound or have impairment of the spine or long bones. There are specific devices for measuring sitting height that are comprised of a stadiometer attached to a base of a known height. The base is a box wide enough for a child to be comfortably seated. The child is placed on the base as erect as possible. Buttocks, shoulders and back of head should be against the back of the height rule. The head should be positioned in the same manner as standard stadiometer measurement. Total height is measured from the floor to the top of the child's head. The length of the sitting base is then subtracted to obtaining the sitting height measurement. The measurement can be plotted on the CDC stature-for-age

chart so that a trend can be ascertained over time. There are references for sitting heights, however the standards are from the early1970s and did not include children with special healthcare needs (14).

Growth Velocity

Growth velocity refers to the rate of change in height over time. Growth velocity is helpful in ascertaining the overall health status of a child as well as the effectiveness of therapy to improve growth. The accuracy and usefulness of measuring height velocity depends on the accuracy of the underlying height measurements and on the time interval over which the measurements are recorded. There is seasonal variation in growth velocity throughout the year in healthy children. Growth velocity charts examine mean change in height in centimeters over 1 year; thus, measurements should be obtained as close to a 1-year interval as possible. The height velocity charts that are most commonly used today are based on large longitudinal studies and are thus more accurate than cross-sectional estimates of rate of change in height (*Figs. 22-8* and *22-9*) (15). Often, in the practice setting, measurements are taken over a shorter time period and extrapolated for 1 year estimates. While this method is more practical clinically, it increases error in calculation and may lead to clinical misinterpretation. Any growth velocity estimates based on data from less than 1 year should be used with caution.

The time period over which the measurements are taken should be marked along the x-axis of the growth chart and the mean growth velocity should then be plotted at the midpoint of the time period. It is also helpful when interpreting the results to record the timing of important clinical events, such as initiation of recombinant human growth hormone (rhGH), on the height velocity chart. This permits assessment of pre- and post-treatment growth response. When calculating height velocity, time periods with and without rhGH treatment should have separate height velocities calculated and should not be averaged together. Averaging will lead to underestimation of the response to rhGH. Height velocity is informative in ascertaining whether a child experiences catch up growth in response to treatment. Catch up growth describes height velocity that exceeds the limits of normal for age for at least 1 year after a period of depressed growth.

Growth velocity patterns are not static. Patterns vary widely with pubertal maturation stages (16, 17). Growth velocity charts provide centile lines that allow a practitioner to assess whether a child is growing within the normal variation for age, regardless of maturation stage. Pubertal growth is characterized by an acceleration in growth followed by a deceleration and then stabilization with closure of the epiphyses. Peak height velocity during puberty is reached earlier in girls than boys. In general, girls reach peak height velocity at 11.5 years whereas boys reach peak height velocity at 13.5 years. Whole-year peak height velocity is on average 8.3 cm per year in girls versus 9.5 cm per year in boys.

Interpretation of height velocity differs significantly from the interpretation of absolute height. Since height velocity is variable, a child may remain at the fifth percentile for absolute height, but the height velocity should change based on the maturational stage. A child needs to maintain a height velocity at the 50th percentile in order to maintain a height at the 50th percentile. If a child maintains height velocity at the 25th percentile throughout maturation, for example, that child will significantly drop percentiles in absolute height. Such a drop would be quite concerning and should prompt evaluation for growth impairment. In a cohort of 1,949 prevalent pediatric patients with ESRD, for each 1 SDS decrease in growth velocity, the risk of death increased by 12% (5). Thus, measurement of height velocity may be a valuable prognostic indicator for overall morbidity in pediatric ESRD.

Head Circumference

It is recommended that occipitofrontal circumference be assessed in all children regularly up to 3 years of age. The measurement of head circumference in infancy and early childhood is particularly important because it reflects the time of most rapid brain growth and neurocognitive development. Head circumference measurements should coincide with the routine clinic assessment schedule after birth. To obtain an accurate head circumference measurement, a flexible but non-stretchable measuring tape should be used. Head circumference or OFC (occipitofrontal circumference) should be measured over the occiput and just above the supraorbital ridges to ensure measurement of the area of greatest circumference.

Just as with height measurements, head measurements should be obtained serially to assess trends in growth and development. A single head measurement may appear normal, but when interpreted in the context of head measurements at other points in time may reveal an abnormal pattern. Rapid growth in head circumference may be concerning for the development of hydrocephalus. Poor growth in head circumference may be indicative of a genetic or metabolic disorder. Head circumference which

Figure 22-8

Height velocity in boys 2 to 19 years of age (From (15) with permission).

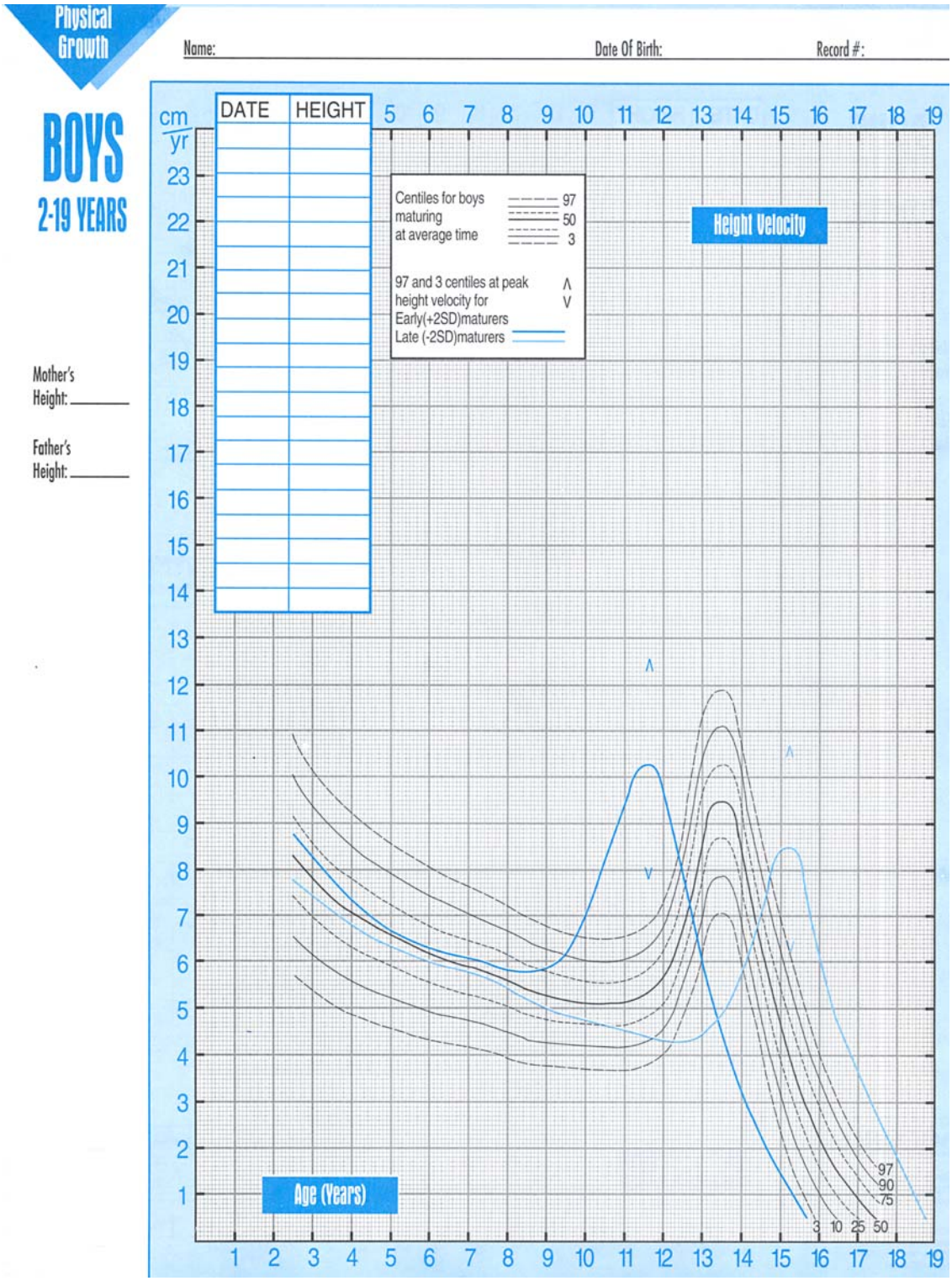
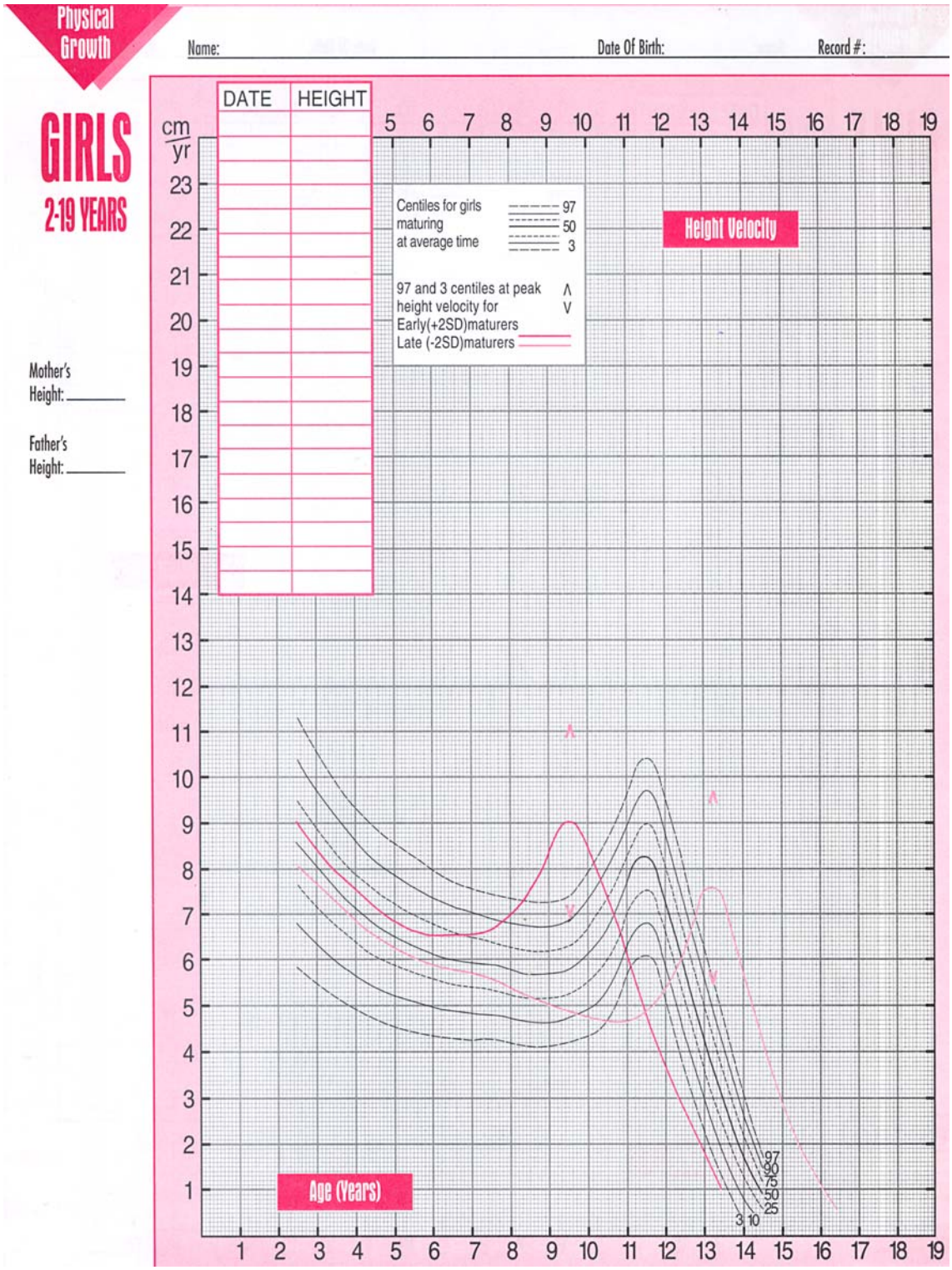


Figure 22-9

Height velocity in girls 2–19 years of age. (From (15) with permission).



is stable in growth but at the lower or upper percentile margins may be familial. It is often helpful to obtain head circumference measures on the parents.

In most nutritional conditions and chronic diseases, head circumference is not severely affected. Brain growth is generally separate from somatic growth. Thus, head circumference cannot be used to assess presence or progression of chronic kidney disease. When occipitofrontal circumference is altered in the context of kidney disease, this generally suggests that there is a systemic process that has affected the brain as well as the kidneys.

In 1968, Nelhaus reported head circumference means and standard deviations for age and gender in children from birth through 18 years, calculated from published reports in international literature over the preceding 20 years (18). These normative values are still used today and are reflective of a multi-racial and multi-ethnic population of healthy children. No significant racial, ethnic or geographical differences were found in head circumferences and thus the norms have widespread applicability (*Figs. 22-10* and *22-11*).

Body Mass Index

The updated 2000 CDC growth charts include body mass index charts to assess weight correlated with stature (1). Standards are detailed in *Figs. 22-12* and *22-13*. Body mass index is calculated as weight in kilograms divided by height in meters squared. Body mass index provides a better assessment of body mass than absolute weight. According to the body mass index growth charts, overweight is defined as body mass index greater than or equal to the 95th percentile for age and gender. Underweight is defined as body mass index below the fifth percentile. Both too little and too much body mass pose health risks. In prevalent pediatric ESRD patients, an association has been shown between both extremely low and high body mass index measures and increased risk for death (5).

There is increasing prevalence of overweight status in children and adolescents (19). There are significant differences in obesity prevalence by race and ethnicity, with the greatest prevalence being Mexican Americans and non-Hispanic blacks (20, 21). Increases in overweight are notable as young as 2–4 years of age. In a Canadian cohort of over 6,000 children aged 0–19 years who were referred to a tertiary pediatric nephrology center, an increase in body mass index coincided with a significant increase in chronic kidney disease (22).

Body mass index is a practical method of body mass assessment, however, it is not a particularly good measure of overall body composition. To better understand body composition, it is necessary to differentiate between muscle mass and fat mass. There are several other methods for assessing body composition that may be more accurate. Waist to hip ratio has recently been shown to be reliable for assessing cardiovascular and metabolic risk in adults (23). In overweight children, a waist circumference of >90% or a waist to height ratio greater than 0.5 has been associated with increased metabolic and cardiovascular risk (24). Skinfold thickness is also often purported to be a useful anthropometric measure for fat mass, however recent pediatric studies have suggested skinfold thickness to be less informative than body mass index for fat assessment in children (25, 26). Lean body mass may also be assessed using dual-energy X-ray absorptiometry (DEXA) scans. DEXA scans can detect subtle changes in bone mass over time. They also provide data on total fat mass. Use of DEXA scans in children for assessment of body composition may show significant variability across different body sizes and health states and can be particularly problematic in children with chronic kidney disease in whom lean body mass assessment can be confounded by fluid overload (27–29). Bioelectrical impedance measures the conductive resistance of a biological tissue exposed to an electrical current. This method can be used to detect total body water and fat-free mass. Bioelectrical impedance use has been validated in children, including children on dialysis and with kidney transplants (30). Bioelectrical impedance may have utility in assessing dry weight of dialysis patients. It is important to use prediction equations that have been validated in children as the validity of adult commercial software for bioelectrical impedance in children is quite poor (30).

Bone Age

Skeletal development is primarily assessed by radiography. Commonly, left hand radiographs are compared with images of standards in the Greulich and Pyle skeletal age atlas (31). The bone age is reported as the standard that looks most similar to the images in the atlas. There is thus frequent interobserver variability. There are also gaps of an entire year between standards so it is difficult to assess subtle changes in bone maturation. Additionally, the atlas does not account for different bones growing at different rates. There is often a discrepancy between the maturity of bones in the hand versus wrist. Moreover, the Greulich and Pyle atlas was derived from bone standards of white children of upper socioeconomic status living in the United States in the 1930s. Although, the Greulich and

Figure 22-10

Head circumference weight for length percentiles in boys from birth to 36 months.

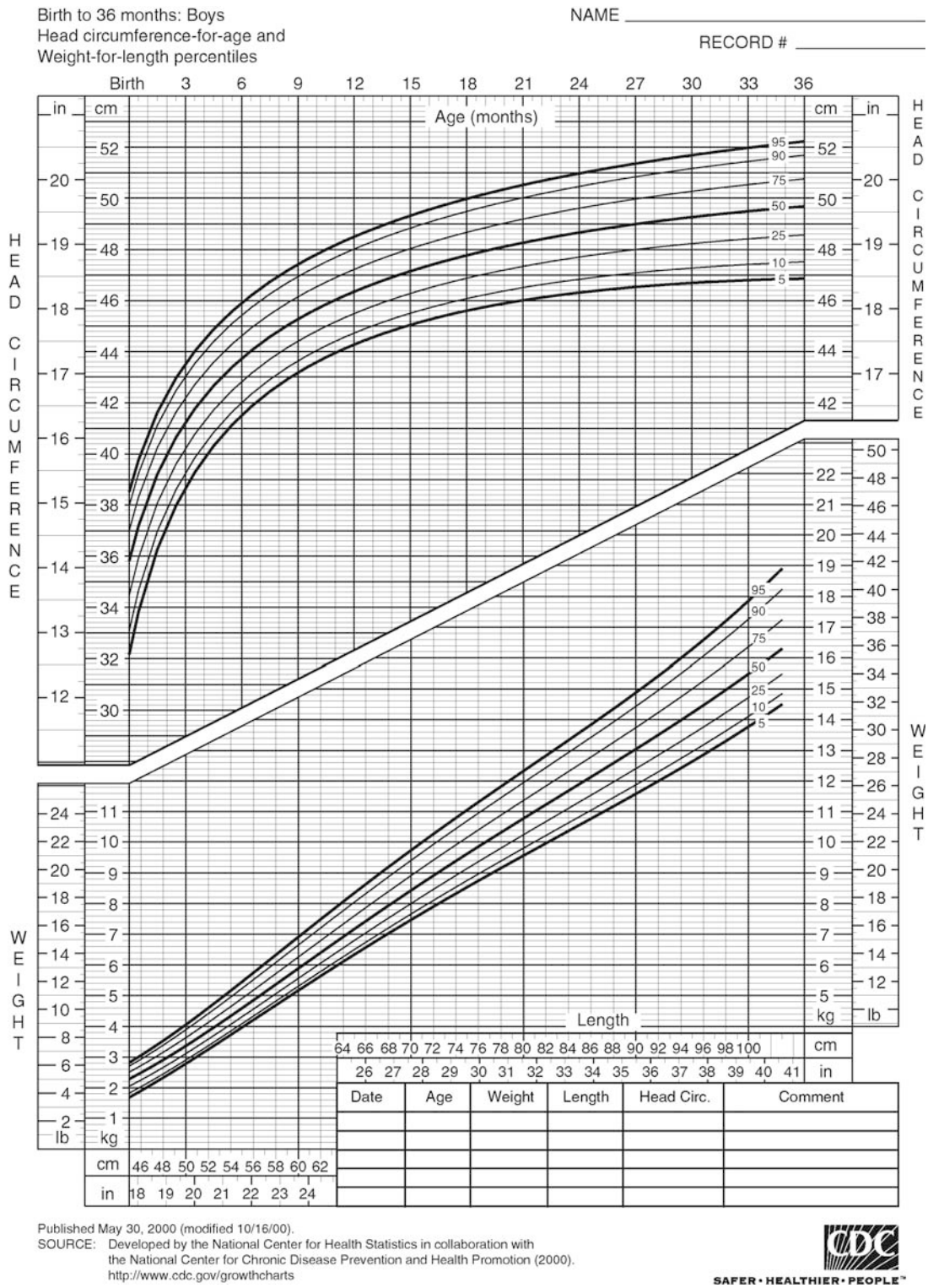


Figure 22-11

Head circumference weight for length percentiles in girls from birth to 36 months.

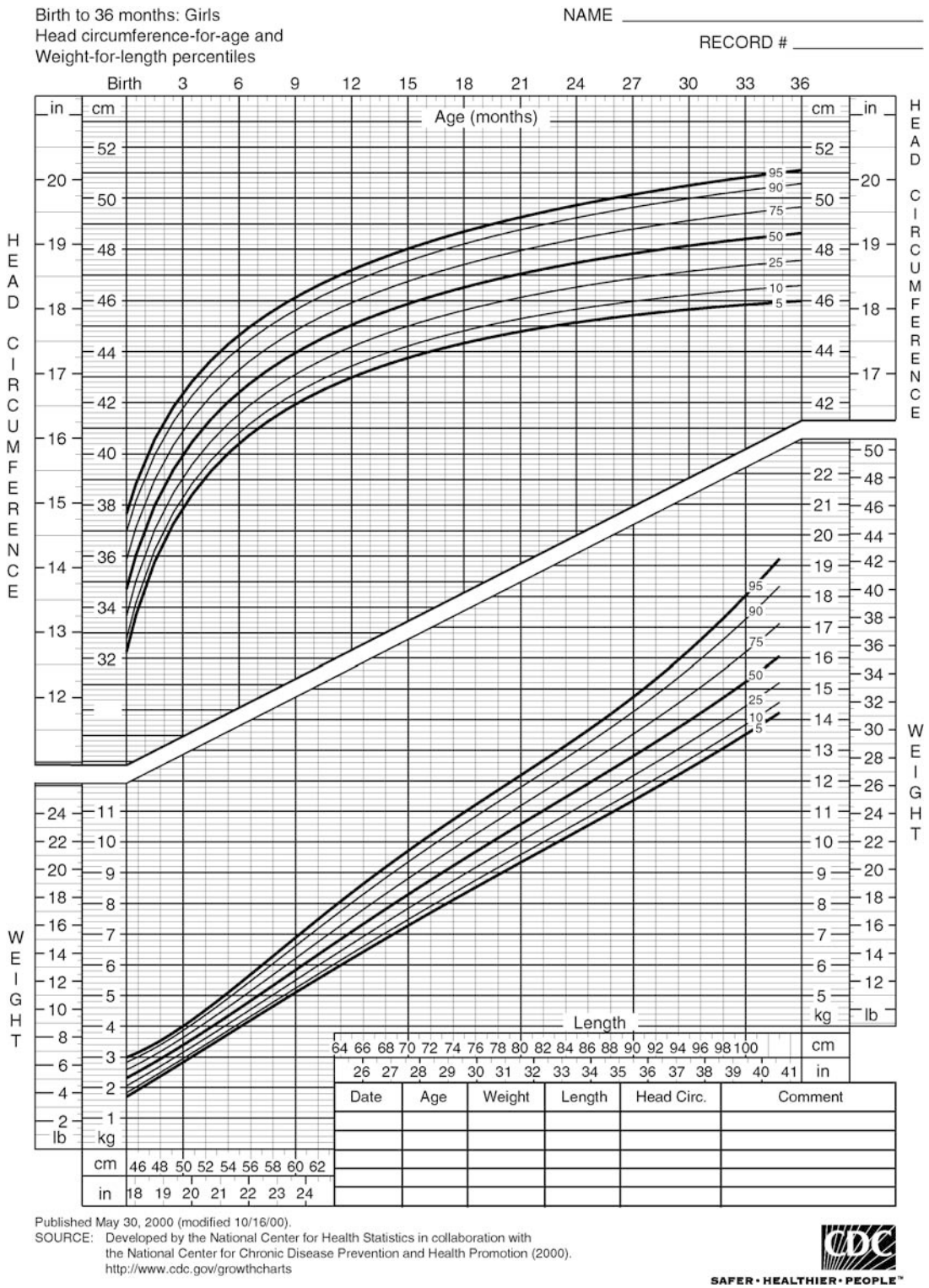


Figure 22-12

Body mass index (BMI) percentiles for boys 2–20 years of age.

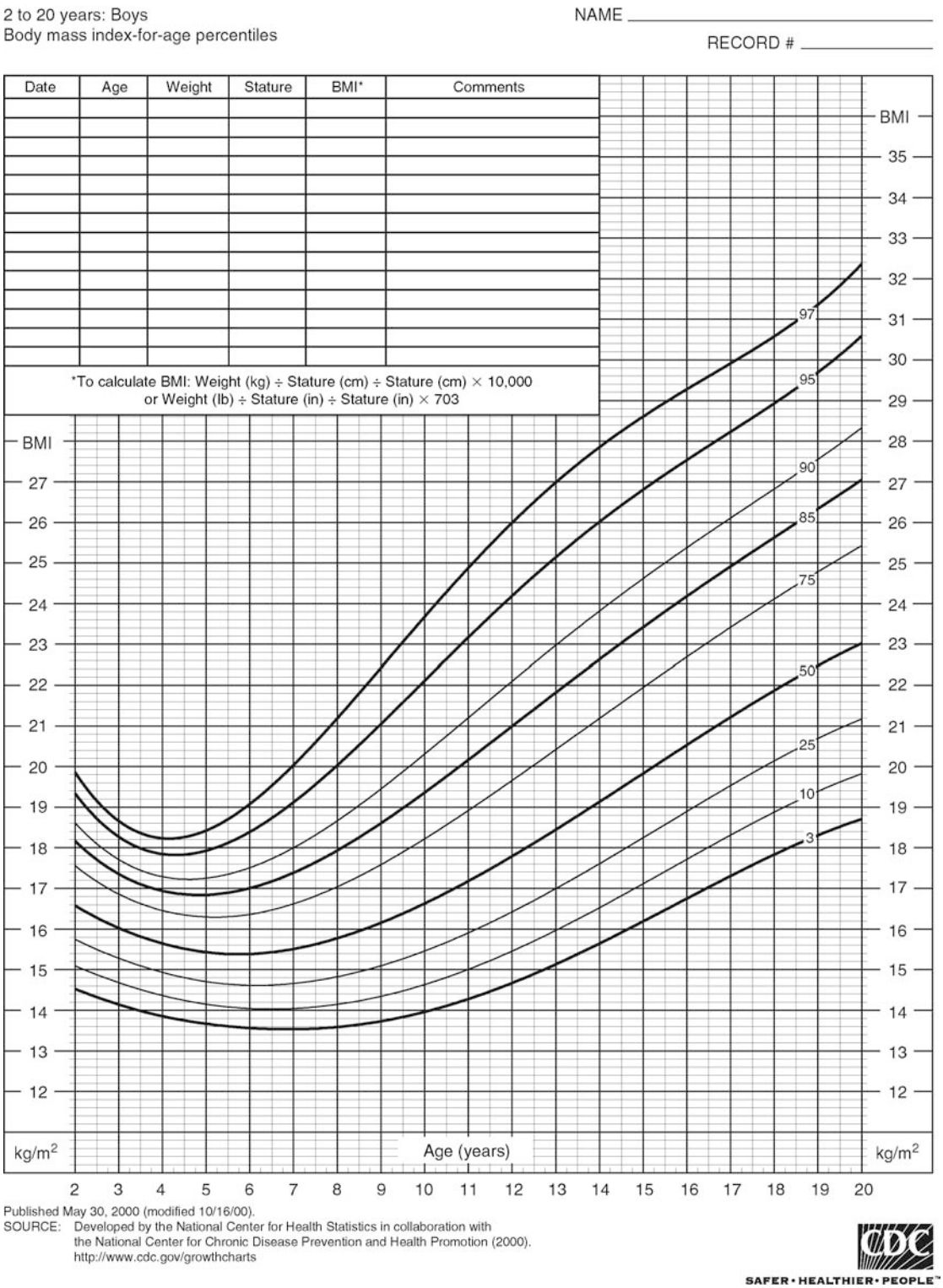
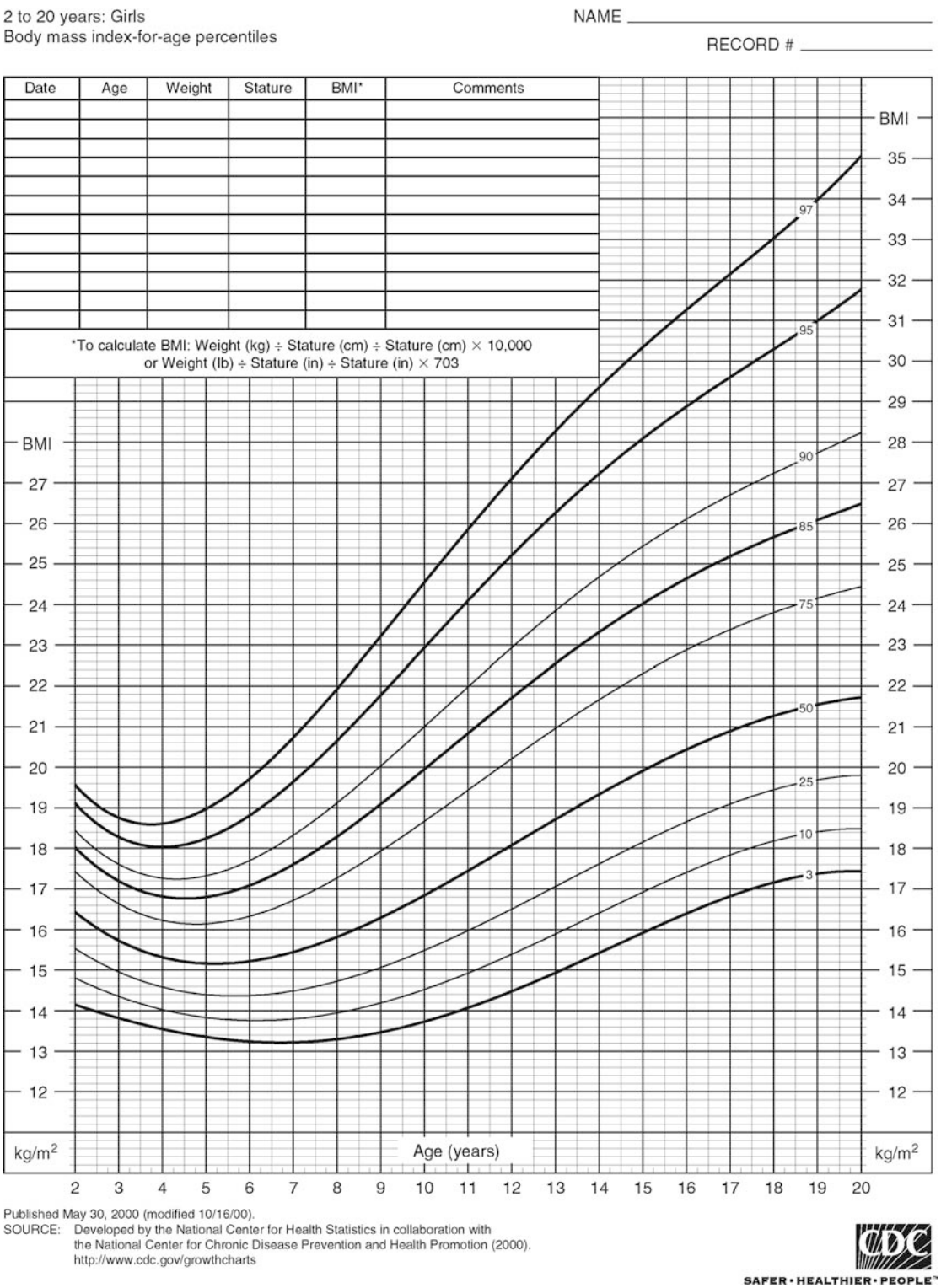


Figure 22-13

Body mass index (BMI) percentiles for girls 2–20 years of age.



Pyle atlas is a fairly quick and simple assessment, there is debate regarding current applicability of the atlas for diverse pediatric populations. Several studies of children of European and African descent have demonstrated ethnic differences in bone age and variability depending on pubertal status (32–34).

Little is known about the applicability of the Greulich and Pyle atlas for children with chronic kidney disease. One study of 40 children with chronic kidney disease and ESRD in the United Kingdom compared bone age using the Greulich and Pyle atlas with the Tanner and Whitehouse method (35). The Tanner and Whitehouse method showed better repeatability than the Greulich and Pyle atlas. The Tanner and Whitehouse method uses a more detailed examination of each individual bone in the hand (36). In this method, 20 bones of the hand are scored into one of eight categories. The numerical score is translated into a bone age. The advantage of this method is that it accounts for differential maturity of different bones. It also enables assessment of bone age at 0.1 year increments, thus it is more sensitive for detecting less dramatic changes in bone maturation. In most populations, the Greulich and Pyle atlas and the Tanner and Whitehouse method yield comparable results. Thus, since the Tanner and Whitehouse method is significantly more time-consuming, it is much less readily performed by radiologists.

Some newer methods for bone age assessment that involve decreased radiation risk are currently being explored. MRI of the wrist has been proposed as a method of skeletal maturity assessment (37). In MRI, skeletal age interpretation is based on fusion of the left distal radial physis. Studies are also being conducted in ultrasonography of the hand and wrist for children (38, 39). Ultrasonography evaluates the relationship between the velocity of sound passing through the distal radial and ulnar epiphysis and growth, using gender and ethnicity based algorithms. None of these methods is yet validated in a large pediatric population and should be used with caution.

Since bone age has been shown to have significant variability depending on pubertal stage and ethnicity, interpretation of bone age is not completely straightforward. A patient's bone age should not be interpreted dichotomously, as normal or abnormal. Rather bone age should be interpreted within the context of the physical maturity and overall health status of the patient.

Pubertal Stages

Since patterns of height and growth velocity vary widely with stages of pubertal maturity, it is important to accurately assess pubertal stages. Monitoring of pubertal development also provides information regarding general health and potential growth impact of kidney disease. *Figure 22-14* describes the Tanner pubertal staging method which is the universal standard for assessment of pubertal development (39a). For females, there are separate stages for breast development and pubic hair development. These stages may be discordant. For males, there are five stages of genital development. Assessment of male genital development includes accurate assessment of testicular size and penile length in addition to pubic hair development. Testicular size, or volume, should be compared to a standard Prader orchidometer with a gradation of size from 1 to 25 mL. A testicular volume of greater than or equal to 4 mL generally indicates onset of puberty. Both testicles should be measured for accurate assessment (40).

Onset of puberty varies significantly not only between genders but also among individuals of the same gender. There are ethnic differences in pubertal onset as well, with black females experiencing earlier pubertal development than whites. Additionally, both genetic and environmental factors may impact pubertal development. A family history regarding timing of pubertal onset in parents should be obtained if there is any concern of delayed sexual development.

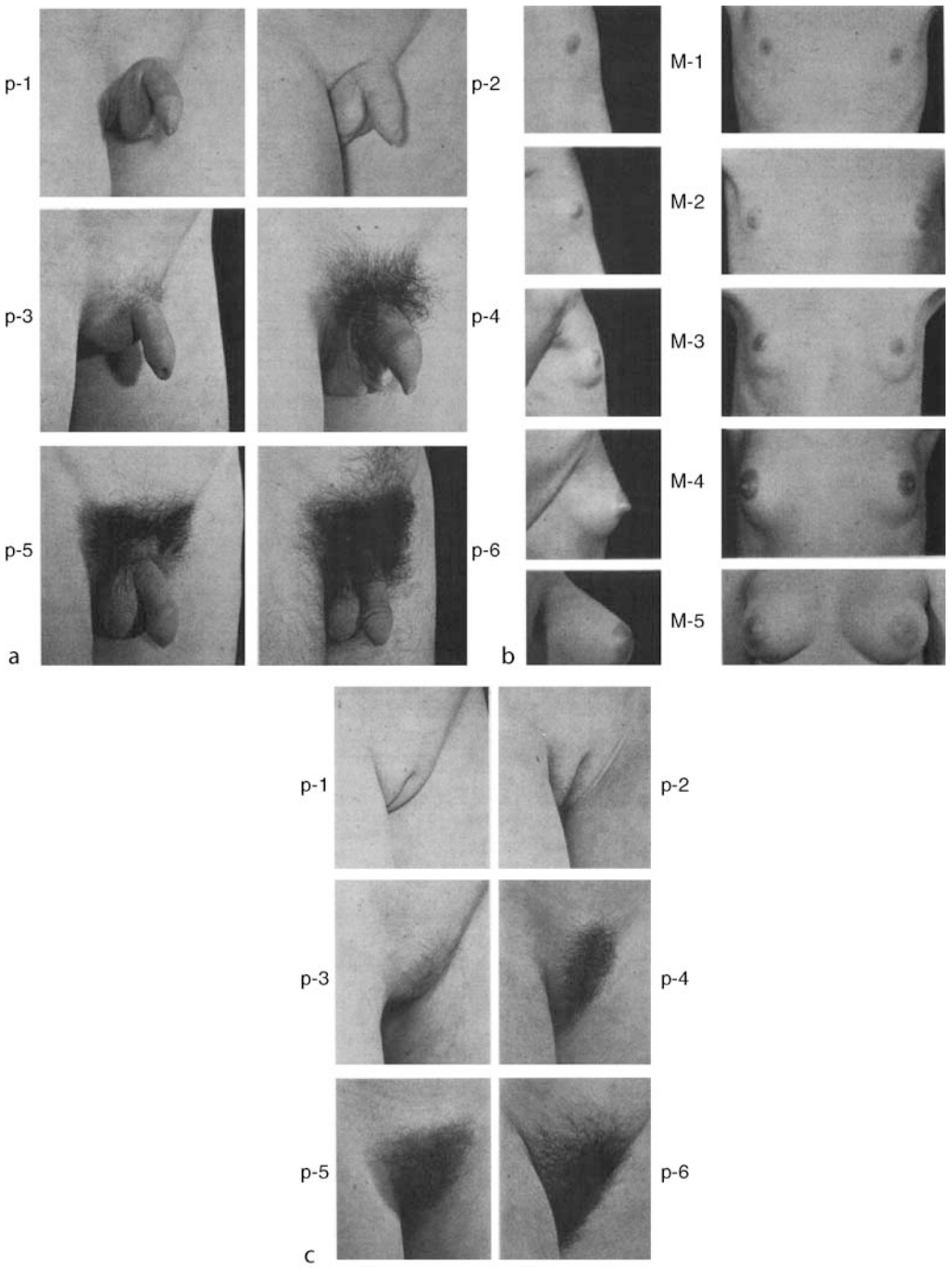
Any child with delayed sexual development should be evaluated by an endocrinologist. For girls, delayed sexual development is diagnosed when there is lack of any breast development by 14 years of age or when there is more than a 5 year delay between breast development and menarche. Girls who fail to reach menarche by 16 years of age should also be evaluated. For boys, delayed sexual development is defined as no testicular enlargement by age 14 or greater than 5 years between the initial and complete maturation of the genitalia (41).

In pediatric patients with kidney disease, accurate assessment of puberty is particularly important. The clinical history, physical exam and growth chart in conjunction with bone age studies can help identify abnormal growth patterns and potential for intervention.

Normal Growth-Phases and Drivers of Growth

There are several phases of growth from infancy through adulthood. The first phase of growth begins in utero. Although chromosomal and genetic abnormalities play a role in intrauterine growth, the primary contributor to adequate fetal growth is appropriate nutrition. Adequate nutrition for the fetus is dependent on both adequate

Figure 22-14 (Continued)



maternal nutrition and adequate delivery of nutrition via a viable placenta. If either of these conditions is not met, intrauterine growth restriction (IUGR) occurs. IUGR has been associated with increased cardiovascular disease, learning difficulties, and insulin resistance in adulthood (42, 43). Approximately 10% of children born with intrauterine growth restriction do not experience catch-up growth (44). It is postulated that there is intrauterine growth programming that occurs in which the in utero environment is permanently altered as an adaptive response. Arterial endothelial dysfunction and arterial stiffness have been demonstrated in term infants, children and young adults born with IUGR (45, 46). The mechanisms for these adaptive changes are poorly understood, however, insulin-like growth factor signaling is believed to play an important role (47, 48).

The second phase of growth occurs post-natally over the first 2 years of life. During this period, there is rapid growth, although the growth velocity gradually declines over time. A newborn generally gains 2 pounds per month in the first 3 months of life. By 1 year of age, this slows to an 8 ounce monthly weight gain. During infancy, there is a shift in growth toward genetic potential and many children will cross percentiles on their growth chart. Mechanisms for infantile growth are not well understood, however, it is evident that nutrition plays a primary role. In a study of interventions for child undernutrition and survival, improved nutrition reduced growth stunting by 36% and mortality by 25% at 36 months of life (49). Nutrition drives insulin and insulin-like growth factors, the primary regulators of growth during this phase of life.

After the second year of life, somatic and brain growth slows, commensurate with declines in appetite and nutritional needs. At this stage, growth is more dependent on growth hormone and is less influenced by nutrition. Between the ages of 2 and 5 years, the average child gains approximately 2 pounds in weight and 7 cm in height annually. Growth velocity essentially stabilizes until the onset of puberty. Growth velocity for any given child may be inconsistent, thus repeated measures during this time period can be invaluable in the assessment of growth disorders.

The last phase of growth constitutes pubertal maturation, driven by sex hormones and increased growth hormone production. Pubertal growth patterns vary by gender. In girls, the growth spurt is greatest in the first 2 years of pubertal onset, followed by an additional 2–3 years of slower growth. For boys, pubertal growth begins slowly. Growth is not spurred immediately by rising levels of testosterone and some boys may experience a decline in growth velocity for 12–18 months before a pubertal growth spurt is noted. As levels of testosterone gradually increase, boys will experience increased growth velocity at mid-puberty. Similarly to females, this increased velocity

Figure 22-14

Stages of pubertal development for boys and girls (a–c). Stages of genital development for boys (a): (1) Preadolescent – testes, scrotum, and penis are of about the same size and proportions as in early childhood. (2) Enlargement of scrotum and of testes – the skin of the scrotum reddens and changes in texture; little or no enlargement of penis at this stage. (3) Enlargement of penis, which occurs at first mainly in length – further growth of testes and scrotum. Increased size of penis with growth in breadth and development of glans; further enlargement of testes and scrotum; increasing darkening of scrotal skin. Stages of breast development for girls (b): (1) Preadolescent – elevation of papilla only. (2) Breast bud stage – elevation of papilla as a small mound; enlargement of areolar diameter. (3) Further enlargement and elevation of breast and areola, with no separation of their contours. (4) Projection of areola and papilla to form a secondary mound; the development of the areolar mound does not occur in all girls. (5) Mature stage – projection of papilla only, caused by recession of the areola to the general contour of the breast. Stages of pubic hair development for boys and girls (a–c): (1) Preadolescent – the vellus over the pubes in not developed further than over the abdominal wall. (2) Sparse growth of long, slightly pigmented downy hair, or only slightly curled, appearing chiefly at the penis or along the labia. (3) Considerably darker, coarser, and more curled; the hair spreads sparsely over the junction of the pubes. (4) Hair now resembles adult in type but the area covered is still considerably smaller than in the adult; no spread to the medial surface of the thighs. (5) Adult in quantity and type with distribution of the horizontal (classically feminine) pattern; spread to the medial surface of thighs but not up linea alba or elsewhere above the base of the inverse triangle. (From (39a) Tanner J. Growth at adolescence. Oxford: Blackwell, 1962, with permission).

lasts about 2 years and is followed by 2–3 additional years of gradual growth. Pubertal development ceases with closure of the epiphyses of the long bones. Epiphyseal fusion is driven by estrogen in both males and females.

Because rates of growth velocity change dramatically from the pre-pubertal stage through puberty, it is vital to assess pubertal development when assessing normalcy of growth. Pubertal parameters tend to correlate better with bone age than chronologic age. Thus, if there is concern of growth delay, bone age is often quite helpful. When an individual shows delayed bone age, this suggests potential for growth. Individuals with constitutional growth delay will generally achieve their genetic potential albeit later than expected. In patients with chronic illness, however, full growth potential may not be achieved, even with therapy.

Determination of Genetic Target Range and Correction of Growth Expectations for Parental Heights

Ultimate, or final, adult height is significantly impacted by genetic potential. Any assessment of absolute height, therefore, must consider the target height range, which is best estimated statistically by parental heights. A height prediction based on the sex-adjusted midparental height can be determined using calculators that are readily available on the internet. For girls, 13 cm is subtracted from the father's height and averaged with the mother's height. For boys, 13 cm is added to the mother's height and averaged with the father's height. For both boys and girls, 8.5 cm on either side of the calculated target height represents the 3rd to 97th percentiles for anticipated adult height (50).

Regulation of the Growth Hormone Axis

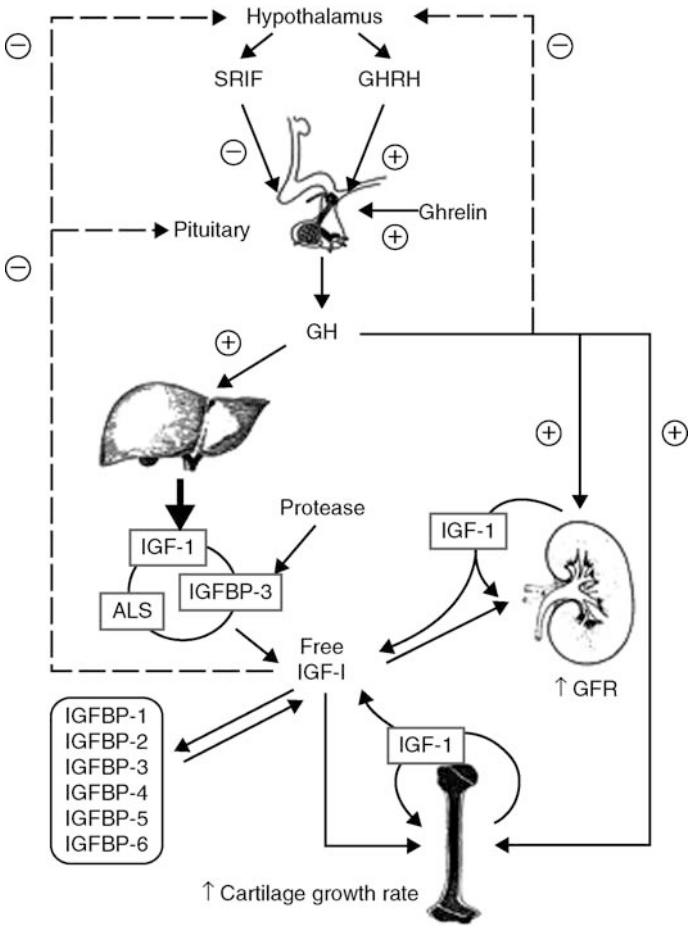
As mentioned above, growth hormone (GH) is the key endocrine influence for postnatal growth. Although a complete discussion of the regulation of the GH/insulin-like growth factor (IGF) axis, shown in *Fig. 22-15*, is beyond the scope of this text, chronic kidney disease is associated with significant alterations in this axis and, therefore, a basic understanding of the regulation of the GH/IGF axis is important when evaluating growth in children with chronic kidney disease (51). Growth hormone is released by the anterior pituitary in a pulsatile fashion under the influence of growth-hormone releasing hormone (GHRH) from the hypothalamus, which stimulates secretion, and somatostatin, which inhibits secretion. GH release is also stimulated by ghrelin, a peptide produced primarily in the stomach whose secretion is dependent on both acute and chronic changes in nutritional status (52, 53). Once released into the blood stream, GH binds to GH receptors to stimulate the production of IGF-I, formerly known as somatomedin C (51). Although GH receptors are located in many organs, the liver produces 75% of IGF-I circulating in plasma (54). Both GH and IGF-I serve in a negative feedback loop to inhibit further GH release (*Fig. 22-15*) (51, 55). The majority of IGF-I in the circulation is bound to IGF binding-protein-3 (IGFBP3) in a complex with an acid-labile subunit, while a smaller fraction is bound to 5 other IGFBP's (IGFBP1, 2, 4, 5, 6) (51). Free IGF-I in the circulation mediates most of the biologic effects of GH, by binding to the IGF-I receptor in many tissues and organs. Binding of IGF-I to IGFPB limits its bioactivity and usually less than 1% of IGF-I in blood remains in the bioactive-free form (51). Serum levels of IGF-1 vary with age, with low levels at birth and peak levels during puberty. Levels of IGF-1 decline with increasing age and may also be low in states of malnutrition, catabolism and chronic disease (56).

Growth Failure and Its Impact in Chronic Kidney Disease

Growth failure has long been recognized as a ramification of CKD (57). The typical growth pattern in a child with congenital kidney failure is shown in *Fig. 22-16*, and demonstrates that while growth during the growth-hormone-dependent growth phase in mid childhood parallels the normal curve, significant loss in terms of linear growth is seen in infancy, the nutrient-dependent growth period, and during the sex-hormone dependent pubertal growth spurt (58, 59). Reports from the North American Pediatric Renal Trials and Cooperative Study (NAPRTCS, previously known as the North American Pediatric Renal Transplant Cooperative Study) reveal that among children with CKD in the registry, 37% of all patients and nearly half of patients under the age of 5 years were less than the third percentile for height at registry enrollment (60, 61). Once end-stage is reached, growth deficits worsen and data from North America, the Netherlands and Australia consistently demonstrate that height standard deviation scores fall further from the mean over time among children who remain on dialysis (62–65). Although the restoration of kidney function with transplantation improves linear growth, data from NAPRTCS

Figure 22-15

The growth hormone (GH) and insulin-like growth factor-I (IGF-I) axis. Pituitary release of GH is controlled by GH-releasing hormone (GHRH) and somatostatin (SRIF), which in turn are regulated by feedback (dashed lines) from circulating GH and IGF-I. Ghrelin, which is produced in the stomach, also stimulates GH release. GH stimulates IGF-I production from the liver and other organs. Most of the IGF-I in the circulation is bound to IGF-binding protein-3 (IGFBP-3) in a complex with acid-labile subunit (ALS); a smaller fraction is bound to the five other IGFBP. Less than 1% of the total IGF-I in blood is in a bioactive-free fraction. Reproduced with permission from (51).

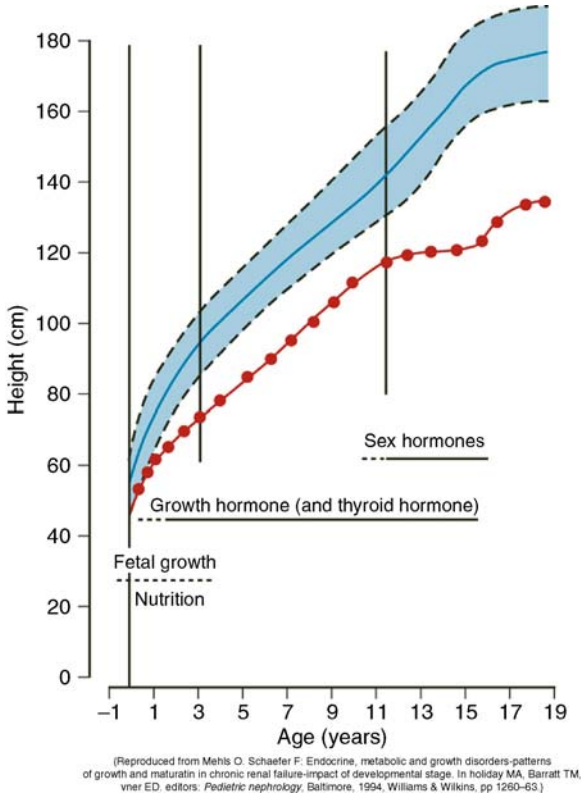


suggest that "catch up" growth is seen only in patients younger than age 6 years, thus transplantation alone does not adequately address growth impairment in children with CKD (66).

The growth failure seen in children with CKD often results in short stature in adulthood. Early data from the NAPRTCS registry revealed that among patients with CKD who were 18–20 years of age, 18.6% were less than the third percentile for height, suggesting that many patients had significant short stature as they approached their final adult height (60). International data have supported this earlier finding, with reduced adult height in 30–50% of patients with childhood CKD (67–71). Recent data, however, suggest that there may be a trend for improvement in the last decade (72, 73). In the NAPRTCS 2007 annual report, transplanted pediatric patients in the 2000–2006 cohort had an average terminal height of −0.98 z-score versus −1.97 in the 1987–1990 cohort. Studies of short stature in adulthood have demonstrated significant negative impact on quality of life (74–76). Broyer and colleagues evaluated long-term social outcomes in 244 adults who had received a kidney transplant in childhood and demonstrated that each 1 cm increase in final adult height was associated with a significantly increased likelihood of being married or living outside of the parent's home (74). In addition, patients in this

Figure 22-16

Typical growth pattern in patients with congenital chronic kidney failure is shown in red. Loss of statural height predominates in infancy and adolescence, with growth parallel to the normal curves in mid-childhood. The shaded area represents the normal range (3–97%). Reproduced with permission from (58).



cohort whose final adult height was more than 2.5 standard deviations below the mean were much less likely to be employed full time (74). Rosenkranz et al. surveyed 39 adult patients with early onset of ESRD regarding perceived quality of life (76). Thirty-six percent of patients reported dissatisfaction with their height compared to 4% of healthy age-matched controls. Positive perception of quality of life was significantly correlated with satisfaction with adult height (76).

The importance of identification and treatment of growth retardation in pediatric patients with CKD goes beyond the need to maximize final adult height, however. In fact, two retrospective cohort studies using large pediatric ESRD registries have demonstrated an association between short stature and increased risk for morbidity and mortality (77, 78). Using data from the Pediatric Growth and Development Study of the United States Renal Data Systems (USRDS), Furth and colleagues categorized pediatric dialysis and transplant patients according to growth velocity over a 1 year period; a height velocity SDS of less than -3 denoted severe growth failure, a height velocity SDS between -3 and -2 was moderate growth failure and height velocity SDS greater than -2 was normal growth (77). Over the 5 year follow up period, after adjusting for age, gender, race, cause and duration of ESRD, and treatment modality (dialysis or transplant), patients with moderate and severe growth failure had higher hospitalization rates than patients with normal growth velocity [relative risk (RR)1.24 (95% Confidence interval (CI) 1.2, 1.3); and RR 1.14 (95% CI 1.1, 1.2), respectively]. In addition, after adjustment, moderate and severe growth failure was associated with a higher risk for death (RR 2.01 (95% CI 1.1, 3.6); RR 2.9 95% (CI 1.6, 5.3), respectively) (77). Similarly, a study of 2,306 pediatric dialysis patients in the NAPRTCS registry demonstrated that short stature patients, defined as patients who were more than 2.5 standard deviations below age and gender norms for height (less than 1%) at the time of dialysis initiation, had significantly more hospital days per month of dialysis follow-up than patients with heights closer to the normal range (78). Height less than 1% was also associated with a twofold higher risk of death, even after controlling for patient age, race, gender, cause of ESRD, whether or not the patient was listed for a kidney transplant, and dialysis modality 78. Although these studies can not prove causality, they both suggest that short stature may be a marker for patients who are at increased risk for morbidity and mortality.

Etiology of Growth Impairment in Chronic Kidney Disease

Although multiple conditions are associated with poor growth in children with CKD, the mechanisms responsible for the influence of many of these factors on growth and whether there is true causality for some factors remains unclear (79–81). CKD can result in impairment in each phase of growth, from in utero to adolescence, and studies suggest that age of onset and duration of CKD are two of the most important predictors of growth failure (82–85). Not surprisingly, congenital/urologic diseases are also a leading predictor of poor growth (83). Among the other chronic kidney conditions, cystinosis and primary hyperoxaluria are associated with the most significant impairment in growth (86, 87). Multiple other factors that have been associated with poor growth in CKD are listed in *Table 22-1* (79–81, 83). A correlation between

Table 22-1

Factors Contributing to or Associated with Growth Failure in Children with Chronic Kidney Disease
Age at onset of CKD
Etiology of CKD
Stage of CKD
Protein-energy malnutrition
Inflammation
Fluid and electrolyte imbalance, including acidosis
Anemia
Disorders of bone and mineral metabolism
Renal replacement therapy modality
Treatment with corticosteroids
Disturbances in growth hormone/insulin-like growth factor I axis

Adapted from (79), (80), (81), (83)

degree of renal impairment in terms of glomerular filtration rate (GFR) and short stature has been demonstrated in some studies, but is not a consistent finding (61, 80, 81, 88, 89). This may reflect the inaccuracy of the methods used to measure GFR and/or the fact that other measures of kidney function that might affect growth, including tubular function, are not typically taken into account in these studies. Correction of acidosis and replacement of salt and fluid losses in children with tubular dysfunction has been shown to improve linear growth (90). Protein and calorie malnutrition are commonly seen in children with CKD. The impact of malnutrition on growth is particularly important in infancy, at which time growth is primarily nutrition dependent. Not only can malnutrition contribute to poor growth and development, but it may be associated with increased mortality in children with CKD. Studies using data from USRDS and the Center for Medicare & Medicaid Services' (CMS) Clinical Performance Measures Project have demonstrated an association with low serum albumin and increased risk for hospitalization and death in pediatric dialysis patients (91, 92). It should be recognized, however, that albumin is a relatively poor nutritional indicator, and the low albumin seen in children and adults with ESRD may be related to chronic inflammation, the so-called malnutrition-inflammation complex syndrome (93).

Abnormalities in bone and mineral metabolism can also result in growth impairment. While treatment of hyperparathyroidism with 1,25 dihydroxyvitamin D has been shown to improve growth, some studies suggest that normalizing intact parathyroid hormone levels may result in low bone turnover (i.e., adynamic bone disease) which, in turn, may diminish linear growth (94–96). Finally, although chronic anemia has long been recognized as a contributor to poor growth, many studies of erythropoietin treatment in children with CKD have been unable to demonstrate persistent catch-up growth (97–99). One retrospective cohort study of 47 children who initiated chronic dialysis between 1994 and 2004 demonstrated catch-up growth during predialysis care in 40% of children (100). Catch-up growth was independently associated with both hemoglobin and erythropoietin therapy at first referral. The relationship between anemia and growth requires further exploration in multi-center large studies.

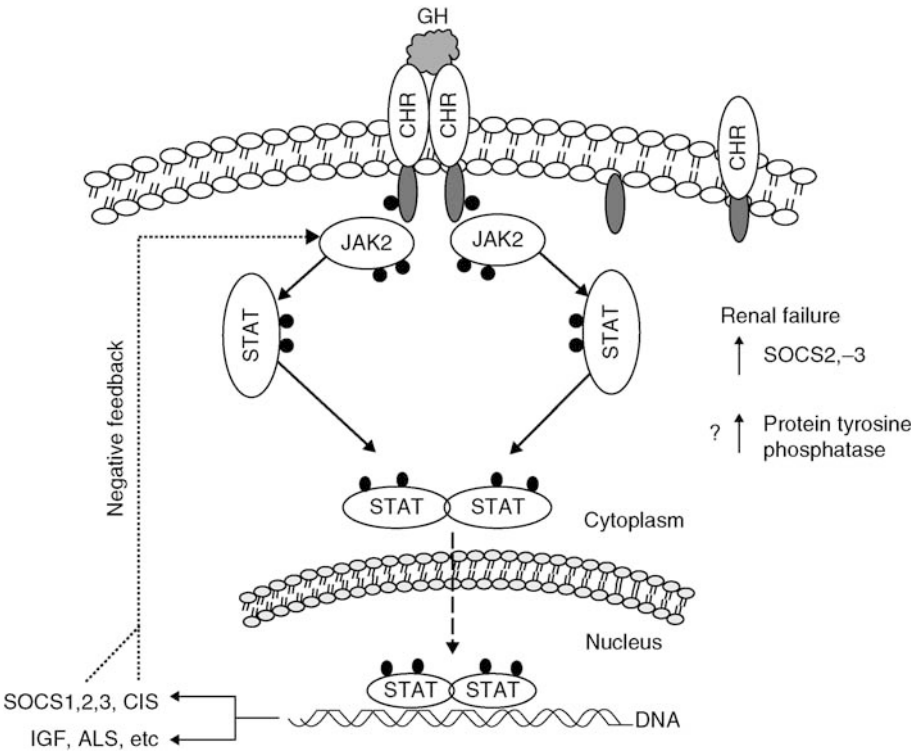
Pertubations of Growth Hormone/ Insulin-Like Growth Factor Axis in Chronic Kidney Disease

Abnormalities of the GH and IGF axis play a major role in growth impairment in CKD (51, 83, 101, 102). Circulating levels of GH are normal or even increased in children with CKD, depending on the degree of renal impairment (103). GH is primarily cleared from the circulation via the liver, reticuloendothelial system and the kidney and the metabolic clearance rate of GH correlates linearly with GFR (104, 105). GH release in children with CKD is variable, with normal or increased release documented in pre-pubertal children, but release is reduced in pubertal children possibly related to decreased stimulation of GH by gonadal hormones (104–107).

The normal to high levels of GH in CKD suggest a state of relative resistance to GH. GH resistance can be due to either reduced density of GH receptors in organs or a postreceptor signaling defect resulting in decreased IGF-I production in response to GH binding. GH receptor binding protein (GHBP) is a product of the proteolytic cleavage of the extracellular domain of the GH receptor. Circulating GHBP levels are reduced in children and adult CKD patients, and GHBP levels correlate with spontaneous growth rate and response to recombinant human growth hormone therapy in children with CKD (108, 109). However, whether GHBP levels are a reliable marker of GH receptor expression is controversial (110). Binding of GH to the GH receptor leads to activation of Janus kinase 2 (JAK2), a receptor-associated tyrosine kinase (*Fig. 22-17*, 111). JAK2, in turn, self-phosphorylates and phosphorylates signaling (signal transducer and activator of transcription or STAT) proteins. Once activated, the STAT proteins move to the nucleus and activate GH-regulated genes. The GH-induced activation of JAK2 and

Figure 22-17

Growth hormone (*GH*)-mediated JAK2/STAT signal transduction. Binding of GH to its receptor (*GHR*) activates Janus kinase2 (*JAK2*), which then self-phosphorylates followed by phosphorylation of the GHR and subsequently STAT 1a, 3, 5a, and 5b. These phosphorylated STATs form dimers that enter the nucleus where they activate target genes including insulin-like growth factor-1 (*IGF-1*) and some suppressors of cytokine signaling (*SOCS*). Phosphorylation of JAK2 and the downstream signaling molecules STAT5, STAT3, and STAT1 and the nuclear levels of phosphorylated STAT proteins are impaired in uremia. Uremia is also associated with upregulation of SOCS. Reproduced with permission from (111).



several of the downstream STAT proteins are reduced in chronically uremic rats (111). In addition, the JAK2/STAT pathway is in part regulated by suppressor of cytokine signaling (SOCS). The term SOCS refers to proteins that are induced by GH and serve as negative feedback by binding to JAK2 and inhibiting STAT phosphorylation (111, 112). SOCS are up regulated in inflammatory states, and it has been suggested that this may be one of the mechanisms involved in the malnutrition-inflammation complex syndrome observed in patients with CKD (113).

In addition to GH resistance, there is evidence that resistance to IGF-I plays an important role in the growth retardation seen in CKD (83, 101). Circulating levels of IGF-I are normal in children with CKD and only mildly decreased in children with ESRD (109). However, IGFBP levels are increased, thereby reducing the amount of bioactive-free IGF-I available (109, 114, 115). The increase in the levels of the IGFBPs is inversely correlated with level of kidney dysfunction, and the molar excess of IGFBPs as compared with IGFs is 200% in children with ESRD and 150% in children with CKD, compared to 25% in healthy children (116). Not only is the level of bioactive-free IGF-I reduced, but there is also evidence that there may be a post-receptor defect of IGF-I signaling which could contribute to the state of IGF-I resistance (83, 117).

Lastly, growth and pubertal development in kidney disease are also impacted by abnormalities in gonadotropin hormones. Normally, leutinizing hormone (LH) is released from the pituitary gland in pulsatile bursts in response to hypothalamic GnRH secretion. Like GH, LH is metabolized by the liver, macrophages and kidney (105). Consequently, in CKD and ESRD, renal clearance of LH is reduced and LH levels may be elevated (118). However, pituitary LH secretion is also altered in kidney disease such that pubertal patients on dialysis produce 70% less immunoreactive LH and 60% less bio-active

LH overnight than their healthy peers (105, 118). The ratio of bioactive LH to immunoreactive LH is also decreased in uremic children (118). Additionally, it has been shown that patients with kidney disease have decreased frequency of plasma LH pulses. This is believed to be secondary to blunted GnRH secretion from the hypothalamus (119). In rat models, uremic serum has been shown to selectively inhibit the hypothalamic GnRH pulse generator (119).

Treatment of Impaired Growth in Chronic Kidney Disease

Although abnormalities of the GH/IGF-I axis are significant contributors to growth retardation in chronic kidney disease, given the multifactorial nature of this problem, correction of any of the possible contributors to growth failure should be attempted. In fact, the National Kidney Foundation (NKF) Kidney Disease Outcomes Quality Initiative (KDOQI) guidelines recommend correction of any nutritional deficiencies or metabolic derangements prior to initiation of recombinant human growth hormone (120). This may include dietary supplementation, including tube feedings in those patients with insufficient intake of protein, energy or other nutrients (120). Delivery of adequate alkali therapy, salt and water to correct deficits should be provided if necessary (120, 90). Finally, abnormalities of bone and mineral metabolism should be treated, although as stated previously, the optimal level of parathyroid hormone control to promote maximum growth is unclear (94–96, 102).

Several studies have demonstrated that treatment with recombinant human growth hormone (rhGH) in children with CKD is safe and efficacious and its use is now considered standard of care (102, 121, 122). A recent Cochrane review included 15 randomized control trials of rhGH treatment in children with CKD, including those on dialysis and status post-kidney transplant, and demonstrated that rhGH treatment resulted in significant improvement in height SDS at 1 year and a significant increase in height velocity at 6 months and 1 year (123). Collectively in these studies, the frequency of reported sides effects were not different between rhGH-treated subjects and the control group (123). However, the impact of rhGH treatment on adult height in pubertal patients has not been clearly delineated, and growth response in pre-pubertal patients in multiple studies has been variable (123–127). In a recent report of the Pfizer International Growth Database (KIGS), Nissel and colleagues evaluated growth and near-final adult height in 240 children with CKD who had received rhGH for at least 1 year and who had reached near-final adult height, defined as height velocity less than 1 cm/year, advanced clinical signs of puberty in boys with an age of at least 16 years and in girls of at least age 14 years (124). The study demonstrated that 40% of patients achieved a final adult height within 2 standard deviations of the norm. Near-final height was positively associated with initial growth deficit and duration of rhGH therapy, but was negatively associated with proportion of time spent on dialysis (vs. conservative therapy or transplant), delayed puberty, female gender and age at initiation of rhGH. The authors commented that the finding that delayed puberty and dialysis therapy are associated with a clear reduction in response to rhGH should prompt care-providers to consider treatment early in the course of CKD and in early childhood. Unfortunately despite these warnings and the well-documented success of rhGH treatment in children with CKD, underutilization of this medication has been repeatedly documented (84, 128). A recent Consensus Committee statement recognized that the lack of clear guidelines for the evaluation and treatment of growth failure in children with CKD are contributing to this underutilization, and provided a proposed algorithm in an effort to address this important need (*Fig. 22-18*, 102).

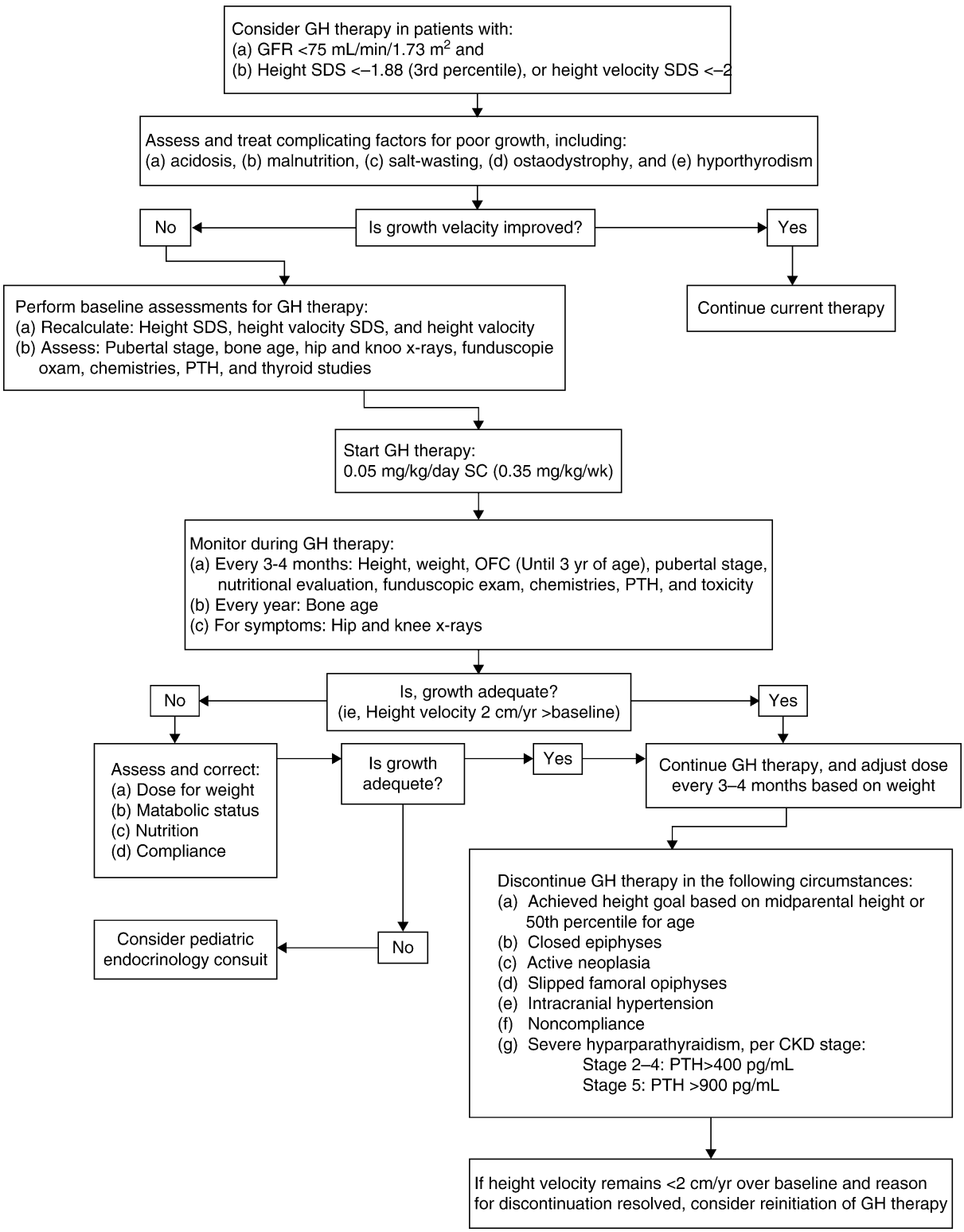
Future targets for treatment of GH resistance include use of recombinant IGF-I (rhIGF-I), either alone or in combination with rhGH or recombinant IGFBP3, which might mitigate the possible suppression of endogenous GH, IGFBP3 or ALS production by rhIGF-I (101, 129–131). A final potential therapeutic agent is IGFBP3 displacers which could increase free, or bioactive IGF-I levels (132).

Conclusion

In summary, kidney disease has a significant impact on growth. Impaired growth in turn stems from many complex and interacting factors and may lead to increased morbidity and mortality. Thus, proper and frequent assessment of growth in children with kidney impairment is vital. Recognizing growth perturbation in its early stages and treating its causes can have significant influence on the long-term medical and psychosocial outcomes of children with kidney disease. Recombinant growth hormone use should be instituted when appropriate and further research should focus on other potential targets for treatment of GH resistance.

Figure 22-18

Proposed algorithm for evaluation and treatment of growth failure in children with CKD. Reproduced with permission from (102).



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23 Diagnostic Imaging

Stephen F. Simoneaux · Larry A. Greenbaum

Diagnostic Procedures

Imaging is essential for the diagnosis and management of many disorders of the urinary tract (UT). Clinicians should be familiar with the available techniques, including their indications, limitations, and potential complications.

Ultrasound (US) is the most widely used modality because of the information provided, safety, and low cost. Intravenous pyelography (IVP) is currently seldom utilized, but voiding cystourethrography (VCUG) and nuclear medicine studies continue to have important roles. Computed tomography (CT) is readily available and can acquire images rapidly, generally obviating the need for sedation. Magnetic resonance imaging (MRI) generates anatomically precise images without radiation exposure, but at high direct financial cost, and with the need for sedation in young children. Imaging studies should be carefully selected, with consideration of diagnostic utility, cost, and potential complications such as contrast nephropathy and radiation exposure.

Ultrasound

US is the principal imaging modality for visualization of the UT. US provides a rapid assessment of kidney shape and size, and it is especially good at identifying hydronephrosis. Studies are completed without discomfort or need for sedation, and there are no known complications or side effects. In addition, US is relatively inexpensive and readily available. The skill of the examiner can significantly influence the information obtained via US. Lack of patient cooperation and large body habitus may also limit image quality.

Because of its low cost and the absence of side effects, US is the optimal modality for clinical situations requiring repeated evaluations. For example, US can assess hydronephrosis before and after an intervention such as a catheter, stent, or nephrostomy tube placement. US is also ideal as a screening examination for children who need surveillance for kidney stones or malignancy.

Technique

Images are generally obtained with the patient supine, which optimizes visualization of the upper poles, using the spleen and liver as acoustic windows. The contralateral decubitus position may permit better visualization in some patients. In certain situations, placing the patient in the prone position may allow for better visualization of the lower pole if subtle pathology is present. Ideally, the bladder should be visualized while filled with urine. A cooperative child should be asked not to void for a few hours prior to the exam. In a patient who is not toilet trained, the bladder should be visualized first to allow imaging the bladder before the patient voids.

US transducers produce sound waves of varying frequencies. High frequency sound waves do not penetrate tissue as deeply, but provide greater resolution. In most situations, the sonographer should use the highest frequency that penetrates to the desired depth. Hence, high-frequency US permits excellent resolution in infants and small children. Conversely, resolution is decreased in very large or obese individuals.

US evaluation of the UT should include measurement of renal lengths and assessment of renal shape, thickness and echogenicity of the cortex, the presence of corticomedullary differentiation, and evaluation of the renal pelves, ureters, and bladder. The ureters are not usually visible, but may be seen when dilated due to underlying pathology.

There are published standards for US determination of renal length for fetuses, preterm infants, full-term neonates, infants and older children (1–6 ❯ *Table 23-1*). Length measurements should be made with the patient supine or in the contralateral decubitus position; length may be underestimated with the patient prone (7, 8). Gestational age and birth weight correlate with kidney length in newborns (1, 4). In older children, age is a convenient parameter, but length or height is a better predictor of normal kidney length (3, 9, 10). (❯ *Fig. 23-1a* (10)) A multivariable approach allows for improved prediction equations for renal length (11), but such equations are not widely used in clinical practice.

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Table 23-1
Renal length

Age	Mean length (cm)	Range (±2 SD in cm)
Term newborn	4.48	3.86–5.10
2 months	5.28	3.96–6.60
6 months	6.15	4.81–7.49
1.5 years	6.65	5.57–7.73
2.5 years	7.36	6.28–8.44
3.5 years	7.36	6.18–8.54
4.5 years	7.87	6.87–8.87
5.5 years	8.09	7.01–9.17
6.5 years	7.83	6.39–9.27
7.5 years	8.33	7.31–9.35
8.5 years	8.90	7.14–10.66
9.5 years	9.20	7.40–11.00
10.5 years	9.17	7.53–10.81
11.5 years	9.60	8.32–10.88
12.5 years	10.42	8.68–12.16
13.5 years	9.79	8.29–11.29
14.5 years	10.05	8.81–11.29
15.5 years	10.93	9.41–12.45
16.5 years	10.04	8.32–11.76
17.5 years	10.53	9.95–11.11
18.5 years	10.81	8.55–13.07

Adapted from Robenbaum et al. (6)

In adults and children, the average left kidney is slightly longer than the average right kidney (3, 4, 9), although this is only observed in slightly more than 50% of measurements of individual patients (the remainder have kidneys of equal length or a longer right kidney) (10). In infants and adults, the median left renal length is about 3 mm longer than the median right renal length (3, 12). In adults and older teenagers, males have longer kidneys, which is explained by their increased height compared to females. Black children have shorter kidneys than white children (11).

Measurement of renal length in children has a fairly high rate of intraobserver and interobserver variation (13); hence, US measurement of renal length lacks the accuracy to allow assessment of renal growth over limited time periods. Measurement of the left kidney length is less accurate, probably because the spleen provides a smaller acoustic window than the liver. Nevertheless, renal length discrepancy in children can be used to predict abnormalities on Technetium 99m-dimercaptosuccinic acid (^{99m}Tc-DMSA) renal scintigraphy (14).

The appearance of the kidneys changes with age. The relative volume of medullary tissue compared to cortical tissue is increased in neonates and infants (*Fig. 23-1b*) (15). Cortical echogenicity is increased in the term newborn kidney, with the majority having the same echogenicity as the liver or spleen (5). Renal cortical echogenicity is further increased in premature infants, with some kidneys having cortical echogenicity greater than the echogenicity of the liver or spleen (16). Cortical echogenicity decreases after birth, and only a small percentage of normal kidneys of term infants have cortical echogenicity equivalent to the liver or spleen by 6 months. Cortical echogenicity is compared to the liver or spleen, but this comparison may not be valid if there is underlying liver disease. Transient increased cortical echogenicity of the kidneys may occur in children with a variety of non-renal illnesses (17).

The kidneys of term newborns have prominent medullary pyramids that are relatively hypoechoic (15). This results in marked corticomedullary differentiation. The echogenicity of the medullary pyramids increases after birth and prominent medullary pyramids are not normally present by 1 year (*Fig. 23-2*). In contrast, the central sinus echo is minimal at birth and gradually increases during the first 10 years of childhood (5).

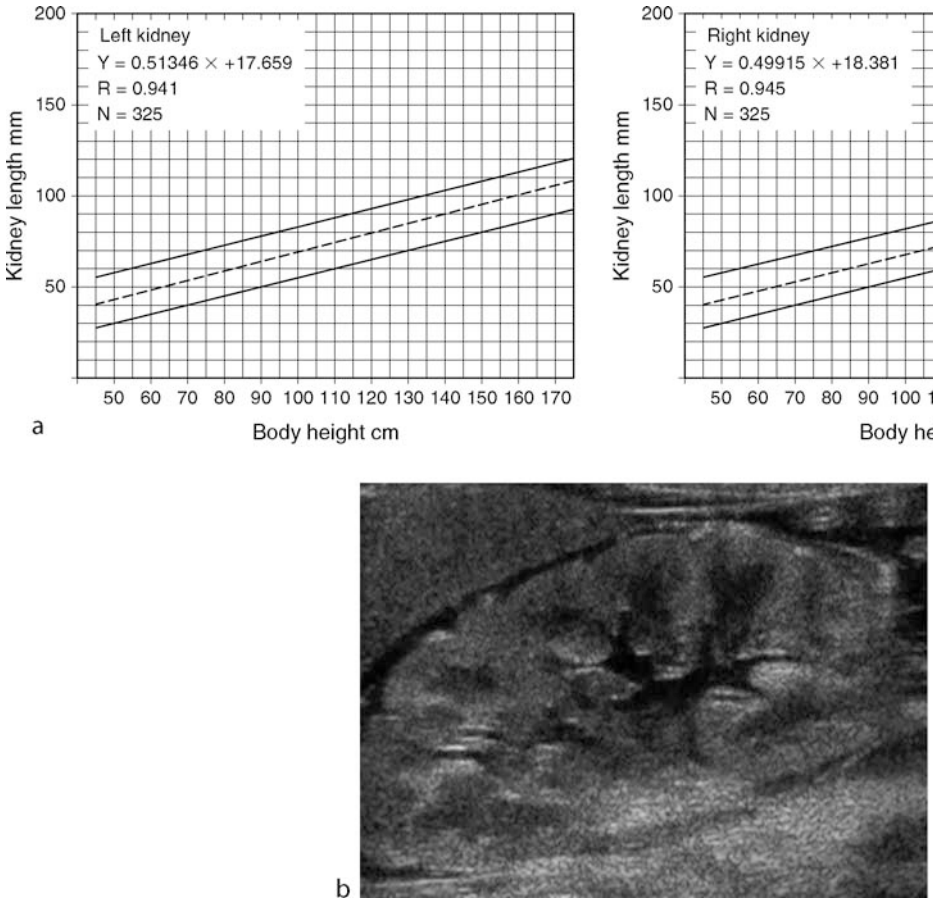
Hydronephrosis may indicate obstruction or vesicoureteral reflux (VUR), or it may be a normal variant, especially when mild. The Society for Fetal Urology developed a grading system for hydronephrosis that is predictive of the presence or absence of obstruction (*Table 23-2*) (18, 19). Mild hydronephrosis is common in newborns, and occurs in a higher percentage of males than females (20).

An increase in bladder wall thickness may be an indication of pathology, especially chronic increased bladder pressure. Measurement of bladder wall thickness is highly dependent on the degree of bladder filling, but does not appear to be dependent on patient age or gender (21), although male neonates may have slightly greater bladder wall thickness than females (20). The upper limit of normal is 5 mm for an empty bladder and 3 mm for a well distended bladder (21).US can be used to accurately estimate bladder volume (22, 23). US measurement of bladder volume after voiding is a useful parameter for assessing bladder function. US may detect ureteroceles or bladder masses.

Doppler US permits evaluation of blood flow, and has been utilized to identify renal artery disease, renal vein thrombosis, tumor thrombosis in the renal vein

Figure 23-1

(a) Length of both kidneys related to body weight. Mean values and the 95% regions of tolerance are determined by routine statistical analysis of 325 children; (b) Normal ultrasound of a neonatal kidney (prone view). Medullary pyramids are hypoechoic with a relatively thin cortex.



and inferior vena cava, and arteriovenous fistulas (24–26). Resistance to blood flow can be quantified by measuring the resistive index (RI), which is calculated from the formula: RI = (peak systolic velocity-end diastolic velocity)/peak systolic velocity. A normal RI is below 0.7 in adults and children who are 6 years of age or older, but higher values are observed in children less than 6 years (27).

Power Doppler mode allows increased sensitivity for blood flow detection, but does not provide information on flow direction or velocity. Power Doppler US is superior to color Doppler US in detecting intrarenal blood flow (28), and in identifying areas of decreased perfusion within the kidney (29). Power Doppler increases the sensitivity of US for detecting acute pyelonephritis (30). Power Doppler is very sensitive to motion artefact and hence requires a very cooperative patient, limiting its utility in young children.

Voiding urosonography (VUS) is an alternative technique for detecting VUR that uses intravesical instillation of an US contrast agent (31, 32). The principal advantage is the elimination of radiation exposure, but bladder catheterization is still necessary. VUS does not provide adequate imaging of the bladder and urethra, and thus should only be used for assessment of VUR. The results with VUS correlate well with conventional VCUG (31, 33–36). It is not yet clear if operator skill or other barriers will limit the widespread use of this technique.

Figure 23-2

Normal ultrasound of the kidney in a 17 year old child (prone view). The renal pyramids are more isoechoic to the pyramids than in a neonatal kidney (*Fig. 23-1b*).

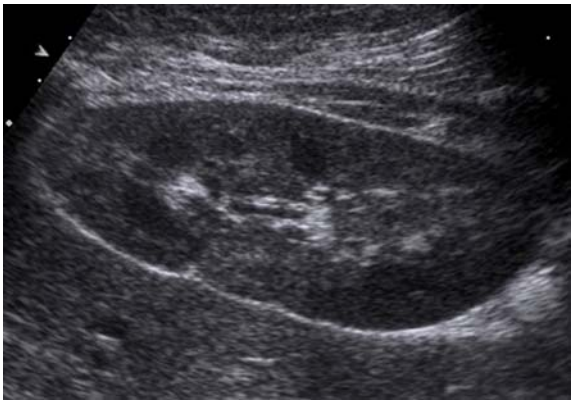


Table 23-2

Society of Fetal Urology grading system for hydronephrosis

Grade	Ultrasound
0	No hydronephrosis
1	Only renal pelvis is seen
2	Renal pelvis and a few calices are seen
3	Virtually all calices are seen
4	Similar to grade 3, but parenchymal thinning present

Application

US is the initial imaging modality in a variety of clinical situations, including evaluation of renal failure, kidney transplant dysfunction, hematuria, screening for renal malignancy, abdominal mass, and follow-up of abnormal prenatal US.

Voiding Cystourethrography

VCUG is the gold standard for the diagnosis of VUR, and most clinical studies of VUR have utilized VCUG. Moreover, VCUG is the only modality that detects VUR and provides detailed information about the bladder and urethra.

Radiation exposure is the most important side effect of VCUG. The radiation dose has been reduced through technological innovations, and a variety of additional approaches are available to further reduce radiation exposure (37, 38). A known allergy to contrast material is a contraindication to the study (39). Because of the need for catheterization, VCUG creates a small risk of iatrogenic infection and trauma to the urethra, although these risks are low (40). In addition, many children and families find catheterization frightening and emotionally traumatic. Sedation with midazolam appears safe and effective (41–43).

Technique

VCUG is done using fluoroscopy. An initial film prior to bladder catheterization detects calcifications or bony abnormalities; an abnormal bowel gas pattern may suggest a mass. The bladder is sterilely catheterized using a feeding tube (5 F in young infants; 8 F in older children) and urine can be obtained for culture. After emptying the bladder of urine, contrast material is infused to fill the bladder based on estimates of bladder capacity, but using gravity to avoid overfilling. The bladder must be filled to capacity to insure an adequate study.

An early film during filling of the bladder is useful for identifying filling defects, such as caused by a ureterocele or bladder tumor. Films are taken before and after voiding, with VUR graded based on the International Reflux Study in Children (44). In young children, who may not allow their bladder to fill to capacity, the sensitivity of VCUG for detecting VUR improves with multiple cycles of bladder filling and voiding (45, 46), but this increases the length and radiation exposure of the procedure. A film during voiding permits visualization of the urethra and is essential in male children (*Figs. 23-3* and *23-4*). At the end of voiding, a final film identifies residual urine and previously unidentified VUR.

Intravenous Pyelography

The availability of US and other imaging modalities has dramatically decreased the use of intravenous pyelography (IVP) (47). The risks of IVP include radiation and contrast exposure; intravenous access is necessary.

Technique

An initial supine film is performed prior to contrast injection. Approximately 5–7 min after contrast, a film

Figure 23-3
Voiding cystourethrogram. Frontal voiding image of normal 8 month old girl.

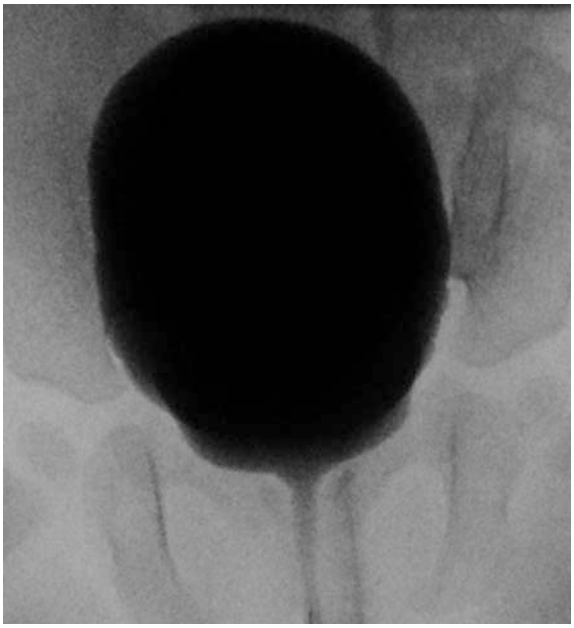
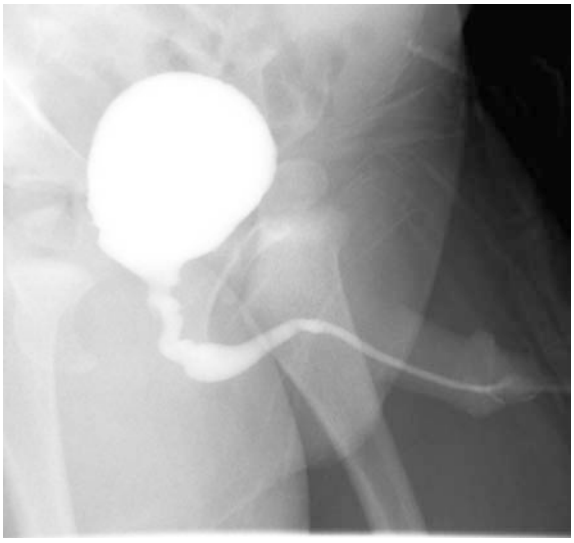


Figure 23-4
Voiding cystourethrogram. Oblique voiding image of a normal 5 month old boy.



centered on the kidneys allows visualization of the nephrogram and the calices. Subsequent films are full length and taken 15 and 30 min after injection. Delayed films are useful in cases of urinary obstruction. Bowel preparation does not improve image quality (48, 49), although a short fasting period is usually recommended. The radiation exposure of IVP can be reduced by adjusting the number of films based on the clinical question and the results of initial films. Nonionic contrast medium improves image quality and decreases the risk of contrast nephropathy.

Application

Poor renal function limits the utility of IVP due to inadequate contrast accumulation in the kidneys. Moreover, poor renal function increases the risk of contrast nephropathy. IVP is currently rarely used in the evaluation of obstructive disease; US and nuclear medicine studies are the preferred imaging modalities. ^{99m}Tc-DMSA has replaced IVP for detection of renal scarring, but IVP provides excellent detail and is certainly an acceptable substitute (50, 51). IVP is useful for evaluation of ectopic ureters, ureteroceles, and fusion anomalies, although alternative imaging (US, MRI, CT) is generally utilized when available.

Computed Tomography

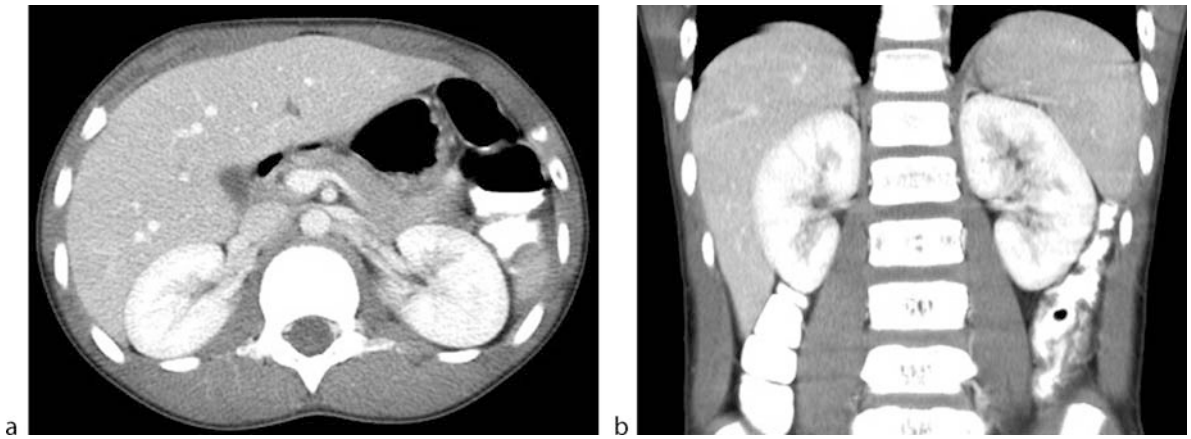
CT provides excellent anatomic resolution of the UT (*Fig. 23-5*), and CT has many applications. Radiation exposure is an important risk of CT, and should limit its use to cases where the detail provided outweighs the risk of the study (52, 53). IV contrast is necessary for optimal studies in most situations. Multiple strategies and innovations have reduced the radiation dose with CT (52, 54), but it remains an important concern (55). Sedation is currently rarely necessary due to rapid image acquisition (56, 57), which also decreases motion artifact (54).

Technique

Thin-section helical imaging, now performed on all CT scanners, allows for multiplanar reformatted images to be obtained quickly and displays the kidneys, ureters, and bladder in three planes. Oral contrast, while often unnecessary for evaluation of the UT, is useful for examinations where differentiating bowel from pathology is necessary. Time constraints (acute trauma) or risk of aspiration may prevent the use of oral contrast. For younger children,

Figure 23-5

CT of a normal 11 year old boy. (a) Axial image through the midpole of both kidneys, (b) coronal image.



excessive movement can be prevented by using blankets or adhesive tape.

Intravenous contrast is usually indicated, with the notable exception of nephrolithiasis evaluation. Low osmolality nonionic contrast is preferred; contrast is dosed based on weight (54, 58). Neonates and infants require a longer delay from contrast injection to imaging.

Application

CT is the preferred modality for initial evaluation of possible symptomatic nephrolithiasis, although US is generally utilized for surveillance to minimize radiation exposure. CT is used for renal tumor staging, and is the optimal modality when trauma of the UT is suspected. CT provides excellent resolution of renal scarring, but ^{99m}Tc-DMSA is more commonly used. CT angiography is useful in assessing for renal artery stenosis (59, 60).

Nuclear Medicine

A variety of radiotracers are utilized for imaging of the UT (61, 62). The radiotracers emit photons, which are detected by a scintillation detector. Nuclear medicine imaging does not provide anatomic detail; it provides functional data that is typically utilized to answer a specific question. Nuclear medicine is often a complementary technique. While there is radiation exposure, it is less than other modalities such as VCUG, IVP, or CT.

Technique

Dynamic Renal Scintigraphy

Technetium 99m-diethylene triamine pentaacetic acid (^{99m}Tc-DTPA) and Technetium 99m-mercaptoacetyltriglycine (^{99m}Tc-MAG$_3$) are the radiotracers currently utilized for dynamic renal scintigraphy. ^{99m}Tc-DTPA is primarily excreted via glomerular filtration; it is not reabsorbed or secreted by the tubules; its clearance is slightly lower than inulin. ^{99m}Tc-MAG$_3$ is predominantly excreted via tubular secretion. For most indications, ^{99m}Tc-MAG$_3$ is superior to ^{99m}Tc-DTPA because ^{99m}Tc-MAG$_3$ is more rapidly excreted and thus provides a superior renal/background ratio.

Patients are encouraged to consume liquids immediately prior to the procedure and should ideally void before injection of isotope. The uncooperative child should be immobilized, but sedation is rarely necessary. Images are obtained with the patient supine; the scintillation detector is below the patient for native kidneys and above the patient for renal transplants. After bolus injection of radiotracer, dynamic images are recorded for 30 min. The initial images assess renal perfusion, and later images evaluate excretion. Differential renal function is calculated using the early images, typically during the second minute, prior to radiotracer appearance in the collecting system.

Diuresis scintigraphy is performed to evaluate a patient for renal obstruction (63, 64); use of a diuretic is only necessary if radiotracer does not rapidly drain from the renal pelvis during initial imaging. A dilated renal pelvis that is not obstructed may have slow drainage of radiotracer due to the increased pelvic volume. Administration

of diuretic allows differentiation of an obstructed kidney from a non-obstructed dilated renal pelvis. Furosemide is given 15–20 min after administration of radiotracer.

Following administration of diuretic, radiotracer drains rapidly from a non-obstructed renal pelvis (*Figs. 23-6* and *23-7*); there is limited washout of radiotracer if obstruction is present. A half-life of radiotracer disappearance greater than 20 min indicates obstruction (65). There is a good correlation between the test results and histological changes (66), and the test is sensitive and specific (65, 67). Diuresis scintigraphy is accurate in newborns, despite their lower glomerular filtration rate (67, 68), although follow-up imaging of normal results is necessary to ensure that obstruction does not subsequently develop (69). Potential causes of false positive results are a massively dilated renal pelvis or a kidney with very poor function (70), but technical modifications may allow for more accurate assessment of a poorly functioning kidney (71–73). False positive results can also be decreased by obtaining images after the patient voids to avoid bladder pressure effects and after placing the child in the erect position to maximize gravitational drainage (74–76).

Cortical Scintigraphy

^{99m}Tc-DMSA is the preferred radiotracer for imaging of the renal cortex (77, 78). ^{99m}Tc-DMSA is taken up by the cells of the proximal tubule, where it binds to sulfhydryl groups (*Fig. 23-8*). The accumulation of ^{99m}Tc-DMSA in the kidney is relatively slow; hence, imaging is typically performed 1.5–3 h after injection. Diagnostic accuracy may improve with an increased delay in imaging if renal function is significantly impaired. Posterior and left and right posterior oblique views should be obtained (77, 78), but there is minimal loss in diagnostic accuracy if only a posterior view is possible in a restless child (79). Anterior imaging is preferred for pelvic or horseshoe kidneys. Additional pinhole images are useful for improving resolution when imaging infants.

A variety of factors may complicate interpretation of ^{99m}Tc-DMSA imaging. Fetal lobulations may give the false impression that there are areas of decreased radiotracer uptake. The splenic impression explains flattening of the superolateral surface of the left kidney (78). The poles of normal kidneys may have relatively decreased uptake of radiotracer. Nevertheless, there is good interobserver agreement in the interpretation of ^{99m}Tc-DMSA scans for scarring and acute pyelonephritis (80, 81).

^{99m}Tc-DMSA is not specific for scars or acute pyelonephritis. Alternative explanations for defects such as cysts, hydronephrosis, infarcts, masses, or a dysplastic half of a duplex kidney can only be identified using alternative imaging strategies (e.g., US). Children with proximal tubulopathies have decreased uptake of ^{99m}Tc-DMSA and increased urinary excretion (82).

Single photon emission computed tomography (SPECT) is an alternative to planar scintigraphy. SPECT

Figure 23-6

Posterior image of a normal ^{99m}Tc-MAG$_3$ renal scan in an 8 year old with hydronephrosis by ultrasound.

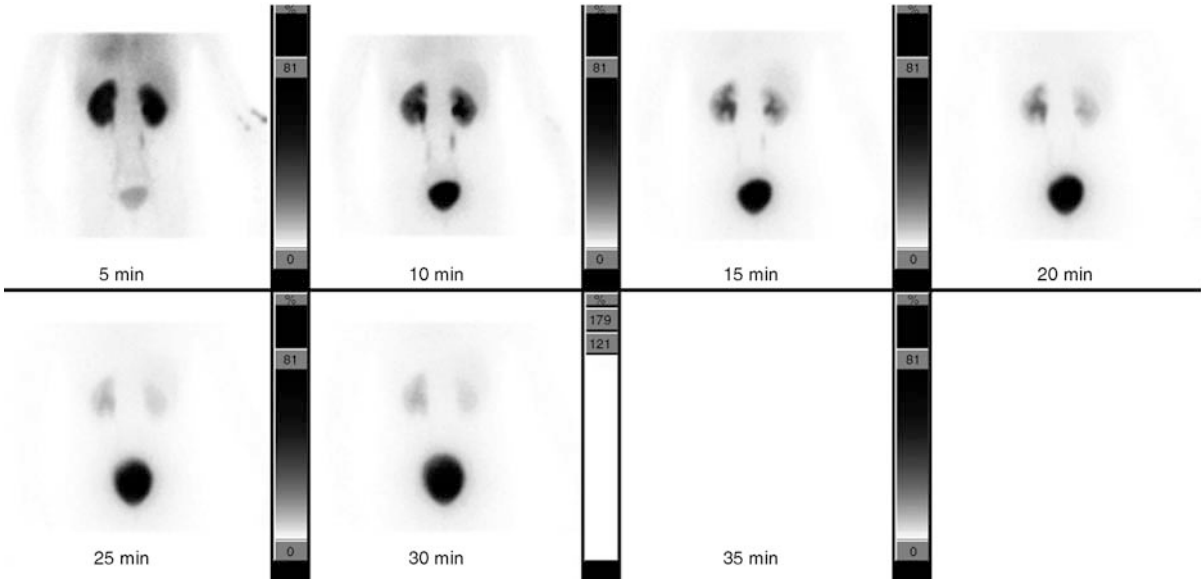
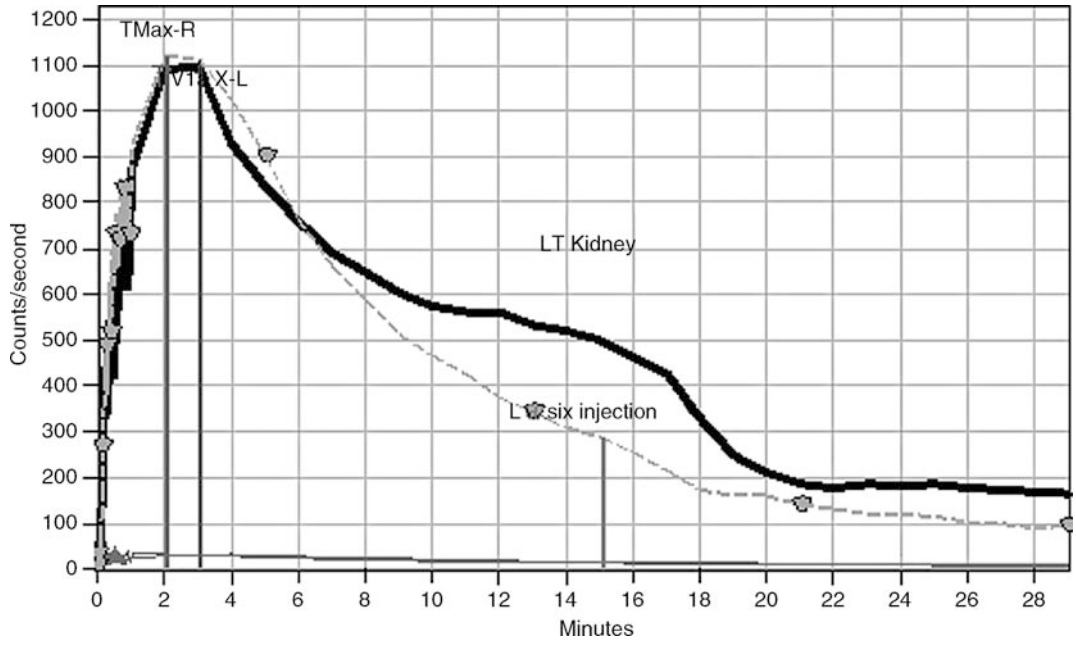


Figure 23-7

Time-activity curve from ^{99m}Tc-MAG$_3$ renal scan showing symmetric prompt excretion of radiotracer, indicating no obstruction.



appears to offer superior sensitivity for detecting renal scars (83, 84), although with a higher rate of false positive results (85, 86). The clinical relevance of the increased detection of small scars via SPECT is not clear. An intrarenicular septum, a common normal variant, may lead to a photopenic area with SPECT, but can be differentiated from a scar because of preserved cortical uptake (87). The increased imaging time required with SPECT may necessitate sedation in young children.

Split function is determined by outlining the appropriate regions of the kidneys and subtracting the activity of an appropriate background area in the posterior image. Normal split function is 50% +/−6%. Estimates of split function must be corrected for attenuation if there is an anteriorly placed ectopic kidney. Such correction is not accurate with a pelvic kidney due to the effect of the pelvic bone. Imaging may need to be delayed for up to 24 h in an obstructed kidney because isotope accumulation in the renal pelvis may cause an error in estimating split function, with an overestimation of the function of the obstructed kidney if the image is obtained prematurely. A similar approach may be necessary for a nonobstructed, severely dilated renal pelvis, although injection of furosemide, with the subsequent diuresis clearing radiotracer from the dilated pelvis, is an alternative strategy. Estimates of split function are reliable, with good interobserver agreement, except in cases where renal function is significantly impaired (88).

Adrenal Gland Scintigraphy

Meta-iodobenzylguanidine (MIBG), when labeled with either ^{131}I or ^{123}I, is used for the detection of catecholamine-secreting tumors, including pheochromocytomas and neuroblastomas (89–91). MIBG, a guanethidine analog that structurally resembles norepinephrine, is taken up by sympathetic nerve cells. The patient should receive oral nonradioactive iodine before administration of radiolabeled MIBG to compete with free radioactive iodine, minimizing radioactive exposure to the thyroid gland. A variety of medications interfere with MIBG uptake into tissue and should be discontinued prior to MIBG imaging (*Table 23-3*). MIBG radiotracer is injected, with images obtained at various times depending on the isotope. MIBG accumulates in a variety of organs, including the liver, heart and salivary glands; isotope is detected in the kidney and bladder due to renal excretion. Tumors can be identified because of the stronger radiotracer uptake, which is typically focal. ^{123}I has a much lower radiation burden than ^{131}I and results in superior images (92).

Figure 23-8

Posterior image of normal kidneys on DSMA scan in a 3 year old with a urinary tract infection.

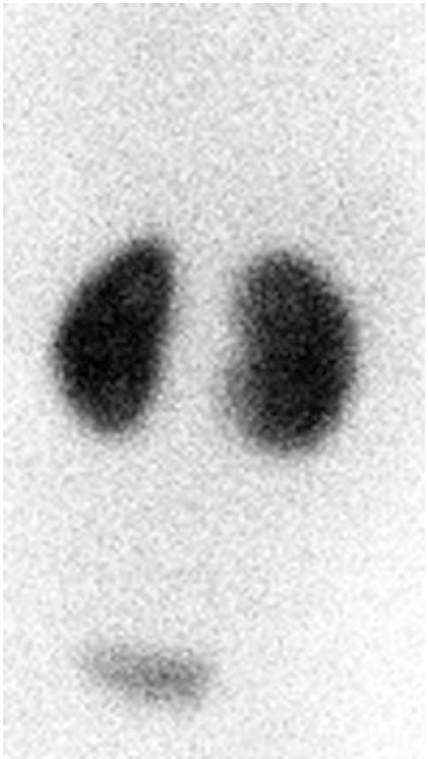


Figure 23-9

Radionuclide Cystogram. Posterior image from a nuclear cystogram in a 4 year old demonstrating left vesicoureteral reflux.

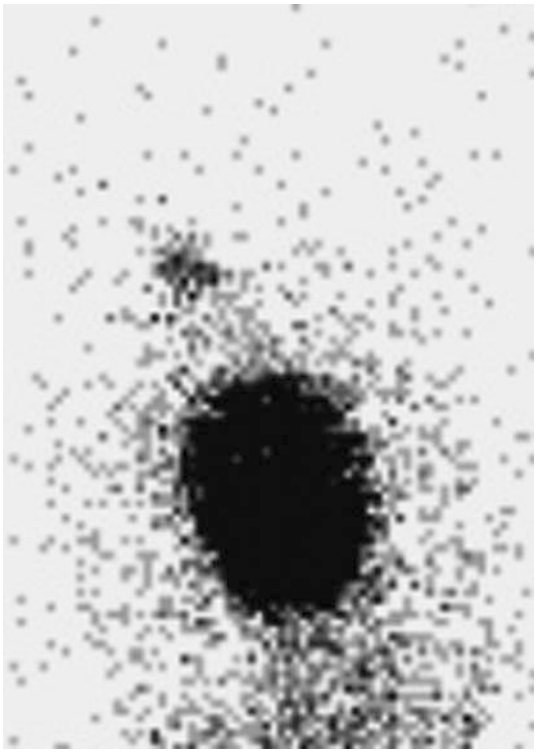


Table 23-3

Drugs that interfere with uptake of MIBG

Propranolol
Labetalol
Calcium channel blockers
Phenylephrine
Pseudoephedrine
Tricyclic antidepressants
Phenothiazines
Monoamine oxidase inhibitors

Radionuclide Cystography

Radionuclide cystography (RNC) is an alternative to VCUG for the detection of VUR (*Fig. 23-9*) (93–95). In RNC, a catheter is placed into the bladder and a solution containing a radioisotope is infused, with complete filling of the bladder. RNC, because it allows for continuous monitoring, is more sensitive at detecting VUR than VCUG, which cannot utilize continuous monitoring due to radiation concerns. Furthermore, the radiation burden is considerably less than VCUG. However, radionuclide cystography does not permit evaluation of the bladder or the urethra.

Applications

^{99m}Tc-DMSA is the gold standard for the identification of pyelonephritis and renal scars. ^{99m}Tc-DMSA can be utilized to determine split renal function and to identify and characterize renal infarcts or anatomically abnormal kidneys such as horseshoe kidneys, pelvic kidneys and crossed fused ectopia (96, 97).

Dynamic renal imaging with ^{99m}Tc-MAG or ^{99m}Tc-DTPA permits evaluation of renal blood flow, split renal function, kidney location, and urinary drainage. Dynamic renal imaging allows determination of whether hydronephrosis is secondary to renal obstruction or an alternative

etiology such as VUR, high urine flow, or relieved obstruction (98). Dynamic renal imaging can be utilized to determine split renal function and to identify renal artery stenosis when used with captopril (99).

Magnetic Resonance Imaging

MRI, with its ability to image the abdomen in multiple planes and with sequences showing different signal characteristics, provides excellent resolution of the UT without radiation exposure (*Figs. 23-10* and *23-11*) (100). Gadolinium-based contrast at standard doses is significantly less nephrotoxic than iodinated contrast (101, 102), but gadolinium-based contrast is nephrotoxic when used at radiographic doses for angiographic procedures (103–105). Gadolinium-based contrast is the dominant risk factor for the development of nephrogenic systemic fibrosis (NSF) (106), an entity that occurs almost exclusively in patients with end stage renal disease or severe renal impairment. Most cases of NSF have been associated with gadodiamide, although other gadolinium-based contrast agents may lead to NSF (107). Gadolinium-based contrast should be used cautiously in patients with moderate to severe renal impairment (108).

Sedation is typically necessary for infants and young children. MRI is an expensive imaging modality, but it may replace multiple other studies and provide superior resolution in certain clinical situations.

Technique

Magnetic resonance urography (MRU) provides superb anatomic detail combined with functional information (109). In this technique, a gadolinium chelate (Gd-DTPA) is injected and the kidneys are imaged over time. With post-processing of data derived from the study, differential renal function, transit time of the agent through the kidney, and an estimate of glomerular filtration rate can be determined. These techniques are new and depend on meticulous technique, but have great promise for the future of renal imaging.

Application

MRI is valuable in evaluating masses, renal vascular disease, cystic lesions, and congenital abnormalities.

Congenital Kidney and Urological Disorders

Fetal Ultrasound

The widespread use of fetal US has led to a dramatic increase in postnatal imaging of the UT. Most congenital abnormalities of the UT are now detected prenatally, and

Figure 23-10

Renal MRI of a normal 5 year old. (a) Axial T2-weighted image through the mid-pole of the kidneys, (b) coronal T2-weighted image.

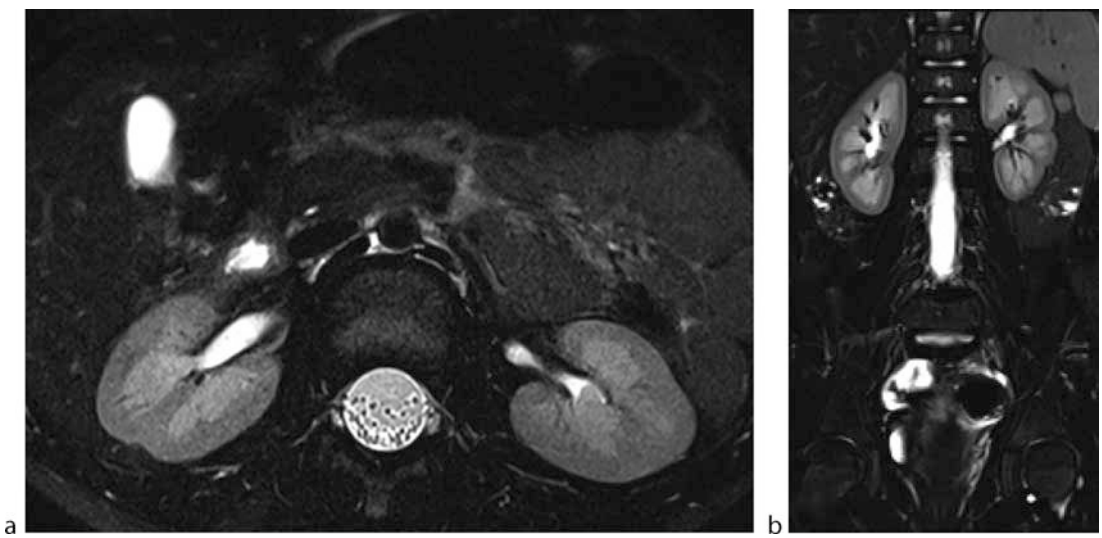
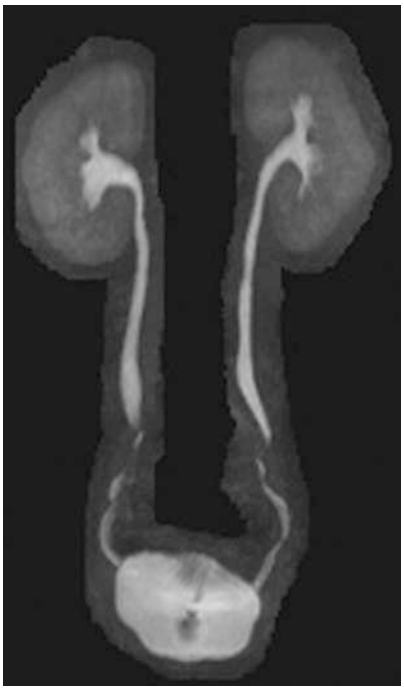


Figure 23-11

Renal MRI. 3-D image of the kidneys and ureters in a normal 12 year old.



this has led to a decrease in the diagnosis of obstructive uropathies as a consequence of urinary tract infection (UTI), potentially diminishing the role of renal US in children with UTI (110, 111).

Fetal kidney length relates to gestational age, and normal values are available (2) (*Table 23-4*). Fetal US is excellent at identifying hydronephrosis, and this may identify infants with UT obstruction or VUR. Oligohydramnios, which may indicate poor urine output due to renal insufficiency, is easily quantified and followed. Persistent oligohydramnios is associated with pulmonary hypoplasia. The fetal bladder, which may be distended with impaired bladder emptying, and the echogenicity of the renal parenchyma are also visualized via fetal US.

Hydronephrosis is the most common renal abnormality on fetal US, and ureteropelvic junction (UPJ) obstruction is the most common pathologic condition, although a high percentage of kidneys are ultimately diagnosed as normal (112). There is a high rate of false negative results when a postnatal US to follow-up on prenatal hydronephrosis is done in the first few days of life, presumably because of low urine output due to physiologic dehydration (113). Hence, the initial postnatal US should either be done at least 4 weeks after birth or the child should have a second US.

Table 23-4

Mean renal lengths for various gestational ages

Gestational age (weeks)	Mean length (cm)	SD	95%CI	n
18	2.2	0.3	1.6–2.8	14
19	2.3	0.4	1.5–3.1	23
20	2.6	0.4	1.8–3.4	22
21	2.7	0.3	2.1–3.2	20
22	2.7	0.3	2.0–3.4	18
23	3.0	0.4	2.2–3.7	13
24	3.1	0.6	1.9–4.4	13
25	3.3	0.4	2.5–4.2	9
26	3.4	0.4	2.4–4.4	9
27	3.5	0.4	2.7–4.4	15
28	3.4	0.4	2.6–4.2	19
29	3.6	0.7	2.3–4.8	12
30	3.8	0.4	2.9–4.6	24
31	3.7	0.5	2.8–4.6	23
32	4.1	0.5	3.1–5.1	23
33	4.0	0.3	3.3–4.7	28
34	4.2	0.4	3.3–5.0	36
35	4.2	0.5	3.2–5.2	17
36	4.2	0.4	3.3–5.0	36
37	4.2	0.4	3.3–5.1	40
38	4.4	0.6	3.2–5.6	32
39	4.2	0.3	3.5–4.8	17
40	4.3	0.5	3.2–5.3	10
41	4.5	0.3	3.9–5.1	4

Gestational age is an average of the gestational ages in weeks determined on the basis of biparietal diameter, femoral length, and abdominal circumference. *SD* standard deviation; 95% *CI* 95% confidence interval; *n* number of fetuses. At distribution was used when $n < 30$ (From Cohen HL et al. (2).)

Deferral of the initial US is inappropriate if the prenatal US suggests the presence of disease that would require immediate intervention (e.g., bilateral hydronephrosis or oligohydramnios). The degree of prenatal hydronephrosis is highly predictive of the likelihood of pathology on postnatal evaluation (114).

Prenatal US can detect children with a variety of other pathologic conditions, including posterior urethral valves, multicystic dysplastic kidney, VUR, megaureter, duplex anomalies with upper-pole hydronephrosis, single kidney, polycystic kidney disease, and Eagle-Barrett syndrome (115).

Megaureter

Megaureter may be primary or secondary to VUR or obstruction. VCUG permits identification of VUR and diuretic scintigraphy establishes the presence of obstruction. MRU provides excellent anatomic detail, often allowing identification of the site of obstruction (116).

Ureterocele

Ureteroceles may be demonstrated by US, VCUG, IVP, or MRU (*Fig. 23-12*). An everting ureterocele may be confused with a bladder diverticulum on VCUG (117). Ureteroceles may obstruct the ureter during voiding.

Ureteropelvic Junction Obstruction

UPJ obstruction is one of the most common congenital kidney disorders, and most cases are now diagnosed by fetal US. Dynamic renal scintigraphy remains the gold standard imaging technique for diagnosing UPJ obstruction (*Fig. 23-13*). MRI is an alternative approach that provides functional information and superior anatomic detail without exposure to radiation, albeit with the need for sedation (*Fig. 23-14*) (118, 119).

A second alternative technique for diagnosing obstruction is the measurement of the resistive index (RI) by Doppler US, with an elevated RI suggesting obstruction. The sensitivity and specificity of this approach is improved by administering normal saline and furosemide prior to US (120). The principal advantage is the elimination of radiation exposure, but this approach is not yet widely utilized.

Abnormal Kidney Number or Location

Unilateral renal agenesis must be distinguished from an ectopic kidney, which can be located anywhere along the course of renal ascent. Such kidneys are often smaller in size. Detection by US is possible, but MRI, IVP or DMSA are often necessary (*Fig. 23-15*). IVP may have limited sensitivity if the ectopic kidney has poor renal function.

Horseshoe Kidney and Crossed Fused Ectopia

Horseshoe kidney, with an incidence of 1 of 400 births, is the most common fusion anomaly. Crossed ectopia

Figure 23-12

Ectopic ureterocele with vesicoureteral reflux. (a) Voiding cystourethrogram shows rounded filling defect in bladder base from ureterocele, (b) voiding cystourethrogram shows reflux into the lower pole moiety of the left duplex system with eversion of the ureterocele (*asteric*). Opacification of the kidney indicates intrarenal reflux.

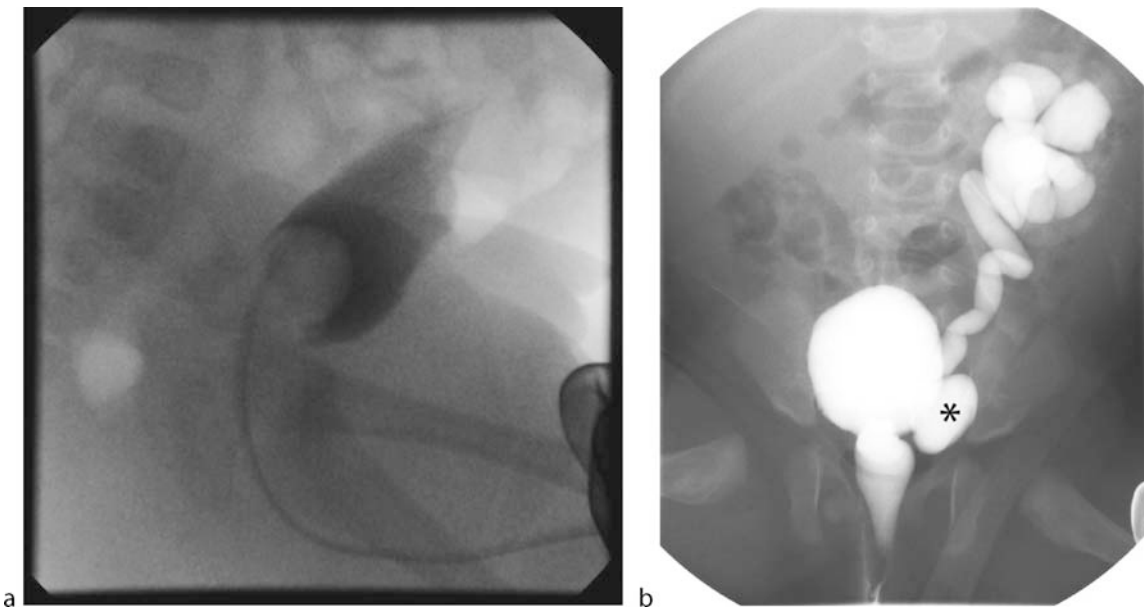
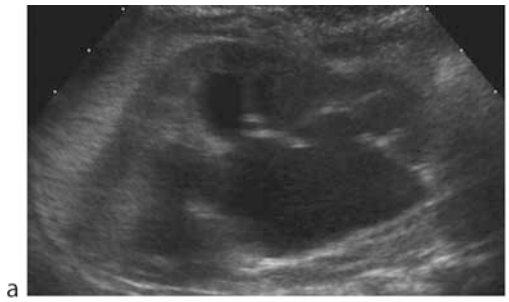
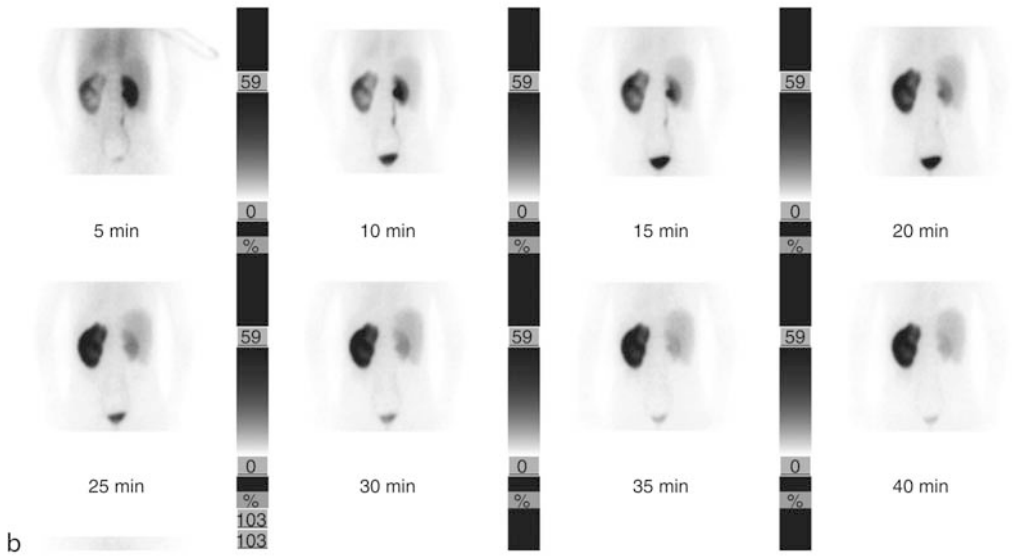


Figure 23-13

Left ureteropelvic junction obstruction. (a) US image of left kidney shows dilatation of renal pelvis and calices, (b) ^{99m}Tc-MAG$_3$ renal scan shows retention of radiotracer in left kidney, (c) time-activity curve from ^{99m}Tc-MAG$_3$ scan shows a normal right kidney. On the left, little drainage occurs in response to furosemide. T ½ exceeds 20 min.





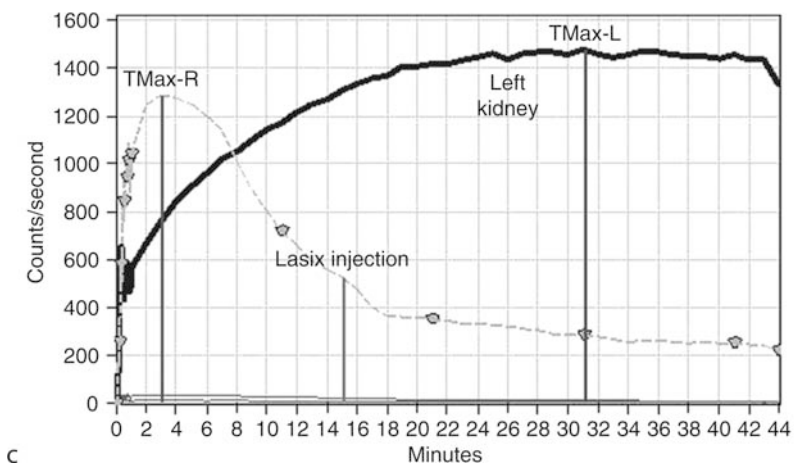


Figure 23-14

Left ureteropelvic junction obstruction. MR Urogram in patient with UPJ obstruction shows dilatation of the left renal pelvis and calices.

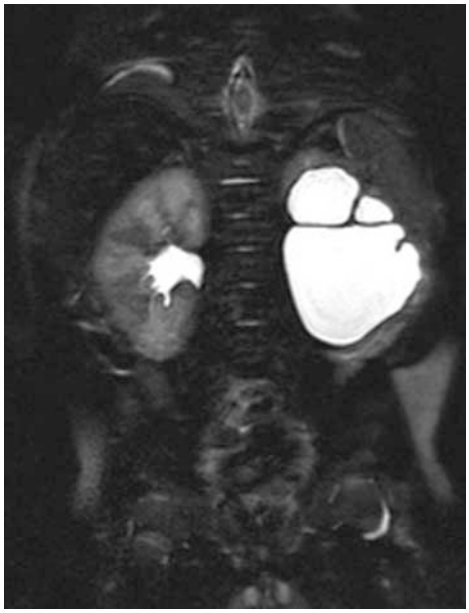


Figure 23-15

Crossed-fused ectopia. DMSA renal scan anterior image demonstrates a single fused renal moiety on the right.

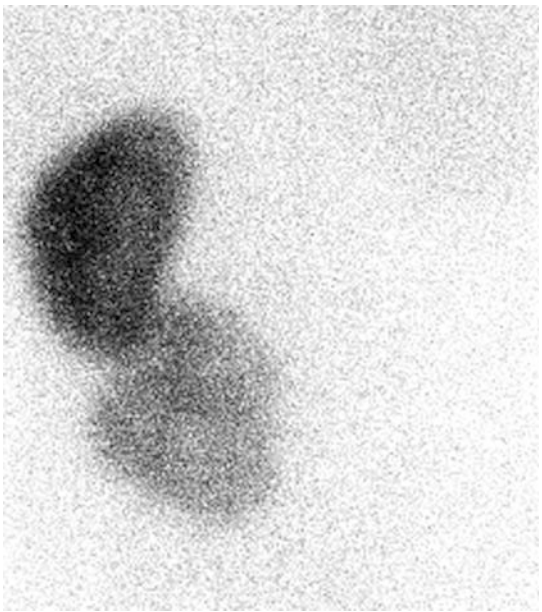
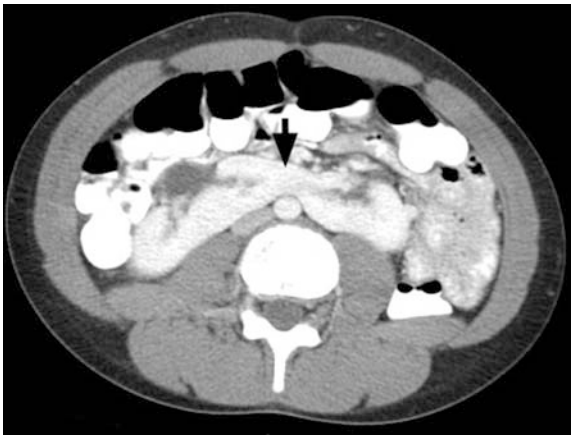


Figure 23-16

Horseshoe kidney. CT scan of abdomen shows a horseshoe kidney with a bridge of renal tissue fused across the midline (*arrowhead*).



occurs in an estimated 1 of 1,000 to 2,000 births. While US can be diagnostic, additional imaging, including IVP, MRI, CT, or DMSA, is often necessary to establish the diagnosis (*Fig. 23-16*).

Renal Dysplasia

Renal dysplasia is a histological diagnosis characterized by primitive ducts and cartilage. US evaluation demonstrates increased echogenicity; most dysplastic kidneys are decreased in size. US may detect cysts, which may vary in size and number.

The incidence of multicystic dysplastic kidney (MCDK) is estimated at 1 in 2,500 newborn (121); it is one of the common prenatally detected fetal anomalies. Postnatal US shows cysts of varying size that do not appear to communicate with each other or the collecting system, and a small amount of abnormal-appearing renal parenchyma. It may be difficult to differentiate MCDK from severe hydronephrosis (122). In MCDK, there is typically no communication between cysts, and the larger cysts are not medial (*Fig. 23-17*). In hydronephrosis, the calyces extend outward from the dilated renal pelvis, and there is functional renal parenchyma surrounding the central cystic structure. If the diagnosis is unclear, a DMSA shows uptake of tracer if hydronephrosis is present, but usually no uptake with MCDK (*Fig. 23-18*). Alternatively, MRI can distinguish these entities.

Figure 23-17
Left multicystic dysplastic kidney. Renal US in an infant shows multiple non-communicating cysts in the left kidney.

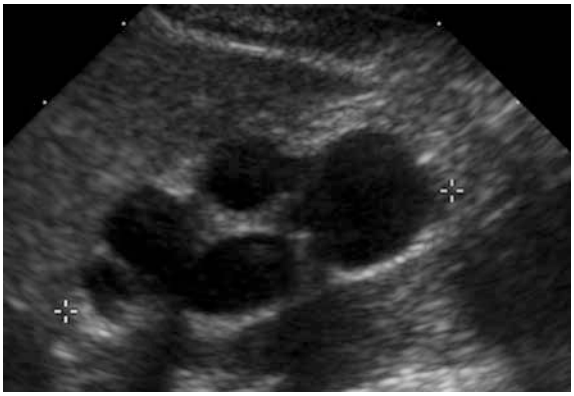


Figure 23-18
Left multicystic dysplastic kidney. DMSA renal scan (*posterior image*) shows no uptake in the left multicystic dysplastic kidney.

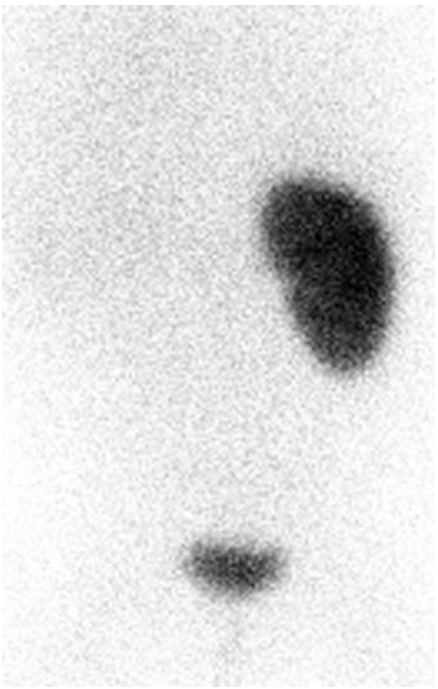


Figure 23-19
Duplex system with ectopic ureterocele. Renal US shows mild hydronephrosis (*arrowheads*) of the upper and lower poles. A band of renal tissue extends across the midpole, indicating a duplex system.

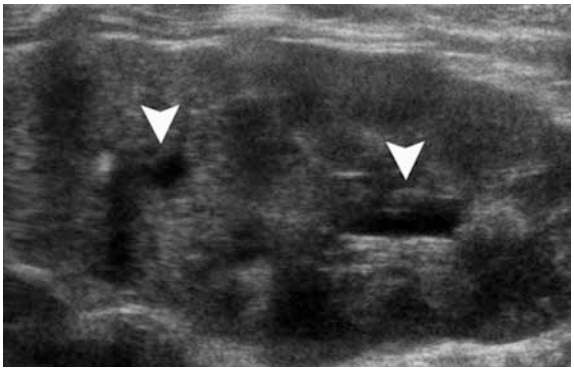
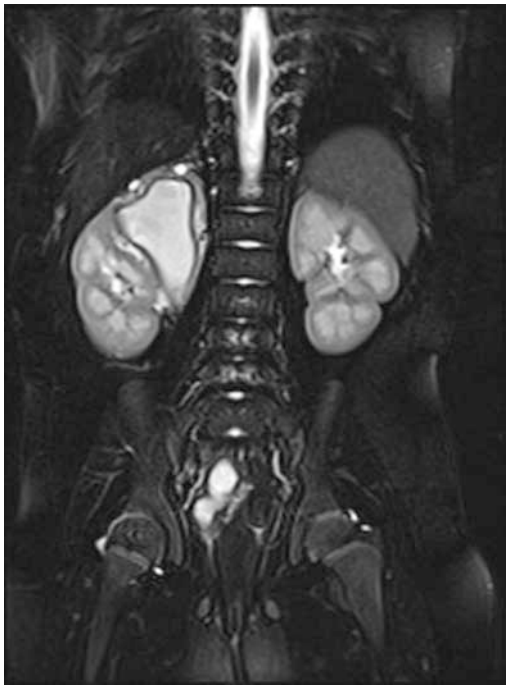


Figure 23-20
Duplex system with ectopic ureterocele. MRU shows hydronephrosis of the right upper pole.



Duplex Collecting System

A duplex collecting system is a common incidental finding and usually of no clinical significance unless hydronephrosis is also present. Associated anomalies include ureterocele, VUR, ectopic ureters, renal dysplasia, and UPJ obstruction (*Figs. 23-19* and *23-20*) (123). The upper pole is more likely to be associated with obstruction at the distal ureter while the lower pole is associated with VUR or UPJ obstruction. US can usually demonstrate

division of the kidney, but is not reliable in distinguishing between bifid ureter and complete ureteral duplication. IVP is being supplanted by MRU for delineating the anatomy of the ureteral duplication when such information is necessary for planning surgery (116). MRU is especially useful for characterization of the distal ureter (124). VCUG may demonstrate VUR or ureterocele.

Ectopic Ureters

Ectopic ureters are three times more common in girls, and approximately 80% are associated with a duplicated collecting system and ectopic insertion of the upper pole ureter. Greater distance from the normal ureter insertion site is associated with more severe ipsilateral renal abnormalities (125). US can demonstrate duplication, hydronephrosis, megaureter, and abnormal renal parenchyma. IVP is useful for delineating the course of the ectopic ureter, although lack of contrast may limit the study if the associated renal unit is poorly functioning. MRU may allow detection of the ectopic ureter if other studies are unsuccessful (124). VCUG is useful for showing reflux, and may indicate the insertion point of the ureter if it is proximal to the external sphincter. DMSA is useful for identifying an ectopic kidney that is the source of an ectopic ureter in a patient with unexplained incontinence (96, 97). By indicating relative renal function, DMSA may also help with surgical planning.

Bladder Abnormalities

US and VCUG are the standard imaging modalities for patients with a neurogenic bladder (*Fig. 23-21*). VCUG readily identifies bladder diverticula, which are easily missed by US. Bladder wall thickening, consistent with high bladder pressures, can be quantified by US.

Urethral Abnormalities

VCUG is the definitive test for diagnosing posterior urethral valves (*Fig. 23-22*). The prostatic urethra is dilated, the membranous urethra is stenotic, and the distal urethra appears normal. Secondary VUR, hydronephrosis, and bladder abnormalities are often present (*Fig. 23-23*). Other urethral abnormalities (anterior urethral valves, urethral duplication, Cowper's duct cyst, urethral diverticulum, megalourethra) are also best visualized via VCUG.

Figure 23-21
Neurogenic bladder in a 16 year old with a spinal cord injury. Voiding cystourethrogram shows pear-shaped bladder with trabeculations.

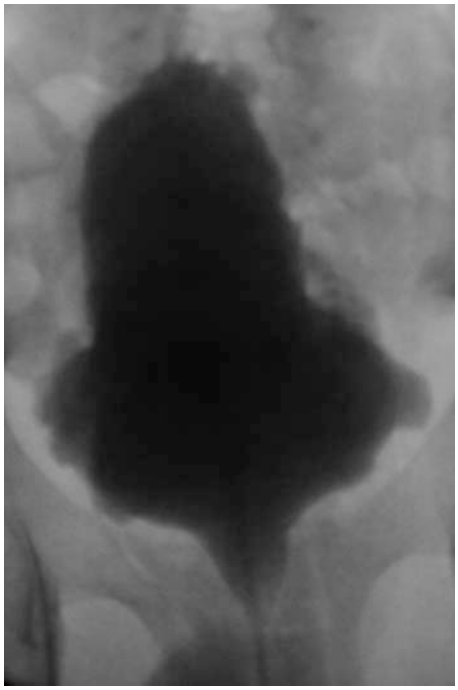
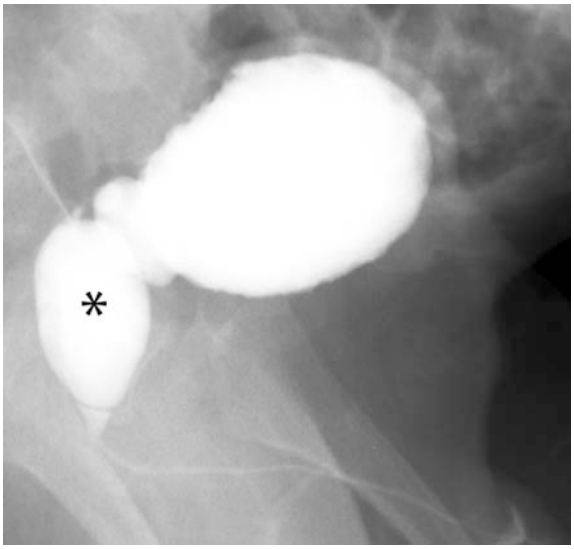


Figure 23-22
Voiding cystourethrogram shows dilatation of the posterior urethra (*asteric*) in a boy with posterior urethral valves.



Vesicoureteral Reflux

VCUG is the gold standard for diagnosing VUR, with VUR graded based on the International Reflux Study in Children (*Fig. 23-24*) (44). RNC or VUS, because of decreased or no radiation burden, respectively, are appropriate substitutes for VCUG in clinical situations where the purpose of the study is solely to assess the patient for VUR. Examples include follow-up studies in children with previously diagnosed VUR, screening siblings of children with VUR, and potentially for the initial evaluation of girls with urinary tract infections. Renal US lacks adequate sensitivity and specificity for diagnosing VUR (126–129), although high grade VUR is more likely if hydronephrosis is present (130).

Figure 23-23

Renal US shows dilatation of the right renal pelvis, calices, and ureter in a boy with posterior urethral valves.

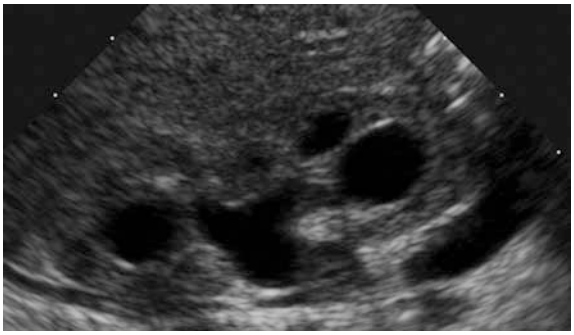
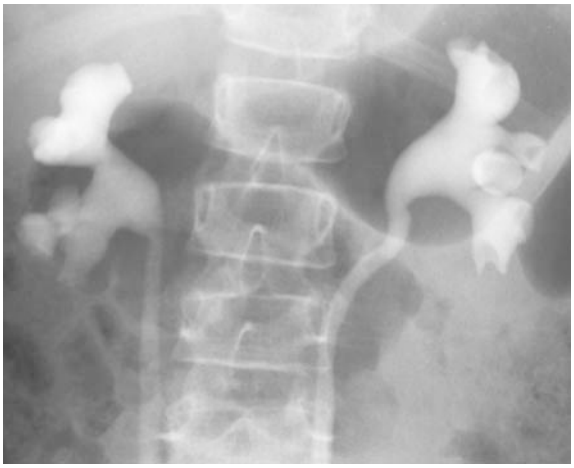


Figure 23-24

Voiding cystourethrogram shows bilateral grade 4 vesicoureteral reflux.



Hereditary Disorders

Cystic Kidney Diseases

In autosomal recessive polycystic kidney disease, intravenous pyelogram (IVP) shows enlarged kidneys with a delayed nephrogram (131). Radial streaks due to contrast in the dilated collecting ducts are usually present in infancy, but may not be visible in older children. Because of concerns regarding intravenous contrast, a renal US is now the usual initial diagnostic test (*Fig. 23-25*). US shows enlarged kidneys with increased echogenicity and poor corticomedullary differentiation; a hypoechoic rim is often visible (132). Older children have increased medullary echogenicity, which may resemble nephrocalcinosis (133). Multiple small cysts may be visible by US (133, 134), but this is quite variable. Macrocysts are sometimes visible in older children (134).

In autosomal dominant polycystic kidney disease, US, CT and MRI are useful for detecting macroscopic cysts (*Figs. 23-26* and *23-27*). The number and size of cysts in children increases with age, and those children with more cysts have increased kidney size (135). In adults, kidney size measured by MRI predicts decline in renal function (136). Increased renal echogenicity may be seen in some children (134). Occasionally, children have marked disease asymmetry, which can create diagnostic confusion (137).

US in nephronophthisis shows hyperechoic kidneys with loss of corticomedullary differentiation; they are of normal or slightly decreased size. Medullary cysts are a hallmark of the disease, but they are not always detected by US. CT is a more sensitive method for identifying medullary cysts and thin-section CT is recommended (138).

The diagnosis of medullary sponge kidney is based on characteristic IVP changes: stagnation of contrast in one or more renal papillae due to dilation of the collecting ducts. The resultant image has been described as a "pyramidal blush." In medullary cystic disease, the kidneys are usually normal or small, and US or CT may show a few medullary cysts (139).

Tuberous Sclerosis

Angiomyolipomas are the most common renal lesion in TS and are readily seen by CT or ultrasound (*Figs. 23-28* and *23-29*). Children with disruption of the contiguous *PKD1* and *TSC2* genes usually have severe cystic disease and are at high risk for hypertension and early kidney failure (140). Renal malignancies, including renal cell carcinoma and malignant angiomyolipomas, are a serious

Figure 23-25

Autosomal recessive polycystic kidney disease in an infant. (a) Longitudinal view shows diffuse enlargement of the right kidney, which is in increased in echogenicity, (b) transverse image through the mid-pole of both kidneys.

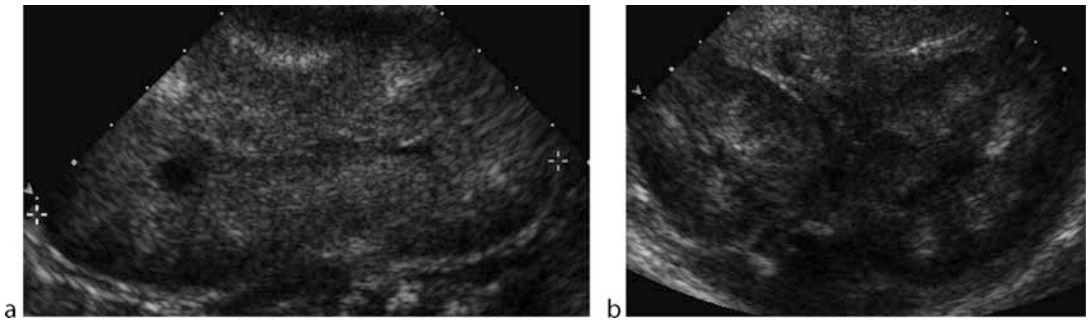
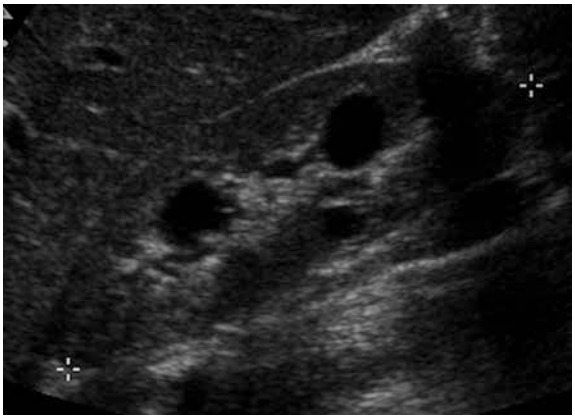


Figure 23-26

Autosomal dominant polycystic kidney disease. Renal US, longitudinal image, renal cysts of varying sizes in a 15 year old.



concern in TS and both are seen in children, sometimes in early childhood (141, 142). All children with TS should have a renal ultrasound at diagnosis and follow-up renal ultrasounds every 1–3 years, with frequency dictated by the specific clinical situation (143). Patients with extensive or rapidly changing lesions require more frequent follow-up. Those with more severe kidney disease may require CT or MRI to screen for malignant changes. Differentiating angiomyolipomas from malignancy requires careful comparison of sequential images.

Infections of the Urinary Tract

Clinical and laboratory criteria do not reliably differentiate pyelonephritis from cystitis (144–146). DMSA is the gold standard for diagnosing acute pyelonephritis, although MRI and CT provide similar sensitivity (147). US may detect loss of corticomedullary differentiation and renal enlargement, but lacks adequate sensitivity and specificity when compared to DMSA (148–151).

The decreased uptake using DMSA with pyelonephritis may be focal, multifocal or diffuse. Acute pyelonephritis must be differentiated from renal scaring, which is associated with sharper borders and loss of cortical volume. DMSA may allow diagnosis of pyelonephritis in children who have received antibiotics prior to urine culture or who have a negative culture despite clinical and laboratory evidence suggesting pyelonephritis (152).

DMSA is the current standard modality for diagnosing renal scarring (*Fig. 23-30*) (153), with increased sensitivity when compared to US or IVP (154). Scars appear as photopenic areas with volume loss. DMSA should be deferred at least 6 months after an acute infection to differentiate a transient lesion due to infection from a scar (95, 154, 155). MRI has the potential to differentiate acute pyelonephritis from renal scarring, perhaps avoiding the need for radiation exposure and obviating the need for two diagnostic tests (156).

All boys and young girls with a first UTI are conventionally evaluated with a renal US and VCUG (157). The necessity of the this approach has been questioned, especially given the lack of evidence that prophylactic antibiotics are effective in preventing UTIs or renal damage (158). Moreover, because of the widespread use of prenatal US in many countries, the diagnostic yield of US examination in children with UTI is low (159). In girls, the radiation burden of the initial imaging can be reduced by using RNC instead of VCUG. An alternative strategy is to perform DMSA scintigraphy as an initial test in children with UTI, and only perform VCUG or RNC in children with an abnormal DMSA (160).

Figure 23-27

CT in a 15 year old with autosomal dominant polycystic kidney disease and bilateral cysts of varying sizes. (a) axial image, (b) coronal image.

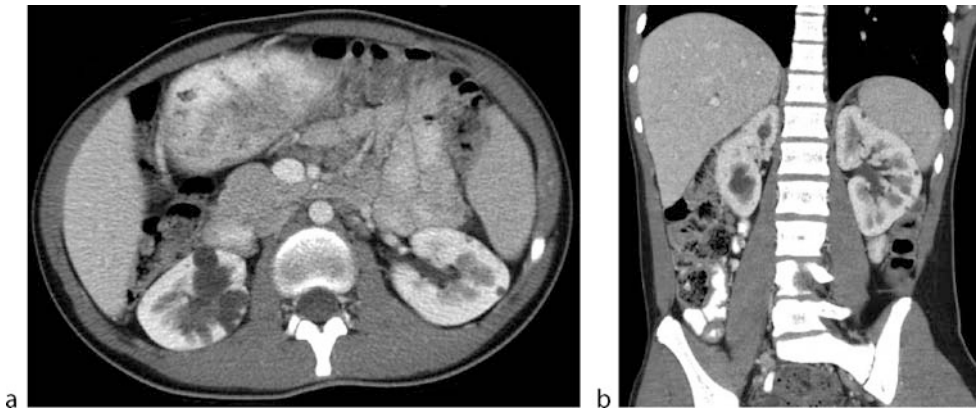


Figure 23-28

Angiomyolipomas in tuberous sclerosis. Renal US, longitudinal image, demonstrates multiple small echogenic lesions throughout the kidney.

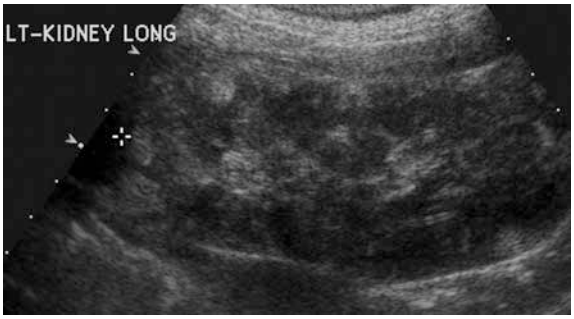
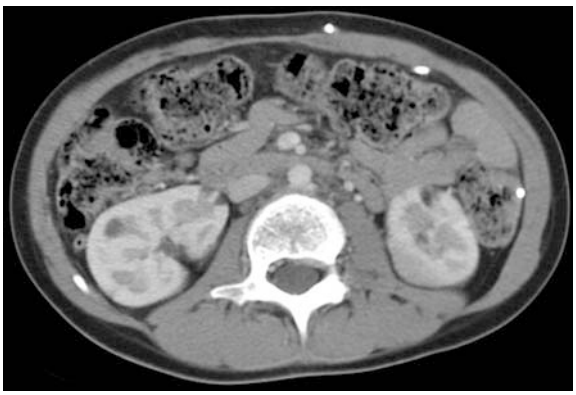


Figure 23-29

Angiomyolipomas in tuberous sclerosis. Renal CT, axial image, with multiple fat-containing lesions in the kidneys.



Renal Failure

US is the initial imaging modality when a patient presents with unexplained renal failure. This generally allows differentiation of chronic renal failure (small, echogenic kidneys with a thin cortex) from acute renal failure. The kidneys may be large if chronic renal failure is secondary to polycystic kidney disease. US readily detects hydronephrosis, which suggests that renal failure is due to an obstructive etiology. Hydronephrosis may also be secondary to VUR, papillary necrosis or high urine output; such hydronephrosis is rarely severe. Renal parenchymal disease is often associated with increased echogenicity (*Fig. 23-31*) (161–163), although a normal US examination does not exclude this possibility. The kidneys generally have a normal appearance in prerenal azotemia. The kidneys may be normal or have increased echogenicity in acute tubular necrosis. Echogenic kidneys, which may be moderately enlarged, are often seen in glomerular disease (162, 164). The kidneys are enlarged and very echogenic in HIV nephropathy(165). The US findings in renal parenchymal disease are rarely specific (163, 164).

Renal Transplant

US is the primary imaging modality for evaluation of the renal transplant (166, 167). US identifies abnormal fluid collections (e.g., lymphoceles, urinomas) and obstruction (*Fig. 23-32*). Doppler US can detect vascular disease after kidney transplant (renal artery thrombosis, renal

Figure 23-30

Renal scarring in a 10 year old with vesicoureteral reflux. DMSA renal scan shows a small right kidney with diffuse renal scarring. Uptake in the right kidney is 20%.

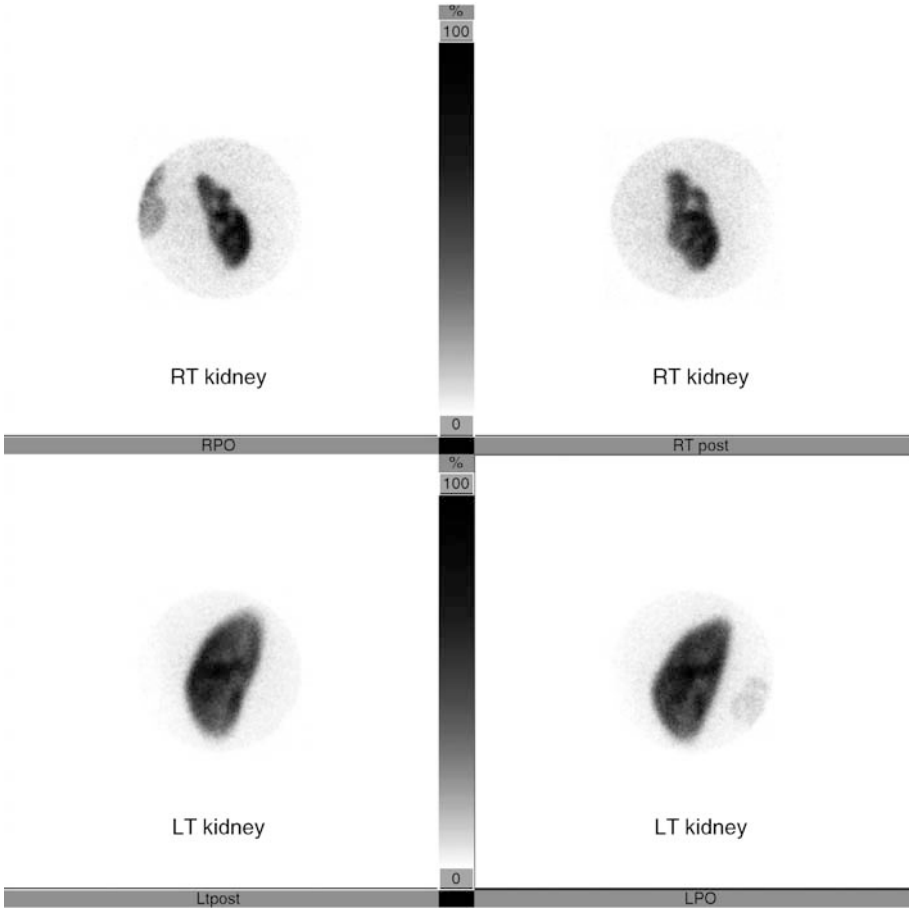
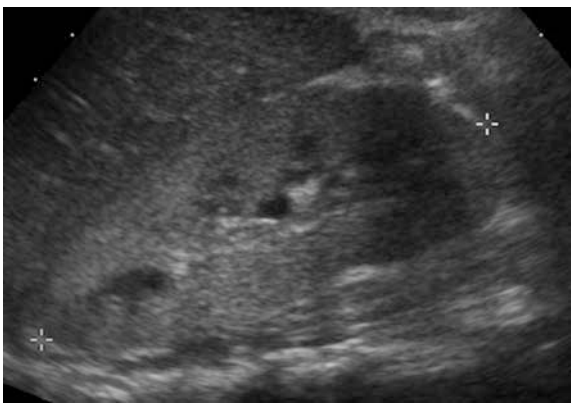


Figure 23-31

Acute glomerulonephritis in a 3 year old. Renal US shows large echogenic right kidney.



artery stenosis, renal vein thrombosis, arteriovenous fistula) (26, 167–169). There are a number of potential US abnormalities seen in patients with acute graft rejection (increased renal size and echogenicity, decreased or absent central sinus echoes) (170), but US does not have adequate sensitivity or specificity; kidney biopsy remains necessary for the diagnosis of rejection.

Radionuclide imaging (^{99m}Tc-DTPA or ^{99m}Tc-MAG$_3$) is useful when US does not provide an explanation for graft dysfunction. Absence of flow to the kidney occurs with arterial or venous obstruction or with hyperacute rejection. In contrast, a graft with ATN has normal renal perfusion, but delayed or no excretion. Radionuclide imaging is useful for demonstrating urinary obstruction or that a perinephric fluid collection is due to a urine leak (171–173).

Urolithiasis and Nephrocalcinosis

Urolithiasis

US is a sensitive test for detecting calculi in the kidney and the proximal and distal ureter. US does not easily detect stones in the middle portion of the ureter, although hydronephrosis due to obstruction may suggest the diagnosis. Acoustic shadowing is a useful finding, but may not be present with smaller calculi. US is less sensitive than CT in detecting calculi in symptomatic children (*Fig. 23-33*) (174). CT is also superior to US for identification of bladder stones, especially if the bladder is augmented.

Nephrocalcinosis

Nephrocalcinosis is typically detected by US (*Fig. 23-34*), although it may be seen via CT. Plain x-ray only detects nephrocalcinosis in severe cases (175). Causes of cortical nephrocalcinosis include primary hyperoxaluria(176) and cortical necrosis, while there are many entities that may cause medullary nephrocalcinosis (175, 177).

Trauma to the Urinary Tract

CT with contrast is the primary modality for evaluating a patient with suspected trauma to the kidney (*Fig. 23-35*) (178, 179). CT angiography permits detailed evaluation of the renal artery and vein. US is an alternative imaging modality that may identify large lacerations, hematomas, and urinomas; US with Doppler readily identifies significant injuries of the renal artery or vein. US suggests bladder laceration when there is a large amount of fluid in the cul-de-sac. When indicated, VCUG can potentially identify the site of bladder leak and detect urethral injury.

Figure 23-32

Lymphocele in a 15 year old with renal transplant. Renal US shows a complex, loculated fluid mass adjacent (L) to the transplanted kidney (T).

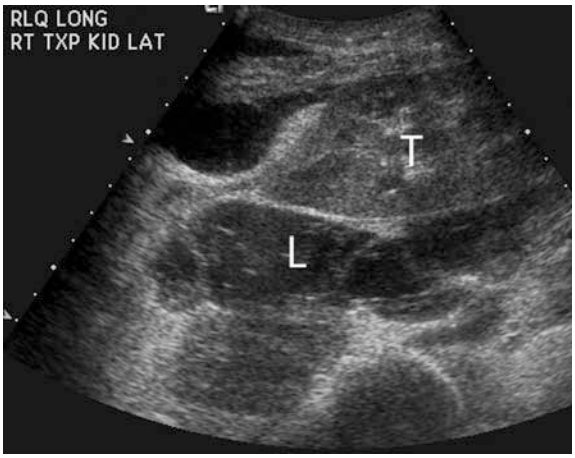
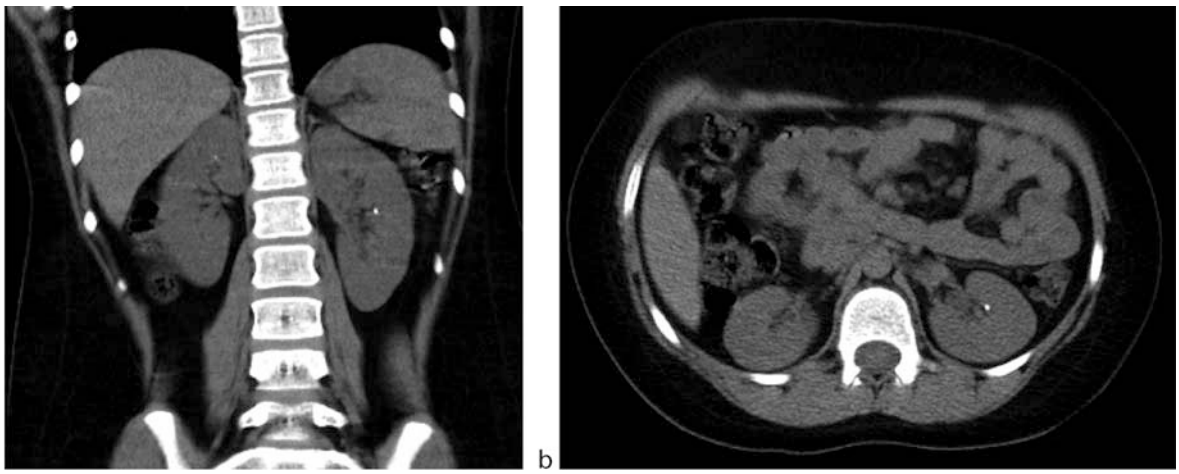


Figure 23-33

Renal calculus in the mid-pole of the left kidney. (a) Coronal image, (b) axial image.

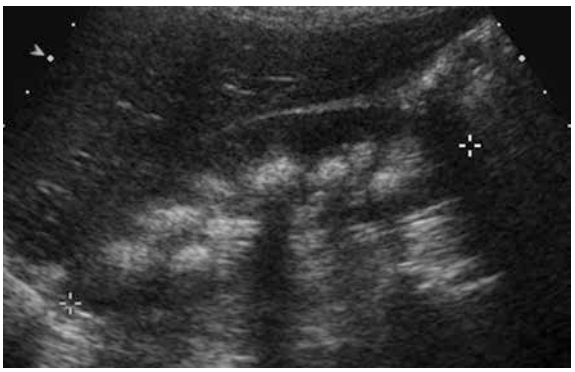


Renal and Urinary Tract Tumors

Role of Imaging

US is especially suited for initial evaluation, while CT or MRI are necessary for defining tumor extent and the presence of metastases. CT without contrast allows for identification of tumor calcifications; contrast allows for better differentiation of tumor from normal tissue. US is sensitive for the detection of bladder tumors in children (180).

Figure 23-34
Medullary nephrocalcinosis in a 12 year old with renal tubular acidosis. Renal US, longitudinal image, shows echogenic renal pyramids.



Imaging of Specific Renal Tumors

Wilms' Tumor

US and CT are complementary in the evaluation and follow-up of Wilms' tumor (*Figs. 23-36* and *23-37*). US with Doppler may provide superior imaging of tumor in the inferior vena cava in some patients (181). Radiologic staging of Wilms' tumor includes chest x-ray, US of the abdomen and pelvis, and CT of the chest, abdomen, and pelvis. Chest x-ray appears to be an appropriate substitute for chest CT to identify pulmonary metastases (182). MRI is also an effective modality for evaluation of Wilms' tumor (183). US is a sensitive approach for screening patients for tumor recurrence, although CT or MRI is necessary to identify nephrogenic rests (184).

US is the preferred modality for Wilms' tumor screening, which is necessary in children with syndromes that significantly increase the risk of Wilms' tumor (e.g., hemihypertrophy, Denys-Drash syndrome, Beckwith Wiedemann syndrome) (185). Screening should occur every 3–4 months until 8 years of age.

Lymphoma

Children with lymphoma may have renal involvement. By US, renal lymphoma has decreased echogenicity (186).

Figure 23-35
Motor vehicle collision resulting in extensive left renal laceration with a large perinephric hematoma. (a) Axial CT, (b) coronal CT.

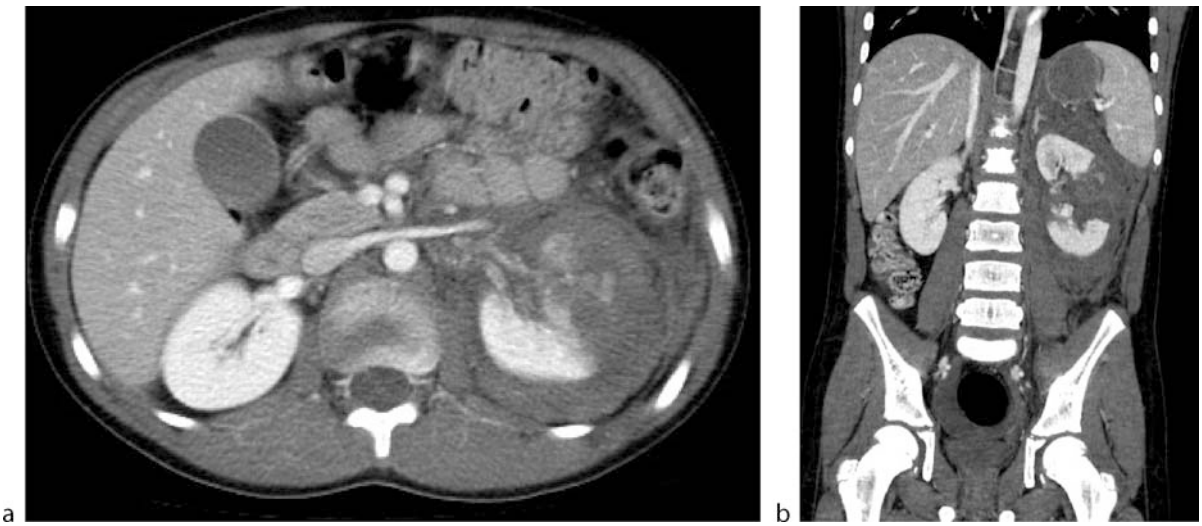
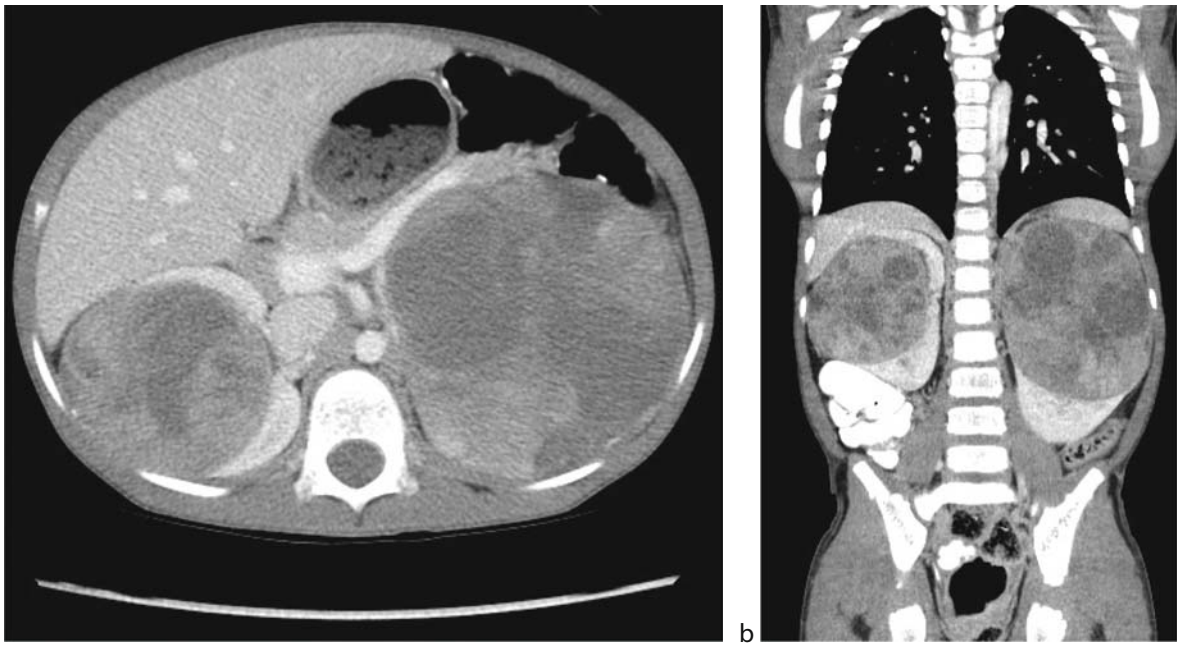


Figure 23-36

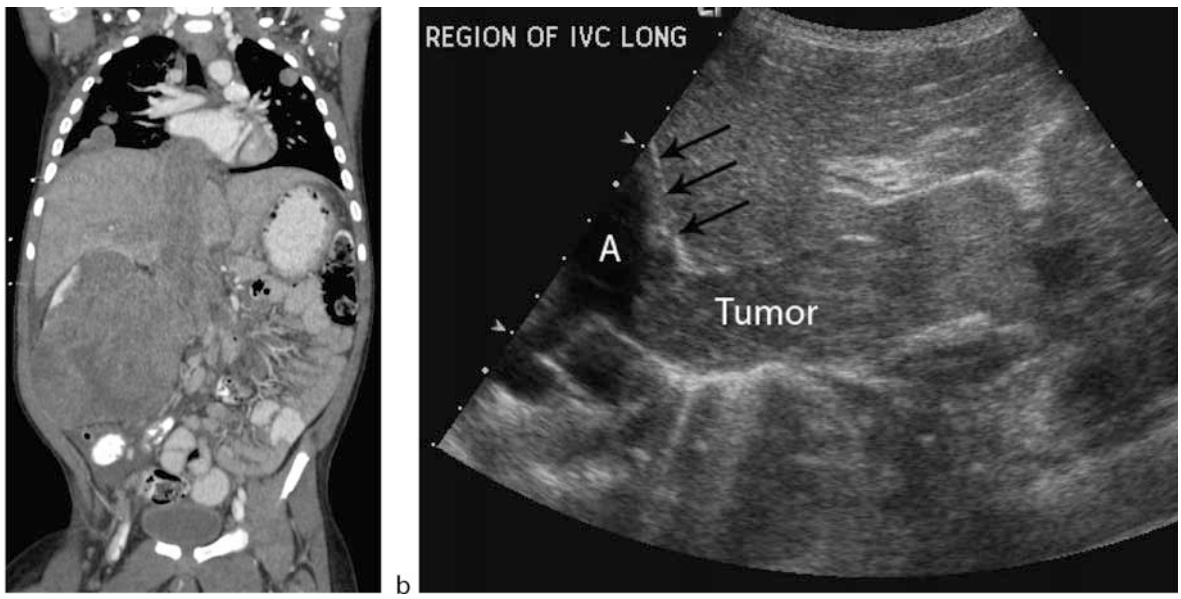
Bilateral Wilms' tumor in a 5 year old with a palpable abdominal mass. (a) Axial CT, (b) coronal CT.



a b

Figure 23-37

Wilms' tumor in a 2 year old. (a) Coronal CT image shows a large right renal mass, pulmonary nodules, and tumor extension into the IVC to the diaphragm, (b) longitudinal US image with tumor filling the dilated IVC extending to the diaphragm (*arrows*) and entering the right atrium (A).



a b

CT may demonstrate "contrast inversion" of the lesions in renal lymphoma: increased density compared to normal parenchyma without contrast and decreased density with contrast (186).

Vascular Disease and Hypertension

Renal Artery Stenosis

Doppler US lacks adequate sensitivity for diagnosing renal artery stenosis in children (187–189), although it may be a useful screening test in adults (190). US may demonstrate a small affected kidney if the stenosis is severe and long-standing; the contralateral kidney may have increased echogenicity (191). Renal scintigraphy before and after captopril has good sensitivity in identifying renovascular disease in children (99). CT and MRI angiography provide excellent visualization of the renal artery (*Fig. 23-38*), but do not visualize distal branch vessel lesions. When clinical suspicion is high, conventional angiography remains necessary to evaluate children with suspected hypertension due to renal artery disease.

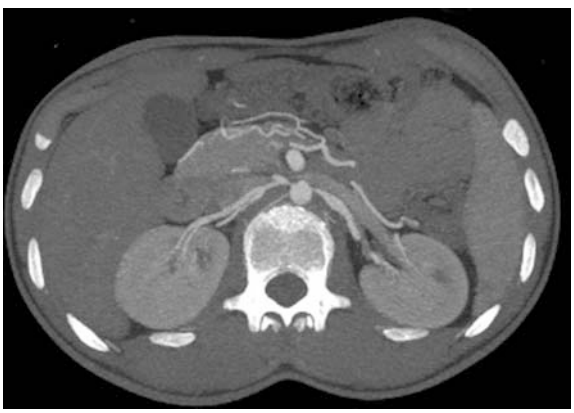
Renal Vein Thrombosis

US is utilized to diagnose renal vein thrombosis. The appearance on US varies depending on the timing of imaging. Within the first few days of clot formation, imaging may demonstrate pathognomonic echogenic streaks due to clot in interlobular and interlobar veins (192, 193). Renal enlargement occurs during the first week, and is associated with an echogenic cortex and less echogenic medullary pyramids. Beyond the first week, there is a loss of corticomedullary differentiation and echogenic streaks are no longer visible (192). An additional later finding is calcification of thrombi within renal veins, including large or small veins (194). Long-term imaging outcomes vary from a normal appearing kidney to a small, echogenic kidney with little or no function. In perinatal renal vein thrombosis, renal length is negatively correlated with outcome (195).

US may directly demonstrate clots in the renal vein and inferior vena cava. Doppler US may show absent renal venous flow and pulsatility. The arterial RI is frequently elevated. Clot retraction and formation of collaterals after a few days may create diagnostic confusion due to a return of some venous flow and a decrease in arterial RI. In neonates, adrenal hemorrhage, which is readily seen by US, frequently accompanies renal vein thrombosis and assists in confirming the diagnosis (196, 197). This association is more common on the left due to the common drainage of the renal vein and adrenal vein.

Figure 23-38

Renal artery stenosis in a 13 year old with neurofibromatosis and hypertension. Axial maximal intensity projection CT image shows narrowing of both renal arteries near their origins from the aorta.



Pheochromocytoma

Pheochromocytoma may be strongly suspected based on clinical and laboratory evaluation, but imaging is necessary to confirm the diagnosis and allow for staging and resection, which is usually curative. US may identify a pheochromocytoma in or near the adrenal gland, but US lacks the sensitivity of CT or MRI, especially for small lesions or tumors in other locations (*Fig. 23-39*). Hypertensive crisis may occur if ionic contrast is utilized, but nonionic contrast appears safe at the doses used for CT (198). Chest CT or MRI is indicated if an abdominal lesion is not detected or for staging of malignant disease. Pheochromocytomas enhance on contrast CT, although nonenhancing foci of necrosis and hemorrhage may be present.

MIBG scintigraphy, a complementary imaging modality, may be appropriate as an initial test, to confirm the diagnosis in equivocal cases, or to search for disease when the CT or MRI is negative (*Fig. 23-40*) (199, 200). Positron emission tomography (PET) scan is a potential alternative if all other studies are negative and clinical suspicion remains high (201).

Figure 23-39

Pheochromocytoma in a 14 year old. (a) Axial CT image shows a right pelvic mass (P), (b) sagittal T2-weighted MR image shows a right pelvic mass extending into the adjacent neural foramen (*arrow*).

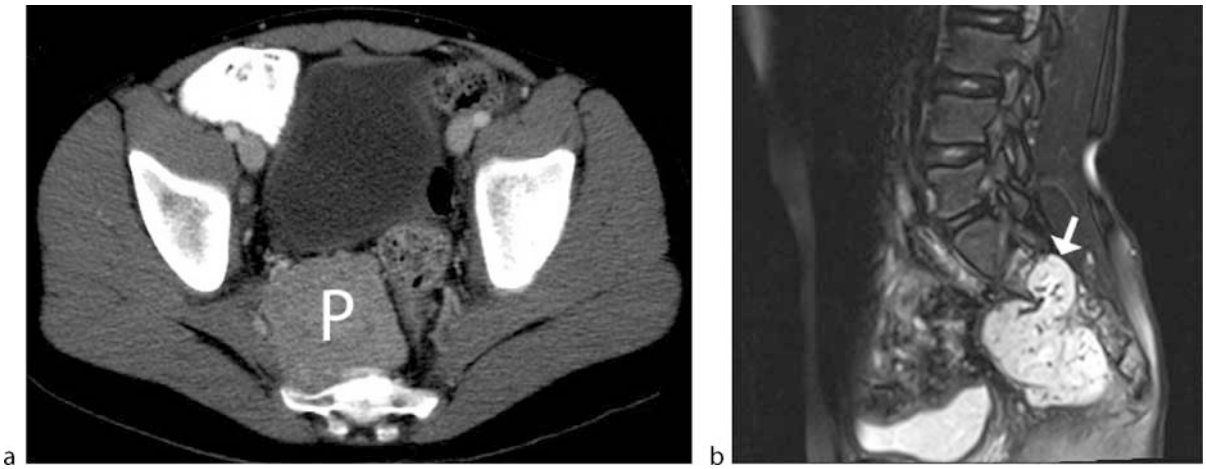
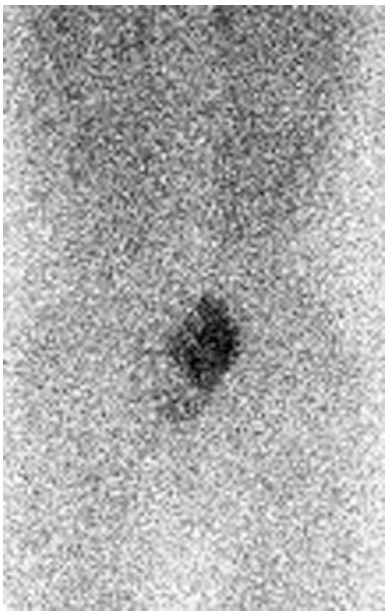


Figure 23-40

Pheochromocytoma. Posterior ^{123}I-MIBG image shows tumor uptake in the pelvis.



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24 Renal Pathology

Agnes B. Fogo

Introduction

This chapter reviews the usual circumstances in which biopsies are obtained, methods of obtaining the biopsy material and analyzing the tissue, and the distinct characteristic morphologic findings in various diseases. Last, experimental techniques that may provide important pathogenic, prognostic, or diagnostic information are discussed.

Renal Biopsy Indications

The indications for renal biopsy vary according to the ethnic and age characteristics of the population studied and the geographic location because these factors influence the incidence of various renal diseases. The indications discussed below present the most common settings in children for which renal biopsy is undertaken.

Hematuria

Isolated hematuria (i.e., without proteinuria and with normal function of the kidney) may be due to hypercalciuria or familial or urologic disease (1–3). Once these disorders are ruled out, a glomerular origin of persistent isolated hematuria should be considered. Red blood cell casts or dysmorphic red blood cells indicate glomerular origin of hematuria. Renal biopsy may define the underlying abnormality in these patients. The most common findings are mesangial proliferative disease or IgA nephropathy (Berger's disease). Less common disorders include hereditary nephritis (Alport syndrome) and thin basement membrane lesion. The latter may be familial (benign familial hematuria) or sporadic. One-quarter to nearly one-half of the patients with isolated hematuria have normal biopsies (1–4). Renal biopsy may, therefore, define the pathology and provide assurance of a benign prognosis in some patients or diagnose a possible hereditary disease, which would initiate screening of other family members. Last, the information obviously can be of importance in avoiding further repeated invasive evaluation in the patient.

Proteinuria

Isolated proteinuria may be postural or due to tubulointerstitial disease. These possibilities should be evaluated completely over time before renal biopsy is considered. Any glomerular disease may cause mild to moderate proteinuria as the only manifestation, and biopsy may yield the diagnosis even at an early stage.

Nephrotic Syndrome

Numerous children with nephrotic syndrome (NS) were studied when renal biopsy first became available. The biopsies showed so-called minimal-change disease (MCD) in the vast majority of cases. The efficacy of corticosteroids in this setting has obviated the need for renal biopsies in most of these cases. Therefore, young children with NS will typically undergo a therapeutic trial of corticosteroids without a biopsy. However, in infants with NS, in older children, or in those with evidence of nephritis (hypertension, hematuria, low C3, or decreased renal function) or failing corticosteroid therapy, renal biopsy is often performed. In these patients, disease other than MCD (e.g., focal segmental glomerulosclerosis [FSGS], membranoproliferative glomerulonephritis [MPGN], IgA nephropathy, membranous glomerulopathy, or more rarely, in infants less than 1 year, Finnish-type nephrotic syndrome or diffuse mesangial sclerosis) is often present (4–9). Children with steroid-resistant NS and FSGS on biopsy may have a podocyte gene mutation, such as podocin, as cause of their disease. Genetic testing is important to identify these children, as 10–30% of children with sporadic steroid-resistant NS and FSGS have such mutations, and do not typically respond to continued immunosuppression (10, 11). These genetically-induced lesions do not show specific renal biopsy morphologic findings.

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Acute Nephritis

The child with acute glomerulonephritis may need a biopsy when the course is not typical of acute poststreptococcal disease or if urinary abnormalities persist. Although the primary disease process may be evident in systemic conditions, such as Henoch-Schönlein purpura or systemic lupus erythematosus (SLE), renal biopsy often is indicated to assess severity of injury, to guide therapy and prognosis. Differentiation of specific types of proliferative lesions such as MPGN type I and dense deposit disease (DDD) is made by renal biopsy. This distinction has important implications for eventual treatment because the morphologic lesions of DDD invariably recur in transplants, although the clinical course is less severe than in the native kidney (12, 13).

Acute Renal Failure

The cause of acute renal failure may be clinically obvious, or there may be multiple potential culprits. When prerenal and obstructive causes are not apparent, renal parenchymal disease should be considered. When acute renal failure is associated with nephritis, NS, or evidence of vasculitis or systemic diseases, biopsy is usually performed. Other common causes include acute tubular necrosis or injury, often caused by drug or ischemic injury, vascular disease, and interstitial nephritis. These conditions can often be diagnosed without renal biopsy. However, when the cause remains uncertain after complete evaluation, renal biopsy may be necessary for diagnosis (14).

Rapidly Progressive Glomerulonephritis

Renal biopsy may be considered an urgent procedure in the patient with rapidly progressive glomerulonephritis (RPGN). Various systemic vasculitides that may be distinguished only by specific serologic studies (see below) or renal biopsy must be treated urgently to avoid severe chronic renal damage. Although anti-glomerular basement membrane (GBM) antibody or anti-neutrophil cytoplasmic antibody (ANCA) titers may provide useful information, the ANCA test in particular is not diagnostic of a specific condition, rather it is a screening test for necrotizing vasculitides (15, 16). ANCA positivity, whether in a perinuclear (p-ANCA) or cytoplasmic (c-ANCA) pattern was present in approximately 60% of patients with immune-complex glomerulonephritis with crescents in a study of more than 200 renal biopsies (17). Furthermore, specialized confirmatory ELISA ANCA assays may have a longer turn-around time than the renal biopsy, from which preliminary information from immunofluorescence (IF) and light microscopic studies can be available within hours after biopsy.

Chronic Renal Insufficiency

Patients with chronic renal insufficiency of uncertain etiology are candidates for renal biopsy. Although renal biopsy of the small, shrunken kidney is more risky because of the greater incidence of bleeding complications, the diagnosis of primary disease can be important. This information allows assessment of existing severity of morphologic lesions, determination of risk of recurrence in eventual renal transplant, and suitability of cadaveric vs. living-related donor transplantation. If the disease has a familial basis or recurs frequently with resultant graft loss, cadaveric transplantation may be preferable to living-related donor transplant (13).

Systemic Diseases

The severity of renal involvement in systemic disease, such as hemolytic-uremic syndrome (HUS), Henoch-Schönlein purpura, diabetes mellitus, or SLE, may not be apparent without renal biopsy. The trend is now toward early biopsy in patients with diabetes and renal abnormalities. Severity of lesions and stage of chronicity and activity impart prognostic information and may affect therapeutic decisions (see below). The most extensively studied disease in this regard is SLE. Differentiation of specific class of lupus nephritis by WHO or International Society of Nephrology/Renal Pathology Society (ISN/RPS) class (see below) may be difficult without renal biopsy (18–20). Overall, evidence indicates that renal biopsy findings may be more sensitive than clinical assessment alone in evaluating the severity of renal involvement in SLE (21, 22).

Follow-Up of Disease

With improved therapeutic modalities available for intervention in chronic progressive renal disease, sequential or follow-up biopsies is becoming increasingly necessary to evaluate therapeutic efficacy. On the other hand, additional cytotoxic therapy with its side effects may be withheld if the biopsy shows end-stage histology. Intervention with, for example, low-protein diets or angiotensin-converting enzyme (ACE) inhibitors or angiotensin

type 1 receptor blockers (ARBs) has been shown to alter the course of chronic progressive renal disease (23).

Transplantation

Renal transplant biopsies are useful in assessing episodes of clinically suspected rejection, investigating the cause of decreased renal function or urine output, and detecting the development of de novo or recurrent disease. Occasionally, infection may be diagnosed by renal biopsy. Drug toxicity may be diagnosed by morphologic findings. The absence of lesions in a patient with a rise in creatinine supports calcineurin inhibitor toxicity because this drug commonly causes a decline in the glomerular filtration rate (GFR) by vasoconstriction and not overt structural lesions. The absence of findings of acute rejection in renal biopsy or by needle aspiration (see below) thus can assist in avoiding unnecessary immunosuppressive therapy with its potential for increased morbidity and mortality. Even a diagnosis of chronic allograft nephropathy (CAN) which is not amenable to immunosuppressive therapy, has important therapeutic implications for the patient.

Diseases that recur in the transplant with high frequency include IgA nephropathy, MPGN type I, dense deposit disease (DDD, also known as MPGN type II), FSGS, HUS, and membranous glomerulopathy. The latter two also occur de novo in the transplant. Metabolic diseases such as oxalosis and diabetic nephropathy can also cause recurrent disease in the renal transplant, if liver or pancreas transplantation does not cure the primary abnormality (13). Alport syndrome is caused by mutation in one of the type IV collagen genes, resulting in abnormal GBM assembly and structure. Patients with Alport may develop anti-GBM antibody disease in the transplant because of antibodies against its normal type IV basement membrane collagen (24, 25).

Obtaining Tissue

General Considerations

Percutaneous renal biopsy is the most common method for obtaining tissue for the kidney. In large series, major complications are rare. The technique, first done in 1951 by Iverson and Brun, allows tissue yield in 93–95% of biopsies, with more than 87% of these being adequate (23–25). The biopsy findings altered diagnoses in half of the cases in one series, indicating different therapeutic approaches in approximately one-third of those cases (26). Although some renal diseases show diagnostic features by light microscopy (LM) (*Table 24-1*), special studies add to the sensitivity of the study. For the renal biopsy to be most useful, it must be evaluated appropriately by an experienced renal pathologist. Biopsies must be examined by special LM, IF, and electron microscopy (EM) for the most accurate diagnosis (27). If the nephrologist's hospital does not provide these services, arrangements must be made to send tissue in appropriate fixatives (see below) to a reference laboratory with these capabilities. If such services cannot be provided, it is doubtful whether the institution should be undertaking renal biopsies.

Contraindications

Contraindications to percutaneous biopsy are solitary, ectopic, or horseshoe kidney; bleeding diathesis; abnormal renal vascular supply; and uncontrolled hypertension (26–29). In the era of ultrasound guidance and automated biopsy instruments, solitary kidney may be biopsied safely in selected patients, however (30). Relative contraindications include obesity, uncooperative patients, hydronephrosis, ascites, and small shrunken kidneys, all associated with greater risk for complications. Open biopsy is preferable if the biopsy information is crucial in these conditions. Percutaneous biopsy is contraindicated if the kidney has tumors, large cysts, abscesses, or pyelonephritis because the needle track may facilitate spread of malignant cells or infection. Open biopsy allows selection of specific areas for biopsy in these situations.

Biopsy Technique

The patient may be brought to the hospital on the day of the biopsy. Laboratory evaluation must include a complete blood cell count with normal platelet count, partial thromboplastin and prothrombin times. On rare occasions, infusions of platelets or fresh frozen plasma may be necessary to allow renal biopsy in critical clinical situations in which histopathologic diagnosis is essential. Adequate control of hypertension before the procedure is important as hypertension is a risk for post biopsy bleeding (31). Before biopsy is done, ultrasound examination must confirm that there are two kidneys in normal position. The biopsy optimally is timed so that an experienced technician or pathologist can attend to ensure prompt processing of the biopsy tissue.

Food and drink should have been withheld for at least 6 h before biopsy, and the child should be lightly sedated. The child lies in the prone position with a sandbag or

Table 24-1

Characteristic abnormalities of glomerular diseases

Disease and typical clinical presentation	LM pattern	IF Staining			EM, Other Findings
		Mesangial	Subepithelial	Subendothelial	
Hematuria/nephritis					
Alport's syndrome	Early: normal Late: sclerosis	–	–	–	Thin and thick, split GBM
Mesangial lupus nephritis ISN/RPS II	Mesangial proliferation	+ All Igs, C3, C4	–	–	Immune deposits by EM, reticular aggregates in endothelial cells
Focal lupus nephritis ISN/RPS III	Proliferative, <50% of glomeruli	+	+ (few)	+ (Scattered)	Immune deposits by EM, reticular aggregates in endothelial cells
		–All Igs, C3, C4–			
Diffuse lupus nephritis ISN/RPS IV	Proliferative, >50% of glomeruli wire loops	+	+	+ (wire loop)	Immune deposits by EM, reticular aggregates in endothelial cells
		–All Igs, C3, C4–			
IgA nephropathy	Mesangial proliferation	+ Predominantly IgA	–	–	Immune deposits by EM
Henoch-Schönlein purpura	Mesangial and ± endocapillary proliferation, ± crescents	+ –	+/– Predominantly IgA	+/– –	Immune deposits by EM
Post-infectious GN	Endocapillary proliferation, PMNs	+ Coarsely granular IgG, C3	+ Coarsely granular IgG, C3	–	Irregular, hump-like deposits on top of GBM by EM
Hemolytic-uremic syndrome	Glomerular/arteriolar thrombosis	–	–	–	Increased lamina rara interna by EM, swollen endothelial cells, no deposits
MPGN I	Endocapillary proliferation, lobular double contour GBMs	+ IgG, C3	–	+ IgG, C3	Subendothelial immune deposits by EM, cellular interposition
MPGN II (dense deposit disease)	Mesangial, ± endocapillary proliferation, ribbon-like capillary wall	± C3 globular	– Ribbon-like, C3	Discontinuous	Intramembranous, mesangial non-immune dense deposits by EM
Nephrotic syndrome					
Minimal change disease	Normal	–	–	–	Effacement of podocyte foot processes No deposits
Focal segmental glomerulosclerosis	Segmental glomerulosclerosis, glomerular hypertrophy	+/– IgM, C3	–	–	Effacement of podocyte foot processes, no deposits
Diabetic nephropathy	Increased mesangial matrix, ± nodular, thick GBM, hyalinized arterioles	–	–	–	Thick GBM without deposits

Table 24-1 (Continued)

Disease and typical clinical presentation	LM pattern	IF Staining			EM, Other Findings
		Mesangial	Subepithelial	Subendothelial	
Membranous lupus nephritis ISN/RPS V	Thick GBM, spikes on Jones' stain	Scattered	+	–	Subepithelial, mesangial immune deposits
		+	–All Igs, C3, C4–	–	Reticular aggregates in endothelial cells
Idiopathic membranous GN	Thick GBM, spikes on Jones' stain	–	+	–	Subepithelial immune deposits
		–	IgG, C3–	–	
RPGN					
Anti-GBM disease	Focal segmental necrosis of glomeruli, crescents	–	Linear staining of GBM	–	No deposits by EM
			IgG, C3		
Wegener's granulomatosis	Focal segmental necrosis of glomeruli, crescents	–	–	–	No deposits by EM
Microscopic polyangiitis	Focal segmental necrosis of glomeruli, crescents	–	–	–	No deposits by EM

rolled sheet under the abdomen, and the skin of the flank is "prepped" and draped in sterile fashion. Although the left kidney is usually preferred, either side can be chosen for biopsy. The lower pole of the kidney is marked on the skin with a pen after localization by any of several imaging techniques, such as fluoroscopy, radionuclide scanning, intravenous urography, computed tomography (CT), or ultrasound, the last being the most commonly used method. Local anesthetic is infiltrated first in the skin and then in deeper tissues, taking care not to enter the kidney. In younger children who cannot reliably cooperate in this manner, biopsy is done under anesthesia. Conventional or spring-loaded needles are used for renal biopsies. Most now use the spring-loaded so-called "biopty gun". For conventional needles, the biopsy needle is inserted to the desired position as the patient again holds his or her breath, advancing the cannula over the obturator once the needle is in correct position. The entire needle with the core of tissue is then removed.

The use of an automatic spring-loaded biopsy system has been used widely in the last years because of the simplicity and ease of the technique (32, 33). The kidney is localized with ultrasound guidance, and the depth of the kidney as judged by ultrasound. The biopsy needle is advanced to the kidney capsule under ultrasound observation and guidance. The patient may hold his or her breath for only a few seconds while the spring-loaded needle is activated, causing the obturator to automatically advance into the kidney, and the entire needle is then removed with the tissue core. The speed of automated biopsy needles, however, minimizes the need for the patient to hold respirations, required with conventional needles. It is important to note that the caliber of the needle used with any of these techniques directly impacts the adequacy of the specimen (34). When 18-gauge needles are used with this method, the resulting cores are very small and there is artifact along the edges. The use of a 16-gauge needle thus is more likely to provide an adequate tissue sample without distortion and with fewer passes necessary to obtain adequate tissue.

Usually two cores of tissue are necessary for optimum evaluation, or three cores if 18-gauge needles are used. If tissue cannot be obtained after several passes, the biopsy should be attempted on another day. After biopsy, a dressing is applied, and the child is kept supine in bed for 6 h and monitored with frequent checks of vital signs and urine for hematuria.

Increasingly, percutaneous native and transplant renal biopsies are performed as same day procedures in pediatric patients so that hospital admission is not required. Those with post biopsy perinephric hematomas, post biopsy gross hematuria, or very young children can be observed overnight so that hemostasis is assured (35–37). Open biopsy can be performed under local or general anesthesia. The kidney can be directly visualized even through a small incision. Although a larger sample may be obtained with a wedge biopsy, it is preferable to also perform a needle biopsy to sample deeper cortex and

medulla for assessment of diseases that preferentially involve juxtamedullary glomeruli (see below).

Aspiration Biopsy

Fine-needle aspiration (FNA) biopsy technique is used most often in the transplant setting for analysis of immunologically activated cells. FNA can be useful in evaluating type 1 acute rejection (38). FNA can also obtain material for culture. A modified FNA technique has been described for collection of glomeruli from either native or transplant kidneys to analyze glomerular lesions. This FNA technique has limitations in sample size and is obviously not suitable for study of vascular or fibrotic processes. The less invasive nature of the procedure makes it amenable to serial monitoring of interstitial cellular infiltrates in transplanted kidneys (39).

Complications

Important complications occur in 5–10% of patients (26, 40–48). Major complications, usually bleeding, leading to nephrectomy occurred in 5 patients in a review of 8,081 (48), and 1 patient in a series of 5,120 biopsies. In a series totaling 1,820 biopsies in children, 1 nephrectomy resulted (40–45). Transient microscopic hematuria is universal after biopsy, although macroscopic hematuria is seen in only 5% and requires transfusion in up to 2.3% of patients. Perirenal hematoma is most often asymptomatic and can be seen by CT in up to 85% of biopsies. Symptomatic hematoma is rare, occurring in less than 2%. Arteriovenous fistulae are symptomatic with hematuria, hypertension, or cardiac failure in only 0.5% of biopsies, although bruits may be detected in as many as 75% of patients. Most fistulae heal within a few months. Other complications have been reported, including inadvertent puncture of other viscera or major renal vessels, sepsis, renal infection, and seeding of cancer. Death is rare, less than 0.1% in reviews of large series. Complication rates appear to be slightly higher in less developed countries (40, 42). Complications for the spring-loaded needle biopsy system appear to be similar to conventional needles if the same gauge is used (49). Complications relate in part to the number of passes made to obtain tissue. Therefore, the lower rate of complication with an 18-gauge spring-loaded needle in some studies is offset largely by the need for more passes for adequate samples and the distortion of tissue and edge artifact with use of an 18-gauge needle (see above).

Assessment of the Renal Biopsy

Adequacy of Sample

Cores of fat and connective tissue will float when placed in saline, but a core of renal parenchyma will sink. The biopsy sample should also be visually inspected with a dissecting microscope or hand lens. Glomeruli are visualized as small red dots in the biopsy core. Scarred glomeruli may be difficult to identify since they are not perfused. In diffuse disease, such as membranous glomerulopathy, one glomerulus may be adequate for diagnosis. However, in other diseases, such as crescentic glomerulonephritis, FSGS, or lupus nephritis, disease may be focal. The greater the number of glomeruli sampled, the lower the probability of missing a focally distributed lesion (50). If only 10% of glomeruli in the kidney are involved by the focal process, a biopsy sample of only 10 glomeruli has a 35% probability of missing the lesion, decreasing to 12% if the biopsy contains 20 glomeruli. When one-fourth of glomeruli are involved in the kidney, there is only a 5% chance of missing the abnormal glomeruli in a biopsy of 10 glomeruli. A biopsy of 20–25 glomeruli is sufficient to distinguish between mild disease (less than 20% of glomeruli involved), moderate disease (20–50% of glomeruli involved), or severe disease (more than 50% of glomeruli involved). Unfortunately, the widespread use of small gauge spring-loaded biopsy needles often results in smaller samples, which make the above assessments difficult or impossible. The sample site must also be considered in evaluating the adequacy of tissue. Even a large biopsy, consisting only of superficial glomeruli, cannot exclude the presence of early FSGS, in which the initial involvement is in the juxtamedullary glomeruli. Likewise, although nephronophthisis is most often diagnosed clinically, a juxtamedullary biopsy would be necessary for its morphologic diagnosis.

Allotment of Tissue

Renal tissue should be studied by light microscopic techniques with special stains (hematoxylin and eosin, modified silver stain [periodic acid/methenamine or Jones' stain], periodic acid-Schiff), IF, and EM (51–53). The tissue is divided so that glomeruli are present in each portion of the sample. Optimally, the pathologist or an experienced histotechnologist will attend the biopsy and inspect and allot tissue for each study. If this is impossible, tissue may be placed in saline and brought directly to the laboratory for prompt processing. It is important to handle the tissue gently so that artifacts do not occur.

The fresh tissue must not be picked up with forceps because this will crush and distort the morphology. The core can be handled carefully with a wooden stick or pipette. The core should not be placed on a sponge or gauze pad because this may cause a divotlike artifact as the unfixed tissue molds to the holes of the underlying surface. The biopsy specimen is therefore placed on a clean smooth surface, such as a wax board, for cutting.

It may be difficult to identify the cortical end of the specimen even after inspection with a hand lens or dissecting microscope when scarring or severe injury is present. Therefore, we recommend cutting two 1-mm pieces with a sharp blade from each end of the core for electron microscopic studies. The remaining core is then divided into specimens for IF and LM. Of note, use of an 18-gauge needle yields tissue cores that are too thin to divide lengthwise, leading to a greater chance of problems in allocation of tissue for each method of study. This tissue should be cut across into two pieces for IF and LM. When two cores are obtained, we prefer to duplicate this process, rather than allocating one complete core to one study to maximize chances of adequacy of tissue for each study. When the tissue sample obtained is very small, the nephrologist and the pathologist should consider the differential diagnosis and allocate tissue accordingly. For example, in a case of suspected IgA nephropathy, tissue for IF is most important. Although electron microscopic studies can be done on other portions of tissue (as long as mercury-based fixatives have not been used), IF studies on fixed tissue are not as reliable. When tissue for EM is inadequate, portions of the paraffin-embedded tissue left after light microscopic examination may be cut out from the block and processed for EM. Although the quality is not optimal, diagnostic findings can still be discerned. In special circumstances, when no tissue remains in the paraffin block and a focal lesion in one section must be studied, one may attempt to process the tissue section from a glass slide for EM.

Light Microscopy

Numerous fixatives are used for light microscopic examination, and they vary from institute to institute. Satisfactory results may be obtained with Zenker's, Bouin's, formalin, Carnoy's, or paraformaldehyde. Material for IF studies may be snap frozen immediately at −20°C in solutions of isopentane, dry ice, acetone, or freon and embedded in Tissue-tech, OCT, or other compounds for frozen sections. If tissue cannot immediately be snap frozen, it may be placed in Michel's tissue media, where it may be stored for up to 1 week before freezing. This allows tissue to be sent to reference laboratories for appropriate processing. Tissue for EM may be fixed in glutaraldehyde, formaldehyde, or other appropriate non-mercury fixatives. Tissue placed in glutaraldehyde should be promptly processed, or if stored for future possible processing, should be transferred to an appropriate buffer solution within a week to avoid artifacts. Tissue for LM is routinely processed, embedded in paraffin, and cut into 2- to 3-μ thick sections. Serial sections with multiple levels are then prepared for examination.

If water-soluble compounds are expected, such as urate or uric acid, the tissue should be fixed in ethanol. Lipids are best detected in frozen sections because they are extracted during xylene processing for paraffin sections. Hematoxylin and eosin stains are most useful for overall assessment of the interstitium and crystals. This stain also allows particularly good visualization of infiltrating cells, especially eosinophils. In addition, fibrin may be easily visualized by this stain. Periodic acid-Schiff (PAS) stains glycoproteins and accentuates basement membranes and matrix material and allows definition of the brush border of proximal tubular cells. Areas of hyalinosis and protein precipitation, including cryoglobulin (which usually is dominantly IgM), are also accentuated with PAS. Silver stains, such as Jones' stain, stain basement membrane material but not deposits, thus allowing distinction of these components. Masson's trichrome stain detects areas of collagen deposition by staining bluish. Other special stains, such as immunohistochemistry for specific molecules, may be indicated. Congo red stain detects amyloid. Special stains can detect bacterial or fungal organisms and acid-fast bacilli. Special techniques may also be used on the light microscopic material. These include polarization to detect crystals or foreign bodies and morphometry to assess glomerular size (see below) and severity of interstitial fibrosis quantitatively.

Immunofluorescence

IF studies are most commonly done by direct IF on frozen tissue sections with application of fluorescein-conjugated antibodies directed against IgG, IgA, IgM, and complement component C3. Additional antisera may be used as clinically indicated. These include antisera to kappa or lambda light chain, antisera to hepatitis B antigen, thyroglobulin, fibrinogen, C1q, C4 cyantisera to type IV collagen chains and C4d. Some of these antigens are recognized by antisera even after fixation and can be detected by immunohistochemical techniques (e.g., immunoperoxidase) on the formalin-fixed tissue sections and then studied

with a light microscope. This technique requires enzyme pretreatment of tissue, which must be tailored exactly depending on length and type of fixation, section thickness, and antigen one wishes to study. Direct observation of the digestion process, stopping when all plasma is removed from capillary loops has been used to achieve reliable results (54). These challenges have prevented widespread use of this technique in the USA.

Frozen tissue sections stained by the commonly used method of fluorescein-conjugated antibodies are viewed by IF microscopy, evaluating staining in glomeruli, vessels, tubules, and interstitium. The pattern of glomerular staining is assessed to define granular or linear capillary wall staining or mesangial deposits. Arteriolar staining may be of diagnostic significance. Tubules may show deposits in lupus nephritis, light chain deposition disease or linear staining in the rare anti-TBM antibody disease. Nuclear staining can be seen in lupus or lupuslike diseases as a tissue manifestation of the patient's positive ANA. IF is more sensitive than EM in identifying immune deposits, although EM provides more detailed information on the exact localization of those deposits (52, 53). Lesions of interest should be photographed because fluorescence fades on storage and with light exposure.

Electron Microscopy

Tissue for EM is processed with postfixation in 1% osmium tetroxide, which enhances contrast of the tissue, and then dehydrated and embedded. With new, more rapidly polymerizing embedding media, such as Spurr, tissue may be ready for examination within 1 day. Electron microscopic study was found to add information in 6–11% of renal biopsies in a study from 1983 (27). In a 1997 study, EM was needed to make a diagnosis in 21% of cases, and provided important confirmatory data in approximately 20% of cases (52). EM showed diagnostic pathological abnormalities in 18% of patients with "normal" light microscopic findings. Larger, so-called thick scout sections (1 μ) are stained with toluidine blue to select smaller areas for thin sectioning for the electron microscope. Usually, the glomerulus containing the most representative lesion is chosen, but sclerotic glomeruli are not useful to examine by EM. If there are irregular or focal proliferative lesions, several glomeruli may be sampled, including the proliferative ones. The 60–90 Å thin sections are stained with uranyl acetate and lead citrate before viewing in the EM scope, to enhance contrast.

Immune complex deposits are nonmembrane-bound and denser than basement membrane or matrix materials. In specific diseases, such as cryoglobulinemia, amyloid, immunotactoid or fibrillary glomerulopathies, or lupus nephritis, specific substructure of deposits may be seen. Specific localization of immune complexes is done by EM examination, indicating whether deposits are subendothelial, subepithelial, mesangial, or in all of the above compartments. In some diseases, such as light chain deposition disease or lupus nephritis, deposition may also be seen in vessels and tubules. So-called fingerprint deposits, with substructure reminiscent of fingerprinting, are present in some cases of lupus nephritis. Reticular aggregates (or so-called tubular arrays) of membrane material in endothelial cell cytoplasm throughout the body are characteristically seen in large numbers in patients with SLE or HIV infection, thought to reflect a response to high levels of interferon (53, 55).

EM also delineates specific basement membrane abnormalities. For instance, small subepithelial deposits without surrounding new basement membrane material do not result in spikes, and therefore cannot be visualized by Jones' stain on LM, but can still be detected directly by EM. The lamina densa of the GBM is markedly thickened in diabetic nephropathy. Circumferential cellular interposition is defined by extension of monocytes and mesangial cell cytoplasm into the subendothelial space, with newly formed basement membrane interpositioning between the advancing infiltrating cells and the endothelium with intervening basement membrane material, thus giving rise to the classic double contours seen with silver stain by LM in MPGN. Increased lucent material is present in the expanded lamina rara interna in transplant glomerulopathy, HUS, ecclampsia and other diseases presumed to involve endothelial injury/coagulopathy. In these conditions, deposition of fibrin, fibrinogen and their degradation products may also occur. Fibrin is recognizable by its dense, coarse sheaflike structure by EM, with periodicity observed in favorable sections. Morphometry of the GBM from EM prints is used to diagnose thin basement membranes in hereditary nephritides. EM also allows structural assessment of changes of specific cells (see below). Diagnostic inclusions are seen in various storage/metabolic disease, such as Fabry's disease.

Basic Renal Lesions

Normal

During fetal maturation, the glomerular capillary tufts are initially covered by large, cuboidal, darkly staining epithelial

cells with only small lumina visible (Fig. 24-1). The cells lining Bowman's space undergo similar change from initial tall columnar to cuboidal to flattened epithelial cells, except for those located at the opening of the proximal tubule, where cells remain taller. Immature nephrons may occasionally be seen in the superficial cortex of children up to 1 year of age. Glomerular growth continues until adulthood, with average normal glomerular diameter approximately 95 μ in a group of patients less than 5 years old (average age 2.2 years) and 140–160 μ in adulthood (57, 58).

The normal mature glomerulus consists of a complex branching network of capillaries originating at the afferent arteriole and draining into the efferent arteriole. The glomerulus contains three resident cell types: mesangial, endothelial, and epithelial cells (Fig. 24-2). The visceral epithelial cells (podocytes) cover the urinary surface of the GBM with pseudopodlike extensions called foot processes, with intervening filtration slits. Endothelial cells are opposed to the inner surface of the GBM and are fenestrated. At the stalk of the capillary, the endothelial cell is separated from the mesangial cells by intervening mesangial matrix. The term *endocapillary* is used to describe proliferation filling up the capillary lumen, contributed to by proliferation of mesangial, endothelial and infiltrating inflammatory cells. In contrast, *extracapillary* proliferation refers to proliferation of the parietal epithelial cells that line Bowman's capsule.

The mesangial cell is a contractile cell that also has phagocytic properties. It lies embedded in the mesangial matrix in the stalk region of the capillary loops, attached to anchor sites at the ends of the loop by thin extensions of its cytoplasm. Normally up to three mesangial cell nuclei per lobule are present. The basement membrane consists of three layers distinct by EM, the central broadest lamina densa and the less electron-dense zones of lamina rara externa and interna. Thickening occurs with maturational growth. Most investigators have found thicker basement membranes in boys, with normal range from 220 to 260 nm at 1 year of age, 280 to 327 nm at age 5 years, 329 to 370 nm at age 10 years, and 358 to 399 nm at age 15 years (59, 60). In our laboratory, we found a range of GBM thickness in children with normal kidneys from approximately 110 nm at age 1 year to 222 ± 14 nm at 7 years of age.

The glomerulus is surrounded by Bowman's capsule, which is lined by parietal epithelial cells. These are continuous with the proximal tubule, identifiable by its

Figure 24-1

Schematic illustration of glomerulus, with glomerular capillary attached to a mesangial stalk area. The glomerular endothelium *(E)* is fenestrated and lines the glomerular basement membrane *(GBM),* which covers the mesangium. The outside of the GBM is covered by the epithelial cell and its foot processes *(Ep)*. The mesangial cell *(M)* is embedded within the mesangial matrix *(MM),* with processes connecting to the GBM (Provided courtesy of Professor Wilhelm Kriz with permission from (56).

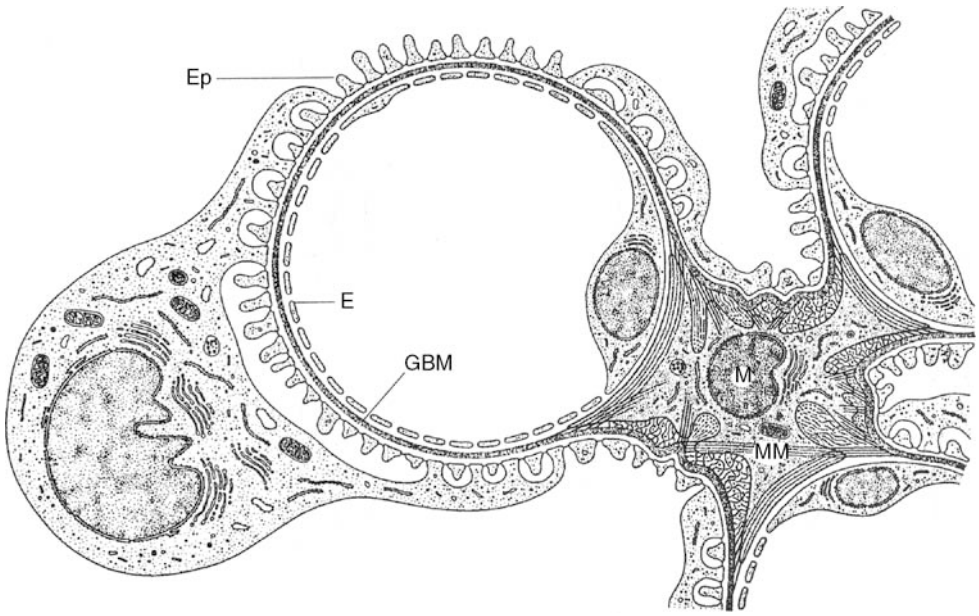
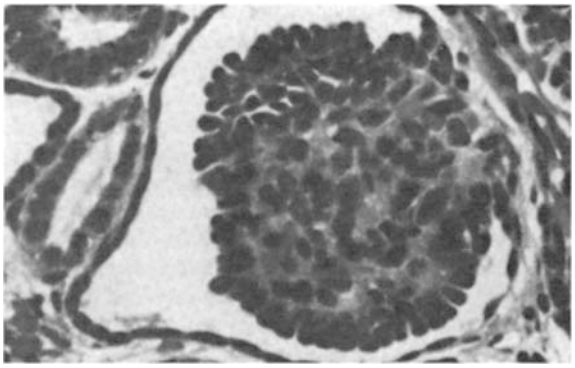


Figure 24-2
Immature glomerulus with plump, dark epithelial cells from biopsy of a 29-week-gestation baby (PAS, ×670).



PAS-positive brush border. The efferent and afferent arterioles can be distinguished morphologically in favorably oriented sections or by tracing their origins on serial sections. Segmental, interlobular, and arcuate arteries may also be present in the renal biopsy specimen. The cortical biopsy also allows assessment of the tubules and interstitium. Proximal tubules are readily identified by their PAS-positive brush border, lacking in the distal tubules. Collecting ducts show cuboidal, cobblestonelike epithelium. Tubules are normally back-to-back with minimal interstitial cells and the peritubular capillaries intervening. The medulla may also be included in the biopsy.

Overall Pattern

Assessment of the biopsy specimen must include inspection of all sections from different levels because additional glomeruli may be sampled on deeper cuts of the biopsy core and many diseases are characterized by focal lesions. The severity and patterns of lesions are assessed, and normal and affected glomeruli are counted. Lesions are classified as focal if only some (less than half) glomeruli are involved, diffuse if all (or most) glomeruli are involved, segmental if only portions of glomeruli are involved, and global if entire glomerular tufts are involved. Characteristic glomerular disease patterns include lobular proliferation in MPGN, nodular proliferation of mesangial cells and abundant matrix material in characteristic Kimmelstiel-Wilson lesions, focal and segmental sclerosis, intraglomerular/arteriolar fibrin thrombi, necrotizing lesions and crescent formation (*Table 24-1*). The crescent, a lesion due to proliferation of mostly parietal epithelial cells, owes its name to its shape in well-established lesions.

Glomeruli are assessed for alterations in size (see below). It is important to compare with a normal control for a given age group because marked glomerular maturational growth occurs in children. Glomerular hypertrophy may be an important predictor of increased risk of FSGS in children with apparent MCD (see below). Maturational pattern of glomeruli (see above) should be noted. Occasional fetal glomeruli may be found in children beyond infancy and are not of specific diagnostic significance.

Glomeruli are assessed for glomerulosclerosis, that is, the presence of segmental obliteration and scarring of glomerular capillary tufts. Sclerosis may be in a segmental or global pattern (*Table 24-2*). Previous studies suggested that up to 10% of glomeruli may be normally globally sclerosed in people younger than 40 years of age (61). This number may be even smaller in children, with less than 1–3% global sclerosis expected normally up to age 40 or 56, respectively (62, 63). These occasional globally sclerotic glomeruli are thought to represent errors of nephrogenesis. The percentage of global sclerosis increases even with normal aging, up to half the patient's age, minus 10 (63). Globally sclerosed glomeruli in greater percentage indicate the possibility of renal disease (focal global sclerosis) (64).

The pattern of tubulointerstitial fibrosis, whether proportional to glomerular sclerosis or not, whether diffuse or present in a "striped" pattern following the medullary rays, or in broad patchy zones, has diagnostic significance (see below).

Specific Glomerular Cells

Podocytes

The podocytes (glomerular visceral epithelial cells) may show vacuolization in various diseases with severe proteinuria. Although more extensive vacuolization of podocytes has been seen in FSGS compared with patients with MCD (65), these changes are only seen after established sclerotic lesions are identifiable by LM and do not permit distinction of these two disease processes in the early phase in which segmental sclerosis may be undetected. Hypertrophy of podocytes is prominent in MCD and FSGS. Effacement of the foot processes of the podocytes by EM is common to any disease with marked proteinuria, and the podocyte may also show microvillous transformation, with long, attenuated pseudopods. Podocytes are limited in their ability to proliferate. However, the early sclerotic lesion of FSGS is characterized by prominence and apparent increase of the overlying podocytes

Table 24-2

Definitions of common morphological terms

Light microscopy	
Focal	Involving some glomeruli
Diffuse	Involving all glomeruli
Segmental	Involving part of glomerular tuft
Global	Involving total glomerular tuft
Lobular	Simplified, lobular appearance of capillary loop architecture (MPGN)
Nodular	Acellular areas of mesangial matrix (diabetic nephropathy)
Sclerosis	Obliteration and scarring of capillary loop
Crescent	Proliferation of parietal epithelial cells
Spikes	Projections of glomerular basement membrane intervening between subepithelial immune deposits (membranous GN)
Endocapillary proliferation	Increase in mesangial and/or endothelial cells
Hyaline	Descriptive of glassy, smooth appearing material
Hyalinosis	Hyaline-appearing insudation of plasma proteins (focal segmental glomerulosclerosis)
Mesangium	Stalk region of capillary loop with mesangial cells surrounded by matrix
Subepithelial	Between podocyte and glomerular basement membrane
Subendothelial	Between epithelial cell and glomerular basement membrane
Tram-track	Double contour of glomerular basement membrane due to deposits and/or CMIP (see below)
Wire loop	Thick, rigid appearance of capillary loop due to subendothelial deposits
Activity	Score of possible treatment sensitive lesions, based on e.g., extent of crescents, cellular infiltrate, necrosis, proliferation
Chronicity	Score of probable irreversible lesions, based on e.g., extent of tubular atrophy, interstitial fibrosis, fibrous crescents, sclerosis
Immunofluorescence microscopy	
Granular	Discontinuos flecks of staining along capillary loop producing granular pattern
Linear	Smooth continuous staining along capillary loop
Electron microscopy	
Foot process effacement	Flattening of foot processes so that they cover the basement membrane
Microvillous transformatiom	Small extensions of epithelial cells with villus-like appearance
Circumferential mesangial interposition (CMIP)	Extension of cell cytoplasm with interpostion between endothelial cell cytoplasm and basement membrane and underlying new basement membrane formation
Reticular aggregates	Organized arrays of membrane particles within endothelial cells
Immunotactoid GP	Large, organized microtubular deposits, >30 nm diameter
Fibrillary GP	Fibrils 14–20 nm diameter without organization

GP glomerulopathy

("capping" lesion), often associated with endocapillary foam cells. This cellular variant of FSGS may be more common in children than in adults with FSGS (66). The idiopathic collapsing variant of FSGS and HIV-associated nephropathy both show prominent hyperplasia and protein droplets of the podocytes, with overlying segmental collapse of the glomerular capillary tuft. In the situation of recurrent FSGS in the renal transplant, NS and foot process effacement may be seen within weeks after biopsy, with sclerosis becoming apparent at a later date (67). In Fabry's disease, there is accumulation of glycosphingolipid because of deficiency of alpha-galactosidase. Podocytes show marked vacuolization by LM with characteristic whorled, laminated electron-dense myelin bodies by EM. In Fabry's disease, these inclusions may also be present in endothelial cells, tubular epithelial

cells, some interstitial cells, and the vessels in early lesions in children (27, 53, 68).

Mesangial Cells

Hyperplasia of mesangial cells is recognized by LM when more than three mesangial cell nuclei are present per mesangial region. Increased mesangial prominence may be due to increased cellularity, increased matrix, deposits, or a combination. Large mesangial deposits appear on Jones' stain as pinkish areas surrounded by the light silver-staining areas of mesangial matrix. So-called interposition results when the monocyte or mesangial cell cytoplasm extends outward between basement membrane and endothelial cells and new matrix accumulates between the mesangial and endothelial cell bodies.

Endothelial Cells

Extreme proliferation and swelling of endothelial cells can obliterate capillary lumina in conditions characterized by abnormalities of coagulation. Endothelial cells usually contain characteristic reticular aggregates in lupus nephritis and HIV-associated nephropathy (53, 55). Endocapillary cell proliferation is characteristic of for example diffuse proliferative lupus nephritis and MPGN type I.

Crescents

Crescents consist primarily of proliferating parietal epithelial cells with some infiltrating macrophages and are a manifestation of severe glomerular injury. The name reflects the often crescent-shaped sheet of cells filling up part or nearly all of Bowman's space. Crescents result from injuries that break the GBM, leading to exudation of plasma protein and formation of fibrin within Bowman's space, which then induces proliferation of the parietal epithelial cells and infiltration of macrophages. When crescents are a prominent histologic feature, the patient most often presents clinically with a rapidly progressive glomerulonephritis.

Crescents may occur in a variety of diseases. Diseases with crescents as a primary manifestation include antibody-mediated injury (anti-GBM antibody disease), severe immune-complex diseases (e.g., lupus nephritis) and pauciimmune diseases. The latter are often, but not invariably, associated with positive ANCA tests, and may be associated with systemic disease, or be renal limited. The p-ANCA (anti-myeloperoxidase) pattern is most often associated with microscopic polyangiitis, whereas the c-ANCA (anti-proteinase-3) pattern is typical in Wegener's granulomatosis. Of note, positive ANCA tests are not sensitive in distinguishing these categories (15–17). Renal biopsy is therefore critical for accurate diagnosis. Diagnosis and appropriate treatment must occur rapidly in this clinical situation to optimize chances of recovery of renal function. The early lesion of cellular crescents is responsive to cytotoxic therapy. Biopsy indications of irreversible renal damage include breaks of Bowman's capsule and fibrous transformation of the cellular crescents, periglomerular fibrosis, and scarred glomeruli and tubulointerstitium.

Glomerular Basement Membrane

GBM abnormalities are best evaluated by EM. The basement membrane is abnormally thick in diabetic nephropathy (53). Diffuse abnormally thin GBMs, less than 250 nm in adults, are seen in familial hematuria (59, 60). GBM thinning cannot be accurately from EM processed from paraffin tissue, as this back-up technique causes artifactual and variable thinning of GBMs (69). In children, the diagnosis of thin basement membranes is more difficult than in adults because GBM increases in thickness with normal maturation. Glomerular basement membrane thickness should be compared with normal for age and sex (see above; 59, 70, 71). In Alport syndrome, the basement membrane in established lesions is characterized by irregular areas of very thin and very thick, with splitting and splintering of the basement membrane (25, 72). The GBM in nail-patella syndrome is irregular, thickened, and split, with electron-lucent areas containing banded collagen type I fibers (53).

Immune deposits may localize on either side of the GBM. Subepithelial immune deposits are characteristically seen in membranous glomerulopathy. Subendothelial immune deposits are seen for example in proliferative lupus nephritis or MPGN type I.

The basement membrane may appear split by LM in diseases other than MPGN type I or dense deposit disease. In transplant glomerulopathy, the split appearance results from varying degrees of cellular interposition and widening, with increased lucent material in the lamina rara interna. This is also a characteristic finding in preeclampsia, transplant glomerulopathy and chronic HUS or other chronic thrombotic microangiopathies (53).

Tubules

Morphologically evident tubular necrosis correlates poorly with the clinical extent of acute kidney injury (AKI).

The changes vary from nondiagnostic vacuolization to frank necrosis with sloughing of tubular epithelial cells (toxic type) and flattened epithelium characteristic of regeneration (ischemic type). In cortical necrosis, zones of cortex, including glomeruli, are necrotic. In chronic kidney disease, tubules are atrophied with dilation and flattened epithelium, presumably secondary to lesions affecting the glomerulus, although primary tubular and interstitial injury mechanisms may also be involved in these changes. Tubular atrophy is also present in primary tubulointerstitial diseases. Tubulointerstitial fibrosis is an important manifestation of calcineurin inhibitor toxicity. The fibrosis occurs along the medullary rays due to the more severe ischemia occurring in these areas, resulting in a striped, rather than diffuse, pattern of fibrosis, with intervening preserved tubules.

Nonspecific casts of Tamm-Horsfall protein are seen in chronic kidney disease. Other casts may have a diagnostic appearance, such as the giant cells surrounding tubular casts in light chain cast nephropathy (so-called myeloma kidney). Casts of myoglobin with characteristic reddish-brown appearance are seen in rhabdomyolysis, often with associated acute tubular necrosis. Crystals, for example oxalate or cysteine, may be identified by examination under polarized light (73). Tubules contain characteristic inclusions in Fabry's disease (68).

Polymorphonuclear neutrophils (PMNs) within collecting ducts and proximal tubules are diagnostic of acute pyelonephritis. In chronic pyelonephritis, there is tubular atrophy and interstitial fibrosis, characteristically in a patchy, regional distribution (geographic or "jigsaw" pattern). The combination of segmental glomerular sclerosis with ischemic changes of corrugation and thickening of the GBM and periglomerular fibrosis, and patchy, regional interstitial fibrosis and tubular atrophy is also characteristic of reflux nephropathy (74).

Cysts may be demonstrated by biopsy, although the diagnosis of specific cystic diseases is usually made by combination of clinical and ultrasound findings. The segment of the nephron giving rise to the cysts can be identified by histochemical stains (75). Areas of low cuboidal epithelial-lined structures surrounded by a cuff of immature mesenchyme are present in the dysplastic kidney, often with cartilage, fat, or abnormal blood vessels in the interstitium. The deep medullary cystic dilation with thickened, lamellated TBM characteristic of nephronophthisis can be identified with a deep biopsy. Dilation of proximal tubules with microcyst formation in conjunction with extensive foot process effacement by EM is characteristic of congenital nephrotic syndrome of Finnish type. FSGS in a collapsing patten with podocyte hyperplasia, numerous reticular aggregates by EM, and with tubular cystic dilation and interstitial fibrosis out of proportion to the severity of glomerular lesions are highly suggestive of HIV-associated nephropathy (55).

Viral infections, including BK and CMV, result in characteristic nuclear inclusions in tubular epithelia. Specific diagnosis is made by immunostaining for viral proteins. Characteristic viral particles may also be detected by EM (76).

Interstitium

Interstitial edema is a nonspecific change, present, for example, in early acute transplant rejection, renal vein thrombosis, or inflammatory processes. Identification of eosinophils in an infiltrate is suggestive of drug-induced interstitial nephritis, although eosinophils are also present in some cases of idiopathic interstitial nephritis (77). Eosinophils can also be part of acute cellular rejection in the transplant. Nonnecrotizing granulomas, with or without eosinophils, most often reflect drug-induced hypersensitivity reaction. A pleomorphic infiltrate of lymphocytes, plasma cells and PMNs suggests possible BK nephropathy in the transplant, confirmed by finding viral nuclear inclusions and positive immunostaining in tubules (see above) (76). Fibrosis results in increased spacing of tubules because of the accumulation of PAS-positive collagenous material. The collagen also stains specifically blue with Masson's trichrome stain. Fibrosis in a striped pattern suggests calcineurin inhibitor toxicity (78, 79). Occasionally, the interstitium is infiltrated by malignancy. Hematopoietic neoplasms are especially prone to involve the kidney. In cystinosis, characteristic rectangular or trapezoid crystals are present mostly in monocyte/macrophages, and can be recognized by EM or when polarized on frozen sections (73).

Vessels

Arterioles and larger segmental and interlobular arteries are evaluated for changes in the intima and media; the presence of deposits, fibrin, hyalin, amyloid, or other material; or the presence of vasculitis. Arterioles show inclusions early in children with Fabry's (68). Larger vessels typically are not sampled by a biopsy, and diseases such as classic polyarteritis nodosa that affect these large vessels are therefore best evaluated by other methods (e.g., arteriography). Intimal fibrosis and medial thickening with hyperplasia and hypertrophy of media are characteristic of hypertensive injury. Concentric medial necrosis

with nodular protein deposition suggest acute calcineurin inhibitor nephrotoxicity (79). Fibrin thrombi, when present in glomeruli and/or arterioles, are the essential lesion of the thrombotic microangiopathies caused by e.g. HUS (53). Fibrin localizes predominantly within glomerular lumina in disseminated intravascular coagulation and hyperacute rejection.

Clinical Pathological Correlations

After evaluation of the structural changes of the renal biopsy in conjunction with the clinical history, a diagnosis may be obvious. In some cases, the biopsy specimen may show overlap features, or there may be elements that do not correlate clearly with the clinical setting. Close collaboration by nephrologists and pathologists is essential in arriving at the diagnosis. The pathologist must be familiar with clinical manifestations of renal disease, and the nephrologist should be familiar with the terminology used by the pathologist to describe the biopsy findings (*Table 24-2*).

When lesions are evaluated, the balance of all elements must be considered. When a typical disease pattern is not present, one must consider whether more than one process is taking place. For instance, drug-induced interstitial nephritis may be superimposed on other glomerular disease. This is especially true in the transplant setting, where multiple disease processes may occur at one time. In some instances, the biopsy findings do not correlate with the patient's renal function. When such apparent discrepancies are found, one possibility is that the biopsy specimen is not representative of all the nephrons of the kidney. The number of glomeruli necessary to estimate the severity of diseases that show focal distribution has been discussed above. However, the extent of glomerulosclerosis may in and of itself not correlate with renal function. Tubular atrophy and interstitial fibrosis may be more closely correlated with extent of renal damage and renal function (80–82). One must also consider the elements other than structure that influence the patient's renal function (i.e., blood pressure, filtration properties of the GBM, and the glomerular filtering surface area). Patients with enlarged glomeruli, either caused by compensatory hypertrophy or by a primary pathological process, may show less deterioration of renal function than expected based on the extent of glomerular scarring. Similarly, treatment of the patient with antihypertensive agents that affect glomerular filtration rate (e.g., ACE inhibitors, which preferentially dilate efferent arterioles) may actually increase serum creatinine levels in the short run. Compensation by remaining nephrons may mask ongoing severe disease processes such that creatinine levels may remain near normal until late in the course of disease when therapy is less likely to have an impact on chronic progressive injury.

Diagnostic Findings in Selected Renal Diseases

Minimal Change Disease/Focal Segmental Glomerulosclerosis

MCD is diagnosed only after the exclusion of abnormal findings at the light microscopic level, with diffuse foot process effacement as the only abnormality by EM. The disease is characteristically sensitive to glucocorticoid therapy. However, repeated renal biopsies in patients with apparent MCD initially have shown progression to FSGS, which has a high incidence of progression to end-stage renal disease (ESRD) (83, 84). As discussed above, a small sample may not include the segmentally sclerotic glomerulus, diagnostic of FSGS. In FSGS, there is often also hyalinosis, an exudation of plasma proteins and lipids with a glassy, smooth (hyaline) appearance on LM. There are no immune deposits, and foot process effacement is present in all glomeruli by EM (*Fig. 24-3*). Mesangial expansion in the native kidney FSGS biopsy may be associated with increased risk for recurrence in the transplant (85).

The presence of IgM by IF without deposits by EM in a biopsy that otherwise appears to be MCD (so-called IgM nephropathy) does not have prognostic value (86). Some variants of FSGS may have prognostic value (87). A recently proposed working classification of FSGS aims to examine whether morphological patterns of FSGS have prognostic implications (*Table 24-3*) (88). The usual type is diagnosed when no special features are present. The collapsing type of FSGS, characterized by collapse of the glomerular tuft, either segmental or global with associated podocyte hypertrophy/hyperplasia, shows a rapid progression to end-stage disease (89). The cellular lesion, with endocapillary proliferation with frequent foam cells and often with podocyte hyperplasia, may represent an early stage of FSGS, and appears to occur more often in children with FSGS than adults (66, 90). The tip lesion, that is adhesion, sclerosis or endocapillary foam cell lesion localized to the proximal tubular pole, appears to have better prognosis (91–93). The perihilar variant, with sclerosis and hyalinosis localized to the vascular pole, likely more often represents a secondary sclerosing process (88).

Figure 24-3

Minimal change disease. The foot processes are effaced (×11,000).

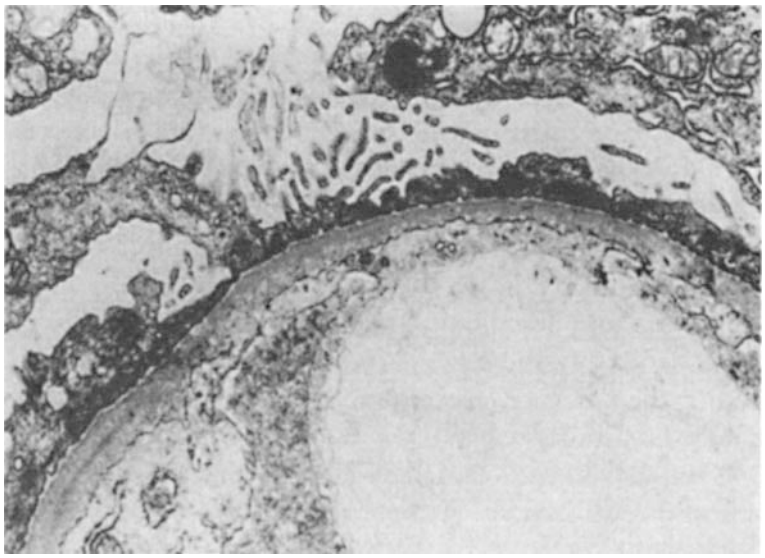


Table 24-3

Working classification of FSGS

Type	Key histologic feature	Possible prognostic implication
FSGS, nos	Segmental sclerosis	Typical course
Collapsing FSGS	Collapse of tuft, GVEC hyperplasia	Poor prognosis
Cellular FSGS	Endocapillary proliferation, often GVEC hyperplasia	?Early stage lesion
Tip lesion	Sclerosis of tuft at proximal tubule pole	?Better prognosis
Perihilar variant	Sclerosis and hyalinosis at vascular pole	?May reflect a secondary type of FSGS

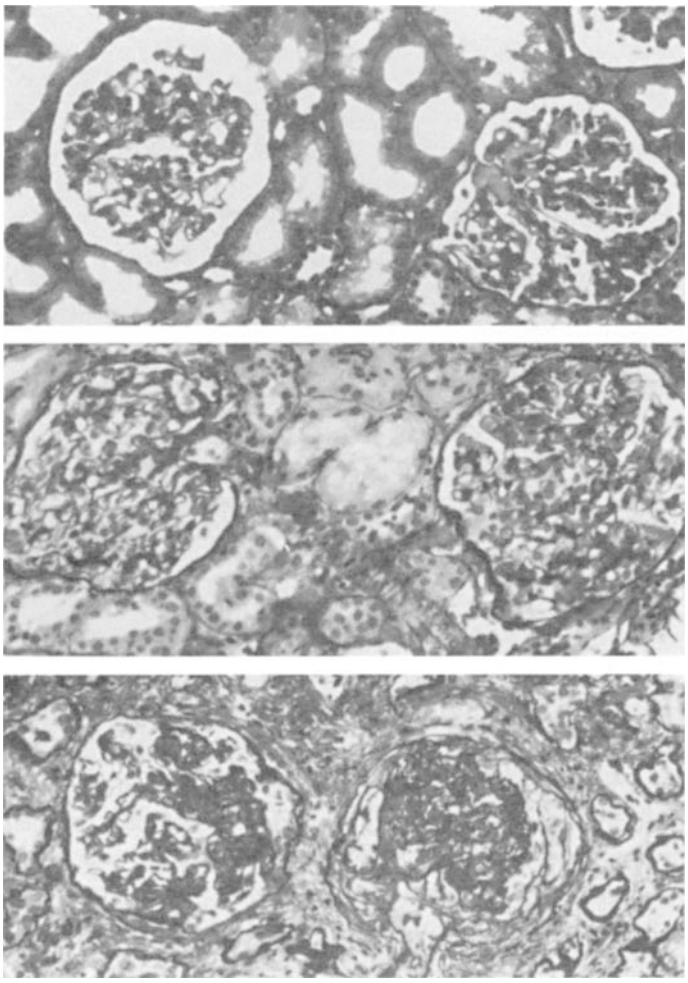
nos not otherwise specified; *GVEC* glomerular visceral epithelial cell

C1q nephropathy is characterized by either no sclerosis or segmental glomerulosclerosis by LM, with mesangial C1q deposits and lesser immunoglobulin components without dominant or codominant IgA (94). EM shows mesangial and paramesangial dense deposits but a lack of reticular aggregates. Patients typically are adolescents and have steroid-resistant NS and do not have clinical evidence of SLE. Although these findings suggest a distinct clinicopathologic entity, the prognostic significance of these lesions has not yet been established. Progression to ESRD has occurred in some patients with sclerosis at biopsy, but long-term outcome of those without sclerosis at presentation has not yet been established.

Because therapy and prognosis are different for MCD vs. FSGS, early distinction of these two entities is of primary interest. We studied pediatric patients with steroid-resistant nephrotic syndrome and MCD on renal biopsies and compared to patients with apparent MCD on biopsy who subsequently progressed to overt FSGS (57). Morphometric analysis of initial biopsies showed that glomerular size at the onset of disease, before sclerosis was apparent, was remarkably larger in patients who subsequently progressed to FSGS (*Fig. 24-4*) (95, 96). There was a higher risk for development of FSGS in patients <5 years old with glomerular area greater than 1.5 times that of normal age-matched controls. On the other hand, glomerular size equal to or less than normal controls in this group of patients indicated a good prognosis. Calculated glomerular diameter for increased risk of FSGS in these patients less than 5 years old was more than 118 μ vs. 95 μ glomerular diameter in age-matched controls. Depending on processing and fixation,

Figure 24-4

Apparent minimal change disease (MCD) with subsequent progression to focal segmental glomerulosclerosis (FSGS). The first biopsy from this 5-year-old girl, *middle panel,* was indistinguishable from MCD, except for marked glomerular hypertrophy vs. age-matched typical MCD with subsequent benign clinical course *(top panel).* The patient's later biopsy, *bottom panel,* 50 months later, showed segmental sclerosis, diagnostic of FSGS (Jones' stain, ×160).



these values may vary (our values are based on paraffin-embedded tissue fixation in formalin or Zenker's fixative). Normal ranges should be established in each laboratory assessing glomerular size.

From these studies, abnormal glomerular enlargement suggests a high probability of development of FSGS in pediatric patients with apparent MCD. Causes of abnormal glomerular enlargement other than idiopathic FSGS, such as diabetes mellitus, cyanotic cardiovascular disease, and massive obesity, must be excluded before such inferences can be made. Interestingly, the incidence of FSGS may be increased in these diseases. The association of abnormal glomerular growth with development of glomerulosclerosis may reflect a pathogenic linkage, in that processes leading to excess matrix and sclerosis may be manifested as glomerular growth. This view is supported by the coexistence of these two processes in many other diseases, including sickle cell diseases, HIV infection, and reflux nephropathy (97).

Alterations of dystroglycans, specific proteins that are expressed along the GBM, may be of use in differentiating MCD in FSGS. α- and β-dystroglycans were decreased in MCD, but preserved in the nonsclerotic areas of FSGS in a small study (98). Recently, molecular studies also indicate distinct gene expression profiles in MCD vs FSGS, with much higher ratio of podocin to synaptopodin in mRNA in the former (99).

Recently, specific gene mutations of podocyte-specific genes have been identified in some forms of familial FSGS. The slit diaphragms are crucial for regulation of permselectivity, and are decreased in density in proteinuric conditions. Mutations of several slit diaphragm genes cause proteinuria and FSGS. The gene for autosomal dominant FSGS has now been localized to ACTN4, at chromosome 19q13. ACTN4 encodes alpha-actinin-4, and a gain-of-function mutation with possible altered actin cytoskeleton interactions has been proposed. The prognosis of this form of familial FSGS has been poor, with progression to renal disease in 50% of patients by age 30. Recurrence of NS in the transplant has been very rare, presumably related to immune events, as the transplant does not carry the mutated gene (100). Autosomal recessive FSGS with early onset and rapid progression to end stage is caused by mutations in NPHS2, which encodes podocin (101). Podocin is expressed only in podocytes and is an integral stomatin protein family member. Its function is not determined. Mutations in NPHS2 have been described in sporadic steroid resistant FSGS (11). TRPC6, a receptor, is mutated in other kindreds with FSGS, with variable penetrance and adult onset (102). Phospholipase C epsilon mutation (NPHS3) recently was found to underly many cases of DMS or rare cases of FSGS, with rare steroid response (103–105). These familial forms do not have specific morphological features of the segmental sclerosis.

Congenital nephrotic syndrome (CNS) of Finnish type shows mesangial hypercellularity or no glomerular lesions and dilated proximal tubules by light microscopy. The gene mutated is nephrin and is also in the 19q13 region (106). Nephrin localizes to the slit diaphragm of the podocyte, and is tightly associated with CD2 associated protein (CD2AP). Nephrin is thought to function as a zona occludens type junction protein. CD2AP plays a crucial role in receptor patterning and cytoskeletal polarity, and its absence resulted in sclerosis and foot process effacement in mice, supporting a role for CD2AP in the function of the slit diaphragm. Rare case reports of CD2AP mutation in human FSGS exist (107). Laminin-β2 (LAMB2) is mutated in Pierson syndrome, often with microcoria and CNS abnormalities, with mesangial sclerosis (108, 109).

FSGS associated with mitochondrial cytopathy, due to a mutation of mitochondrial DNA in tRNAleu(UUR), may show multinucleated podocytes and have abnormal mitochondria by electron microscopic examination. Patients also have unusual hyaline lesions in the arterioles. Some patients with FSGS without full-blown features of mitochondrial cytopathy (i.e., myopathy, stroke, encephalopathy, occasionally diabetes mellitus, hearing problems, cardiomyopathy) have also been reported to have this mitochondrial mutation (110). For further discussion of MCD and FSGS, see Chapters 45 and 46.

Hemolytic-Uremic Syndrome

HUS is the most common disease in children that is manifested by injury to the microvasculature and is typically due to E. coli 0:157 verotoxin and associated with diarrhea. In adults, thrombotic thrombocytopenic purpura, related to ADAMTS13 deficiency, and postpartum renal failure may produce similar morphologic changes. The characteristic lesion of these conditions is thrombotic microangiopathy (TMA). By LM, thrombi in glomeruli and arterioles are present (*Fig. 24-5*). The renal biopsy findings, rather than clinical parameters, have recently been found to best predict long-term prognosis (111). Patients with cortical necrosis have a particularly ominous prognosis. The extent of glomerular vs. arteriolar involvement is also of prognostic significance. Generally, both the long-term prognosis and the clinical presentation are more severe if larger vessels are involved. Arterial involvement was not seen in biopsies performed during the first 2 weeks of hospitalization (112). The glomerular endothelium is markedly swollen, nearly occluding capillary lumina. Fibrin thrombi are visualized easily. With chronicity, these areas may progress to segmental ischemic collapse with sclerosis, especially when arterioles are involved. The arterioles can become completely occluded by thrombi, with necrosis of vessel walls. IF shows occasional nonspecific entrapment of C3 and IgM in injured areas with fibrin and fibrinogen. EM shows extreme swelling of the glomerular endothelium with increased lucent material in the lamina rara interna of the basement membrane with entrapped platelets, fibrin, and red cell fragments, without immune deposits (*Fig. 24-6*). De novo thrombotic microangiopathy in the renal transplant is indistinguishable morphologically from HUS in the native kidney. Cyclosporine and FK506 have both been implicated in its pathogenesis (113, 114). Mutations of complement regulatory genes are implicated in familial and some diarrhea-negative sporadic cases. The morphologic characteristics of the TMA lesion are largely the same regardless of etiology. For further discussion of HUS, see Chapter 48.

Henoch-Schönlein Purpura/IgA Nephropathy

Henoch-Schönlein purpura is often viewed as the systemic variant of IgA nephropathy (Berger's disease) (4, 115).

Figure 24-5

Arteriolar fibrin thrombi with minor areas of thrombi in capillary loops in hemolytic uremic syndrome (Jones' stain, × 270).

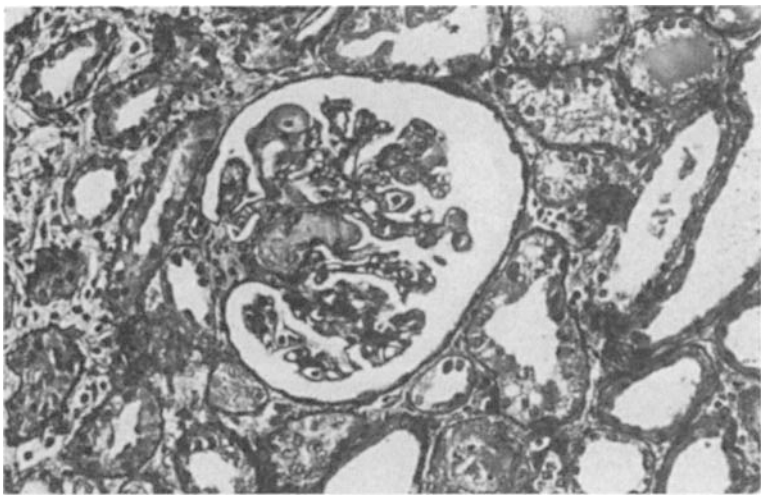
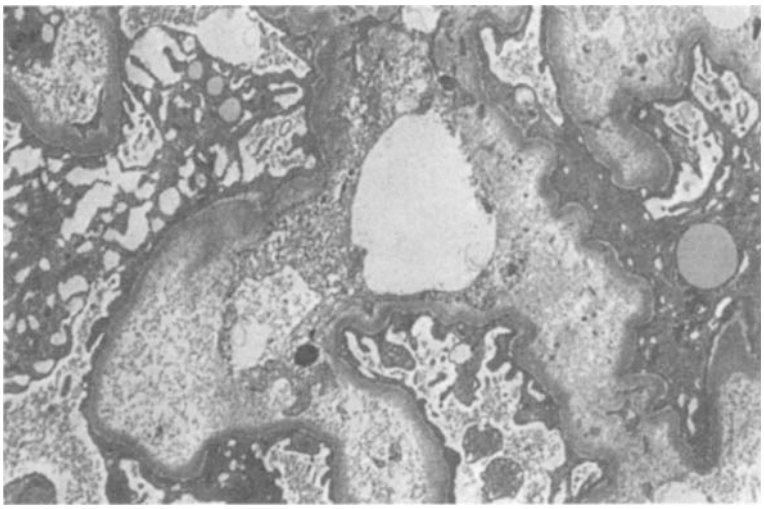


Figure 24-6

Hemolytic uremic syndrome. The lamina rara interna (endothelial side of the glomerular basement membrane) is widened with increased lucent material. No immune deposits are present (× 3,300).



The glomerular manifestations are similar, and vary in both, likely depending on extent and location of deposits. In some cases, there is only focal or even diffuse mild to moderate mesangial proliferation (*Fig. 24-7*) (116). In more severe cases, there is focal or even diffuse endocapillary proliferation. In severe cases, there may also be necrosis of glomerular tufts with crescents in Bowman's space. IF by definition shows predominance or codominance of mesangial IgA, with capillary loop deposits in more severe cases. IgG, IgM, and C3 deposits may also be detected. The immunoglobulin deposits are present diffusely, even in glomeruli that appear normal by LM. By EM, electron-dense mesangial deposits are present, with occasional "spill-over" of deposits to subendothelial regions in the regions adjacent to the mesangium, particularly in cases with endocapillary proliferative lesions (*Fig. 24-8*). Deposits are decreased when clinical remission occurs (117). In Henoch-Schönlein purpura, deposits are often present in subendothelial as well as mesangial areas, associated with more severe glomerular lesions, including crescents, and worse outcome (116). Classification schemas analogous to those for lupus nephritis have

Figure 24-7
Mesangial prominence, segmental sclerosis, and small organizing crescent with adhesion in Henoch-Schönlein purpura. Immunofluorescence demonstrated IgA mesangial deposits (Jones' stain, × 430).

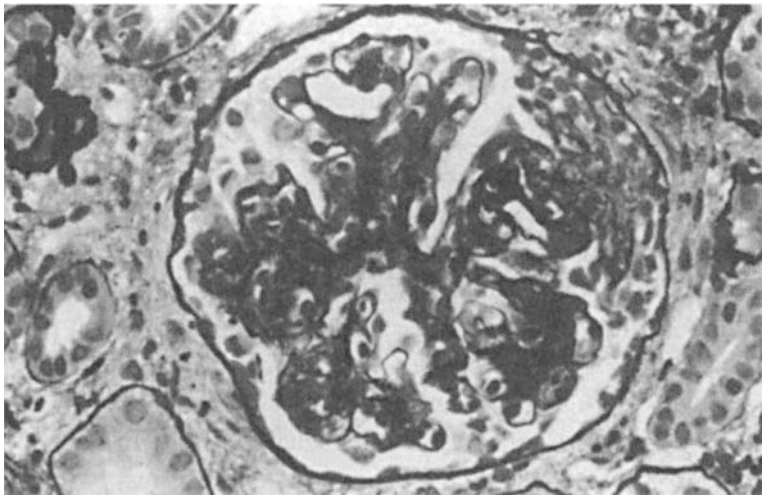
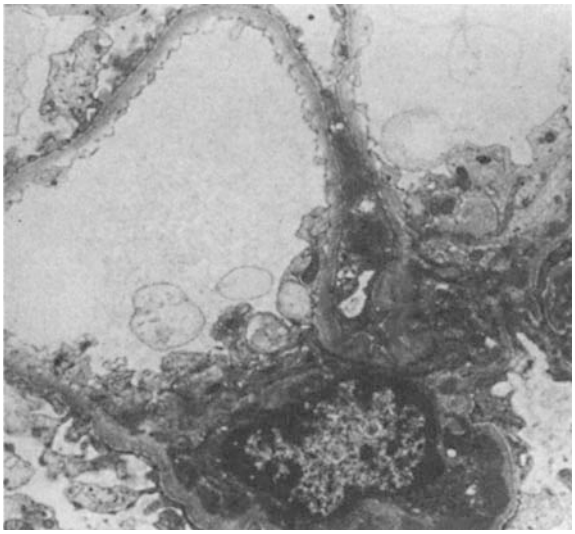


Figure 24-8
Immune complex deposits surrounding mesangial cell in Henoch-Schönlein purpura (× 3,400).



been proposed, but have not had widespread use (118, 119). Therefore, a recent International Study Group of IgA Nephropathy composed of nephrologists and pathologists reviewed a large number of cases to determine which biopsy lesions have prognostic implications (120). Mesangial hypercellularity, i.e., more than half the glomeruli with more than 3 nuclei in a mesangial area, proliferation (either endo- or extracapillary), segmental sclerosis and increasing interstitial fibrosis were each associated with worse long-term outcome. For further discussion of Henoch-Schönlein purpura/IgA nephropathy, see Chapter 42.

Membranoproliferative Glomerulonephritis Type I

Type I MPGN is characterized by a tram-track appearance of the GBM on silver stain, because of duplication around intramembranous/subendothelial deposits and interposition of mesangial cells and macrophages (*Fig. 24-9*). The glomeruli are enlarged and hypercellular with a lobular appearance by LM (*Fig. 24-10*). There is marked mesangial and endocapillary hypercellularity and occasional PMNs and mononuclear cells may be present. By IF, C3 predominates in a coarse granular pattern along basement membranes, with moderate amounts of IgG and usually lesser IgM. Subendothelial and mesangial immune deposits are seen by EM (4, 53). MPGN may be idiopathic or secondary to any of numerous chronic infections. Hepatitis C positivity, often with associated cryoglobulins, was present in about one fourth of adult cases of MPGN type I in adults in Japan and the United States (121, 122). This association has not been demonstrated in children with apparent idiopathic MPGN (123). MPGN type I recurs in 20–30% of grafts, and may lead to graft loss (13). Secondary MPGN, more common in adults, more often demonstrates a focal

Figure 24-9

Split basement membrane (''tram-tracking'') in MPGN type I (*arrows*), caused by subendothelial/intramembranous deposits and cellular interposition (Jones' stain, × 1,125).

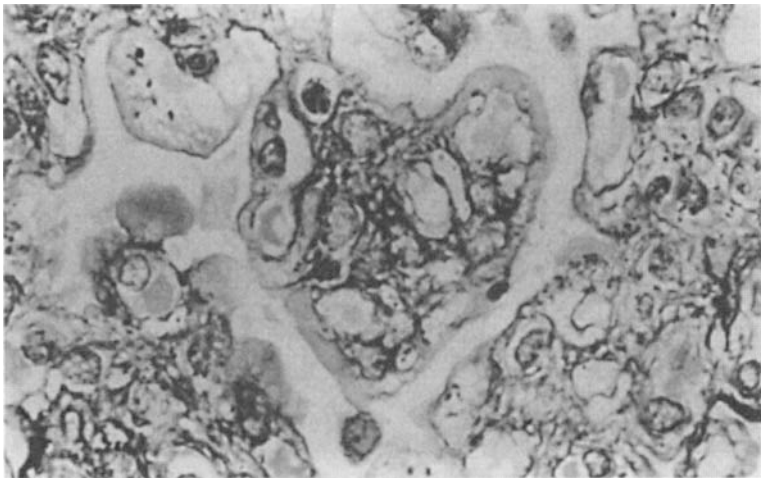
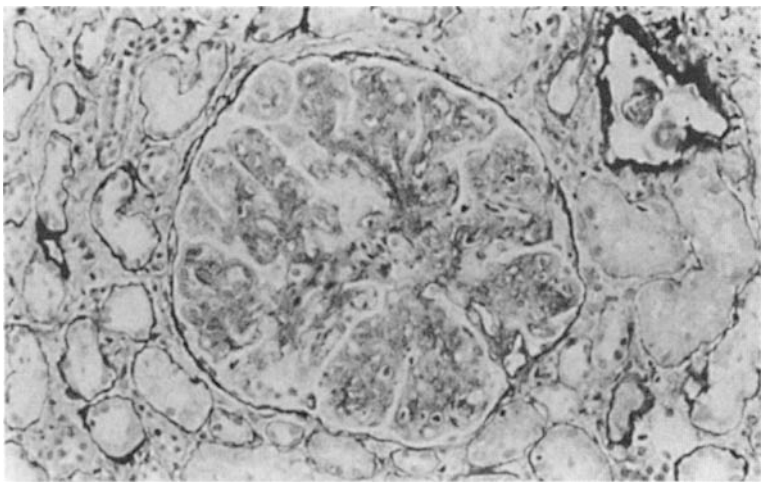


Figure 24-10

Lobular appearance of glomeruli in MPGN type I (× 430).



segmental pattern of proliferation, contrasting the more diffuse involvement seen in idiopathic MPGN, more common in children.

Dense Deposit Disease

In dense deposit disease (DDD), the glomeruli may appear similar by LM to those of type I MPGN, and this disease has therefore also been called type II MPGN. However, the pathogenesis is entirely different. These patients often show circulating IgG autoantibodies, also known as C3 nephritic factor (12, 124, 125). The basement membranes are deeply eosinophilic, often with a ribbon garland or sausage-shaped contour. By IF, discontinuous smooth linear deposits of C3 along the GBM and round globular mesangial deposits are found, typically without immunoglobulin staining. The disease is named dense deposit disease because of the characteristic appearance by EM with strongly electron-dense deposits within the basement membrane. Studies of the dense deposits indicate that these are likely an alteration of basement membrane material and not deposition of circulating immune complexes. Although less specific than

electron microscopic diagnosis, deposits can also be identified by their staining with the fluorescent dye thioflavin T in cases where electron microscopic examination cannot be performed (125). A recent large international study of 69 cases of DDD by the Renal Pathology Society showed mesangial proliferation was most common, followed in order by membranoproliferative appearance with endocapillary proliferation, crescentic or acute proliferative patterns (126, 127). Renal survival may be worse in DDD than in type I MPGN (median survival 8.7 vs. 15.3 years) (128). The distinction between these two diseases is also important since dense deposit disease invariably recurs in renal transplantation, although loss of graft is not always the outcome (12).

Lupus Nephritis

Lupus nephritis is not a single disease but rather there is a spectrum of severity of involvement of the kidney by the immune complexes characteristic of SLE. Most patients with SLE will have morphologic manifestation of renal immune deposition. However, patients who undergo renal biopsy most often have clinical renal manifestations and will have more pronounced changes. Lupus nephritis is characterized by deposits in all anatomic compartments of the glomerulus (i.e., mesangial, subepithelial, and subendothelial regions) (19–21) (*Figs. 24-11* and *24-12*). All immunoglobulin classes with dominant IgG, C3, and smaller amounts of C4/C1q are usually found in lupus nephritis deposits (*Fig. 24-13*), and immune complex deposits are seen by EM. Reticular aggregates (see above) are typically seen in endothelial cells in any class of lupus nephritis (4, 53) (*Fig. 24-14*).

The WHO classifications, either the original or modified, were previously most commonly used (18, 19). However, the International Society of Nephrology (ISN) and the Renal Pathology Society (RPS) put forth a revised lupus nephritis classification to clarify some areas of difficulty in the previous versions (20, 129, 130). Greater interobserver reproducibility occurs with the ISN/RPS classification (129). In this new ISN/RPS classification, Class I has minimal mesangial deposits with normal LM. Class II is characterized by mesangial expansion visible by LM and mesangial deposits, with only scattered peripheral loop deposits. In class III, focal lupus nephritis, deposits are present in mesangial areas and there are lesions of either active endocapillary proliferation, necrosis, cellular crescents, sclerosis, fibrocellular or fibrous crescents. These focal lesions, by definition, involve less than 50% of glomeruli. The subendothelial deposits can result in thick, rigid-appearing capillary basement membranes by LM, the so-called wire-loop lesions. "Hyaline" thrombi (aggregates of immune complexes) may fill capillary lumina. When these lesions affect more than 50% of glomeruli, lupus nephritis is characterized as class IV diffuse lupus nephritis. The proliferative lesions of both class III and IV are typically associated with subendothelial deposits in addition to mesangial deposits, with only rare or scattered subepithelial deposits. IF demonstrates

Figure 24-11

Diffuse lupus nephritis (ISN/RPS Class IV) with massive dense mesangial and subendothelial deposits, and fewer deposits in subepithelial areas (× 5,600).

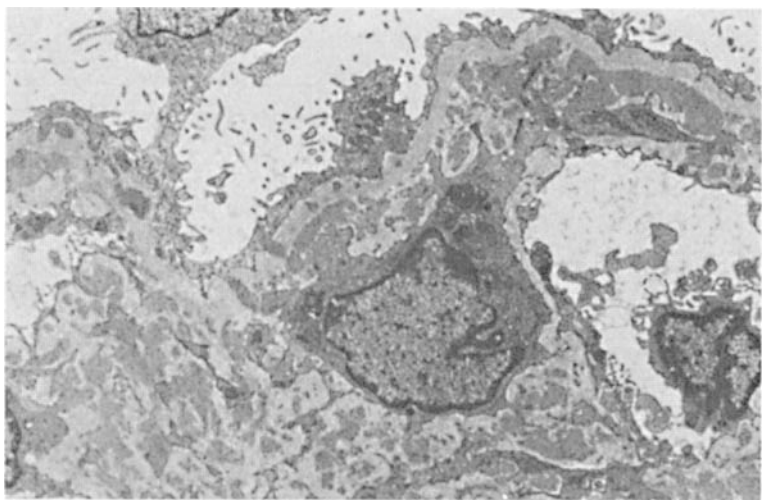


Figure 24-12
Membranous glomerulopathy with subepithelial deposits and intervening lamina densa (seen as spikes by silver stain on LM) (× 15,580).

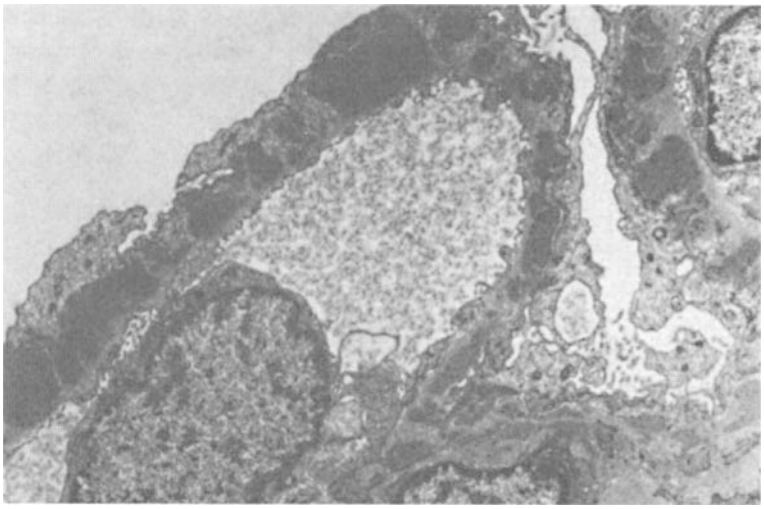
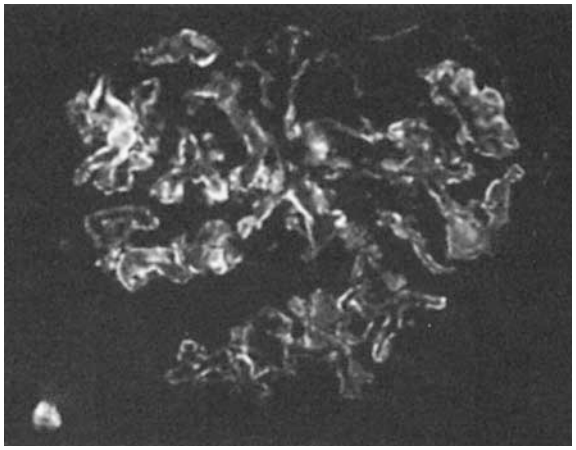
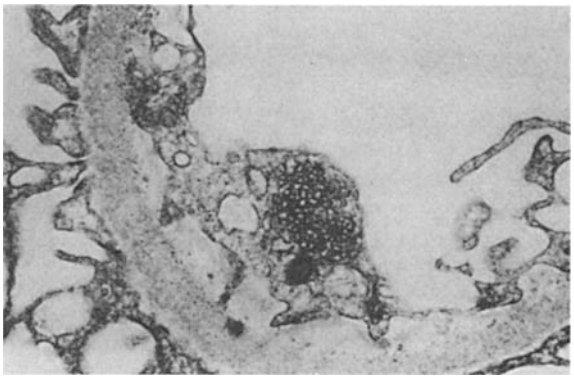


Figure 24-13
Immunofluorescence of granular capillary and small mesangial IgG deposits in diffuse lupus nephritis (ISN/RPS Class IV). The larger segments of capillary loop staining correspond to subendothelial deposits, with a smooth outer edge where deposits are molded underneath the GBM (× 250).



widespread distribution of immune complexes. EM confirms the massive and extensive immune complex deposition (*Fig. 24-11*). For class III and IV, the extent of active vs chronic lesions is specified. The segmental necrotizing lesions in class IV lupus nephritis may have worse prognosis, and thus the presence of these lesions vs global endocapillary proliferation is noted (20, 130).

Figure 24-14
Endothelial cell containing tubular-shaped reticular aggregates in lupus nephritis (× 20,000).



Class V membranous lupus nephritis is characterized by predominance of subepithelial deposits in a pattern similar to that of idiopathic membranous glomerulopathy, with added mesangial deposits (*Fig. 24-12*). Subendothelial deposits are minor components in class V. When there are superimposed focal or diffuse proliferative lesions in addition to membranous changes, both processes are diagnosed, e.g., combined focal or diffuse lupus nephritis and membranous lupus nephritis, ISN/RPS Class III + V or Class IV + V, respectively. Widespread chronic sclerosing lesions in a nonspecific pattern in a case of lupus nephritis are defined as Class VI. Tubular basement membrane deposits can occur in any class of lupus nephritis and

may account in part for the tubulointerstitial injury. Vascular lesions include immune deposits, or thrombotic microangiopathy, often related to anti-phospholipid antibodies. Vasculitis occurs rarely. For further discussion of lupus nephritis, see Chapter 47.

Anti-Glomerular Basement Membrane Antibody Disease

Light microscopic examination shows crescentic glomerulonephritis with focal necrotizing lesions. Patients may not always show detectable serum levels of anti-GBM antibodies, especially after the acute phase of illness. Serology is positive in 95% of patients in the first 6 months after onset. However, in all patients, even those with negative serology, linear IgG staining by IF of glomerular basement membranes is present (*Fig. 24-15*). Standard EM does not visualize the immune deposits, perhaps because of the diffuse distribution of the antigen (α3 type IV collagen NC-domain). Patients with more than 50% crescents have a worse prognosis (131). For further discussion of anti-GBM antibody disease, see Chapter 29.

Wegener's Granulomatosis

By LM, the appearance of Wegener's granulomatosis is the same as for other non-immune complex crescentic necrotizing glomerulonephritides, such as microscopic polyangiitis or anti-GBM antibody disease (*Fig. 24-16*). The lesions are focal and segmental. Granulomas are rare in the kidney, and arteritis is rarely found in the small sample inherent to the renal needle biopsy. IF studies allow differentiation of the lesion from anti-GBM antibody disease. It shows fibrin and fibrinogen in areas of necrosis, and nonspecific trapping of immunoglobulin, especially IgM. By EM, immune deposits are not identified. Distinction from microscopic polyangiitis cannot usually be made by renal biopsy findings. Clinical manifestations must be used to distinguish between these two disorders. For further discussion of Wegener's granulomatosis, see Chapter 45.

Postinfectious Glomerulonephritis

Patients with typical postinfectious glomerulonephritis due to streptococcal infection do not usually undergo renal biopsy. Infectious agents other than streptococci can also cause postinfectious glomerulonephritis. When the diagnosis remains in question, when abnormalities persist, or when the initial disease is severe, renal biopsy may be done. Glomeruli are enlarged and hypercellular with prominent endocapillary proliferation and infiltration by neutrophils and mononuclear cells (*Fig. 24-17*) (132). In severe disease, crescents are present. Occasionally,

Figure 24-15

Anti-glomerular basement membrane antibody disease with linear staining of GBMs by immunofluorescence for IgG. A crescent is present in the glomerulus on the *right* (× 125).

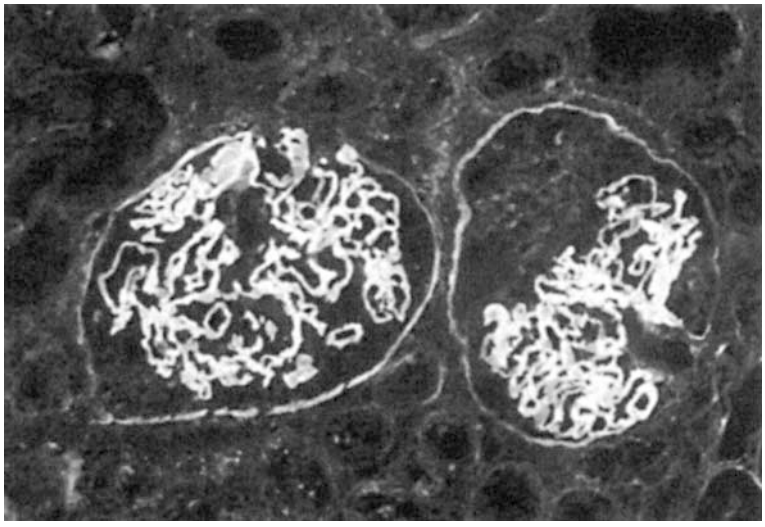


Figure 24-16

Segmental necrosis and crescent in Wegener's granulomatosis. Immunofluorescence was negative (Jones' stain, × 430).

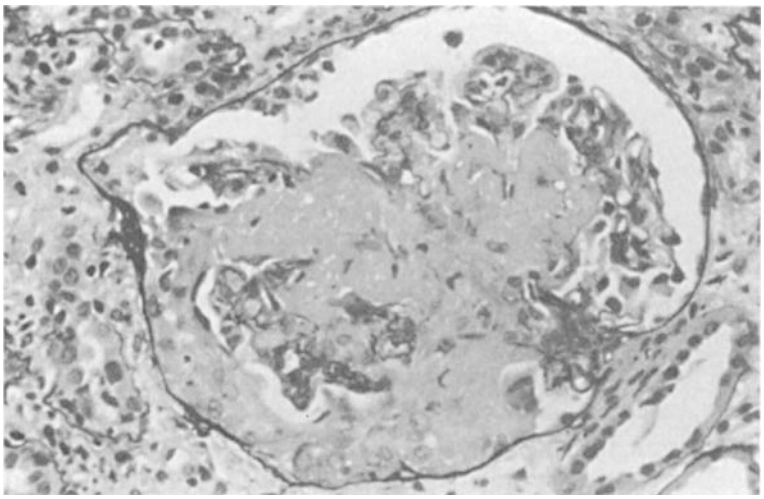
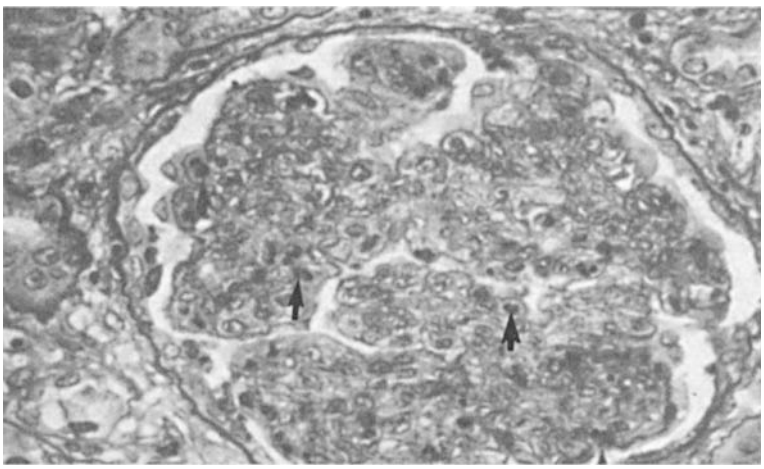


Figure 24-17

Postinfectious glomerulonephritis with endocapillary proliferation and PMN infiltration (*arrows*) (PAS, × 430).



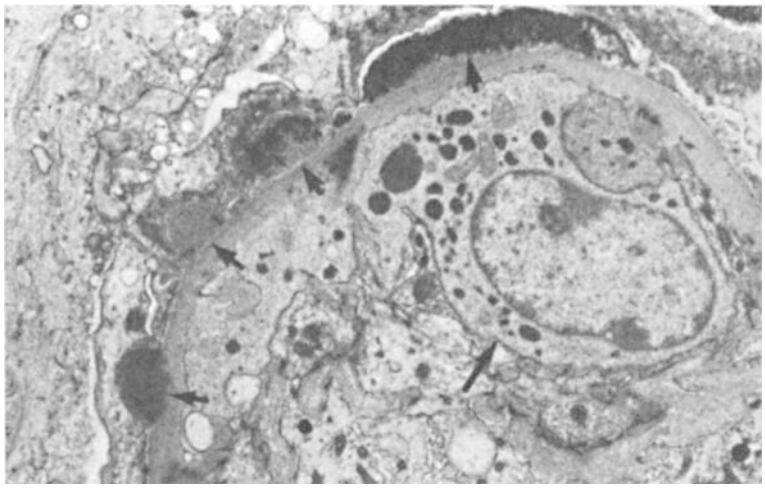
large subepithelial deposits can be visualized by LM. These differ from those typical of membranous glomerulopathy in being more unevenly distributed along the capillary basement membrane and larger in size. The deposits lie on top of the basement membrane, rather than being embedded within it (as in membranous glomerulopathy), and therefore spikes are not usually present. By IF, there are coarsely granular, discontinuous areas of IgG and prominent C3 along the capillary wall and in the mesangium. Postinfectious glomerulonephritis due to staphylococcal infection may have dominant IgA rather than IgG, and can be distinguished from IgA nephropathy by EM appearance of deposits (133). Electron-dense subepithelial deposits are large, variegated, hump- or dome-shaped and irregularly spaced (*Fig. 24-18*). Occasional mesangial deposits are present in many biopsies. For further discussion of postinfectious glomerulonephritis, see Chapter 30.

Diabetic Nephropathy

Diabetic nephropathy affects 30–40% of patients with diabetes mellitus, either type 1 or type 2, with overt clinical nephropathy manifest 15–20 years after onset of diabetes. Therefore, diabetic nephropathy has been

Figure 24-18

Electron micrograph of subepithelial large, irregularly spaced, hump-shaped subepithelial deposits in postinfectious glomerulonephritis. The deposits are variegated and lie on top of the GBM (*small arrows*). There is endocapillary proliferation with PMN (*long arrow*) infiltration (×7,000).



considered a disease of adults. However, adolescents may have diabetic renal lesions even after short duration of disease (134). In addition, obesity and type 2 diabetes mellitus are increasing in children. The structural changes in these diabetic children include GBM thickening and mesangial expansion and were associated with proteinuria, hypertension, and decline in GFR. Overt diabetic nephropathy with nodular glomerulosclerosis and afferent and efferent arteriolar hyalinization was present in several of these young patients. For further discussion of diabetic nephropathy, see Chapter 50.

Alport Syndrome/Thin Basement Membrane Lesion

Early in life in males with classic Alport syndrome, and in female carriers, the renal biopsy may show no significant light microscopic abnormalities. At later stages, glomerulosclerosis, interstitial fibrosis and prominent interstitial foam cells are typical. These foam cells are not specific for this disease, and are found in numerous proteinuric states. Glomeruli can show segmental sclerosis. Immunofluorescence may show non-specific trapping of IgM. By electron microscopy, the diagnostic lesion consists of irregular thinned and thickened areas of the glomerular basement membranes with splitting and irregular multilaminated appearance of the lamina densa, so-called "basket weaving". In between these lamina, granular, mottled material is present. Similar GBM changes by EM, but with normal collagen chain staining (see below) have been reported in Frasier syndrome. This entity is due to WT-1 mutation, and manifests as NS with FSGS by LM, no deposits, pseudohermaphroditism and increased risk of gonadoblastoma (135). At early stages of Alport, i.e., in children or carrier women, the glomerular basement membrane may show only thinning. Some males with classic Alport syndrome only have glomerular basement membrane thinning even at advanced clinical stages (25, 136). Rarely, Alport patients may have proliferative lesions with IF and EM deposits within lamellated GBM areas, suggesting trapping of immunoglobulins rather than a superimposed immune complex disease (137).

Immunostaining for type IV collagen chains can aid in the interpretation of thin basement membranes (136). Heterotrimers of α3, α4 and α5 type IV collagen are a key normal component of the GBM. Mutation of α5 type IV collagen in X-linked Alport syndrome, or of α3 or α4 subchains in autosomal forms, prevents incorporation of the other chains into this heterotrimer of the GBM. Thus, in kidney biopsies, about 70–80% of males with X-linked Alport syndrome lack staining of GBM, distal tubular basement membrane and Bowman's capsule for α3, α4 and α5 (IV) chains. In autosomal recessive Alport syndrome, due to mutations of either α3 or α4, the GBMs also usually show no expression of α3, α4 or α5 type IV collagen. In contrast to X-linked cases, there is normal expression of α5 type IV collagen in Bowman's capsule, distal tubular basement membrane and skin, where the α5 collagen chain are part of other heterotrimers. Female

heterozygotes for X-linked Alport syndrome show mosaic staining of GBM and distal TBM for α3, α4 and α5 type IV collagen chains, and skin mosaic staining for α5 type IV collagen due to the Lyonization effect. Patients with autosomal dominant Alport syndrome have not been studied immunohistochemically. Of note, occasional cases with Alport syndrome clinically and by renal biopsy showed apparent normal α5 type IV pattern of skin IF staining, and about 20% of male X-linked Alport patients show faint or even normal staining of the GBM for α3 and α5, likely because the antigenic site recognized by the antibody has not been altered by the mutation (136).

Thinning of the GBM is also the characteristic finding in benign familial hematuria (70, 71). The diagnosis of thin basement membranes is based on morphometric measurements from electron microscopic prints, and was present in 1.9% of a large native kidney biopsy series (138). LM and standard IF are normal. The GBM thickness normally increases with age. Normal thickness in adults in one series was 373 ± 42 nm in men vs. 326 ± 45 nm in women. GBM thickness < 250 nm has been used as a cutoff in many series (139). In another series, average was 330 ± 50 nm in males and 305 ± 45 nm in women (138). In children, the diagnosis of thin basement membranes must be made with caution, establishing normal age-matched controls within each laboratory. In our laboratory, we found a range of GBM thickness in normal children, from approximately 110 nm at age 1 year to 222 ± 14 nm in seven year olds. As mentioned above, thin GBM (without lamellation) is also the early, or may be the only, manifestation in some kindreds with Alport syndrome and in female carriers of X-linked Alport. Thus, the presence of thin GBM cannot per se be taken to categorically indicate a benign prognosis. Some patients with a clinical diagnosis of benign familial hematuria and α4 or α3 type IV collagen, suggesting that they may represent a carrier state of autosomal recessive Alport (140).

Prognostic Implications of Biopsy Findings

When the biopsy sample is adequate, extensive, severe, and irreversible lesions signify a dismal prognosis for the patient. Globally sclerotic glomeruli are not amenable to treatment, although evidence from human diabetic nephropathy and animal studies indicates that the earlier stages of sclerosis may be affected by some therapeutic interventions, and may even be reversible (141–143). Similarly, active lesions with ongoing cellular crescents, necrosis, and inflammatory infiltrate are potentially dramatically modulated by therapy, allowing subsequent healing. There may be minimal irreversible damage to glomerular structures when intervention occurs early.

Although the renal biopsy may yield a diagnosis, there is less information of prognostic indicators in diseases that have a variable course. Extensive analysis aimed at determining histologic features associated with poor prognosis has been done in some diseases discussed below.

Classification schemes, especially for lupus nephritis and membranous glomerulopathy, imply progression from one stage of disease to the next. Although sequential biopsies have illustrated progression from focal to diffuse proliferative glomerulonephritis in lupus nephritis, there is not clear-cut evidence that progression occurs among all ISN/RPS classes (18, 19, 21, 130). In lupus nephritis, patients with less severe proliferative disease, especially segmental necrotizing lesions, appear to have better prognoses.

Although the presence of cellular crescents is associated with activity of disease clinically, the renal biopsy offers additional prognostic information beyond that gleaned from the clinical presentation (144). Focal and diffuse proliferative lesions (ISN/RPS III and IV) may present very similarly clinically, but only the latter appears to require intense, long-term immunosuppression. Lesions of activity in lupus nephritis include endocapillary proliferation, necrosis, cellular crescents, and interstitial inflammatory cells. Lesions that indicate chronicity include tubular atrophy, interstitial fibrosis, glomerular sclerosis, and fibrous crescents.

Although assessment of activity and chronicity indices is useful for population groups, these appear to have less absolute information to guide assessment in individual patients. Nonetheless, in large series, assessment of indices of activity and severity in patients with lupus nephritis or other diseases has shown some correlation with prognosis and response to therapy. Diffuse proliferative lesions, extensive crescents, segmental necrosis and tubulointerstitial fibrosis are associated with progression to ESRD (144, 145). The best prognostic indicator in a recent study was the proportion remaining of intact glomeruli in follow-up biopsies (146).

IgA nephropathy was previously thought to have a benign prognosis. In a large series of adult patients with IgA nephropathy, poor prognosis was indicated by segmental glomerulosclerosis, adhesions or crescents, and tubulointerstitial fibrosis (147). Progression occurs in 11–15% of pediatric patients (4, 97, 116, 148). Scoring of activity and chronicity of lesions has been correlated with clinical course. Activity is assessed by degrees of

crescent formation, mesangial proliferation, and interstitial infiltrate. Chronicity is scored by degrees of fibrous crescents, segmental and global sclerosis, tubular atrophy, and interstitial fibrosis (149). High indices of chronic injury and focal segmental glomerular changes were associated with a worse prognosis (150, 151). Histologic features that predicted progression in a recent multicenter study in children were crescents, tubulointerstitial fibrosis, and glomerulosclerosis in 20% or more of glomeruli (141). Predominance of matrix expansion appears to be a later stage of injury, associated with a higher percentage of sclerosis and persistent proteinuria (118). Extension of deposits to glomerular basement areas has also been reported as a poor prognostic indicator (149). A recent International Study Group of IgA nephropathy identified four lesions associated with worse long-term outcome, namely mesangial hypercellularity, i.e., more than half the glomeruli with more than 3 nuclei in a mesangial area, proliferation (either endo- or extracapillary), segmental sclerosis and increasing interstitial fibrosis (120).

Focal glomerulosclerosis superimposed on membranous glomerulopathy has been associated with more severe tubulointerstitial nephritis and a worse outcome. This lesion was present in 20% of children with hepatitis B-associated membranous glomerulopathy (152).

Renal Transplant Biopsy

The primary use of biopsy in the renal transplantation is to uncover the reason for altered renal function. Causes of renal dysfunction in the transplant can be broadly divided into those related to rejection, drug toxicity, recurrent or de novo disease, and those related to the procedure itself, such as acute tubular necrosis.

Rejection

Acute rejection is diagnosed by the presence of either interstitial inflammation with lymphocytes and plasma cells infiltrating tubules (tubulitis, the hallmark of acute interstitial type rejection, *Fig. 24-19*), or when more severe, by extension of this process to vessels, with subendothelial arterial or arteriolar infiltration by lymphocytes (endothelialitis, the hallmark of acute vascular rejection, *Fig. 24-20*). The interstitial changes of acute rejection are not pathognomonic. In contrast, the finding of endothelialitis is highly specific for acute vascular rejection. Appropriate stains, such as PAS, must be used to allow visualization of the tubular basement membrane and identification of tubulitis. An adequate specimen for evaluation of possible rejection should contain at least two cores, with at least seven glomeruli and two arteries (153).

Several schemes have been used to diagnose and classify rejection: The Banff scoring system, based on detailed scoring of various components of injury; and the Cooperative Clinical Trials in Transplantation (CCTT) criteria (153, 154). In both classification schemes, acute rejection is based on the presence of tubulitis or endothelialitis, i.e., lymphocytes in the tubule under the tubular basement membrane or underneath the endothelium of arteries. Other inflammatory cells (e.g., eosinophils, neutrophils, and plasma cells), although much fewer in number than T lymphocytes, may also contribute to the infiltrate in acute rejection. Type I rejection in both schemas is diagnosed when interstitial lymphocytic infiltrate and tubulitis are present (>25% of parenchyma infiltrated in Banff, >5% in CCTT) (*Fig. 24-20*). Infiltrate and tubulitis less than specified for type I is called "borderline" by Banff criteria (154). Type II acute vascular rejection is diagnosed in both schemas when there is mild or moderate endothelialitis (arteritis). Severe acute vascular rejection, type III, is diagnosed when there is transmural vascular inflammation and/or fibrinoid necrosis. These types are differentiated not only based on histologic pattern, but also on differences in underlying mechanisms and response to therapies: type I and II are likely T-cell dependent processes and are separated based on the likely greater clinical severity of any rejection when endothelialitis is present, whereas antibody-mediated mechanisms contribute to type III changes.

Identification of acute rejection at earlier stages and thus initiation of treatment at milder levels of injury appear to be clinically important. Thus, mild tubulitis that is borderline by Banff criteria, even in normally functioning grafts, was found to be predictive of higher serum creatinine at follow-up. In contrast, treatment of such subclinical rejection in the early time period after transplantation resulted in better preserved renal function at 24 months (155).

There are no specific IF or electron microscopic immune complexes associated with acute rejection. The recent surge of exciting molecular studies indicates the possibility of earlier, more sensitive, and specific diagnosis of acute rejection using these techniques (see below) (156). In particular, presence of C4d, a complement breakdown product which binds covalently to tissue, in peritubular capillaries, is highly associated with anti-donor antibodies (humoral rejection) (157). Diagnosis of humoral, antibody-mediated rejection has important

Figure 24-19

Acute rejection, classified as type I by Cooperative Clinical Trials in Transplantation criteria. There is interstitial lymphocytic infiltrate with tubulitis, activated lymphocytes, tubular cell injury, and interstitial edema (Jones' stain, × 220).

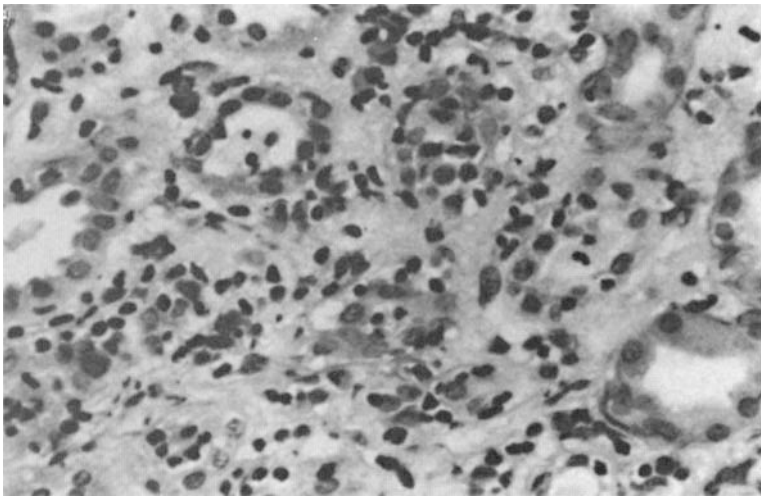
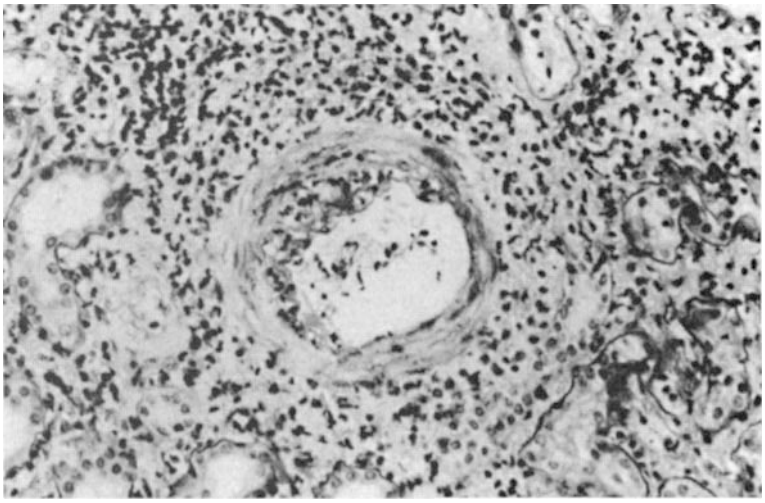


Figure 24-20

Acute vascular rejection, classified as type II by Cooperative Clinical Trials in Transplantation criteria. There is subendothelial infiltration by lymphocytes in this artery, so-called endothelialitis (Jones' stain, × 220).



therapeutic and prognostic implications. C4d staining can be done on frozen or fixed paraffin-processed tissue, although the latter is less sensitive (158).

The changes of chronic rejection include intimal fibrosis of arteries, interstitial fibrosis, and transplant glomerulopathy (154, 159). A previous or baseline biopsy is necessary to prove that intimal fibrosis is de novo and potentially represents chronic rejection, rather than a preexisting, nonspecific change in the graft. Interstitial fibrosis is also a nonspecific finding and may result from various injuries. Transplant glomerulopathy is a more specific lesion indicative of chronic rejection, i.e., scarring injury related to previous immune injury. By LM, the glomeruli show basement membrane splitting and corrugation, and even segmental sclerosis with hyalinosis. The latter lesion likely resulted in erroneous reports of de novo "idiopathic FSGS" in the transplant. However, in transplant glomerulopathy, there is widening of the

lamina rara interna of the GBM with cellular interposition and new basement membrane formation by EM. Reduplication of basal lamina of peritubular capillaries is suggested to be more specific of transplant glomerulopathy but may also occur in some other glomerular diseases and HUS (160).

Calcineurin Inhibitor Toxicity

Calcineurin inhibitor toxicity may manifest in various ways. Tacrolimus (FK506) has much the same spectrum of toxicity as cyclosporine (159, 161). The most common morphologic lesion in patients with a clinical diagnosis of cyclosporine toxicity, as verified by clinical follow-up, is that of a normal kidney biopsy morphologically. In these patients, renal dysfunction is due to reversible, calcineurin inhibitor-induced vasoconstriction and hypofiltration. Morphologic changes of calcineurin inhibitor toxicity include arteriolopathy with injury to the endothelium and vascular smooth muscle cells. In its classic form, this injury results in nodular IgM IF positivity along the apical side of the arteriole, with necrosis and smooth muscle cell injury demonstrated by EM (159, 161). By LM, concentric hyalinosis is present, whereas typically eccentric, more segmental hyalinosis is associated with hypertension. Isometric tubular vacuolization in a patchy distribution, although not specific, is also indicative of cyclosporine toxicity. Chronic calcineurin inhibitor toxicity results in a striped distribution of interstitial fibrosis caused by injury along the medullary rays (159). This pattern often cannot be gleaned in small needle biopsies. FSGS with ischemic, corrugated GBMs in remaining glomeruli may also result from calcineurin inhibitor toxicity and can be associated with significant proteinuria (161).

Calcineurin inhibitors have also been associated with thrombotic microangiopathy lesions (see above) (159, 161). Of note, thrombotic microangiopathy can occur in patients who are recipients of transplants of kidneys or other organs and following radiation, with or without calcineurin inhibitor treatment. In some patients, collapsing type glomerulosclerosis may be associated with cyclosporine toxicity, likely representing a response to severe vascular injury and ischemia (162).

Recurrent and De Novo Disease

Recurrent and de novo disease are important causes of renal allograft injury, affecting approximately 10% of renal allografts (159). Of all graft loss, 2–4% is due to recurrence of disease. IF microscopy should be performed in all transplant biopsies to rule out this possibility. When IF or LM findings in conjunction with the clinical setting indicate an undetermined lesion, electron microscopic study should also be performed.

In children the most common recurrent diseases include IgA nephropathy and Henoch-Schönlein purpura, MPGN, dense deposit disease, and FSGS (163). Although SLE has been reported to recur only rarely, our experience indicates a recurrence rate of approximately 30% (164). However, morphologic recurrence of disease does not necessarily lead to graft loss (159, 163). Dense deposit disease recurs morphologically in nearly all patients, but with only 10 to 20% resultant graft loss. In contrast, HUS has a recurrence rate of 15–25% and 40–50% of these experience graft loss. Although IgA nephropathy recurs in approximately 50% of patients, only 10% of grafts with recurrent disease are lost. MPGN type I recurs in 20–30%, with 10–40% graft loss. FSGS recurs in 20–30% of cases, resulting in graft loss in 30–50% of these. Of note, in recurrent FSGS, the only morphologic change found in the first weeks after recurrence of proteinuria is foot process effacement, with early segmental sclerosis detectable at 6–8 weeks after recurrent NS.

De novo disease may also affect the transplant. Membranous glomerulopathy is the most common de novo glomerulonephritis in the transplant. The etiology remains unknown (159). Glomerulonephritis related to infections, such as hepatitis C-related MPGN, also can occur in the transplant. Early changes of diabetic nephropathy develop much more rapidly in the transplant than in the native kidney and may occur within a few years, whether diabetes preexisted or is corticosteroid-induced (165). Thrombotic microangiopathy may be related to drug toxicity (see above) or be idiopathic in the transplant.

Posttransplant lymphoproliferative disease (PTLD) is due to the unrestrained proliferation of B lymphocytes, most often because of transformation by Epstein-Barr virus, and is an aggressive process, which if untreated, disseminates and may cause death (166). PTLD may respond to decreased immunosuppression. An expansile lymphoid infiltrate with atypical, transformed lymphocytes and serpiginous necrosis are features suggestive of PTLD (166). Immunohistochemical studies can be used to detect Epstein-Barr virus to further support this diagnosis. Typing studies of the lymphocytic infiltrate are not often helpful because most PTLD is polytypic, rather than clonal. Of note, acute rejection and PTLD may be present concurrently.

Polyoma virus nephropathy (PVN), most often due to the BK virus, has increased in the last years in the transplant, perhaps related to increased immunosuppression (76). PVN occurs in both adult and pediatric transplant recipients (167). The biopsy shows a pleomorphic infiltrate with lymphocytes, plasma cells, PMNs and occasional eosinophils, with enlarged tubular cells with smudgy nuclei. Early lesions (stage A) with minimal inflammation, fibrosis or injury have better prognosis than stage B with marked viral changes and inflammation and moderate fibrosis, or stage C, with extensive fibrosis (76). BK infection is confirmed by immunostaining. In some BK nephropathy cases, there may be associated tubular basement membrane deposits staining with IgG and C3 and visualized by EM (168). There may be some response to decreased immunosuppression and antiviral therapy.

New Methods for the Future

With the recent surge of application of molecular biology techniques to the study of renal disease, candidate factors involved in pathogenesis and progression of disease are being studied in animal models. Studies in human beings have also commenced. With further development of such studies, we may identify specific abnormal processes and thus target therapy more specifically. Research techniques that have been advantageously applied to elucidate pathogenesis of disease include immunostaining; identifying specific antigen in deposits of membranous glomerulopathy in some patients (thyroglobulin with Hashimoto's disease, hepatitis B, C antigen); light chains or paraproteins in plasma cell dyscrasia-associated diseases; identification of specific type IV collagen abnormality in Alport syndrome; C4d as a marker of humoral rejection and elucidation of pathogenesis of specific *E. coli*-associated toxins in some forms of HUS.

Current studies are aimed at understanding disease etiologies and mechanisms at a molecular and proteomic level, studying renal biopsies by laser capture microdissection (LCM), with real time reverse transcription polymerase chain reaction (RT-PCR), and in situ hybridization techniques (99, 169–171). Recent efforts have expanded use of these techniques to study mechanisms of injury and progression. Competitive and real time RT-PCR have been used successfully on small cores and even single isolated glomeruli from human biopsies (99, 169). Modulation of growth factors, collagens, cytokines, chemokines and their receptors has been investigated molecularly in renal biopsies. Cytokines and chemokines and their receptors were upregulated in diseases with macrophage influx and mesangial cell proliferation, supporting an important role in initiating and perpetuating injury. Such approaches can offer exciting new mechanistic insights into renal diseases.

In parallel, genetic studies are targeted both at diagnosis and identifying patients at risk for progression in diseases with a variable course, such as diabetes and IgA nephropathy. Genetic screening for podocin mutations is often done in children with FSGS. Polymorphisms of the renin-angiotensin system genes have been implicated as risk factors for progression and also as indices for response to therapies that target this system (172, 173). Noninvasive urinary proteomic studies are also explored to identify particular signature patterns indicative or predictive of specific lesions or risks (174, 175).

Diagnostic use of RT-PCR and in situ hybridization has focused on detection of viruses, including hepatitis B and C, cytomegalovirus, polyoma virus and Epstein-Barr virus. Increased expression of various immune-activated genes, such as perforin, granzyme and fas ligand, quantified by competitive RT-PCR showed initial high predictive value for acute rejection, but subsequent studies have not been as clear-cut (149).

Together these approaches promise to map risks and mechanisms of disease initiation and progression and point to targets to achieve resolution of injury. Sequential biopsies with evaluation of changes in structure and patterns of abnormal factors and modulation by therapy may be necessary to fully understand pathogenesis. Instead of diagnoses of morphologic patterns recognized by current techniques in the renal biopsy specimen, molecular techniques may allow more precise diagnosis of the specific diseases and identification of injury mechanisms.

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Glomerular Disease

25 Congenital Nephrotic Syndrome

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Renal diseases associated with nephrotic syndrome (NS) in the first year of life are uncommon and make up a heterogeneous group of disorders (1). Congenital nephrotic syndrome (CNS) is defined as proteinuria manifesting in the first 3 months of life. NS appearing later during the first year (4–12 months) is defined infantile, and NS manifesting thereafter is called childhood NS. While this classification is used to help the clinical diagnosis, it is arbitrary in the sense that NS caused by a specific gene mutation can manifest soon after birth or later in childhood. However, since the management of CNS is often different from the more common forms of childhood NS, the terminology still seems warranted (*Table 25-1*).

Primary CNS is typically caused by mutations in genes encoding for components of the glomerular filtration barrier (2–4). The classical form is the Finnish type of CNS (CNF), which is caused by mutations in the nephrin gene (*NPHS1*) leading to massive proteinuria, hypoproteinemia and edema in the newborn period. Other known genes causing CNS are podocin gene (*NPHS2*), Wilms' tumor factor 1 gene (*WT1*), laminin β2-gene (*LAMB2*), and PLCe1 gene (*PLCe1*). These disorders have a more widespread age of onset and also more variable clinical manifestations. CNS can also be part of more generalized syndromes, or be caused by neonatal infections, especially in Africa and Asia. Thus, the diagnosis of a patient with early manifestations of NS must be based on several criteria including clinical presentation, family history, laboratory findings, renal histology and genetic testing.

Glomerular Filtration Barrier

Glomerular Capillary Wall and Podocytes

The main feature of primary CNS is the extensive leakage of plasma proteins into urine through the kidney filtration barrier (5, 6). This barrier is located in the glomerular capillary wall and comprises three layers: fenestrated endothelium, glomerular basement membrane (GBM), and epithelial cell (podocyte) layer with distal foot processes and interposed slit diaphragms (SDs) (*Fig. 25-1*). The filter is a highly sophisticated size- and charge-selective molecular sieve, and normally only water and small plasma solutes pass into Bowman space (7, 8). As 100–200 l of protein free filtrate (primary urine) is formed daily, one can calculate that 3–6 kg of albumin has to be prevented from crossing the glomerular filtration barrier each day.

The flow of glomerular filtrate follows the extracellular route, passing across the GBM and SD, which bridge adjacent foot processes just above the GBM. The GBM is a protein network formed by type IV collagen, laminin, nidogen and negatively charged proteoglycans. The role of GBM in glomerular permselectivity has been debated, but it is now known that a primary defect in a GBM component results in heavy proteinuria (9). Podocytes and SD are, however, even more important in preventing proteinuria than the GBM (4, 10, 11). Podocytes are specialized cells that consist of the cell body and primary, secondary and tertiary processes. The cytoskeleton of the cell body and major processes consist of primary of microtubules and intermediate filaments, whereas actin is the major cytoskeletel component of foot processes. Actin network and the interacting proteins, such as α-actinin-4, maintain the architecture of the foot processes and responds to signals from the outside (e.g., SD and GBM) modifying the cellular functions accordingly (12–14).

Slit Diaphragm Components

The precise molecular structure of SD is still unresolved (*Fig. 25-2*). The first SD component identified was nephrin, which is a 1,241-residue cell adhesion protein of the immunoglobulin family (15–18). The extracellular part of nephrin contains eight immunoglobulin like modules and one type III fibronectin domain (*Fig. 25-3*). The intracellular domain contains nine tyrosine residues, which may become phosphorylated by protein kinases. This phosphorylation modulates the interaction of nephrin with other proteins, such as adaptor protein Nck and phophoinositide 3-kinase (PI3K) (19–21). These proteins link nephrin to actin cytoskeleton, suggesting an important role for nephrin in the regulation of the actin cytoskeleton and podocyte morphology. Nephrin takes part also in other cell signaling processes involved in the podocyte cell survival (22, 23).

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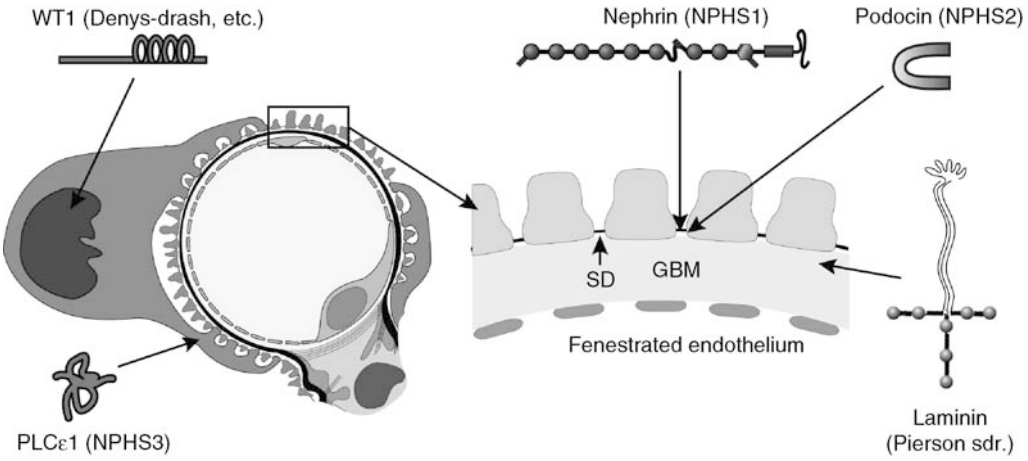
Table 25-1

Classification of Congenital Nephrotic Synrome (CNS)

Primary CNS
Nephrin (*NPHS1*) gene mutations (CNF, NPHS1)
Podocin (*NPHS2*) gene mutations
Phospholipase C epsilon 1 (*PLCE1*) gene mutations (NPHS3)
Wilms tumor suppressor 1 (*WT1*) gene mutations (Denys-Drash, isolated NS)
Laminin β2 (*LAMB2*) gene mutations (Pierson syndrome, isolated NS)
Laminin β3 (*LAMB3*) gene mutations (Herlitz junctional epidermolysis bullosa)
Lim homebox transcription factor 1β (*LMXB1*) mutations (Nail-patella syndrome)
Mitochondrial disorders
Syndromic CNS with brain malformations (Galloway-Mowat; gene defects not yet known)
Syndromic CNS without brain involvement (gene defects not yet known)
Secondary CNS
Congenital syphilis
Toxoplasmosis, malaria
Cytomegalovirus, rubella, hepatitis B, HIV
Maternal systemic lupus erythematosus (SLE)
Neonatal antibodies against neutral endopeptidase
Maternal steroid-chlorpheniramine treatment

Figure 25-1

A cross-sectional image of one glomerular capillary (*left*) and glomerular capillary wall (*right*), which comprises three layers: fenestrated endothelium, glomerular basement membrane (GBM), and epithelial cell layer with podocyte foot processes an interposing slit diaphragms (SD). The figure also depicts the five important molecules in the pathogenesis of CNS. WT1 is a transcription factor important for podocyte functions. PLCε1 is a cytoplasmic enzyme involved in cell signaling. Nephrin is a major component of SD and podocin is an adapter and scaffold protein for the SD components. Laminin is an important component of GBM.



In addition to nephrin, several other new podocyte proteins have been identified in the SD, such as Neph1, Neph2, Neph3, FAT 1 and FAT 2 (24, 25). According to the present understanding, nephrin associates with the Neph-proteins extracellularly and form the backbone of SD (26–28). Neph-proteins are structurally related to nephrin and they are also involved in the cell signaling (28). Mice with genetic defects in Neph1 develop heavy

Figure 25-2

A hypothetical model of the major components of the slit diaphragm and the basal membrane of the podocyte foot process anchoring it to glomerular basement membrane (GBM). See the text. The backbone of the SD is formed by nephrin, Neph- and FAT-proteins. The adapter proteins, such as podocin, Nck and C2AP, link SD to actin cytoskeleton as well as take part in the cell signaling.

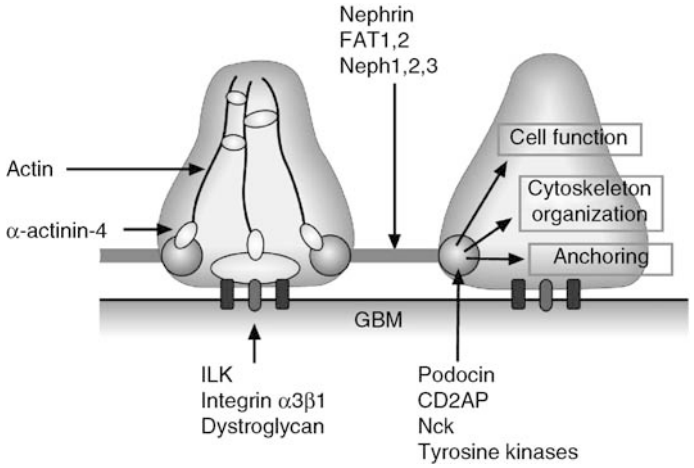
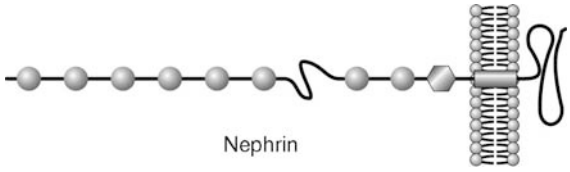


Figure 25-3

The nephrin molecule is a transmembrane cell adhesion protein with small intracellular and transmembrane domains and a larger extracellular domain. The extracellular domain contains eight Ig-like modules and one fibronectin type module adjacent. The extracellular part contains three cysteine residues taking part in molecular interactions. The intracellular part contains several tyrosine residues phosphorylated by protein kinases, which is important for cellular signaling.



proteinuria, but no humans with mutations in Neph1 have so far been identified (24).

Nephrin and Neph-proteins interact with the adapter proteins podocin, CD2AP and ZO-1, that are located in the cytosolic part of the podocyte foot process (29–34). Podocin is encoded by the *NPHS2* gene and it is a small hairpin-like protein belonging to a raft-associated stomatin family. It is a scaffolding protein and serves in the structural organization of the SD as well as in the signal transduction from the SD into podocytes (13, 19, 33, 35). Genetic mutations in podocin result in CNS as well as NS manifesting later in life.

Primary Nephrotic Syndrome

Nephrin Gene Mutations

CNF originally denoted to a severe form congenital nephrotic syndrome typically seen in the Finnish newborns (36, 37). The disease (also called as NPHS1) is highly enriched in Finland, the incidence being 1:8,200 live births (38). However, patients are reported all over the world among various ethnic groups (39–43). A very high incidence (1/500 live births) of CNF has been reported among the Old Order Mennonites in Lancaster County, Pennsylvania (41).

Genetics (42)

CNF is inherited as an autosomal-recessive trait (16). The *NPHS1* gene is located to chromosome 19q13.1 and exon sequencing analyses has revealed two important mutations in over 90% of the Finnish patients (Fin-major and Fin-minor) (16). Both mutations result in a stop codon and a truncated nephrin protein not expressed in SD. Several reports on *NPHS1* mutations in non-Finnish patients have been published (39–46). The patients come from Europe, North America, North Africa, Middle East and Asia. Most non-Finns have individual mutations including deletions, insertions, nonsense, missense, and splicing mutations spanning over the whole gene. Missense mutations are all located within the extracellular part, and clustering to

exons coding for the Ig-like motifs two, four and seven have been reported.

The Fin-major and Fin-minor mutations are rare outside Finland, but enrichment of other mutations has been reported also in non-Finns. In Mennonites, 1481delC mutation is common and leads to a truncated protein of 547 residues (41). On the other hand, a homozygous nonsense mutation R1160X in exon 27 has been found in all Maltese cases (42). Importantly, six of the 16 cases with this mutation had an atypically mild disease. The same mutation has been reported in six French patients and two of them had a mild disease.

Pathogenesis

Mutations in the *NPHS1* gene result in defective nephrin molecule and disturbed structure and function of the glomerular filtration barrier. The Fin-major and Fin-minor mutations cause a complete absence of nephrin in SD, and these kidneys lack the filamentous image of SD in electron microscopy (*Figs. 25-4* and *25-5*). Similarly, nephrin knock-out mice lack the slit diaphragm filaments in electron microscopy and die of severe proteinuria soon after birth (47). The findings suggest that the absence of nephrin leads to leakage of plasma proteins into urine through the "empty" podocyte pores. Liu et al. have shown that many missense mutations cause misfolding of nephrin protein and defective intracellular nephrin transport with absence of nephrin in SD as well (48). This most probably explains why even "mild" mutations cause heavy proteinuria and a severe form of CNS.

The expression of nephrin was first reported to be restricted to glomerular podocytes and this was later confirmed (16, 49). The data obtained especially from

Figure 25-4

Electron microscopy of the glomerular capillary wall. (a) Normal kidney showing the podocyte foot processes, the glomerular basement membrane, and the fenestrate endothelium. (b) CNF kidney with irregular podocyte foot processes (effacement) typical for all proteinuric kidney diseases. (c) Immunoelectron microcopy of a podocyte pore in a normal kidney. The filamentous image of the slit diaphragm (S) is seen (*arrow*). Antibodies against the intracellular part of nephrin show the localization of nephrin in the SD (gold particles). (d) Immunoelectron microscopy of the podocyte slit pore in a CNF kidney. No SD or nephrin is seen.

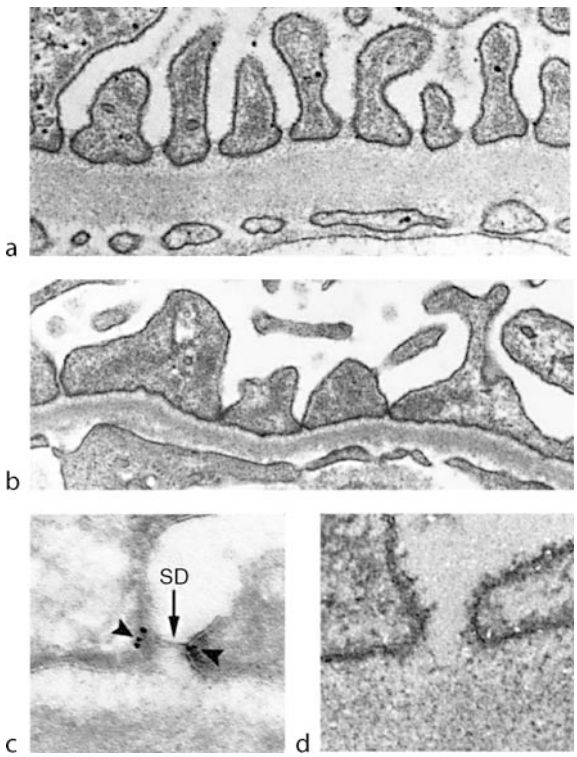
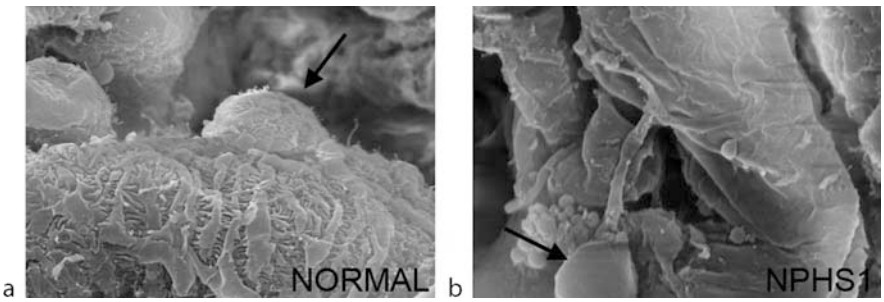


Figure 25-5

Scanning electron microscopy of a capillary wall in a normal and a CNF(NPHS1) kidney. (a) The podocyte cell body (*arrow*) and the primary, secondary and tertiary processes are clearly seen. (b) In the NPHS1 kidney, the podocyte structure is distorted. The cell body (*arrow*) "hang" in a primary process and the ramification of secondary processes is absent.



mice, however, suggest that nephrin may also be expressed in some areas of the central nervous system, pancreatic β-cells, and testis (47). No constant extrarenal manifestations, however, are observed in CNF patients speaking against a significant role of nephrin in other organs than kidney (50).

Renal Pathology

The CNF kidneys develop quite normally in the fetal period (51, 52). At the 16–22 gestational weeks, the glomeruli show little changes in light microscopy, but occasional dilated tubules may be seen (51, 53). On electron microscopy the mature glomeruli show effacement of podocyte foot processes and the SD image may be completely missing in cases with severe *NPHS1* mutations (46). It is remarkable that also carriers of *NPHS1* mutations can have these "proteinuric" changes making the pathological diagnosis difficult (54).

After birth, the CNF kidneys are large with the mean kidney weight of almost twice that of age-matched control children (51). The glomeruli show a slight to moderate increase of mesangial matrix and mesangial hypercellularity (*Fig. 25-6*). Degenerative changes such as shrinking of the glomerular tuft, fibrotic thickening of the Bowman's capsule, and glomerular sclerosis become evident with time (55). Electron microscopy shows effacement of foot processes, irregulariry in the GBM, and some swelling of the endothelial cells (peliosis) (56). Dilated proximal tubules are few at first, but radial dilations of the tubules become obvious with time (*Fig. 25-7*). Also, interstitial fibrosis and lymphocyte collections especially around sclerotic glomeruli increase substantially within the first 1–2 years (57).

Clinical Features

The basic problem in CNF is severe renal loss of plasma protein, which may begin already in utero (38). The early signs and symptoms are secondary to this protein deficiency (36, 38, 46). In a recent survey, over 80% of the children were found to be born prematurely (<38th week), with a mean birth weight of 2,600 g (1,500–3,500). However, only two of the 46 newborns were small for gestational age (46). Most neonates do not have major pulmonary problems, although amniotic fluid is often meconium stained. The placenta is larger than normal and almost invariably weighs more than 25% of the baby's birth weight. The mean ratio of placental to infant weight (ISP) is 0.38 in babies with CNF, compared with 0.18 in normal babies. The reason for this is not known.

In typical CNF, edema and abdominal distension become evident soon after birth. NS was diagnosed within the first week in 82% of the Finnish patients and within 2 months in rest of the cases (46). In a recent survey of European and Turkish patients, CNF manifested within the first 90 days in all 18 cases (2). Protein losses lead to severe hypoalbuminemia (<10 g/l) before protein substitution is started (58). Without albumin substitution and nutritional support, the classical features of CNF, such as generalized edema, abdominal distension, ascites, and widened cranial sutures and fontanelles, may develop during the first weeks or months of life (36).

Infants with CNF do not have extrarenal malformations. Minor functional disorders in central nervous system and heart, however, are quite common. Most children have muscular hypotonia, and mild atrophic changes in the brain were observed by computer tomography (CT) or magnetic resonance imaging (MRI) in a third of the Finnish infants (46). In 8% of the Finnish

Figure 25-6

Light microscopy of two glomeruli in a kidney with CNF. The histological structure is normal with only slight increase of mesangial matrix and mesangial cell proliferation (×400).

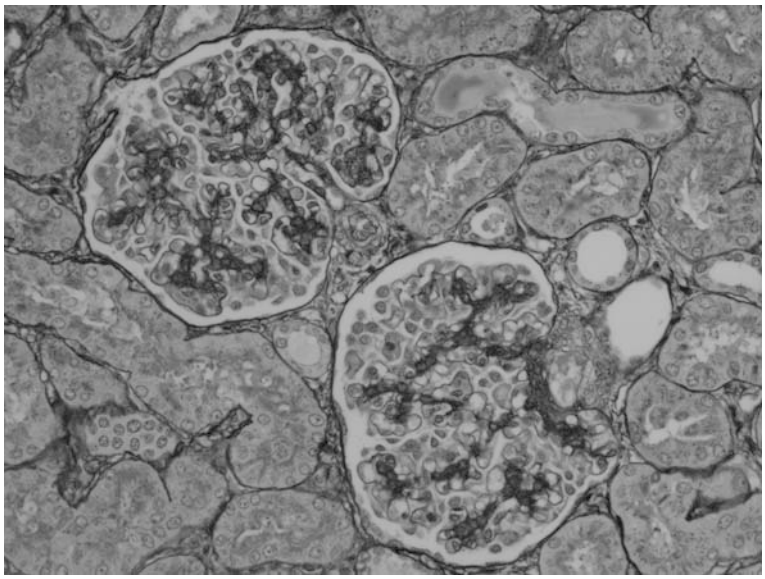
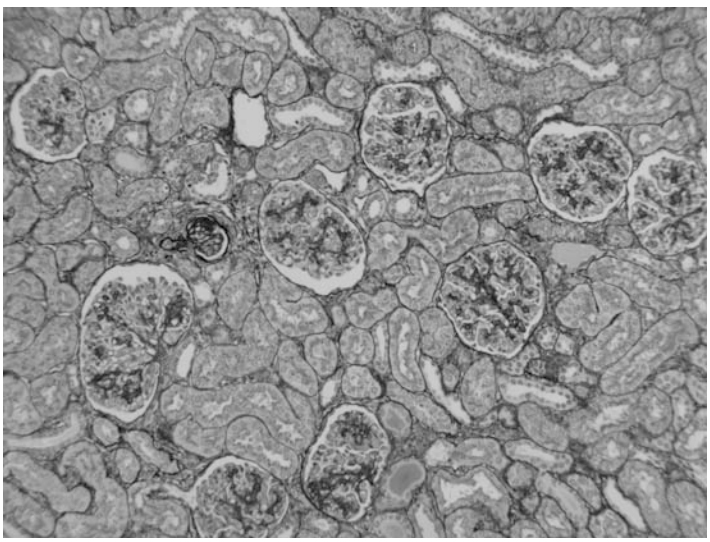


Figure 25-7

Histological picture of a CNF kidney at 7 months of age. The general structure is almost normal and the tubules are focally dilated. Arteriolar wall thickening is also evident (×100).



patients dystonic cerebral palsy has been diagnosed; the etiology of this is not known. Minor cardiac findings such as hypertrophy and mild pulmonary stenosis has been reported in one fourth of the Finnish patients (46). In a report form Malta, pulmonary valve stenosis was found in three cases and a subaortic stenosis in one patient (59).

Laboratory Findings

The magnitude of proteinuria depends on the "severity" of the *NPHS1* mutations. Typically, the first urine analysis already shows proteinuria, microscopic hematuria, and some leukocyturia. If the serum albumin concentration is

not low (<15 g/l), the urinary protein concentration usually exceeds 20 g/l (58). In addition to albumin, many other proteins are lost in the urine: immunoglobulin G (IgG), transferrin, apoproteins, lipoprotein lipase (LPL), antithrombin III (ATIII), ceruloplasmin, vitamin D binding protein, and thyroid binding globulin (60). The serum levels of these and their ligands (e.g., thyroxin) are low in serum, leading to secondary metabolic disturbances. The low thyroxin concentration leads to an increase in thyroid-stimulating hormone (TSH) (61).

Low serum albumin and postheparin plasma LPL activities and high free fatty acid concentrations lead to hypertriglyceridemia. Total and low-density lipoprotein (LDL) cholesterol levels are high but high-density lipoprotein (HDL) levels are low and the LDL and HDL particles are enriched with triglycerides (62). These lipid abnormalities and arteriolar changes seen already during the first year of life may lead to an increased risk of arteriosclerosis. In a recent analysis, deficiency in polyunsaturated fatty acids was especially prominent in infants with CNS, and this was regarded as a possible risk factor for the developing brains (63).

Podocin Gene Mutations

Genetics

Mutations in the *NPHS2* gene, encoding for a podocyte protein podocin, are a common cause of a steroid resistant NS (SRNS) in children and adults, accounting for up to 28% of sporadic and over 40% of familial cases of SRNS manifesting at various ages (29, 64–70). The podocin gene mutations, however, are also an important cause of CNS. In a recent report, recessive *NPHS2* mutations accounted for half of the CNS cases in 80 European families, while *NPHS1* mutations were responsible for only one third of the cases (2). The *NPHS2* mutations have also been found in CNS patients from Japan and elsewhere.

Hinkes et al. recently found *NPHS2* mutations in 18% of over 400 patients from a worldwide cohort of SRNS cases with the disease onset ranging from birth to 21 years (71) They found that severe, truncating mutations (nonsense or frameshift) resulted in early onset of NS (from birth to 9.1 years of age; mean 1.75 years). This was also true for homozygous R138Q mutation (from birth to 5.4 years; mean 1.77 years), which is common in European patients. The fact that identical mutations may result in onset of SRNS over spectrum of several years suggests that additional modifying factors are involved. Since podocin is a podocyte adapter protein required for the proper targeting of nephrin into SD, also nephrin expression may be distorted in CNS caused by *NPHS2* mutations (42, 72). Co-existence of *NPHS1* and *NPHS2* mutations with two homozygous mutations in one gene and one heterozygous mutation in the other gene resulting in a triallelic hit has been reported in CNS patients (42, 73).

Clinical Features

No systematic analysis of the clinical findings in CNS patients with *NPHS2* mutations has been published. The severity of proteinuria and the clinical findings are more variable than in CNF patients. According to the report by Hinkes et al., the patients develop end stage renal disease (ESRD) on average 6.6 years after diagnosis (2). As podocin is only expressed in kidney glomerulus, extra-renal manifestations are unlikely. The founder mutation R138X was, however, recently associated with cardiac anomalies in six consanguineous Arabs (74). In another cohort of 12 patients, 10 had a normal heart condition and two presented mild anomalies with mitral insufficiency and left ventricular hypertrophy (75).

In general, most patients with *NPHS2* mutations show histology of focal segmental glomerulosclerosis (FSGS). However, in the recent report, the CNS patients with *NPHS2* mutations often had minimal histological changes (MCNS) or NPHS1-type histology, and FSGS was present in only a third of the patients. Thus, it is clear that CNS caused by *NPHS1* or *NPHS2* mutations cannot be differentiated by the renal histology (71).

CNS patients with *NPHS2* mutations do not respond to steroid therapy and kidney transplantation is the only curative treatment option. Importantly, patients with *NPHS2* mutations have a reduced risk of recurrence of the nephrotic syndrome after kidney transplantation (64).

WT1 Gene Mutations

Genetics

Wilms' tumor suppressor gene (*WT1*) is located on chromosome 11p13 (76). It encodes for a nuclear WT1 protein, which is a transcription factor of the zinc finger family presumed to regulate the expression of numerous target genes through DNA binding (77). WT1 plays a crucial role in the embryonic development of the kidney and genitalia. In mature kidney, WT1 is abundantly expressed in podocytes and it is believed to control the expression the SD proteins, such as nephrin (78). The *WT1* gene

contains ten exons, the first six of which encode a proline/glutamine-rich transcriptional regulatory region. Exons 7–10 encode the four zinc fingers of the DNA-binding domain. Up to 24 different isoforms of WT1 may result from the combination of alternative translations sites, alternative RNA splicing, and RNA editing. The biological role of all these isoforms is not known.

A variety of *WT1* mutations, which either affect development or induce tumor formation, have been identified. Developmental defects include the Denys-Drash syndrome (DDS), Frasier syndrome (FS), and WAGR syndrome (Wilms tumor, aniridia, genitourinary anomalies, and mental retardation) (79). Missense mutations mostly cause DDS, donor splice site mutations in intron 9 are associated with FS, and constitutional deletion of one whole copy of *WTI* is associated with WAGR. FS is characterized by the association of male pseudo-hermaphroditism and glomerulopathy (80, 81). There is complete male to female gender reversal in 46,XY patients. FS is associated with gonadoblastomas but not with Wilms' tumor. Proteinuria is detected in childhood, usually between 2 and 6 years of age, and kidney biopsy reveals FSGS. The renal disease does not respond to medical therapy but it has a slower progressive course to renal failure than DDS.

Denys-Drash Syndrome

DDS is caused by heterozygous mutations in *WT1* (82, 83). More than 60 germline mutations (both familial and de novo) have been described in DDS patients. Most are missense mutations within exon 8 and 9, coding for zinc finger domains 2 and 3, which leads to alteration in the DNA-binding capacity of WT1 (84). Also deletions, insertions and nonsense mutations have been described in DDS patients leading to a truncated protein (85). The most prevalent mutation is R394W located at the zinc finger 3. The mutant protein probably suppresses the normal allele, which explains the more severe phenotype seen in DDS compared with children with complete deletion of one WT1 allele. In most patients the nuclear expression of WTI is absent or reduced in podocytes. The podocyte function is affected and, at the molecular level, up-regulation of PAX2 and down-regulation of nephrin have been reported (86, 87). Also, the expression of growth factors that regulate glomerular capillary development is affected in DDS (88).

DDS is a combination of NS with DMS, male pseudohermaphroditism and Wilms' tumor (89, 90). DDS can be divided in three clinical categories: (1) genotypic males with all three abnormalities, (2) genotypic males with nephropathy and ambiguous external and/or internal genitalia only, and (3) genotypic females with nephropathy and Wilms tumor only (85). While male pseudohermaphroditism is an important diagnostic sign, the diagnosis in females may be difficult and delayed. The renal tumor and nephropathy may be clinically simultaneous, but in some cases, the nephropathy either precedes the tumor or remains the only abnormality of the disease (91, 92).

The nephropathy begins with proteinuria and NS. It is usually discovered at the age of a few months, sometimes at birth. Renal failure is often noted concomitantly with NS and progression to ESRD before the age of 4 years is the rule. The characteristic glomerular lesion is DMS (93). No correlation between the genotype and the severity of renal disease has been observed. Because the renal disease in DDS is resistant to drug treatment, kidney transplantation remains the only therapeutic alternative, and favorable results without recurrence of DMS have been reported. In order to avoid the development of Wilms' tumor, bilateral nephrectomy at the onset of terminal renal failure is recommended. Based on a recent report by Hu et al., removal of native kidneys should be performed to all patients with nephropathy caused by *WT1* mutations (94).

Isolated NS with DMS

Mutations in *WT1* can also cause an isolated kidney disease with NS appearing at a various age. In many cases, the kidney biopsy reveals DMS histology. Three studies reported *WT1* mutations in eight of 21 patients with isolated DMS (95–99). These mutations were localized on exons 7, 8, 9 and on intron 9 in the *WT1* gene.

The connection between CNS and DMS was described already in 1970s (1, 100). As the clinical picture appeared to be distinct, DMS was thought to represent a specific entity. In the published reports, proteinuria was often moderate and NS was detected soon after birth or later in infancy, often in the second and third years. The disorder did does not respond to medical therapy and terminal renal failure often develop within a few months or years.

It is clear now that isolated DMS is not a single entity but probably caused by several gene defects. Besides *WT1*, mutations in the *PLCE1* gene may result in DMS, as discussed later.

Laminin β2 Gene Mutations (Pierson Syndrome)

Pierson syndrome is a recently identified form of primary CNS caused by loss-of-function mutations in the *LAMB2* gene (101–104). This gene encodes for the

laminin β2-chain, that is specifically expressed in the GBM, ocular structures, and neuromuscular synapses. Laminins are extracellular matrix proteins with important roles in cell adhesion, differentiation and proliferation. The deficiency of laminin β2-chain results in the GBM alterations and impairment of the function of the glomerular filtration barrier. In an animal model, lack of laminin β2-chain resulted in ectopic deposition of laminin and mislocalization of anionic sites in the GBM (9). These changes and proteinuria preceded the lesions in podocytes indicating that the GBM lesions were responsible for protein leakage.

Pierson syndrome comprises CNS with the histological lesions of DMS, associated with complex ocular maldevelopment. The renal disease may start prenatally resulting in terminal renal failure during the first few weeks or months of life (103, 104). The most characteristic ocular feature in Pierson syndrome is microcoria, a fixed narrowing of the pupils due to a defect of the dilatator muscle. Ocular manifestations also include abnormalities of the lens, cornea and retina, often leading to blindness. Patients who survive the newborn period due to renal replacement therapy develop neurological deficits, consistent with the known expression of laminin β2 in the nervous system. These include severe muscular hypotonia and psychomotor retardation (105).

Pierson syndrome is typically caused by biallelic truncating mutations in the *LAMB2* gene. Recently, several reports have revealed new *LAMB2* mutations causing variants of Pierson syndrome. Hasselbacher et al. described children with CNS and early development of ESRD, but without eye anomalies or with minor ocular changes different from those observed in Pierson syndrome (106). Kagan et al. described a patient with CNS, high-grade myopia, and minor structural eye anomalies, but no microcoria (107). The patient had normal renal function at the age of 16 months. Choi et al. presented a girl with congenital microcoria and infantile NS, with normal renal function still at the age of 6 years and another female patient with isolated CNS without ocular involvement. Her renal function deteriorated progressively over several months, and retinal detachment developed at the age of 10 months (108).

Since missense mutations in the *LAMB2* gene present with a spectrum of symptoms, genetic analysis of *LAMB2* gene is indicated in CNS patients with no mutations in the *NPHS1* and *NPHS2* genes. *LAMB2* gene mutations, however, are much less frequent as indicated by the recent European report on early-onset NS revealing missense *LAMB2* mutations in 2 out of 46 patients (4.4%) with CNS (2).

PLCε1 Gene Mutations

Mutations in the phospholipase C epsilon 1 (*PLCE1 or NPHS3*) gene were recently identified as a new cause of autosomal recessive form of early-onset NS (109). PLCe1 is a phospholipase enzyme and a signaling protein for many G protein-coupled receptors, including angiotensin II. PLCe1 promotes the downstream activation of protein kinase C enhancing calcium signaling events. PLCe1 is abundant in kidney glomerulus and localizes to the cytoplasm of the podocyte cell body and major and secondary processes. It interacts with IQ motif-containing GTPase-activating protein 1 (IQGAP1), which also interacts with nephrin (110). This suggests that PLCe1 may be involved in the SD signaling and the function of the glomerular filter (111). PLCe1 is highly expressed in the developing glomerulus and the absence of PLCe1 may halt kidney development leading to DMS-type glomerular lesions. Of note, this is associated with a major reduction in the expression of nephrin and podocin (112).

Mutations in *PLCE1* were originally reported in 14 patients from seven families (109). Most patients were Turkish and the age at the onset of the disease ranged from 2 months to 9 years. The patients had no extrarenal manifestations. Twelve patients had homozygous truncating mutation, and all developed proteinuria with DMS. Nine of these 12 patients progressed to ESRF by the age of 5 years. Interestingly, two patients responded to cyclosporine A or prednisone and have remained in remission for several years. Two siblings had nontruncating missense mutations and both had FSGS on the renal biopsy.

Gbadegesin et al. recently analyzed the relative frequency of *PLCE1* mutations in a worldwide cohort of children with "idiopathic" DMS and found truncating PLCe1 mutations in 10 of the 35 families (29%) (113). The age of patients at the onset of the disease varied between 1 and 36 months and most patients progressed rapidly to ESRD, with an age range of 8–60 months. Importantly, three of the 35 families had mutations in the *WT1* gene and none had *LAMB2* mutations. Thus, *PLCE1* mutations were the most common cause of isolated DMS in this cohort.

Other Forms of Primary CNS

Galloway-Mowat Syndrome

In 1968, Galloway and Mowat described two siblings showing congenital microcephaly, NS, and hiatus hernia (114). Several subsequent reports showing various brain malformations and congenital or early-onset NS have been

called Galloway-Mowat syndrome. The classic Galloway-Mowat syndrome is an autosomal recessive disorder, but the gene has not been identified (115). Since podocytes an neuronal cells share common morphology and biology with many proteins expressed in both types of cells, the association of CNS and brain anomalies is not unexpected.

Some patients with CNS and brain malformation show DMS (116, 117). Pathological changes other than DMS have also been reported (116, 117). A wide variety of additional anomalies have also been reported in patients with this association, including dysmorphic facial features (118–121). Other defects include ocular, limb, cardiac, and diaphragmatic anomalies (122, 123).

Other Genetic Forms

Nail-Patella Syndrome is caused by mutations in the gene coding for Lim homebox transcription factor 1β (LMXB1) (124, 125). This protein is expressed in podocytes and regulates the synthesis of α-chains of type IV collagen, podocin and CD2AP. Renal symptoms are found in a third of these patients and a single case with CNS has been described (126). NS has also been described in single patients with Lowe syndrome (127), Herlitz junctional epidermolysis bullosa (128), sialic acid storage disease (129), and mitochondrial disorders (130–133).

Secondary Nephrotic Syndromes

NS has been described secondary to some congenital and infantile disorders. Syphilis, toxoplasmosis, some viral infections, and the infantile form of SLE are currently the most important secondary causes of congenital and infantile NS.

Infections

Congenital syphilis has long been known to cause CNS. It may cause a nephritic or nephrotic syndrome in the newborn (134, 135). Proteinuria and hematuria are present, but severe NS is less common. NS may manifest in the newborn but is more often seen between 1 and 4 months of age. Membranous nephropathy is a common finding in kidney biopsy. Antimicrobial therapy, usually penicillin, is curative, provided that irreversible renal lesions have not developed (136).

An association of neonatal cytomegalovirus (CMV) infection and CNS has also been reported (137, 138). In these cases, DMS type histology is often found and the disease clearly responds to ganciclovir therapy. In these infections, manifestations other than NS lead to the correct diagnosis. Toxoplasmosis has been associated with CNS in a few cases (139). Congenital rubella has been reported to cause CNS with membranous glomerulonephritis (140). The acquired immunodeficiency syndrome caused by the human immunodeficiency virus is associated with nephropathy, including NS. This disease usually affects children older than 1 year, but some infants with nephropathy have been reported (141). Hepatitis B virus may cause membranous glomerulonephritis, and infants with nephrosis associated with hepatitis B infection have been described (142).

Autoimmune Disorders

Although SLE is rarely diagnosed before 5 years of age, an infantile form of SLE has been reported. NS was the major clinical finding in five infants aged 6 weeks to 6 months with SLE (143). These patients had elevated antinuclear antibody titers, hypocomplementemia, and diffuse proliferative glomerulonephritis. Response to the immunosuppressive therapy was poor in many cases. Neonatal membranous nephropathy secondary to feto-maternal alloimmunization with antibodies against neutral endopeptidase present on podocytes have been reported in a few cases CNS (144, 145).

Diagnosis of CNS

Clinical Findings

In severe forms of CNS, generalized edema, urinary protein >20 g/l, and serum albumin level <10 g/l can be detected in the newborn period. The amount of proteinuria, however, varies in different entities and the clinical signs may not be evident during the first weeks or months of life. Also, the true magnitude proteinuria may be detectable only after partial correction of hypoproteinemia by albumin infusions. Small amounts of red blood cells and leucocytes are often present in urine. Serum creatinine and urea levels are variable. Renal function remains normal for the first months in CNF, but in other forms of CNS kidney failure may develop faster. Blood pressure values can be low due to hypoproteinemia and low oncotic pressure, or elevated if renal failure is already present.

In newborns, the placental weight >25% of birthweight is present in CNF, but may be seen in other forms of CNS (46). The kidneys may be of normal size

or larger than normal by ultrasound examination, and the renal cortex is often hyperechogenic. Search for possible non-renal malformations is important, especially since they may give clue to the etiologic diagnosis. These include genital abnormalities (WT1), eye defects (LAMB2), and neurological disorders (Mowat–Galloway). CNS caused by nephrin, podocin or PLCe1 mutations is not associated with extrarenal malformations. Cardiac evaluation, however, often reveals ventricular hypertrophy but structural defects are rare. If infectious etiology is regarded possible, search for specific antibodies (e.g., syphilis, toxoplasma) or DNA/RNA (e.g., CMV, hepatitis B, HIV) in blood samples is indicated.

Kidney Biopsy

Renal biopsy often does not allow the diagnosis but may guide genetic testing. Indeed, a specific gene defect may cause several types of glomerular lesions, such as mesangial expansion, FSGS, MCNS and DMS. Moreover, the same histological glomerular lesion may be due to different genetic defects. Tubular dilatations and interstitial fibrosis and inflammation, can be seen in all forms of proteinuric diseases. Thus, the indications for renal biopsy are not clear. The knowledge of the severity of glomerular sclerosis and interstitial fibrosis may help in the treatment strategies. On the other hand, the lesions are focal and the biopsy findings may be misleading. If immunohistochemistry for nephrin and podocin is available, analysis of their expression in biopsy sample is useful. Total lack of either protein speaks for a severe disorder not responding to antiproteinuric therapy. Immunofluorescence microscopy may reveal the presence of IgG deposits in patients with membranous nephropathy.

Gene Testing

Genetic analysis is the method of choice for the precise diagnosis of CNS. The knowledge of etiology helps in assessing the management and prognosis, in follow up for possible associated symptoms, and in genetic counseling. Analysis of the *NPHS1* and *NPHS2* mutations is warranted in all CNS patients. These analyses are commercially available in Athena Diagnostics. If no mutation is detected in these genes or if clinical findings speak for mutations in *WT1*, *LAMB2,* or *PLCe1* gene, analysis of these genes may be obtained in research laboratories.

Prenatal diagnosis in families with a known risk for CNS should be based on genetic testing whenever possible. The results can be obtained fast if the mutations are already known. In case of no family history or if the mutations in the affected child have not been identified, prenatal genetic testing is a challenge, since sequencing of the *NPHS1* (29 exons) and *NPHS2* (eight exons) genes is time consuming and usually not possible within the short time frame available. Especially CNF can still be suspected prenatally based on an elevated alpha-fetoprotein (AFP) levels in maternal serum and amniotic fluid (146). If the AFP concentration in amniotic fluid is very high and the ultrasound examination does not reveal fetal anencephaly or other malformations, CNF is a likely diagnosis. However, heterozygous fetal carriers of *NPHS1* gene mutations may have temporarily elevated AFP levels in amniotic fluid and maternal serum and repeated measurement of amniotic fluid AFP before the 20th week of pregnancy is recommended in cases with high AFP levels (54).

Management of CNS

In contrast to most cases of childhood NS, immunosuppressive drugs are ineffective in primary CNS (147). This was the case in a recent report, in which only one of the 45 patients with primary CNS achieved a persistent remission following steroid treatment (2). The genetic defect in this child was not identified. Two patients with *PLCE1* gene mutations have also shown improvement after immunosuppressive therapy, but the mechanism behind this is not known (109). In general, CNS children with known disease-causing mutations should be spared unwarranted steroid treatment. In secondary CNS, specific therapy against the infectious agent, of course, is warranted and often effective.

In most patients, the goals during the first months are to control proteinuria and edema, provide good nutrition, and prevent thrombosis and infections, allowing the child to reach a weight and body size consistent with successful kidney transplantation (131, 148, 149). With an optimal therapy growth and development are satisfactory and the patients can spend most of the time at home. In most cases, ESRD is to be expected within the first years of life.

Albumin Substitution

The magnitude of the protein losses into urine is crucial for the therapeutic decisions (*Table 25-2*). If proteinuria is modest, only occasional albumin infusions may be needed. On the other hand, heavy proteinuria (10–100 g/l) inevitably leads to life-threatening edema, protein malnutrition, reduced growth, and secondary complications. In

Table 25-2

Management of Infants with primary CNS

Protein substitution parentally
– 20 % albumin infusions as required
– dosing 3-4 g/kg/day when severe NS
– divided in 1-3 infusions (day or night)
– furosemide (0.5mg/kg) together with albumin
– deep vein catheter needed
Nutrition
– hypercaloric diet (130 kcal/kg/day)
– protein supplementation (4 g/kg/day)
– lipid supplementation (rapeseed/sun flowr oil)
– A,D,E and water soluble vitamins
– calcium, magnesium (potassium) supplementation
– nasogastric tube or gastrostomy often required for proper feeding
Medication
– anticoagulation (warfarin, AT III infusions)
– thyroxin substitution
– parenteral antibiotics when bacterial infection suspected
– antiproteinuric drugs (ACE-inhibitor, AT II R blocker, indomethacin)
Dialysis
– terminal renal failure
– after nephrectomies
Transplantation
– after dialysis/pre-emptive
– extraperitoneal grafting when > 9 kg

these cases, protein substitution by parenteral albumin infusions is mandatory. Our practice in treating CNF patients is to infuse 20% albumin solution together with a bolus of intravenous furosemide (0.5 mg/kg), using deep-vein catheters after 3 weeks of age. The substitution is first divided into three 2-h infusions (starting dose of 1–5 ml/kg/infusion), and after a few weeks albumin is given as one 6-h infusion during the night (up to 15–20 ml/kg/night; which is 3–4 g/kg of albumin). This substitution corrects hypoproteinemia only temporarily, but the patients do not have substantial edema.

Antiproteinuric Medication

A reduction in the protein excretion using an ACE-inhibitor and indomethacin has been reported in infants with primary CNS (150–152). Blockade of renin-angiotensin axis not only reduces glomerular perfusion pressure, but also exerts protective measures on podocytes (153). In a few patients with CNS, adding of angiotensin II receptor (AT II) blocker to ACE-inhibitor medication, has further reduced proteinuria (154). Thus, treatment with these drugs (and indomethacin) is worth trying in many cases of CNS. In our experience, CNF patients with the severe (truncating) Fin-major and Fin-minor mutations do not respond to captopril or indomethacin, but if a mutation leads to a single amino acid change and quite normal nephrin expression, a reduction in the protein excretion may be observed (46).

Nutrition

Infants with severe CNS have traditionally been treated with a high-energy (130 kcal/kg/day) and a high-protein (3–4 g/kg/day) diet (58, 153). Breast milk and milk formulas are first used and the excess protein is given as a

casein-based protein product. Glucose polymers are given to increase energy intake and a mixture of rapeseed and sunflower oil to balance the lipid levels. The children also receive vitamin D_2 (400 IU/day) which can be changed to alpha-calcidiol when an increase of parathyroid hormone level is noticed. Multivitamin preparations are given according to the recommended dietary allowances for healthy children of the same age. Supplementary magnesium (50 mg/day) and calcium (500 –1,000 mg/day) are also given to keep the serum levels within the normal range. The daily water intake is 100–130 ml/kg. Most patients need a nasogastric tube to guarantee their energy intake. Modifications to this treatment are, of course, necessary with deteriorating renal function.

Additional Medication

Patients with NS often have low levels of serum thyroid-binding globulin and thyroxin (155, 156). McLean et al. reported an increase in TSH in four of five patients with CNS and a positive response to thyroxin substitution (61). We have a similar experience. Although serum thyroxin concentration is always low, TSH may be normal in the beginning but increases in most patients during the first months. Thus, we have adopted a policy of routinely giving thyroxin from birth, adjusting the dosage according to TSH.

Increased levels of macroglobulins, fibrinogen, thromboplastin, and factors II, V, VII, X, and XII, contribute to hypercoagulopathy (157). Thromboses and severe coagulation problems have been reported in children with CNS (158) and the use low-dose aspirin and dipyridamole therapy has been recommended. All Finnish CNF patients are treated with sodium warfarin from 3 to 4 weeks of age, and no severe thrombotic complications have occurred since this therapy was commenced. Before surgical or vascular procedures, warfarin is stopped and ATIII (50 IU/kg) is given to temporarily correct the ATIII deficiency.

Bacterial infections may be a major problem in infants with NS. Because of urinary losses of gamma globulin and complement factors B and D (159), nephrotic children are especially prone to infections caused by capsular bacteria such as pneumococci, and prophylactic use of penicillin has been recommended. Ljungberg et al. retrospectively analyzed the incidence and type of infection in 21 infants with CNF during the first year of life (160). The infants suffered from 63 verified and 62 suspected episodes of sepsis. The use of central venous lines had no effect on the incidence, and the prophylactic use of antibiotics or immunoglobulins did not reduce the incidence. Thus, we do not recommend prophylactic medication, but a high degree of suspicion for septic infections is warranted. The symptoms are often vague and masked by signs of focal infections occurring at the same time. Antibiotic therapy should be started promptly on suspicion and should cover the major hospital strains of bacteria. Response to treatment is usually excellent. Because of the urinary loss of IgG, vaccinations are normally given after nephrectomies.

Unilateral Nephrectomy

Some centers have proposed unilateral nephrectomy to reduce protein losses (161, 162). This decreases the need and frequency of the albumin infusions and may help in the every day management so that the start of dialysis and renal transplantation can be postponed to an older age. In children with severe CNF, we perform bilateral nephrectomy and peritoneal dialysis at an early age to avoid the many problems encountered during the nephrotic stage. Prolonged NS is also accompanied by pathological lipid status which leads to vascular changes already during infancy.

Dialysis

Although normal growth can be achieved with optimal therapy during the nephrotic stage, the patients with severe CNS are still malnourished and hypoproteinemic (163). To optimize treatment we perform bilateral nephrectomy and commence peritoneal dialysis (CCPD) when the infant weighs about 7 kg. The aim is to improve the child's nutritional state before the kidney transplantation (58). The general condition improves, muscle mass increases, and catch-up growth can be documented while receiving CCPD (164–166). However, in contrast to our practice, transplantation can be performed also preemptively and the results are satisfactory.

Kidney Transplantation

Renal transplantation has become an established mode of therapy for most children with CNS. The fact, that CNS children are often transplanted at 1–3 years of age using adult-size kidneys, may sometimes be surgically demanding and increase the risk for thrombotic and ureteral complications, as compared to older recipients. A healthy parent with a single mutation in nephrin or podocin gene

can be used as a donor in the transplantation. Postoperatively abundant hydration of the recipient (2,500 ml/m^2) is necessary to maintain optimum aortic and renal artery blood flow and avoid low-flow states that could damage the graft (167).

The use of immunosuppressive medication should be balanced in order to prevent rejection episodes which may be clinically subtle and, on the other hand, avoid the many side-effects associated with these drugs. Recurrence of NS in the graft has occurred in some CNF children who have developed anti-nephrin antibodies after transplantation. Treatment of the recurrence with cyclophosphamide and plasma exchanges (and more recently with anti-CD20 antibodies) normally leads to remission (168). Recurrent NS has also been reported in patients with *NPHS2* mutations, but in these cases no anti-podocin antibodies were detected (169).

Overall, the results of kidney transplantation in CNS are quite good and similar to those obtained in other etiologies. Patient survival at 5 years is over 90% and graft survival over 80% in registry databases and in single centers (170). Also, the quality of life is good in most patients. A great majority (72%) of those who have reached the school attend a normal class. The early CNF patients who suffered from thrombotic complications have neurologic handicaps (171–174). Chronic allograft nephropathy is a major problem in these patients and a second transplantation is inevitable when the patients become young adults.

Concluding Remarks

During the past few years our knowledge on the genetic and molecular basis of CNS has greatly increased. Podocyte proteins play an important role in the glomerular sieving and mutations in the nephrin, podocin, WT1, PLCε1, and lamininB2 account for most cases of CNS. It is to be expected that more genetic defects will be found in CNS patients in the near future. The management of these infants has improved.

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26 Inherited Glomerular Diseases

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In recent years the determined efforts of numerous investigators and the dedicated participation of patients, families and clinicians have led to the mapping and identification of numerous genetic loci involved in inherited glomerular disease and the functional characterization of their protein products. This information has generated important insights into the cell–cell and cell–matrix interactions required for normal glomerular structure and function, and the mechanisms by which genetically programmed disruptions in these interactions produce disease phenotypes. Additionally, our ability to predict prognosis and provide accurate genetic counseling has been greatly enhanced by this expansion of our knowledge base.

Inherited glomerular diseases can be roughly divided into two categories based on clinical presentation. Hematuria is typically the initial symptom of inherited diseases of glomerular basement membranes, with the exception of Pierson syndrome. In patients with inherited podocyte diseases the predominant clinical abnormality at presentation is proteinuria. This chapter will focus on inherited diseases of glomerular basement membranes, particularly Alport syndrome and thin basement membrane nephropathy, which together account for 30–50% of children with isolated glomerular hematuria referred to pediatric nephrology clinics for consultation (1–4).

The Glomerular Basement Membrane

The structural and functional characteristics of the normal glomerular capillary wall are discussed in detail elsewhere in this text. For the purposes of this chapter, this discussion will focus on the major protein components of glomerular basement membrane (GBM), type IV collagen and laminin, which form networks bridged by entactin/nidogen and heparan sulfate proteoglycans (agrin).

Type IV collagen. The type IV collagen protein family comprises six α chains that share several basic structural features: a collagenous domain of ~1,400 residues containing the repetitive triplet sequence glycine-X-Y (Gly-X-Y, with X and Y representing other amino acids); a carboxyterminal noncollagenous (NC1) domain of ~230 residues, including 12 completely conserved cysteine residues; and an aminoterminal noncollagenous sequence of 15–20 residues. The collagenous triplet sequence in each chain contains ~20 interruptions. The secreted form of type IV collagen is a heterotrimer composed of three α chains, resulting from self-association of NC1 domains and folding of the collagenous domains into triple helical structures. Amino acid sequences within the NC1 domains determine the specificity of chain association, resulting in three major trimeric species: α1α1α2, α3α4α5 and α5α5α6 (5). While the interstitial collagens, such as type I collagen, lose their NC1 domains after chain association and form fibrillar networks, type IV collagen trimers form open, nonfibrillar networks through NC1–NC1 interactions between two trimers and aminoterminal interactions of four trimers.

α1α1α2(IV) trimers are present in all basement membranes. α3α4α5(IV) and α5α5α6(IV) trimers are more restricted in their distributions in basement membranes (*Fig. 26-1*). In normal, mature human kidneys, α3α4α5(IV) trimers are found in GBM, Bowman's capsules and the basement membranes of distal tubules. α5α5α6(IV) trimers are present in Bowman's capsules and the basement membranes of distal tubules and collecting ducts of normal kidneys but are not found in GBM (6, 7). α5α5α6(IV) trimers are also found in normal epidermal basement membranes, but α3α4α5(IV) trimers are not. α3α4α5(IV) trimers are also found in several ocular and cochlear basement membranes, as discussed in the section on Alport syndrome below.

Each type IV collagen a chain is encoded by one of six distinct genes, COL4A1 – COL4A6, which are arranged in pairs on three chromosomes (*Fig. 26-2*). COL4A1 and COL4A2 encode the α1(IV) and α2(IV) chains, respectively, and are located on chromosome 1. The α3(IV) and α4(IV) chains are respectively encoded by the COL4A3 and COL4A4 genes on chromosome 2, while the α5(IV) and α6(IV) genes are encoded by the COL4A5 and COL4A6 genes on the X chromosome. The paired genes are arranged in a 5′-5′ orientation, with intervening sequences of varying length that contain regulatory elements.

Laminin. Laminin also forms networks based on heterotrimeric subunits. Each subunit consists of an α, β and

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Figure 26-1

Immunofluorescene microscopy. Normal distribution of the α(IV) chains in renal and epidermal basement membranes. (See color plate 8)

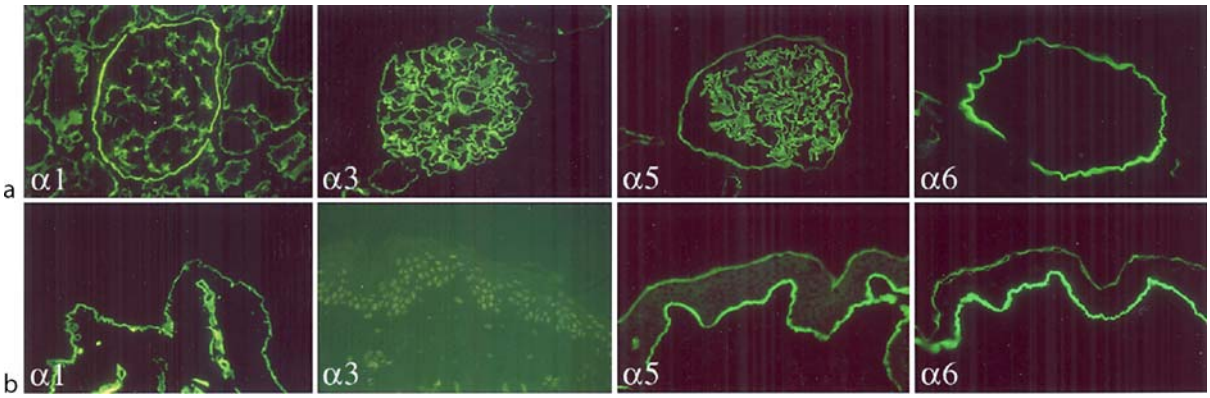
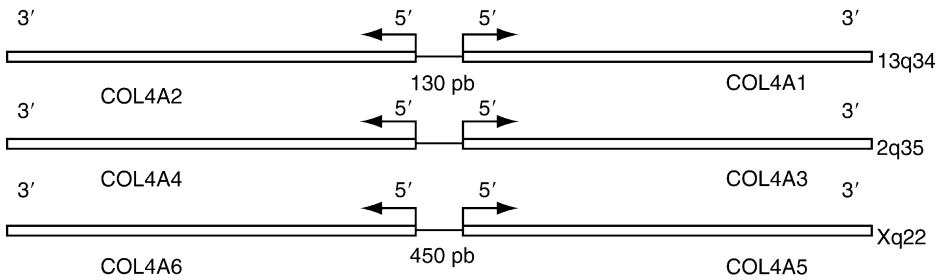


Figure 26-2

Schematic representation of the distribution of type IV collagen gene on chromosomes 13, 2, and X.



γ chain. There are 11 known laminin chains (α1–5, β1–3, and γ1–3), each encoded by a distinct gene, that form 15 heterotrimeric isomers. Laminin-521 (previously known as laminin 11) has the composition α5β2γ1 and is the laminin form found in GBM (8).

Glomerular Basement Membrane Disorders

To date, mutations affecting two of the major proteins of GBM, type IV collagen and laminin, have been identified as causes of inherited glomerular disease. Mutations in type IV collagen cause Alport syndrome, some cases of thin basement membrane nephropathy, and HANAC syndrome, comprising hereditary angiopathy, nephropathy, aneurysms and muscle cramps (*Table 26-1*). Mutations in laminin have been implicated in Pierson syndrome.

Type IV Collagen Disorders

Alport Syndrome

Dominantly-transmitted hematuria was first described as a clinical entity in the early 1900s (9). Over the next 20 years, studies of this condition in successive generations of a single family described development of proteinuria and renal insufficiency in affected individuals, particularly males, and in 1927 Alport reported the association of nephritis with neural deafness in this family (10, 11). Numerous descriptions of families with hereditary nephritis were published in the 1950s and 1960s, leading ultimately to the observations that established Alport syndrome as a heritable disorder of type IV collagen. These signal events included the identification of unique ultrastructural alterations in Alport glomerular basement membranes (GBM) (12–14), the observation that Alport

Table 26-1
Type IV collagen disorders

Disorder	Inheritance	Locus	Gene product
Alport syndrome	X-linked	COL4A5	α5(IV)
	Autosomal recessive	COL4A3 or COL4A4	α3(IV) or α4(IV)
	Autosomal dominant	COL4A3 or COL4A4	α3(IV) or α4(IV)
Thin basement membrane nephropathy	Autosomal dominant	COL4A3 or COL4A4	α3(IV) or α4(IV)
HANAC syndrome	Autosomal dominant	COL4A1	α1(IV)

GBM exhibited abnormal reactivity with anti-GBM sera directed against antigens associated with type IV collagen (15–17), the mapping of the major Alport locus to the X chromosome (18), the discovery of a type IV collagen gene (COL4A5) on the X chromosome (19), and finally the description of COL4A5 mutations in families with X-linked Alport syndrome (20).

Genetics

There are three genetic forms of Alport syndrome. X-linked Alport syndrome (XLAS) is caused by mutations in the COL4A5 gene and is the predominant form of the disease, accounting for ~80% of patients. Affected males are hemizygotes carrying a single mutant COL4A5 allele. Affected females carry a normal COL4A5 allele as well as a mutant allele and are therefore heterozygotes. About 15% of patients with Alport syndrome have the recessive form of the disease (ARAS), due to mutations in both alleles of the COL4A3 or COL4A4 gene. These patients are either homozygotes who have the identical mutation in both alleles of the affected gene (and who may have consanguineous parents) or compound heterozygotes who have inherited different mutations in the affected gene from their parents. About 5% of patients have autosomal dominant Alport syndrome (ADAS) caused by heterozygous mutations in COL4A3 or COL4A4. Most individuals with heterozygous COL4A3 or COL4A4 mutations are asymptomatic or exhibit isolated, nonprogressive microscopic hematuria associated with thin glomerular basement membranes (thin basement membrane nephropathy, or TBMN). For reasons that are as yet uncertain, some people with heterozygous mutations in COL4A3 or COL4A4 have a progressive course leading to chronic renal failure or end-stage renal disease, and are thus considered to have ADAS.

Several hundred different mutations in the COL4A5 gene have been identified in patients and families with XLAS (21–23). While the great majority of these mutations are unique, a small group of missense mutations accounts for a large portion of XLAS patients in the U.S. (24). Reported mutations include large rearrangements (~20%), small deletions and insertions (~20%), missense mutations that alter a glycine residue in the collagenous domain of the α 5(IV) chains (~30%), other missense mutations (~8%), nonsense mutations (~5%) and splice-site mutations (~15%) (21–23). The COL4A5 genotype has a powerful effect on the course of XLAS in affected males (22, 23). Large deletions, nonsense mutations and small mutations that alter the translational reading frame are associated with a 90% probability of progression to ESRD by age 30 (22, 23). This risk is 70% in patients with a splice-site mutation and 50% in those with a missense mutation (22, 23). The position of a glycine substitution may also affect the XLAS phenotype (23). These genotype-phenotype correlations are not apparent in females with XLAS, perhaps as a result of the overwhelming influence of random X-chromosome inactivation on disease course in XLAS females (25).

A similar variety of mutation types has been described in patients and families with ARAS (21, 26–30). Because of small patient numbers, genotype-phenotype correlations in ARAS have not been described.

Clinical Features

Gender and genotype are the major determinants of the severity of renal, cochlear and ocular disease in Alport syndrome. Males with XLAS, and patients of either gender with ARAS, inevitably progress to end-stage renal disease (ESRD) and the majority develop sensorineural deafness. The pacing of these events is influenced by the nature of the underlying disease mutation. While the majority of women with XLAS have mild disease manifestations, ESRD and severe deafness develop in a significant minority. Patients with ADAS exhibit relatively slow progression of renal and cochlear dysfunction, and ocular findings are much less common than in XLAS and ARAS (31, 32).

Renal. Persistent microscopic hematuria is the cardinal clinical feature of Alport syndrome, occurring in 100% of males with XLAS, 95% of females with XLAS

and in all patients with ARAS (22, 25). Hematuria is likely present from infancy in XLAS males and in patients with ARAS. Episodic gross hematuria is not unusual, especially during childhood (33). Some children with Alport syndrome have virtually constant gross hematuria.

Overt proteinuria typically appears during later childhood or adolescence in XLAS males and in ARAS patients and increases progressively, often into the nephrotic range (33–35). About 75% of XLAS females ultimately develop proteinuria of some degree (25). Most children with Alport syndrome have normal blood pressures, but hypertension is common in adolescent males with XLAS and teen-aged ARAS patients.

In XLAS males, the probability of ESRD is 50% by age 25, 80% by age 40 and 100% by age 60 (22). COL4A5 genotype has a powerful effect on rate of progression to ESRD in XLAS males. Large deletions and nonsense mutations confer a 90% probability of ESRD before age 30, compared to a 70% risk with splice site mutations and a 50% risk with missense mutations (22).

Women with XLAS exhibit a lower but substantial risk of progression to ESRD than XLAS males. According to one study of a large cohort of XLAS females, the risk of ESRD is 12% by age 45, 30% by age 60 and 40% by age 80 (25). Proteinuria and sensorineural deafness are risk factors for ESRD in XLAS females (36). COL4A5 genotype does not have measurable effects on the rate of progression to ESRD in XLAS females, perhaps due to the overwhelming influence of X-inactivation balance (25). The epidemiology of ESRD in ARAS is probably similar to XLAS males, although comparable data is lacking. It takes ~50 years for 50% of ADAS patients to develop ESRD, twice as long as XLAS males (31).

Cochlear. Hearing is normal at birth and during early childhood. Symmetrical deficits in sensitivity for high frequency sounds often become detectable by audiometry in late childhood. In XLAS males the probability of hearing loss is 50% by age 15, 75% by age 25 and 90% by age 40 (22). COL4A5 genotype influences the probability of hearing loss, with 90% of those with deletions, nonsense mutations and splice site mutations exhibiting deafness before age 30, compared to 60% of those with missense mutations (22). In XLAS females the probability of hearing loss is 10% by age 40 and 20% by age 60 (25). The majority of patients with ARAS develop deafness, although precise data on timing is not available.

Over time, the hearing deficit progresses into the frequency range of conversational speech. Because the deficit typically does not exceed 60–70 dB and speech discrimination is preserved, hearing aids are effective in most affected individuals.

Ocular. Anomalies of the lens, retina and cornea are common in Alport syndrome, especially among males with XLAS and patients with ARAS, often becoming apparent during adolescence and young adulthood (37). About 15% of XLAS males exhibit anterior lenticonus, in which the central portion of the lens protrudes into the anterior chamber (22). While this lesion is often asymptomatic, it may be associated with reduced visual acuity, and cataracts and even rupture of the lens may occur. Alteration of retinal pigmentation, consisting of whitish-yellowish perimacular flecks, occurs in about 15% of XLAS males (22), often in association with lenticonus. Corneal abnormalities include recurrent corneal erosions (38, 39) and posterior polymorphous dystrophy (40).

Other. The association of XLAS with smooth muscle tumors (leiomyomas) of the esophagus, tracheobronchial tree and, in women, the external genitalia, has been described in several dozen families (41–43). Symptoms such as dysphagia, postprandial vomiting, epigastric or retrosternal pain, recurrent bronchitis, dyspnea, cough, and stridor often appear in late childhood. The Alport syndrome-diffuse leiomyomatosis complex arises from X-chromosomal deletions involving COL4A5 and the proximal portion of the adjacent COL4A6 gene (44).

Mental retardation, midface hypoplasia, and elliptocytosis have been described in a small number of XLAS males who carry deletions that extend downstream of the 3′ end of the COL4A5 gene (45).

Pathology

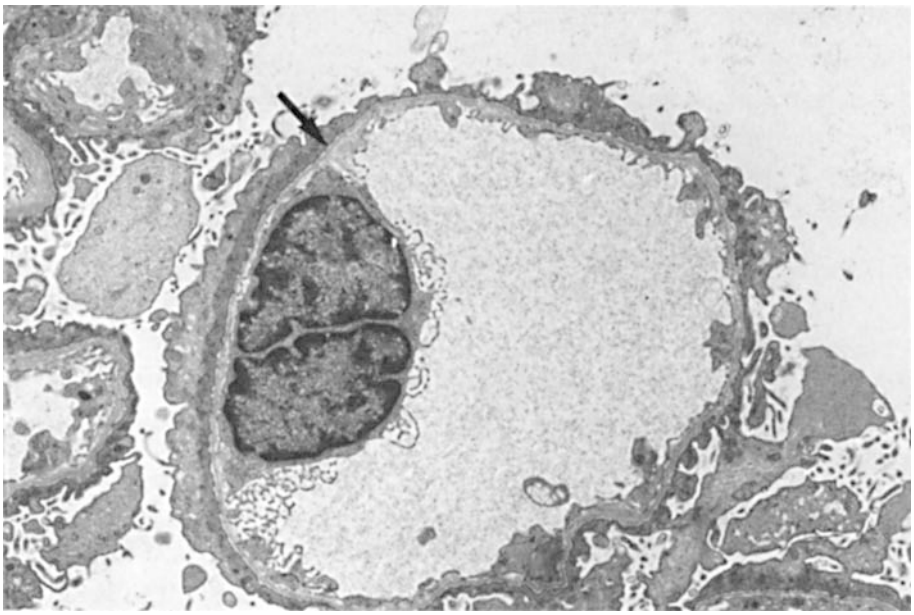
The clinical features of Alport syndrome originate in changes in basement membrane structure and function initiated by absence or abnormalities of α3α4α5(IV) and α5α5α6(IV) networks.

Renal. Light microscopic abnormalities are unusual in children with Alport syndrome who are less than 5 years of age. Mesangial hypercellularity and matrix expansion, and eventually focal segmental glomerulosclerosis, are common in older children and adolescents, especially boys. Tubular atrophy and interstitial fibrosis develop progressively after age 10 in boys with Alport syndrome (46).

Electron microscopy may reveal pathognomonic changes, depending on the patient's age and gender. The earliest abnormality is diffuse attenuation of the GBM; consequently, differentiation of Alport syndrome from thin basement membrane nephropathy by routine renal biopsy processing may be difficult in children (*Fig. 26-3*). To further complicate matters, some families with Alport syndrome due to COL4A5 mutations, GBM attenuation is the only ultrastructural abnormality. However, the great majority of boys with XLAS and both boys and girls with

Figure 26-3

Electron micrograph. Lead citrate and uranyl acetate stain (×4,800). Alport syndrome. Thin and regular GBM (*arrow*) with attenuated lamina densa. Epithelial foot processes are extensively fused.



ARAS develop the classic Alport GBM lesion, consisting of diffuse thickening accompanied by "basket-weave" transformation of the lamina densa, intramembranous vesicles and densities, scalloping of the epithelial surface of the GBM and foot process effacement (*Fig. 26-4*). The percentage of GBM displaying this lesion increases progressively with age in boys with XLAS (34). Females with XLAS display a range of GBM alteration, from focal GBM attenuation to diffuse thickening and basket-weaving, and there is no consistent correlation of GBM findings and age (34).

Routine immunofluorescence is normal or shows nonspecific immunoprotein deposition. Because disease-causing mutations result in abnormal expression of α3α4α5(IV) and α5α5α6(IV) networks in basement membranes in most Alport patients, specific immunostaining for type IV collagen α chains is useful for both diagnosis and differentiation of XLAS and ARAS (47) (*Figs. 26-5* and *26-6*). Expression of the α3(IV), α4 (IV) and α5(IV) chains is completely absent in ~80% of XLAS males, and 60–70% of females with XLAS exhibit mosaic expression of these chains (*Fig. 26-5*). In most patient with ARAS, GBM is nonreactive with antibodies to the α3(IV), α4(IV) and α5(IV) chains, and Bowman's capsules and tubular basement membranes are also negative for the α3(IV) and α4(IV) chains (48) (*Fig. 26-6*). However, immunostaining for α5(IV) chains in Bowman's capsules and tubular basement membranes is positive, because in these basement membranes expression of α5α5α6(IV) networks is preserved. It is important to note that altered immunostaining for the α3(IV)-α6(IV) chains in patients with XLAS and ARAS is not age-dependent. Therefore, this method can provide diagnostic information even in patients who are too young to display characteristic abnormalities in GBM ultrastructure.

Normal epidermal basement membranes (EBM) express the α5α5α6(IV) network but not the α3α4α5(IV) network. Epidermal basement membranes show negative staining for α5(IV) chains in about 80% of XLAS males, and mosaic expression of α5(IV) is observed in 60–70% of XLAS females, allowing diagnosis of XLAS by skin biopsy (49, 50) (*Fig. 26-5*). Skin biopsy is not useful for diagnosis of ARAS, since expression of α5(IV) in epidermal basement membranes is normal (48) (*Fig. 26-6*).

Cochlear. The hearing loss of Alport syndrome arises from cochlear dysfunction (51). Normal cochleae in mice, dogs and humans express type IV collagen α3, α4, and α5 chains in the spiral limbus, spiral ligament and in the basement membrane interposed between the organ of Corti and the basilar membrane (52–55). However, expression of these chains is absent in cochleae of ARAS mice (52), XLAS dogs (53) and men with XLAS (55). Careful examination of well-preserved cochleae from men with XLAS and deafness revealed a zone of separation

Figure 26-4

Electron micrograph. Lead citrate and uranyl acetate stain (×11,250). Alport syndrome. Thickened GBM showing splitting of the lamina densa and presence of granulations.

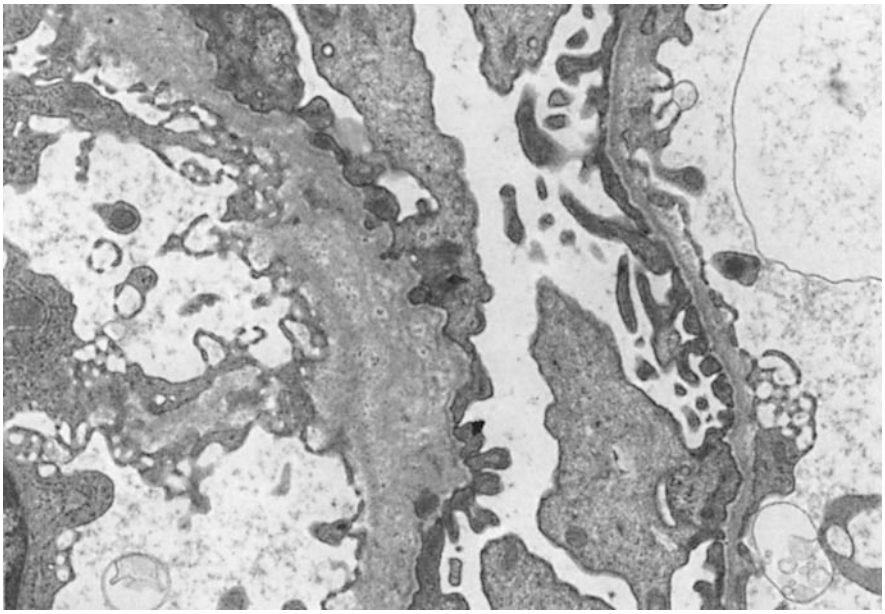
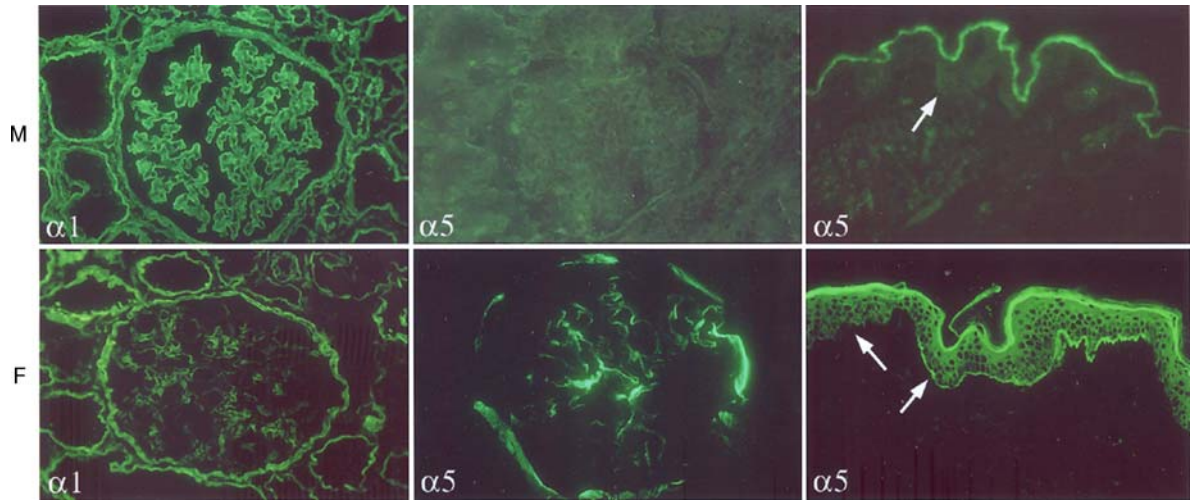


Figure 26-5

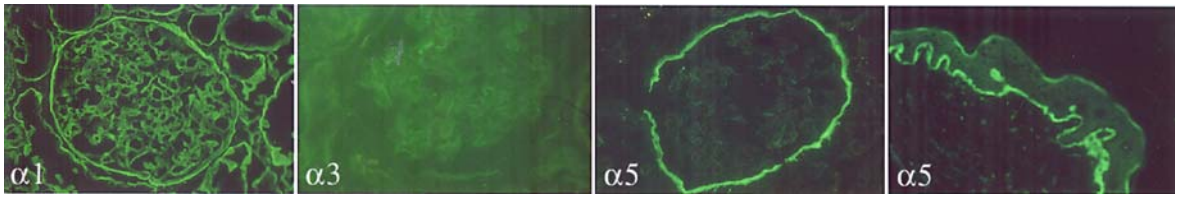
Immunofluorescene microscopy. Distribution of the α(IV) chains in renal and epidermal basement membranes of male (A-C) and female (D-F) patients affected with X-lined Alport syndrome. C: absence of epidermal basement membrane labeling (*arrow*). F: Distcontinuous epidermal basement membane labeling (*arrows*). (See color plate 9)



between the organ of Corti and the underlying basilar membrane, and cellular infiltration of the tunnel of Corti and the spaces of Nuel (56). These changes are not observed in similarly well-preserved cochleae obtained from normal individuals or patients with other causes of deafness. The structural changes observed in Alport cochleae may be associated with defective attuning of basilar membrane motion and hair cell stimulation, resulting in reduced

Figure 26-6

Immunofluorescene microscopy. Distribution of the α(IV) chains in renal and epidermal basement membranes of patients affected with autosomal recessive Alport syndrome. (See color plate 10)



acuity of hearing. Similar changes have not been observed in cochleae from mice or dogs with Alport syndrome, although deafness in these models is minimal or absent.

Ocular. The α3, α4, and α5(IV) chains are normal components of several basement membranes in the eye, including the corneal basement membrane, Descemet's membrane, lens capsule, the internal limiting membrane of the retina, and the retinal pigment epithelium basement membrane (54, 57–59). The ocular manifestations of Alport syndrome likely arise from absence or abnormality of α3α4α5(IV) networks in these basement membranes. The lens capsules of Alport patients with anterior lenticonus exhibit marked attenuation and focal areas of dehiscence, suggesting that the lens capsule lacks the mechanical strength to maintain normal lens shape (58, 60, 61).

Diagnostic Considerations

Accurate diagnosis of Alport syndrome and differentiation of this condition from other causes of familial and sporadic glomerular hematuria are based upon careful clinical evaluation, reliable pedigree data and thoughtful consideration of the relative merits of skin biopsy, kidney biopsy and molecular analysis.

In a child with isolated hematuria, a positive family history of hematuria in the absence of a history of ESRD suggests a diagnosis of thin basement membrane nephropathy (TBMN). Two rare causes of familial hematuria associated with macrothrombocytopenia, Epstein and Fechtner syndromes, can be excluded if the platelet count is normal. Familial IgA nephropathy (62) and membranoproliferative glomerulonephritis (63) are uncommon causes of familial hematuria.

In the absence of a family history of hematuria, the differential diagnosis of glomerular hematuria includes Alport syndrome, TBMN, IgA nephropathy, membranoproliferative glomerulonephritis, membranous nephropathy, lupus nephritis, postinfectious glomerulonephritis and Henoch-Schonlein nephritis. Associated clinical findings (e.g., rash, arthritis) or laboratory findings (e.g., hypocomplementemia) will suggest diagnoses other than Alport syndrome in many of these patients.

While results of hearing evaluation are likely to be normal in young children with Alport syndrome, audiometry may be very useful in children over 6–8 years of age. Ophthalmologic assessment may also provide valuable information, although ocular lesions are more prevalent in Alport patients with advanced disease, and less likely to be present in the population of young patients in whom differential diagnosis of hematuria may be more difficult.

Tissue studies can complement clinical and pedigree information that is insufficient to clearly differentiate Alport syndrome from other diagnoses. Skin biopsy with immunostaining for the α5(IV) chain, as described above, may be diagnostic, especially when clinical and pedigree data strongly suggest a diagnosis of XLAS. Normal expression of the α5(IV) chain in epidermal basement membrane can be explained in several ways: (1) the patient has XLAS, but his or her COL4A5 mutation does not abolish α5(IV) expression; (2) the patient has a form of Alport syndrome (ARAS or ADAS) in which expression of α5(IV) in epidermal basement membranes is not affected; or (3) the patient does not have Alport syndrome. Whereas skin biopsy is useful only if it provides definitive confirmation of a diagnosis of XLAS, renal biopsy carries the advantage of enabling the diagnosis of XLAS, ARAS and non-Alport kidney disease, particularly if type IV collagen immunostaining is applied.

Mutation detection rates of 80–90% are attainable in males with XLAS using direct sequencing of COL4A5 (64). Comparable data for detection of COL4A3 and COL4A4 mutations in patients with ARAS are lacking. COL4A5 mutation analysis has been commercially available in Europe for some time, and has recently become available in the United States. Laboratories providing type IV collagen gene sequencing can be found through the GeneReviews website (www.genereviews.org) and through the website of the Alport Syndrome Foundation (www.alportsyndrome.org).

Treatment

There have been no controlled therapeutic trials in human Alport syndrome. Consequently, treatment recommendations must be derived from animal studies and anecdotal reports. In murine ARAS several interventions have proven efficacious, including angiotensin antagonism (65–67), TGFb-1 inhibition (68), chemokine receptor 1 suppression (69), administration of bone morphogenic protein-7 (70), blockade of matrix metalloproteinases (71) and bone marrow transplantation (72, 73). Inhibition of angiotensin converting enzyme (ACE) treatment resulted in prolongation of survival in dogs with XLAS (74). In uncontrolled studies of human Alport subjects ACE inhibition reduced proteinuria, at least transiently (75, 76).

Cyclosporine treatment also resulted in prolongation of survival in male XLAS dogs (77). Cyclosporine treatment diminished proteinuria and appeared to stabilize renal function in a small, uncontrolled study of Alport males (78). However, apparent acceleration of renal fibrosis was suggested by the results of another study of cyclosporine treatment in Alport patients (79).

At the present time, angiotensin antagonism aimed at suppression of proteinuria appears to be the least risky of available treatment options. There is as yet no evidence that initiation of angiotensin blockade can delay the onset of overt proteinuria in Alport patients who have isolated hematuria. With advancing disease, management of hypertension and other complications of nephrotic syndrome and renal insufficiency is required.

Renal Replacement Therapy

Patients with Alport syndrome typically have excellent outcomes following renal transplantation (80). Two issues require the special attention of transplant physicians involved in the care of Alport patients. First, evaluation of potential related donors must identify affected individuals who may be at risk for development of significant renal insufficiency. Second, posttransplant monitoring must allow early diagnosis of posttransplant anti-GBM nephritis, a complication of transplantation that is unique to Alport syndrome.

Familiarity with the genetics of Alport syndrome and the signs and symptoms of the disease is required for informed donor evaluation. Since 100% of males with XLAS have hematuria (22), the absence of hematuria excludes Alport syndrome in male relatives of XLAS patients. About 95% of females with XLAS have hematuria (25), so there is only a 5% chance that a female without hematuria is affected. Given that by age 60 there is an estimated risk of ESRD of 30% in women with XLAS (25), female members of XLAS families who have hematuria should generally be discouraged from kidney donation.

Anti-GBM nephritis occurs in ~3% of transplanted Alport males (80). The onset of anti-GBM nephritis typically occurs during the first year after transplantation, and usually results in irreversible graft failure within weeks to months of diagnosis. There is a high rate of recurrence in subsequent allografts. In XLAS males the primary target of anti-GBM antibodies is the α5(IV) chain (81, 82). Females with XLAS who require transplantation are at little or no risk of developing anti-GBM nephritis. However, both males and females with ARAS can develop anti-GBM nephritis after transplantation. The α3(IV) chain is the primary target of anti-GBM antibodies in ARAS patients (81, 83). Goodpasture auto-antibodies and anti-GBM antibodies from transplanted Alport patients target distinct epitopes on the carboxyterminal noncollagenous (NC1) domain of the α3(IV) chain (84).

Renal allograft biopsy with routine immunofluorescence should be performed early in the evaluation of Alport patients who develop hematuria or increased creatinine after transplantation, or if circulating anti-GBM antibodies are detected. Anti-GBM nephritis after renal transplantation should be treated with cytotoxic therapy and plasmapheresis, although such therapy has been unsuccessful in the majority of reported patients.

Thin Basement Membrane Nephropathy

Historically, families displaying autosomal dominant transmission of isolated, nonprogressive hematuria were classified as having "benign familial hematuria" (85–87). Affected patients typically exhibited no renal parenchymal abnormalities apart from thinning of glomerular basement membranes (GBM) observed by electron microscopy (88–93). The more inclusive term "thin basement membrane nephropathy" (TBMN) has gradually displaced benign familial hematuria as the preferred designation for hematuria associated with thin GBM, because it encompasses sporadic cases of hematuria associated with thin GBM; familial or sporadic cases of thin GBM in which hematuria is accompanied by proteinuria, hypertension and/or renal insufficiency; and benign familial hematuria.

Thinning of GBM is a pathological description rather than a distinct entity. Depending on the timing of renal biopsy, GBM attenuation may be observed in patients with hemizygous or heterozygous mutations in COL4A5

(X-linked Alport syndrome, or XLAS), biallelic mutations in COL4A3 or COL4A4 (autosomal recessive Alport syndrome, or ARAS), heterozygous mutations in COL4A3 or COL4A4 (the carrier state of ARAS) and mutations at nontype IV collagen loci (94). The natural history of hematuria associated with thin GBM is determined by the underlying mutation, perhaps in combination with remote modifier genes. Hemizygous mutations in COL4A5 and biallelic mutations in COL4A3 and COL4A4 result in progressive GBM thickening, proteinuria and renal failure. Heterozygous mutations in COL4A3 or COL4A4 are usually associated with persistent GBM attenuation, isolated hematuria and benign outcomes. Heterozygous mutations in COL4A5, in women with XLAS, are associated with a wide range of prognostic outcomes, as described in the section on Alport syndrome. Perhaps this spectrum of outcomes reflects differences in the cellular responses provoked by complete absence of α3α4α5(IV) networks from GBM (hemizygous XLAS and ARAS), mixed α3α4α5(IV)-positive and α3α4α5(IV)-negative GBM (heterozygous XLAS) and homogeneous reduction in GBM content of α3α4α5(IV) networks (heterozygous COL4A3 or COL4A4 mutations).

Clinical Features

Persistent microscopic hematuria, associated with normal blood pressure, renal function and urine protein excretion, is the characteristic feature of TBMN in childhood (2, 3). TBMN is not specifically associated with hearing loss, ocular defects or other extrarenal abnormalities.

Proteinuria has been reported in up to 30% of adults with thin GBM (92, 95–99). About 5–7% of adult patients with TBMN have elevated serum creatinine levels (95, 98, 99).

Pathology

Diffuse attenuation of the lamina densa and GBM, with preservation of normal podocyte anatomy, is the characteristic histological abnormality in TBMN. Glomerular obsolescence or sclerosis may be observed in adult patients with TBMN, including some with heterozygous COL4A3 or COL4A4 mutations (98, 100).

GBM width is dependent on age and gender. The lamina densa and GBM increase rapidly in width between birth and 2 years of age, followed by a more gradual increase during childhood and adolescence (101). Adult men exhibit greater GBM widths than adult women (102).

Because different investigators have used different techniques to measure GBM width, a standard definition of "thin" GBM does not exist. In children the threshold is 200–250 nm (1, 3, 103), while the adult threshold ranges from 250 to 330 nm (92, 104).

Routine immunofluorescence studies of renal biopsy material from patients with TBMN are typically unremarkable. Immunostaining using specific antibodies against α3(IV), α4(IV), and α5(IV) chains yields normal results in patients with TBMN (88, 89, 105).

Diagnostic Considerations

IgA nephropathy, TBMN and Alport syndrome together account for the majority of children with glomerular hematuria seen in pediatric nephrology clinics (1–4). Thorough clinical evaluation, including detailed pedigree analysis, can assist in determining which children need tissue studies and which can be followed prospectively without biopsy. Obtaining urinalyses on first-degree family members may provide valuable information, since adults with familial hematuria may be unaware that they are affected (106).

When a child has isolated microscopic hematuria, a family history of dominantly transmitted hematuria and a negative family history for renal failure, a clinical diagnosis of TBMN is reasonable, and renal biopsy is unnecessary. These children should be followed prospectively every 1–2 years. If proteinuria or hypertension develops, renal biopsy should be considered.

In children who have persistent microscopic hematuria but do not have affected relatives, renal biopsy is often informative. In those children whose renal biopsy findings are limited to GBM attenuation, the clinician's challenge is to differentiate TBMN and Alport syndrome. Audiometry and ophthalmologic examination may be helpful in older children but these studies will usually be normal in young children with Alport syndrome. Abnormal results of immunostaining for the α3(IV), α4(IV) and α5(IV) chains suggests a diagnosis of Alport syndrome. While normal immunostaining results cannot entirely exclude Alport syndrome, they can help support a suspected diagnosis of TBMN.

The role of molecular analysis of the COL4A3, COL4A4 and COL4A5 genes in patients with suspected TBMN remains to be determined. Since the finding of a heterozygous mutation in COL4A3 or COL4A4 cannot guarantee a benign prognosis (100, 107–109), the need for follow-up examination of such patients would not be precluded.

Treatment

Since TBMN usually has a benign outcome, treatment is rarely indicated. Patients with TBMN who have proteinuria are theoretically candidates for angiotensin blockade.

Hereditary Angiopathy with Nephropathy, Aneurysms and Cramps (HANAC Syndrome)

Mutations in the COL4A1 and COL4A2 genes have not been found in patients with Alport syndrome or TBMN (110). Recently, missense mutations in the COL4A1 gene were described in three families displaying an autosomal dominant hereditary angiopathy associated with nephropathy, aneurysms and muscle cramps (HANAC syndrome) (111). The mutations affect three highly conserved glycine residues in the collagenous domain of the α1(IV) chain. Retinal arteriolar tortuosity and retinal hemorrhages were common to affected individuals in all three families, as were intracranial aneurysms, leukoencephalopathy and elevated creatine kinase levels. In two families, affected individuals had muscle cramps. Renal findings in affected individuals included microscopic and gross hematuria in one family, mild renal insufficiency in two families and renal cysts in all three families.

Renal biopsy in affected individuals with hematuria showed no abnormalities of GBM structure or type IV collagen expression. However, basement membranes of Bowman's capsules, tubules and interstitial capillaries exhibited irregular thickening, splitting into multiple layers and focal interruptions.

Laminin Disorders

Pierson Syndrome

The association of congenital nephrotic syndrome with eye abnormalities in siblings was first described by Pierson in 1963 (112). Affected infants died within the first year of life with ESRD. Subsequent reports of additional patients described variable abnormalities on fetal ultrasound, including kidney enlargement and hyperechogenicity, oligohydramnios, placental enlargement and/or pulmonary hypoplasia (113, 114). Infants surviving to term exhibited congenital nephrotic syndrome, with renal biopsy findings of mesangial sclerosis and diffuse GBM abnormalities, accompanied by ocular globe enlargement (buphthalmos) with reduction in the size and reactivity of pupils (microcoria). Additional ocular findings in some patients included cataract, posterior rupture of the lens capsule and retinal abnormalities. Some affected children were also found to have muscular hypotonia, central nervous system hemangioma and genital abnormalities (115, 116).

The gene for Pierson syndrome was mapped to chromosome 3p14-p22 and identified as LAMB2, the gene encoding the laminin β2 chain, by Zenker and colleagues in 2004 (113). Subsequent reports indicate that LAMB2 mutations may be associated with isolated congenital nephrotic syndrome and with mild variants of Pierson syndrome (117–121). The absence of the laminin β2 chain in transgenic mice results in massive proteinuria along with retinal and neuromuscular abnormalities (122). In these mice, proteinuria precedes the appearance of podocyte abnormalities, suggesting that the GBM contributes to the barrier function of the glomerular capillary wall.

Type III Collagen Nephropathies

Nail-Patella Syndrome (Hereditary Osteo-Onychodysplasia)

Nail-patella syndrome (NPS) is a rare autosomal dominant disorder characterized by dystrophic nails, hypoplasia or absence of the patellae, dysplasia of the elbows and iliac horns, and renal disease (123, 124). Affected individuals may also exhibit glaucoma and sensorineural deafness (125, 126). The disorder affects about 1 in 50,000 individuals. The severity of renal involvement, which occurs in 30–40% of patients, determines prognosis.

Genetics

Targeted disruption of the LIM homeodomain transcription factor gene Lmx1b in mice resulted in hypoplastic nails and absent patellae as well as renal disease, suggesting a possible locus for human NPS (127). LMX1B, the human homologue of Lmx1b, and the NPS locus were found to map to chromosome 9q34, and heterozygous mutations in LMX1B were identified in NPS patients (128, 129). A variety of LMX1B gene defects have been identified in NPS patients, including missense, splicing, insertion/deletion and nonsense mutations (128–134). The results of *in vitro* experiments in which the transcriptional effects of mixing wild-type and mutant LMX1B protein are measured, along with the variety of observed mutation types, suggest that the NPS phenotype results from haploinsufficiency rather than a dominant-negative mechanism (130, 135). Mutations in the homeodomain of LMX1B protein and female gender may confer an increased risk of renal involvement (126).

LMX1B protein is specifically expressed in glomerular podocytes, beginning at the S-shaped body stage of glomerular development (127). Lmx1b-null mice exhibit marked reduction in GBM expression of the α3 and α4

chains of type IV collagen, and the slit diaphragm proteins podocin and CD2AP (136–138). However, changes in expression of these putative targets of LMX1B were not observed in kidneys of patients with NPS nephropathy (139), perhaps because patients with NPS are heterozygous for LMX1B mutations, leaving the mechanisms for the renal effects of LMX1B mutations uncertain.

Clinical Features

Renal. Less than half of NPS patients have clinical renal disease (140, 141). Symptoms include microscopic hematuria and mild proteinuria, and typically appear in adolescence or young adulthood. Occasional patients develop nephrotic syndrome and hypertension. The nephropathy is typically mild, with about a 10% risk of progression to ESRD. Marked differences in the severity of the nephropathy can be observed in related patients, suggesting the influence of superimposed factors.

Nails. Nail abnormalities occur in over 90% of patients and are usually apparent from birth (142). Fingernails, especially on the thumb and index finger, are more severely affected than toenails. The nails may be absent or dystrophic with discoloration, koilonychias, longitudinal ridges or triangular lunulae.

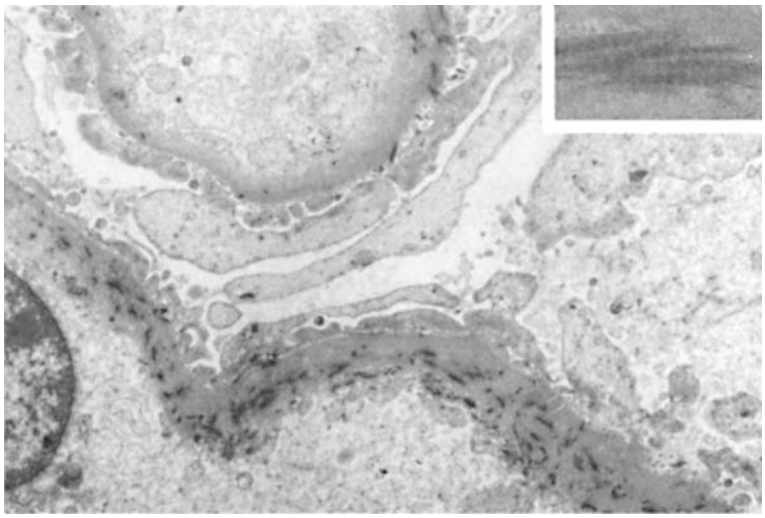
Skeletal defects. Over 90% of NPS patients exhibit aplasia or hypoplasia of the patellae (142). Associated symptoms may include knee pain, effusions, dislocations and osteoarthritis. Dysplasia of the elbows occurs in over 90% of patients (142). Anomalies such as hypoplasia of the radial head with dislocation, posterior processes at the distal ends of the humeri, hypoplasia of the olecranon and elongation of the neck of the radius may be associated with mild to severe limitation in extension, pronation and supination of the forearm. About 80% of patients display osseous processes projecting posteriorly from the iliac wings, known as iliac horns, which are pathognomonic for NPS (142).

Pathology

The nephropathy of NPS has no specific features by light microscopy or routine immunofluorescence. Focal segmental glomerulosclerosis and deposits of IgM and C3 may be observed, depending on the severity of renal involvement. Characteristic lesions are observed by electron microscopy (143, 144). With standard staining techniques the GBM and mesangium exhibit multiple lucencies, imparting a "moth-eaten" appearance. Staining with phosphotungstic acid reveals clusters of cross-banded collagen fibrils within these lucent areas (*Fig. 26-7*). These collagen fibrils have been observed in NPS patients with no clinically evident renal disease, and there is no correlation between the extent of GBM changes and patient age, severity of proteinuria or degree of renal functional impairment (130, 131). Staining of NPS kidneys with antibodies against type III collagen produced irregular, discontinuous labeling of GBM in normal-appearing glomeruli and focally intense staining of sclerotic glomeruli (139).

Figure 26-7

Electron microscopy. Phosphotungstic acidstain (×10,500). Nail patella syndrome. Irregular distribution of fibrillar collagen within the GBM. Inset shows the typical periodicity of interstitial collagen (×48,000).



Treatment

There is no specific therapy for NPS renal disease. Kidney transplantation has been carried out successfully, and recurrence of disease apparently did not recur. Selection of living donors must be performed with care, since NPS is an autosomal dominant disorder.

Collagen Type III Glomerulopathy

Renal disease associated with glomerular deposits of type III collagen has been described in patients who have no extrarenal abnormalities (145–148). In comparison to NPS patients, subjects with collagen type III glomerulopathy have relatively severe renal findings, more extensive histological changes and greater glomerular type III collagen accumulation.

Clinical Features

Patients with collagen type III glomerulopathy can present in childhood or in adulthood. Early presentation is associated with a more severe disease course. Proteinuria is common to the juvenile and adult forms of the disease. In children, proteinuria progresses to nephrotic syndrome and is accompanied by hypertension and the development of renal failure (145, 148). Microangiopathic hemolytic anemia has been described in some patients. Patients presenting in adulthood exhibit more gradual increase in proteinuria and loss of renal function (147).

Pathology

Light microscopy shows enlarged glomeruli with mesangial expansion and glomerular capillary wall thickening due to subendothelial accumulation of poorly staining material. Routine immunofluorescence studies are unremarkable or show nonspecific immunoprotein deposits. Electron microscopy shows deposits of electron-lucent material in mesangial matrix and GBM. Staining with phosphotungstic acid demonstrates fibrillar collagen in these deposits which is identified as type III collagen by specific immunostaining.

Hereditary Nephritis with Thrombocytopenia and Giant Platelets: Epstein and Fechtner Syndromes

Epstein and Fechtner syndromes are allelic, autosomal dominant disorders that have some features in common with Alport syndrome, such as hematuria, progressive nephropathy and sensorineural deafness (149, 150). However, these conditions are distinguished clinically from Alport syndrome by the invariable presence of thrombocytopenia and giant platelets. In addition, granulocytes of patients with Fechtner syndrome display cytoplasmic inclusions known as Dohle-like bodies, and these patients may develop cataracts. In some patients ultrastructural changes in GBM resemble those of Alport syndrome (151, 152). However, glomerular expression of type IV collagen α3, α4 and α5 chains is normal in these patients (152).

Despite the similarities with Alport syndrome, Epstein and Fechtner syndromes are genetically distinct disorders. Following the mapping of the Epstein and Fechtner loci to chromosome 2q11–13 (153, 154), heterozygous mutations in MYH9, the gene that encodes nonmuscle myosin heavy chain IIA (NMMHC-IIA), were identified in patients with these disorders, as well as in patients with two other conditions featuring giant platelets, Sebastian syndrome and May-Hegglin anomaly, and in nonsyndromic hereditary deafness (DFNA17) (155–158). NMMHC-IIA is expressed in podocytes (159), but the mechanism by which mutations in this protein might adversely affect podocyte function is unknown.

Hereditary Metabolic Disorders with Primary Glomerular Involvement

Fabry Disease

Anderson-Fabry disease is a rare X-linked disorder of glycosphingolipid metabolism resulting from deficiency of the lysosomal hydrolase a-galactosidase A. Clinical aspects of this disease are discussed in Chapter 51.

Renal tissue from all hemizygous patients demonstrates characteristic glycolipid accumulation within every glomerular, vascular and interstitial cell and within distal tubular cells, regardless of age at biopsy. Two intermingled cell populations, normal cells and cells exhibiting glycolipid accumulation, are observed in heterozygous females. Degenerative renal changes develop with age. They initially affect vessels and consist of round fibrinoid deposits resulting from smooth muscle cell necrosis. These changes are followed by nonspecific vascular, glomerular and tubulointerstitial lesions (160). Enzyme replacement appears to result in decreased glycolipid storage in renal cells and stabilization of renal function (161, 162).

Other Glomerular Lipidoses

Nephrosialidosis is a rare autosomal recessive condition caused by neuraminidase deficiency. Clinical and radiologic features include dysmorphic facies, visceromegaly, mental retardation, skeletal anomalies, marrow foam cells and cherry-red spot on fundoscopy. Renal involvement consists of proteinuria and progression to ESRD early in life (163). Renal biopsy findings include podocyte and proximal tubular cell vacuolization. By electron microscopy many of these vesicles appear empty, but others contain flocculent or membrane-like electron-dense material. Wheat germ agglutinin binds to cytoplasmic material in podocytes and tubular cells, indicating the presence of compounds with terminal sialic (neuraminic) acid moieties (164).

Silent accumulation of glycolipids or mucopolysaccharides has been described in patients with Gaucher disease, Niemann-Pick disease, I-cell disease and GM1 gangliosidosis (165). Clinical renal disease during childhood is unusual in patients with these disorders.

Hereditary Metabolic Disorders with Secondary Glomerular Involvement

Familial Amyloidosis

Hereditary amyloidosis encompasses a group of autosomal dominant disorders characterized by extracellular accumulation of protein fibrils arranged in an antiparallel β-pleated sheet configuration. These disorders are classified according to the protein composing amyloid fibrils and/or the type of mutation in the corresponding gene. Transthyretin variants have been found in most affected families, but variants of cystatin C, gelsolin, apoliprotein A1, fibrinogen and lysozyme have described in other kindreds (166). Symptomatic renal involvement is unusual during childhood.

Familial Mediterranean fever is an autosomal recessive disorder described primarily, but not exclusively, in several ethnic groups originating in the Mediterranean region. The disease is characterized by recurrent episodes of fever, abdominal pain, joint pain, pleuritis and pericarditis. Renal amyloidosis of the AA type may result in proteinuria and eventual renal failure. Renal amyloidosis of the AA type may be associated with the autosomal dominant disorders *Muckle-Well syndrome* and *tumor necrosis factor receptor-1 associated periodic syndrome (TRAPS)* (167).

Alpha-1 Antitrypsin Deficiency

Chronic liver disease due to deficiency of alpha-1-antitrypsin (α1AT) may be associated with glomerulonephritis in children (168–170). Renal biopsy reveals diffuse or focal segmental membranoproliferative glomerulonephritis, type I in most cases, associated with subendothelial deposits composed of immunoglobulin and complement. Renal manifestations include proteinuria, hypertension and renal failure. Regression of nephrotic syndrome and glomerular lesions after liver transplantation has been observed (171).

Alagille Syndrome

Alagille syndrome is an autosomal dominant disorder with variable penetrance that causes cholestasis in childhood, associated with characteristic facies, cardiac malformations, vertebral abnormalities, posterior embryotoxon, hypogonadism, growth retardation and high-pitched voice. The disease results from mutations in *Jagged1*, which encodes a ligand for the Notch1 receptor (172). Accumulation of lipid vacuoles in mesangial matrix, mesangial cells and GBM has been observed in some patients (173) (› *Fig. 26-8*). The extent of mesangiolipidosis correlates with the severity of cholestasis. Although the glomerular lesions are present early in life, renal symptoms in childhood are unusual. Progression to ESRD may occur in affected adults.

Hereditary Lecithin-Cholesterol Acyltransferase (LCAT) Deficiency

LCAT deficiency is a rare autosomal recessive disorder characterized by the inability to esterify plasma cholesterol, leading to deposition of unesterified cholesterol in tissues, including the kidney (174). Progression to ESRD usually occurs in the fourth or fifth decade. Glomerular lesions include accumulation of foam cells of endothelial and mesangial origin and massive accumulation of lipids within the mesangial matrix and subendothelial GBM (174).

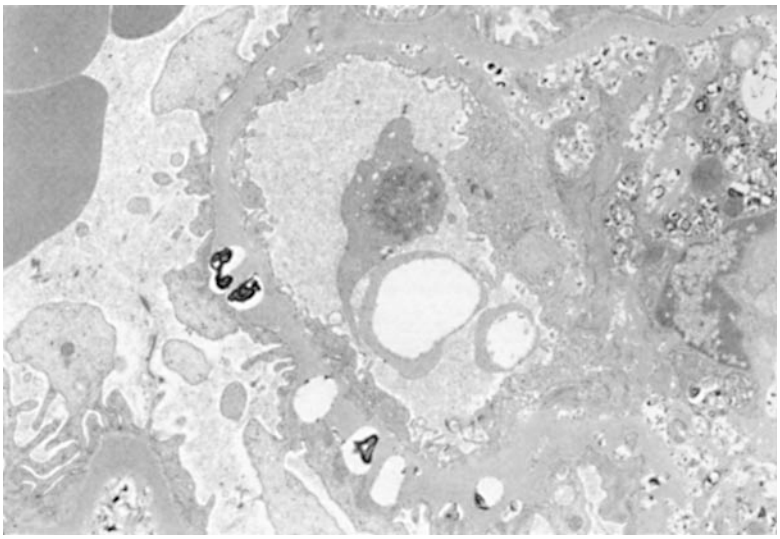
Lipoprotein Glomerulopathy

Lipoprotein glomerulopathy is characterized by intraglomerular lipoprotein thrombosis and high plasma concentrations of apolipoprotein E (175). The disease is usually detected in adults, but onset of symptoms during childhood has been described (176). Renal symptoms range from proteinuria to nephrotic syndrome, and progression to ESRD occurs in some patients. Recurrence of glomerular lesions after renal transplantation has been observed (177). Mutations in apolipoprotein E have been found in patients with this disorder (178).

Glomerular lipidosis has also been reported in patients with *familial hypercholesterolemia* resulting from defects in the LDL receptor, in *type III hyperlipoproteinemia*, and in patients with *cholesterolic polycoria*. Renal symptoms usually appear in adulthood in patients with these disorders.

Figure 26-8

Electron microscopy. Lead citrate and uranyl acetate stain (×8,600). Alagille syndrome. Massive accumulation of lipid vacuoles within mesangial cells and matrix. Irregular distribution of lipid vacuoles within the GBM.



Familial Juvenile Megaloblastic Anemia

Familial juvenile megaloblastic anemia (Imerslund-Grasbeck syndrome) is a rare autosomal recessive disorder caused by selective vitamin B12 malabsorption. Anemia is detected in infancy or early childhood and is associated with mild, nonprogressive proteinuria. Most cases arise from mutation in the gene for either cubilin (chromosome 10) or amnionless (chromosome 14), which are components of the intestinal receptor for the vitamin B12-intrinsic factor complex as well as the receptor that mediates proximal tubular reabsorption of filtered protein (179).

Other Hereditary Diseases with Glomerular Involvement

Charcot-Marie-Tooth (CMT) Disease

CMT disease is a genetically heterogeneous, familial peripheral neuropathy resulting in progressive symmetric atrophy and weakness of distal muscles and sensory deficits. Autosomal dominant and X-linked forms have been described, due to mutations in the myelin protein zero gene on chromosome 1, peripheral myelin protein 22 gene on chromosome 17, and the connexin 32 gene on the X chromosome (180). Proteinuria and progression to ESRD associated with focal glomerulosclerosis occur in some patients (181). Since some of these patients are also deaf, CMT disease could potentially be misdiagnosed as Alport syndrome. No specific ultrastructural changes in GBM have been described in patients with CMT disease.

Cockayne Syndrome

Cockayne syndrome is an autosomal recessive disorder characterized by growth retardation, neurologic abnormalities, premature aging, senile facies, sensorineural deafness, cataracts, retinopathy, sun sensitivity and dental caries (182). The disorder arises from mutations in genes involved in DNA nucleotide excision repair (183). Renal symptoms including hypertension, proteinuria and renal insufficiency occur in about 10% of patients, associated with diffuse, homogeneous GBM thickening (184).

Hereditary Acro-Osteolysis with Nephropathy

Hereditary acro-osteolysis is a rare disorder characterized by arthritic episodes and progressive resorption of carpal and tarsal bones. Familial (dominant or recessive) and sporadic cases have been reported. Hypertension, proteinuria and progressive renal failure occur in some patients (185). Renal biopsy findings include arteriolar thickening and sclerosis and focal glomerulosclerosis.

Other Syndromes with Renal Involvement

Renal abnormalities, typically cystic renal dysplasia and/or tubulointerstitial lesions, are found in most patients with *Bardet-Biedl syndrome (BBS)*, a genetically heterogeneous, autosomal recessive disorder whose cardinal features included obesity, polydactyly, mental retardation, retinal dystrophy and hypogonadism (186). Glomerular symptoms such as proteinuria and questionable glomerular changes have been described in a few patients (187). Mutations in several genes involved in the functioning of primary cilia have been linked to BBS (188).

Alstrom syndrome is an autosomal recessive disorder characterized by cone-rod dystrophy, obesity, progressive sensorineural deafness, dilated cardiomyopathy, insulin resistance syndrome and developmental delay. Renal tubular dysfunction and tubulointerstitial lesions have been observed in some patients (189). Renal disease due to vascular lesions and secondary glomerulosclerosis occurs in some patients with *familial dysautonomia.*

Hereditary Glomerulopathies Without Extrarenal Symptoms

Fibroncectin Glomerulopathy

Fibronectin glomerulopathy is associated with massive accumulation of fibronectin derived mainly from the soluble plasma isoform, transmitted as an autosomal dominant trait (190–192). A substantial fraction of cases arises from mutations in the gene FN1 (193), which is located on chromosome 2 and encodes fibronectin, but another locus appears to exist at chromosome 1q32 (194). Clinically, the disease is characterized by proteinuria of variable magnitude, typically first observed in early adulthood. Subsequently, patients develop hypertension and renal insufficiency, with gradual progression to ESRD.

Other Familial Glomerulopathies

Persistent proteinuria with the abrupt development of rapidly progressive renal failure, malignant hypertension and microangiopathic hemolytic anemia during the third or fourth decade of life, transmitted as an autosomal dominant trait, has been described in several families (195). Renal biopsy findings included diffuse vascular injury, ischemic glomerular changes, and glomerular deposits of IgM and C3. The cause(s) of this disorder is apparently unknown.

Mitochondrial Cytopathies

Mitochondrial cytopathies are a heterogeneous group of disorders resulting from genetic defects in enzymatic complexes of the respiratory chain, leading to impaired oxidative phosphorylation and energy production. The most common renal defect is the de Toni-Debre-Fanconi syndrome, associated with nonspecific abnormalities in tubules and intersititium on renal biopsy, but glomerular lesions such as focal segmental glomerulosclerosis, collapsing glomerulopathy and crescentic glomerulonephritis may also occur (196). Proteinuria has been found in patients with maternally inherited diabetes and/or deafness and in patients with complete or incomplete MELAS syndrome (*m*itochondrial *e*ncephalopathy with *l*actic *a*cidosis and *s*troke-like episodes).

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27 Idiopathic Nephrotic Syndrome: Genetic Aspects

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Introduction

Hereditary forms of nephrotic syndrome (NS) have been considered as infrequent disorders; however, 3–6% of the cases with NS have an affected sibling (1–3). Over the last decade, screening of large cohorts of pediatric patients presenting with steroid-resistant nephrotic syndrome (SRNS) for gene mutations has revealed the importance of genetic disorders in the pathogenesis of proteinuric glomerulopathies. At least 66% of the cases presenting with SRNS during the first year of life have an underlying genetic disease (4). In cases with infantile and juvenile SRNS, the overall proportion of genetic forms appears significantly lower, although the precise frequency remains unknown. Because autosomal recessive diseases may present as sporadic cases, the incidence of hereditary forms of NS is certainly underestimated. From a clinical perspective, most patients with hereditary SRNS will be resistant to immunosuppressive agents and do not experience relapse after transplantation (5–7).

Gene discovery efforts aimed at unraveling the causes of Mendelian forms of nephrotic syndrome have resulted in the identification of mutations in novel genes that encode proteins crucial for the establishment and maintenance of the glomerular filtration barrier. These discoveries have helped decipher the pathophysiologic mechanisms of the glomerular filtration process. Mutations in six genes have been implicated in different forms of non-syndromic SRNS (*Table 27-1*, *Fig. 27-1*). Mutations in *NPHS1*, encoding nephrin, are responsible for most of the cases with congenital nephrotic syndrome (CNS) and might be found in infantile forms of SRNS (8, 9). Mutations in the *NPHS2* gene, encoding podocin, are the most frequent cause of early-onset autosomal recessive SRNS (10), and account for 37.5% of the cases with NS presenting in the first year of life among European populations (4). Some dominant forms of juvenile and adult onset SRNS are due to mutations in *ACTN4* and *TRPC6* (11, 12), encoding the cytoskeletal protein α-actinin-4 and the transient receptor potential ion channel (TRPC) 6, respectively. More recently, mutations in *PLCE1*, which encodes for phospholipase C-epsilon-1, were found in patients with early-onset SRNS and diffuse mesangial sclerosis (DMS) (13).

Syndromic forms of SRNS are less common (*Table 27-1*, *Fig. 27-2*) and may be due to mutations in several genes with varied functions including transcription factors, mitochondrial and lysosomal proteins or constituents of the glomerular basement membrane (GBM). Frasier syndrome (14–16), Denys-Drash syndrome (17–20) and WAGR syndrome (21) are caused by mutations in *WT1*, which encodes for a transcription factor, the Wilms' tumor protein. Furthermore, isolated forms of SRNS may be due to mutations in *WT1* (22). Most cases with Pierson syndrome carry mutations in the *LAMB2* gene, encoding laminin β2, a main component of the GBM (23). Mutations in *LMXB1*, encoding the LIM homeobox transcription factor 1β, are associated with nail-patella syndrome (24). In addition, mutations in the *ITGB4* (epidermolysis bullosa) (25), *SMARCAL1* (Schimke syndrome) (26), *MTTL1* (MELAS syndrome) (27, 28) and *SCARB2* (action myoclonus-renal failure syndrome) genes (29) have been found in patients with diverse extrarenal manifestations associated with SRNS. Patients with primary coenzyme Q10 deficiency due to mutations in *COQ2* and *PDSS2* may develop nephrotic syndrome in addition to neuromuscular symptoms (30–32), although patients with isolated SRNS have been described as well.

This chapter will review the available epidemiologic data, genotype-phenotype correlations and the mechanisms by which mutations in genes implicated in hereditary forms of isolated SRNS lead to proteinuric glomerular disease. A genetic overview on recently discovered genes responsible for rarer cases of hereditary syndromic SRNS and advances in the study of familial Steroid Sensitive Nephrotic Syndrome (SSNS) are presented, as well.

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Table 27-1

Hereditary forms of steroid-resistant nephrotic syndrome

Gene	Locus	Inheritance	Protein	Disease
Non-syndromic forms of nephrotic syndrome				
NPHS1	19q13.1	AR	Nephrin	Congenital nephrotic syndrome of the Finnish type. Early-onset SRNS
NPHS2	1q25-31	AR	Podocin	Early and late onset autosomal recessive steroid-resistant nephrotic syndrome. Congenital nephrotic syndrome
PLCE1	10q23	AR	Phospholipase C epsilon 1	Early-onset SRNS with diffuse mesangial sclerosis and FSGS
CD2AP	6p12.3	AR	CD2 associated protein	Early-onset SRNS and FSGS
ACTN4	19q13	AD	α-actinin-4	Late-onset SRNS with incomplete penetrance and slow progression to ESRD
TRPC6	11q21-22	AD	Transient receptor potential ion channel 6	Adult-onset SRNS with FSGS
Unknown	2p12-13.2	AR	Unknown	Steroid-sensitive nephrotic syndrome
Syndromic forms of nephrotic syndrome				
WT1	11p13	AD	Wilms' tumor 1	Denys-Drash syndrome, Frasier syndrome, WAGR syndrome, isolated FSGS and DMS
LAMB2	3p21	AR	Laminin-β2	Pierson syndrome
SMARCAL1	2q35	AR	SW1/SNF2-related, matrix-associated, actin-dependent regulator of chromatin, subfamily a-like 1	Schimke immuno-osseus dysplasia
SCARB2	4q13-21	AR	Scavenger receptor 2 (LIMP-2)	Action myoclonus renal failure
LMX1B	9q34.1	AD	LIM-homeodomain transcription factor 1, beta	Nail-patella syndrome
COQ2	4q21-q22	AR	Parahydroxygenzoate-polyprenyltransferase enzyme	COQ10 deficiency, early-onset SRNS, with or without encephalomyopathy
PDSS2	6q21	AR	Decaprenyl diphosphate synthase-2	COQ10 deficiency, Leigh syndrome and SRNS
MTTL1	Mitochondrial		Mitochondrial tRNA for leucine (UUR)	MELAS syndrome. Mitochondrial diabetes, deafness and FSGS, with or without nephrotic syndrome
ITGB4	17q25.1	AR	Integrin-β4	Epidermolysis bullosa and FSGS
Unknown	14q24.2	AR	Unknown	SRNS and deafness
Unknown	11q24	AD	Unknown	SRNS and deafness
Unknown	Unknown	AR	Unknown	Galloway Mowat syndrome

AR autosomal recessive, *AD* autosomal dominant. In certain cases, mutations in *WT1*, *LAMB2*, *COQ2* and *PDSS2* can be associated with isolated SRNS

Isolated Steroid-Resistant Nephrotic Syndrome

Mutations in the *NPHS2* Gene Encoding Podocin

Gene Identification and Protein Characterization

In 1995, Fuchshuber et al. mapped a genetic locus on chromosome 1q25-31 (SRN1, MIM #600995) in a group of patients from Europe and Northern Africa who presented with childhood onset SRNS, autosomal recessive inheritance, renal histologic findings of Focal Segmental Glomerular Sclerosis (FSGS) and absence of extra-renal disorders (33). These patients rapidly progressed to end-stage renal disease but no recurrence occurred after renal transplantation. Boute et al. used a positional cloning approach thereafter and identified mutations in the *NPHS2* gene, encoding a novel protein podocin (10). Subsequent studies further defined the phenotype associated with mutations in the *NPHS2* gene, revealing that patients usually develop NS from birth to 6 years of age, do not respond to immunosuppressive agents and reach ESRD before the end of the first decade of life (4–6, 34). Histologic findings range from minimal glomerular changes, in patients biopsied early, to FSGS at later stages (10).

Mutations in the *NPHS2* gene are responsible for 39 to 48% of familial and for 10 to 28% of sporadic cases of SRNS (5, 6, 35–37). Interestingly, *NPHS2* mutations have also been identified in patients presenting with congenital onset of NS (4–6, 38). Hinkes et al. demonstrated that among central European patients with NS presenting in the first 3 months of life, podocin mutations comprise 51.4% of all mutations identified (4). In addition, linkage to the chr 1q25-31 locus and mutations in the *NPHS2* gene have been described in patients with late-onset FSGS; therein further broadening the spectrum of phenotypes attributable to podocin mutations (39, 40).

The *NPHS2* gene spans a 25 kb region, consists of eight exons, and encodes podocin, a predicted 42-kDa protein with 383 amino acid residues. Podocin is a lipid raft-associated protein bearing strong homology with stomatin, an integral membrane protein of human erythrocytes which regulates monovalent cation transport and acts as a cytoskeletal anchor (41). Stomatin is also expressed in vertebrate sensory neurons where it plays a role in mechanotransduction (42–44). Moreover, the podocin orthologue in *C. elegans* is the protein MEC-2 (10), which links a neuronal mechanosensory channel involved in touch with the microtubular and cytoskeletal framework and leads to the opening of ion channels (45). Podocin is predicted to be an integral membrane protein with a single transmembrane domain and intracellular NH2- and COOH-terminal ends; thus, forming a hairpin-like structure (10). Immunolocalization studies have localized podocin exclusively in kidney at the slit diaphragm of podocytes (46, 47).

Podocin Expression and Interactions

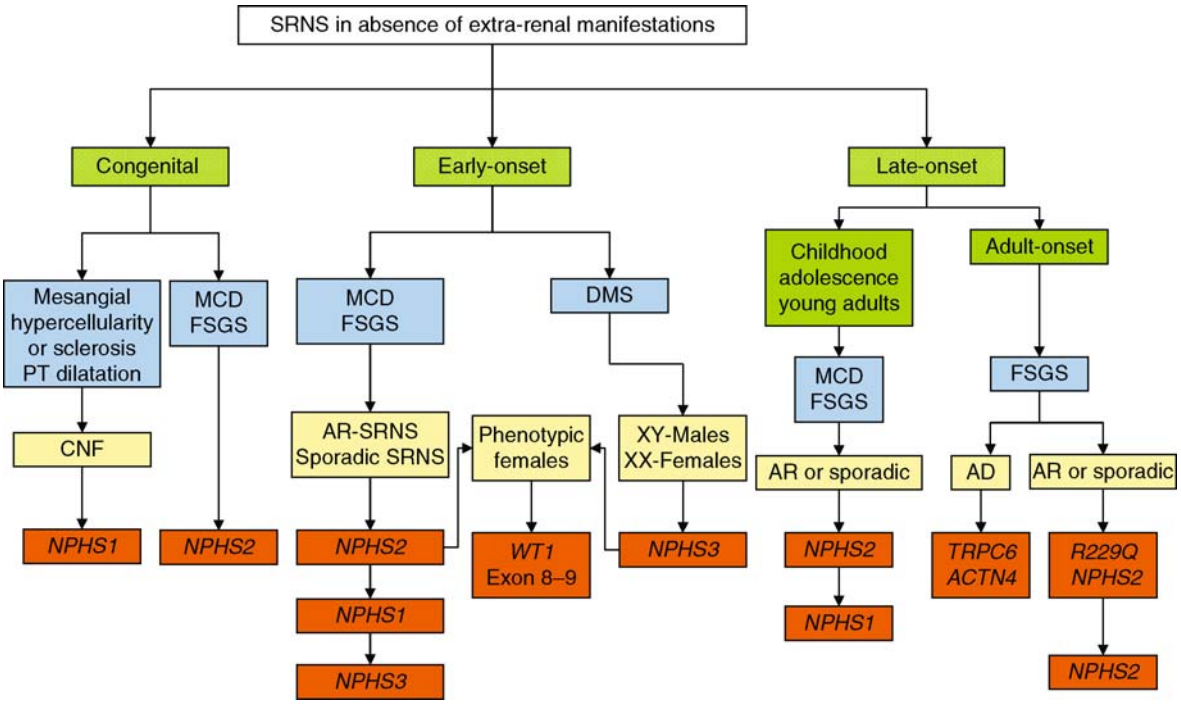
At the mRNA level, podocin is expressed as early as the S-shaped stage, concomitant with vascularization of the interior cleft of the developing nephron (10); whereas protein expression has been documented beginning at the later capillary stage (47). Podocin was demonstrated to accumulate in an oligomeric form in lipid rafts of the slit diaphragm, in complex with nephrin and CD2AP (46), suggesting a potential role of podocin as a scaffolding protein. Nephrin-induced signaling is greatly enhanced by podocin, which binds to the C-terminus of nephrin (48). Interactions of podocin with nephrin, NEPH1, CD2AP and TRPC6 are crucial for structural organization and regulation of filtration function of the slit diaphragm, mechanosensory signaling, podocyte survival, cell polarity and cytoskeletal organization (46, 49–53). In addition, podocin binds cholesterol and creates large protein-cholesterol supercomplexes in the slit diaphragm, thereby regulating the activity of associated TRPC ion channels (51, 52).

Allelic Variants and Genotype/Phenotype Correlations

Mutations in the *NPHS2* gene include a full spectrum of protein-truncating nonsense and frameshift mutations, splice-site variants and missense changes and involve all eight coding exons (6, 10, 35). To date, more than 90 pathogenic mutations and 25 variants of unknown significance have been reported. Mutations are frequently found in pediatric patients with SRNS originating from central Europe and North America, Turkey, Middle East, North Africa and South America (4, 6, 10, 35, 37, 38, 40, 54–61). However, *NPHS2* mutations are rarely detected in cases from Japan (62–65), China (66, 67), Korea and sub-Saharan Africa (68, 69). Several founder mutations have been identified, including p.R138Q in Europe (10, 35), p.R138X in Israeli-Arab population (55), p.V260E in the Comoros island and p.A284V in South America

Figure 27-1

Clinical and genetic approach in patients with non-syndromic SRNS. An exhaustive investigation of extra-renal manifestations must be performed during the first clinical evaluation and subsequent follow-up. In cases in which mutations in *NPHS1*, *NPHS2*, *NPHS3* and *WT1* have been excluded, mutational screening of *LAMB2*, *COQ2* and *PDSS2* might be performed. A first approach genetic screening is proposed according to the phenotype; a negative result implies additional genetic testing if applicable MCD: minimal change disease, FSGS: focal segmental glomerulosclerosis, DMS: diffuse mesangial sclerosis, PT: proximal tubular, AR: autosomal recessive and AD: autosomal dominant.



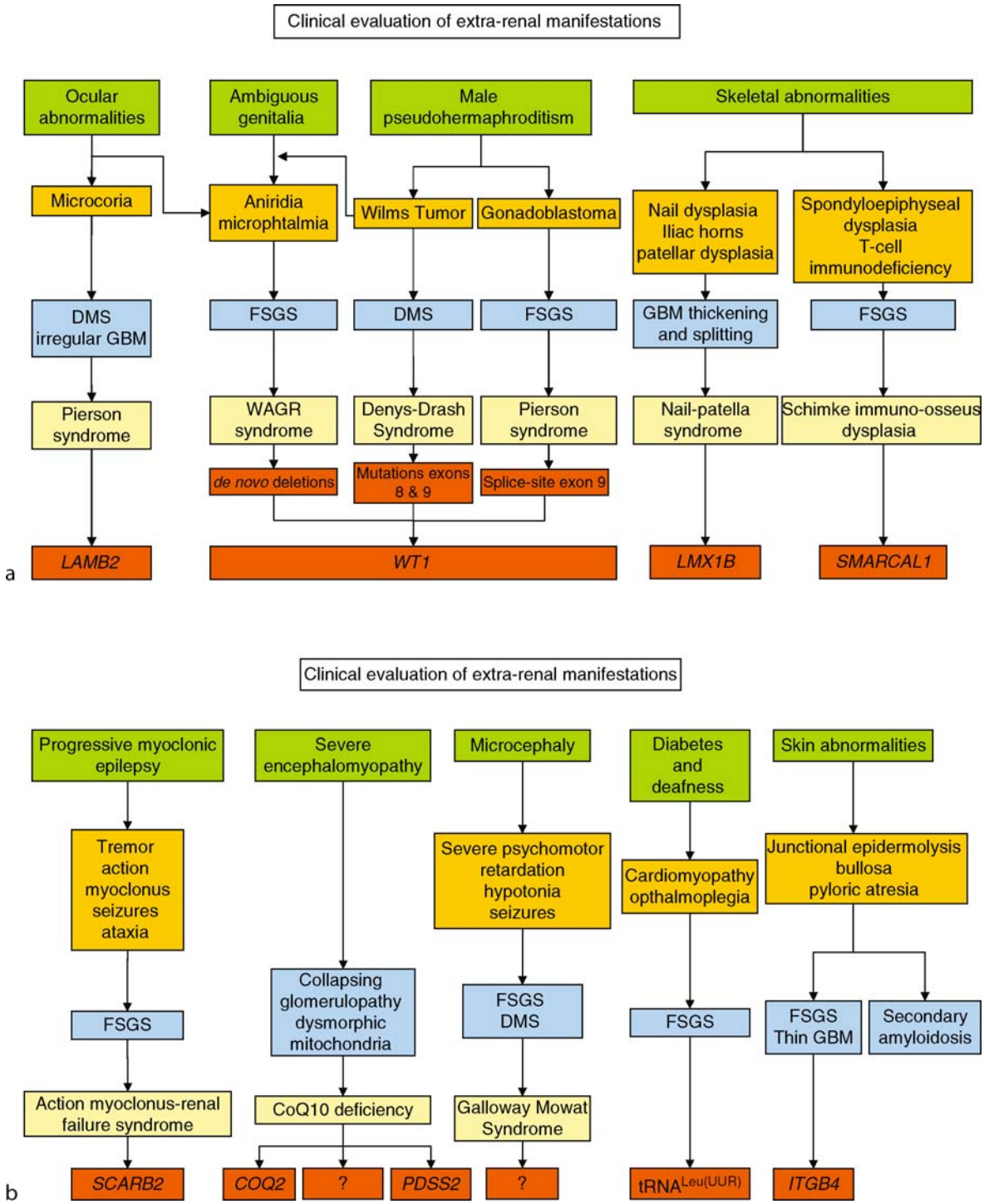
(Antignac, unpublished data). Considering the two largest cohorts comprising pediatric patients with SRNS (5, 37), the most common mutation is p.R138Q, which represents up to 32% of mutant alleles (5).

Individuals bearing pathogenic *NPHS2* mutations in the homozygous or compound heterozygous state manifested earlier than those in whom pathogenic mutations were not identified, for both familial and sporadic cases (5, 37). The mean age at onset of NS in patients carrying two pathogenic mutations ranges between 2.6 and 3.4 years of age (5, 37). Weber et al. also found that patients with frameshift or nonsense mutations in the homozygous or compound heterozygous states led to an earlier onset of nephrotic syndrome than those carrying missense mutations (5). In addition, individuals homozygous for the p.R138Q mutation present with early-onset disease (5, 37). At least two mutations, p.V180M and p.R238S, are associated with a milder phenotype, including later age at onset of NS and age at ESRD (5). The p.R138X mutation has been associated with a high incidence of cardiac abnormalities in children (70), although this finding has not been confirmed in patients carrying other mutations (71).

The p.R229Q variant is the most frequently reported non-synonymous *NPHS2* variant in Caucasians (72), particularly among Europeans, in whom the observed frequency of heterozygotes ranges from 0.03 to 0.13 (5, 6, 40, 72–74). In African-Americans and sub-Saharan populations the p.R229Q allele is infrequent (72). In vitro studies demonstrated decreased binding of the p.R229Q mutant protein to nephrin, suggesting that this variant may be pathogenic (40). Indeed, the p.R229Q variant has been associated with microalbuminuria in a cohort of Brazilian individuals of mixed European and African ancestries (73). Furthermore, the frequency of the p.R229Q allele is significantly higher among individuals of European descent with FSGS compared with controls of similar origin (Machuca et al, in press) (40, 72). This observation has not been confirmed in cohorts with a high proportion of individuals of African-American origin or in patient

Figure 27-2

Clinical and genetic approach in patients with syndromic nephrotic syndrome. Proposed algorithm to decide for directed mutational screening according with the main extra renal manifestations and histological findings in the kidney biopsy.



cohorts with a presumed immune form of nephrotic syndrome (75, 76). In SRNS patients, the p.R229Q polymorphism is frequently found in a compound heterozygous state with a pathogenic *NPHS2* mutation, whereas this association has never been detected among controls (5, 40). These patients present with nephrotic syndrome and ESRD in the second and third decades of life, respectively, markedly contrasting them from patients bearing two pathogenic *NPHS2* mutations (Machuca et al, in press). By contrast, R229Q in the homozygous state has been reported in patients with NS as well as in controls (5, 37, 59), and more likely has a modulatory effect on the risk of developing renal disease. These observations support a pathogenic role of the p.R229Q variant.

Finally, tri-allelic inheritance of *NPHS1* and *NPHS2* mutations has been occasionally reported (36, 54, 77), but additional studies are needed to better understand the complex genetics of renal disease progression in the setting of nephrotic syndrome.

The identification of *NPHS2* mutations in children presenting with nephrotic syndrome may have important clinical implications. Screening of *NPHS2* mutations in patients with SSNS (6), late steroid resistance (78), steroid-dependence or frequent relapses and those with sensitivity to cyclophosphamide have failed to identify pathogenic mutations (6, 36, 79). Patients with mutations in the *NPHS2* gene do not respond to steroid or immunosuppressive therapy; although in a small number of cases a partial reduction of proteinuria has been reported with cyclosporine A (4, 6). Avoidance or withdrawal of immunosuppressive therapies in these patients would spare them from the potential risks and side-effects associated with these drugs.

NPHS2 mutations are rare in patients presenting with FSGS and relapse after transplantation (5). Indeed, patients with two pathogenic *NPHS2* mutations have a significantly lower risk of relapse after transplantation than cases in whom mutations were not identified (8% vs. 30%) (5, 6, 80, 81). Patients bearing mutations in the heterozygous state have a risk comparable to those without mutations (82). In the few patients reported with two *NPHS2* mutations who developed proteinuria post-transplant (5, 6, 83–86), the clinical evolution and renal histology did not correspond to the classic picture of NS relapse after transplantation (5, 83, 85). This potentially suggests de novo glomerulopathy or drug toxicity. In contrast with the mechanism of relapse in cases with *NPHS1* mutations, there is no evidence to support a role for anti-podocin antibodies (5, 83, 87). To-date, the pathophysiologic mechanisms of recurrence of proteinuria in these patients are unclear.

Functional Studies

Functional studies have elucidated some of the mechanism by which missense podocin mutations lead to disease. In vitro studies have shown that podocin missense mutations may either maintain proper intracellular targeting to the plasma membrane or be retained in the endoplasmic reticulum (ER) (88). Interestingly, patients with missense mutations retained in the ER had an earlier onset of disease than patients with mutations that traffic to the membrane (20.8 ± 4 vs. 128.7 ± 9 months) (88). Plasma membrane localization of p.V180M and p.R238S mutations suggests that their deleterious effect could affect the function of the protein by directly modifying its signaling properties and/or altering its interaction with other proteins at the slit diaphragm (88). Moreover, the p.R138X podocin mutant is able to traffic to the plasma membrane (88); however, nephrin is not recruited to lipid rafts, from which downstream signaling events are generated (50). In cells expressing ER-retained podocin mutants, nephrin is similarly retained in the endoplasmic ER (89).

A potential therapeutic strategy that might delay the onset and ameliorate the severity of glomerular disease in patients with missense mutations in genes encoding proteins located in the plasma membrane (i.e., slit-diaphragm) relies on chemical chaperones. Several of these molecules have been used in in vitro systems, allowing for targeting of the mutant protein to the plasma membrane (90).

Animal Models

Podocin-null mice ($Nphs2^{-/-}$) mice are massively proteinuric at birth, NS progresses rapidly, and animals die in the first 5 weeks of life with end-stage renal failure (91). Interestingly, disease progression rate is strongly determined by genetic background and appear to be subject not only to genetic modification, but also to the effects of the maternal environment in which mice are nourished prior to weaning (92). Nephrogenesis appears to be normal in podocin null mice and kidney size at birth is similar to that in wild-type littermates. Unexpectedly, $Nphs2^{-/-}$ mice do not show FSGS lesions, but display typical features of diffuse mesangial sclerosis (DMS). In addition, severe arteriolar lesions characterized by marked thickening of the arteriolar wall, endothelial cell hypertrophy, diffuse dilatation of peritubular capillaries, and multiple foci of interstitial hemorrhages predominating in the superficial cortex are observed. By electron microscopy, podocyte foot processes are only occasionally seen, are abnormal and lack slit diaphragms (91). Resembling the

phenotype of a *Nphs2* null mouse, a mouse model in which the p.R138Q mutant is expressed, leading to mislocalization of the mutant podocin in the ER, develops early-onset severe nephrotic syndrome, display features of DMS, progress rapidly to ESRD and dies at 5 weeks after birth (93). Similarly, mice deficient for CD2AP or NEPH1 develop progressive DMS as the one observed in mice lacking podocin (94, 95).

In addition to mouse models, the zebrafish pronephros has been used as a model of glomerular maturation and development of the filtration barrier. Zebrafish podocin shares 46% identity with the human protein, is specifically expressed in pronephric podocytes, and is required for the development of pronephric podocyte cell structure. Knockdown of podocin expression using antisense morpholino-oligonucleotides results in a loss of slit diaphragms, failure to form normal podocyte foot processes and loss of podocyte barrier function in the mature pronephros (96, 97).

Mutations in the *NPHS1* Gene Encoding Nephrin

NPHS1 has been identified as the major gene involved in congenital nephrotic syndrome of the Finnish type (CNF) (8). However, recent findings have broadened the spectrum of renal disease related to nephrin mutations since patients with childhood-onset SRNS may have *NPHS1* mutations (9, 98). The clinical aspects of CNF, the identification of the *NPHS1* gene and characterization of nephrin are extensively described in chapter 25.

Epidemiologic Overview of NPHS1 Mutations

In the Finnish population, 94% of patients with CNF bear either of two protein-truncating mutations in the *NPHS1* gene (8). The Fin-major mutation (c.121delCT; p.L41fsX91) leads to a frameshift deletion of two base pairs in exon two, resulting in a premature stop codon. The Fin-minor mutation (c.3325C>T; p.R1109X) generates a premature truncation of the terminal 132 amino acids of the protein. The Fin-major and Fin-minor mutations account for 78% and 16% of the mutated alleles in Finnish CNF patients, respectively (8); but are rare in other ethnic groups, therein suggesting founder effects (54). Other founder mutations have been sequenced among Old Order Mennonite patients from Lancaster, Pennsylvania (c.1481delC; p.S494fsX548) and patients from Malta (c.3478C > T; p.R1160X) (54, 99).

More than 100 *NPHS1* mutations have been reported worldwide in patients with CNF, most of which are private mutations found in non-Finnish patients (4, 38, 54, 64, 100–106). In Europe, North Africa and North America, the *NPHS1* mutation detection rate is estimated to be 66% (107). Among central European and Turkish patients presenting with NS in the first 3 months of life, *NPHS1* mutations were found in 34.3% and 54.5% of cases, respectively (4). However, *NPHS1* mutations were identified in only 2/13 patients in Japan (64). The lower frequency of congenital cases attributable to *NPHS1* mutations in these ethnic groups compared to Finnish population points to the genetic heterogeneity of congenital nephrotic syndrome.

Milder and Unusual Phenotypes Associated with *NPHS1* Mutations

The spectrum of *NPHS1* mutations includes protein-truncating nonsense and frameshift insertion/deletion mutations, splice-site changes and missense variants. Most of these mutants are retained in the endoplasmic reticulum (ER), although Liu et al. have demonstrated that in vitro treatment with a chemical chaperone may allow for trafficking to the plasma membrane (108, 109). These mutations lead to a severe CNF phenotype, although some *NPHS1* mutations have been reported in milder cases.

The p.R1160X mutation results in an unexpectedly milder phenotype in about 50% of cases, most of whom were females, suggesting a gender effect (54). This mutation is predicted to form a truncated protein lacking the C-terminal 82 amino acids implicated in the interaction with podocin. Surprisingly, all affected cases were homozygous for this mutation and, among those in which renal biopsy was performed, histologic findings were consistent with CNF. Nevertheless, these patients either had mild proteinuria or were in remission between the ages of 5 and 19 years.

Recently, *NPHS1* mutations were identified in a cohort of 160 patients presenting with SRNS after 3 months of age (9). Mutations in the *NPHS2* gene were excluded, as were mutations in exons 8 and 9 of the *WT1* gene in phenotypically female patients (9). The mean age of onset of NS was 3 years (range 6 months to 8 years). Six patients had preserved renal function after 6 years of age, based on a normal serum creatinine. Renal biopsy performed at the time of presentation revealed that most cases had

MCD or FSGS. All patients were resistant to corticosteroids, as well as other immunosuppressive agents when tried. Nine patients out of 98 with sporadic SRNS, and 1 family with 2 affected siblings among 44 families with familial SRNS, carried pathogenic *NPHS1* mutations. Affected cases were compound heterozygotes for at least one "mild" missense mutation, which exhibited normal trafficking to the plasma membrane and maintained the abilities to form nephrin homodimers and to heterodimerize with NEPH1. These findings may explain the lesser severity of disease observed in these cases.

Finally, Kitamura et al. described the clinical course of two siblings bearing compound heterozygous *NPHS1* missense mutations (98). The severe c.793T>C (p.C265R) mutation leads to ER retention, whereas the mild c.2464G>A (p.V822M) mutation encodes a protein that partially retains plasma membrane targeting. Both patients presented with mild to moderate persistent proteinuria detected from birth to 10 months of age, with several self-limited episodes of nephrotic syndrome triggered by upper airway infections. Kidney histology in both cases revealed minimal changes.

These recent studies highlight the importance of *NPHS1* mutation screening in cases of childhood onset NS, particularly in those in whom mutations in podocin were not found. Studies of large patient cohorts with a broader range of disease onset and ethnic backgrounds are needed to better define the frequency and phenotypic spectrum of nephrin mutations.

Animal Models

Mouse models in which the *Nphs1* gene has been inactivated revealed lesions reminiscent of histological changes observed in CNF patients and absence of slit diaphragms, corroborating the crucial role of nephrin in the establishment and maintenance of the glomerular filtration barrier. *Nphs1* inactivation resulted in massive nonselective proteinuria, edema immediately after birth and death within 24 h (110–112). Histological characterization revealed slightly enlarged kidneys, dilated proximal and distal tubules, and microcysts in the cortex and medulla (110). No prominent changes in the branching morphogenesis of the developing collecting ducts could be found (112). Bowman spaces were enlarged and glomeruli were sclerotic and showed hypercellularity and excessive extracellular matrix deposition (112). Electron microscopy revealed effacement of podocyte foot processes and absence of slit diaphragms (110). The glomerular basement membrane appeared normal, and the expression of several basement membrane proteins including type IV collagen, laminin, nidogen, and perlecan, as well as podocyte-specific proteins such as podocin, CD2AP, α-actinin-4, synaptopodin, integrin α3, and α3, α4 and α5 chains of type IV collagen were normal (111).

The nephrin homologue in zebrafish shares only 36% identity with human nephrin; however, both have a similar predicted secondary structure. Nephrin is expressed in the zebrafish pronephros, specifically in the slit diaphragms of podocyte foot processes (96). Nephrin targeting with morpholino antisense oligonucleotides resulted in pericardial edema progressing to generalized edema (96). Nephrin morphant embryos demonstrated podocyte foot process effacement, lacked slit diaphragms and showed filtration barrier dysfunction in the mature pronephros (96). These findings resemble those found in podocin and CD2AP morphant embryos (96, 97).

Mutations in *PLCE1* Encoding Phospholipase C Epsilon 1

The *NPHS3* gene locus was identified on chromosome 10q23.32-q24.1 in seven consanguineous SRNS families (13). A positional cloning approach coupled to gene expression profiling in rat glomeruli identified the *PLCE1* gene, encoding phospholipase C epsilon 1, as a good candidate. Mutational analysis subsequently revealed truncating and missense mutations in several of its 34 exons (13). In the 12 affected individuals carrying truncating mutations, proteinuria and edema, manifested at a median age of 0.8 years (range 0.2–4.0 yrs) and progressed to ESRD by 5 years of age (13). Furthermore, individuals bearing truncating mutations demonstrated lesions of DMS on renal biopsy, whereas FSGS was found in the affected cases homozygous for missense mutations. In the affected individuals presenting with DMS, immunofluorescence studies revealed that *PLCE1* mutations may lead to an arrest of glomerular development at the S-shaped stage, suggesting a potential role not only in cell junction and signaling events, but in development, as well (13). Interestingly, two patients bearing truncating mutations achieved complete remission when treated early and remain free of proteinuria after several years of follow-up; hence potentially opening a window of opportunity for therapy of some forms of hereditary NS (13, 113). Subsequently these investigators have shown that mutations in the *PLCE1* gene account for 28.6% of cases of isolated DMS in a cohort of 40 patients from 35 families mostly of Turkish origin (114). An additional report has described 4 patients with early-onset SRNS, DMS and

PLCE1 mutations (38). In our cohort of patients with SRNS, we have identified several cases carrying truncating *PLCE1* mutations presenting with early-onset SRNS and exhibiting FSGS on renal biopsy (Antignac, personal observation).

PLCε1 is a phospholipase enzyme that catalyzes the hydrolysis of phosphatidylinositol-4,5-biphosphate and generates two second messengers: inositol 1,4,5-triphosphate (IP3) and diacylglycerol (DAG) (115). IP3 releases Ca^{2+} from intracellular stores, and DAG stimulates protein kinase C. These products initiate a cascade of intracellular responses that result in cell growth and differentiation. Based on the observation that nephrin levels were diminished in the glomeruli of patients bearing mutations in *PLCE1*, it has been shown that PLCε1 interacts with the C-terminal half of IQGAP-1 (13), a cell junction-associated protein and binding partner of nephrin involved in cell morphology and adhesion (116).

The PLCε1 zebrafish orthologue shares 65% identity with the human protein sequence, suggesting conserved function among evolutionarily distant organisms. *Plce1* targeting using antisense morpholino oligonucelotides induced edema and glomerular filtration barrier dysfunction, similar to zebrafish nephrin and podocin loss-of-function morphants (96). Surprisingly, PLCε1 null mice have no obvious developmental defects or evidence of glomerular filtration barrier abnormalities (13, 117).

Mutations in *CD2AP*

CD2AP is a 70-kilodalton adaptor protein that was originally cloned as an interaction partner of CD2, a signaling protein expressed on the surface of T lymphocytes (118). It has been shown that CD2AP is involved several processes including the regulation of the actin cytoskeleton (119–121), endocytosis (122, 123), in the phosphatidylinositol 3-kinase/AKT survival pathway and in the repression of TGF-β induced apoptosis (50, 124).

In the kidney, CD2AP is expressed in podocytes, proximal tubules and collecting ducts. *Cd2ap* null mice developed proteinuria 2 weeks after birth and die by 6 to 7 weeks of age due to advanced renal insufficiency (94). Renal histology showed increased glomerular size and cellularity, foot-process effacement at 1 week, and subsequent abnormal mesangial matrix deposition and glomerular sclerosis 4 weeks after birth (94). *Cd2ap*$^{+/-}$ mice did not develop proteinuria when followed up to 1 year; however, exhibited variable degrees of increased mesangial expansion and hypercellularity at 9 months of age. Two out of 30 patients with idiopathic FSGS were found to carry a heterozygous truncating mutation in the *CD2AP* gene (125), which lead to a reduce expression at the mRNA and protein level. These results suggested that CD2AP could act as a determinant of human susceptibility to glomerular disease.

Recently, Löwik et al. described a patient presenting at 10 months of age with failure to thrive, anemia, hypoalbuminemia and massive proteinuria (126). Renal biopsy showed global glomerular sclerosis and was suggestive of collapsing FSGS. Mutation analysis of the *CD2AP* gene revealed a novel truncating mutation in the homozygous state, which displayed significantly decreased F-actin binding efficiency *in vitro*. The mutant allele was not expressed in the patient's lymphocytes. At the age of 5 years the patient was transplanted, without relapse of proteinuria (126). Both parents were heterozygous for the mutation and had normal glomerular filtration rate and no proteinuria. An additional patient bearing a heterozygous missense mutation in *CD2AP* in association with a missense mutation in *NPHS2* was recently reported (127). This patient presented with SRNS and relapse after transplantation. Both parents had normal serum creatinine and no proteinuria (127). Mutational screening of large cohorts of SRNS patients will be crucial to elucidate the frequency of *CD2AP* mutations and the spectrum of phenotypes.

Autosomal Dominant Forms of SRNS

Autosomal dominant (AD) forms of FSGS are infrequent and generally observed in adults. Variable degree of proteinuria is detected between the third and fourth decades of life and slowly progresses to ESRD (128, 129). So far, two loci have been mapped in cases with non-syndromic AD FSGS; nevertheless, genetic heterogeneity is likely, since no linkage to those loci has been found in several families with similar phenotype (129).

Mutations in *ACTN4* Encoding α-actinin-4

A genome-wide scan performed in a 100-member kindred allowed Mathis et al. to map the first locus of AD FSGS on chromosome 19q13 (130, 131). Linkage analysis including additional families helped to reduce the size of the region and led to the identification of 3 nonconservative missense mutations in the *ACTN4* gene. *ACTN4* encodes for the actin-binding protein α-actinin-4, which is highly expressed in podocytes (11). Affected cases presented with proteinuria starting in the teenage years

or later, slowly progressed to renal insufficiency and developed ESRD in the fifth decade of life (11, 132). Disease was incompletely penetrant, since several individuals from 2 out of 3 families carried a disease allele without clinical symptoms. In affected cases, kidney histology revealed lesions consistent with FSGS and no evidence of a primary basement membrane defect or of immune complex deposition.

Further mutation screening of the *ACTN4* gene in cases with familial and sporadic forms of FSGS allowed the identification of several additional patients carrying mutations (133, 134). In one affected case, proteinuria was diagnosed at 5 years of age and rapidly progressed to ESRD. Unexpectedly, this patient presented recurrence of proteinuria after transplantation. A superimposed immune form of SRNS or a *de novo* glomerulopathy may better explain the outcomes in this patient. Additional screening of small cohorts of patients with sporadic adult-onset FSGS and congenital SRNS have failed to identify *ACTN4* mutations (62, 64, 135). Overall, *ACTN4* mutations seem to account for approximately 4% of familial FSGS (134), although the precise proportion of AD forms in which this gene is mutated is unknown.

The mechanisms by which α-actinin-4 mutations cause disease in humans have been partially elucidated through functional studies and the characterization of mouse models. These studies suggest that the phenotypes in mice and humans involve both gain-of-function and loss-of-function mechanisms (11, 134, 136, 137). Alpha-actinin-4 has a key role in the maintenance of podocyte architecture cross-linking and bundling of actin filaments (138). Disease-associated mutations occur in the actin-binding domain, increasing actin-binding activity *in vitro* and diverting its normal localization from actin stress fibers and focal adhesions *in vivo* (11, 134, 137). Moreover, over-expression of GFP-fusion mutant proteins in cultured podocytes led to the formation of aggregates adjacent to the nucleus, confirming the subcellular mislocalization of mutants (134). Further supporting the hypothesis of loss-of-function, Yao et al. showed increased degradation of α-actinin-4 in cells from knock-in mice carrying an *Actn4* point missense mutation homologue, in comparison to that found in humans with FSGS (136). Finally, a β1-integrin-dependent, α-actinin-4 mediated adhesion is necessary to maintain podocyte attachment to the-glomerular basement membrane (139, 140). Consequently, podocytes from α-actinin-4 deficient mice showed reduced adherence to GBM components type IV collagen and laminin-10 and -11 (140).

The phenotype seen in $Actn4^{-/-}$ mice is more aggressive than the human disease. *Actn4* null mice exhibited abnormalities only in the kidneys. At 5 weeks of age, mice had only focal areas of podocyte foot process effacement, whereas FSGS was evident by 10 weeks. Proteinuria was observed with increasing age in most, but not all mice. Progressive renal insufficiency led to death at 12 weeks after birth. Mice heterozygous for the targeted allele ($Actn4^{+/-}$) showed no obvious phenotype up to 6 months of age (141).

Resembling human disease, a transgenic mouse developed by Michaud et al. (142), which expressed both endogenous wild-type and a K256E-mutant α-actinin-4 transgene, developed proteinuria at 10 weeks, elevated blood pressure and histological features consistent with FSGS. Interestingly, not all *ACTN4* mutant mice were proteinuric, and only a few among those with proteinuria had reduced renal function. Detailed histological analysis revealed segmental sclerosis and tuft adhesion of some glomeruli, tubular dilatation, mesangial matrix expansion, podocyte vacuolization and foot process fusion (142).

A mouse model in which one *Actn4* allele was replaced with a copy bearing a disease-associated mutation in humans (K256E) was developed by Yao et al. (136). Although this model is genetically closer to the human disease, homozygous mutant mice had no glomerular defects evident using light microscopy, although focal areas of foot process effacement and abnormal electron-dense structures in the podocyte cell bodies were observed at the electron microscopic level. Careful assessment of *Actn4* heterozygous mutant mice confirmed that they do not develop evident FSGS, but exhibit focal glomerular hypertrophy and mild glomerular ultrastructural changes (143). The mechanisms underlying the differences between the human and mouse phenotypes remain unknown. Additional modulating factors appear to play a role in the development of *ACTN4*-mediated human disease.

Mutations in *TRPC6* Encoding the Transient Receptor Potential Cation Channel 6

Winn et al. identified a second locus for autosomal dominant FSGS on chr 11q21-22 in a large family from New Zealand (144). Affected cases presented with nephrotic range proteinuria in their third or fourth decade and developed progressive renal insufficiency within 10 years after NS presentation. Using fine-mapping and candidate gene screening, the same group of investigators subsequently detected a missense mutation in the *TRPC6* gene, encoding the transient receptor potential cation channel, subfamily C, member 6 (12). TRP channels are involved in mechanosensation (145), ion homeostasis,

cell growth and PLC dependent calcium entry into cells (146). The proline to glutamine substitution at position 112 (p.P112Q) found in the index family, was shown to enhance TRPC6-mediated calcium signals in response to angiotensin II, suggesting that mutations in this gene disrupt glomerular cell function by amplifying injurious signals triggered by ligands, such as angiotensin II (12).

Subsequently, Reiser et al. identified *TRPC6* mutations in five other unrelated families of diverse ethnic origin (53). Only two of the five mutations were associated with an increase in calcium influx, suggesting that diverse mechanisms may result in dysregulation of the ion channel or may affect the interaction with other slit diaphragm proteins (53). In addition, they demonstrated that *TRPC6* is expressed in podocytes, specifically at the slit diaphragm where it interacts with podocin and nephrin (53).

In our cohort, we have found one patient bearing a *de novo* missense mutation in exon 13 of *TRPC6*, comprising a highly conserved region in the cytoplasmic tail of the protein (Antignac, unpublished data). This patient presented with NS at 6.5 years of age and reached ESRD a few months after diagnosis. Renal histology revealed advanced FSGS. No relapse was observed after transplantation. Mutation screening of large cohorts of patients is needed to evaluate the epidemiologic relevance of *TRPC6* mutations and the phenotypic spectrum of renal disease attributable to the *TRPC6* gene.

The contribution of animal models to understand the mechanisms underlying mutations in *TRPC6* has been limited. Targeted deletion of *Trpc6* in mice was not associated with a renal phenotype; although, mice exhibited an elevated blood pressure and enhanced agonist-induced contractility of isolated aortic rings, as well as cerebral arteries (147). An animal model carrying a missense point mutation in the *Trpc6* gene, homologous to those found in humans, will be required to confirm a gain-of-function mechanism as the triggering event leading to glomerular disease. An evaluation of the role of TRPC6 in kidney disease using zebrafish has not been possible since *TRPC6* was not detected at RNA level in developing and adult zebrafish podocytes (148).

Syndromic Steroid-Resistant Nephrotic Syndrome

Pierson Syndrome: Mutations in *LAMB2* Encoding Laminin β2

In 1963, Pierson et al. described two patients with congenital nephrotic syndrome, unique ocular abnormalities and histopathological features of DMS (149). Subsequently, several isolated case reports appeared in the literature (150–154). Zenker et al. designated this disorder Pierson syndrome, refining the phenotype based on the description of eleven affected cases from two large consanguineous families and after reviewing previous case reports (155). Clinical findings include nephrotic syndrome and oliguria presenting at birth or within the first days of life, enlarged or large appearing cornea in some cases suggesting buphthalmos, extremely narrow, nonreactive pupils (microcoria) and DMS with an irregular basement membrane (155).

In two consanguineous families, a genome-wide scan and homozygosity mapping allowed the identification of a potential locus in chromosome 3p (23). Subsequent positional cloning was greatly facilitated by the previous description of the development of congenital nephrotic syndrome in *Lamb2* null mice (156). Affected cases from five families had truncating or missense mutations of the *LAMB2* gene, either in the homozygous or compound heterozygous states, leading to absent or reduced expression, respectively, of laminin β2 in the kidneys (23). Interestingly, mutation screening in additional families revealed patients bearing missense mutations in *LAMB2* who presented with congenital nephrotic syndrome, minor or no ocular defects (transient fundus hypopigmentation, nistagmus and myopia) and normal psychomotor development (157–159). Childhood onset of Pierson syndrome has likewise been reported in a non-consanguineous family with seven affected individuals, with nephrotic syndrome and ESRD presenting between 5 and 10 years of age (160). Ocular problems paralleled or even preceded the renal symptoms. Visual impairment was progressive since affected individuals had no signs of impaired vision in early infancy, and they all developed blindness around 2 years of age (160).

Laminins are heterotrimeric extracellular matrix proteins that provide the basic scaffold for assembly of the other components of the glomerular basement membrane, including type IV collagen, nidogen/entactin and sulfated proteoglycans (161). The glomerular basement membrane is composed exclusively of laminin-521 (α5β2γ1) (162). The α5 chain is required for GBM integrity and glomerular vascularization (163), whereas the β2 chain is dispensable for glomerulogenesis; concordantly, *Lamb2* null mice displayed no glomerular developmental abnormalities at birth (164).

The clinical features observed in patients with Pierson syndrome are consistent with the phenotype of *Lamb2* null mice, which present with failure to thrive, heavy proteinuria within the first weeks of life (156), and revealed

foot-process effacement and increased GBM permeability (164). In addition, mice show aberrantly formed and functionally impaired neuromuscular junctions (165, 166), and both structural and functional abnormalities in the retina (167, 168). Indeed, these findings reflect the fact that laminin β2 is highly expressed in the glomerulus, the skeletal neuromuscular junction and the retina (167–169).

Denys-Drash, Frasier and WAGR Syndromes: Mutations in *WT1* Encoding the Wilms' Tumor Protein

Through a positional cloning approach, the *WT1* gene was found to be inactivated in Wilms' tumor (170–172). The *WT1* gene is located on chromosome 11p13 and encodes a zinc finger transcription factor that functions both as a tumor suppressor and as a critical regulator of kidney and gonadal development (173–175). The key role of *WT1* in kidney development has been highlighted by the development of animal and *in vitro* models showing the failure of, arrest in or delayed development of nephrogenesis in the absence of *WT1* expression (174, 176, 177). Mutations in the *WT1* gene are associated with varied syndromic forms of glomerular disease and genitourinary abnormalities, as well as isolated cases of SRNS.

Denys-Drash syndrome (DDS, MIM 194080) is a rare urogenital disorder comprising nephropathy due to DMS, associated with male pseudohermaphroditism and Wilms' tumors (17, 18). Nephrotic syndrome presents in the first months of life, may be preceded by isolated proteinuria and is always resistant to steroid therapy. Progression to ESRD occurs before 4 years of age and no recurrence is observed after renal transplantation (178–180). Wilms' tumor may be the first presentation of the disease or may be discovered later during the course of nephropathy by systematic ultrasound screening. Patients with Denys-Drash syndrome bear heterozygous mutations, mostly *de novo*, in exons 8 and 9 of the gene, encoding the second and third zinc finger domains (19, 20). *In vitro* studies have confirmed that missense mutations in the *WT1* gene lead to a change in the structural organization of the zinc finger domains, leading to loss or alteration of their DNA-binding abilities (181). Isolated cases of DMS have also been attributed to mutations in *WT1* (182–184).

Frasier syndrome (FS, MIM 136680) is characterized by male pseudohermaphroditism with normal female external genitalia, streak gonads and 46,XY karyotype. Patients have an increased susceptibility to gonadoblastomas, but do not develop Wilms' tumors. FS is associated with childhood-onset proteinuria, usually between 2 and 6 years of age, slowly progressing to ESRD towards the adolescence or early adulthood, exhibiting histological finding of FSGS on renal biopsy (15, 16). As in Denys-Drash syndrome, inheritance is autosomal dominant, although most cases are sporadic due to *de novo* mutations. The *WT1* gene encodes up to 36 different isoforms, which are products of alternative translation start sites, alternative splicing and RNA editing (185).

Of particular interest are WT1(+KTS) and WT1 (-KTS) variants, which differ by the presence of the three amino acids KTS between zinc fingers 3 and 4. The presence of this insert influences the molecular and biochemical properties of the resulting protein. While WT1 (−KTS) binds DNA efficiently and acts as a transcriptional activator, WT1(+KTS) seems to have higher affinity to RNA (186). Mutations in the donor splice site in intron 9 of the *WT1* gene are causative of Frasier syndrome, and leads to alternative splicing and loss of the +KTS isoform of the protein (14). This results in an alteration of the normal ratio of +KTS/-KTS isoforms in the cell (187).

De novo deletion of the 11p13 locus leads to WAGR syndrome (MIM 194072), characterized by Wilms' tumors, aniridia, genitourinary abnormalities and mental retardation (21). Aniridia is due to the deletion of the *PAX6* gene, which resides in the same locus than *WT1*.

Mutations in *WT1* have also been associated with the development of isolated SRNS with kidney histology consistent with FSGS in some phenotypic females (XX or XY karyotype). Disease onset varies between few months of age to the end of the first decade of life, with rapid progression to ESRD (22, 188–190). Most of the cases carry missense or splice-site mutations in exons 8 and 9 of the *WT1* gene. In these patients, genetic counseling is essential, since a male child from an affected XX female, would either have Denys-Drash syndrome or Frasier syndrome, respectively (188, 189).

Nail-Patella Syndrome: Mutations in *LMX1B* Encoding the LIM Homeobox Transcription Factor 1 β

Nail-patella syndrome (NPS; MIM 161200) is an autosomal dominant disorder with complete penetrance and variable phenotypic expression, characterized by pleiotropic developmental defects of dorsal limb structures. The most characteristic finding is nail involvement. Nails may be absent, hypoplastic or dystrophic. Defects are often bilateral, symmetrical and may be observed at birth. An additional pathognomonic feature of NPS are iliac horns, which are bony processes that project posteriorly and

laterally from the central part of the iliac bones of the pelvis (191). Frequently, patellae may be hypoplastic or absent; involvement of shoulders, elbows and ankles is less common, and may be asymmetrical (192). Nephropathy may occur in 25–50% of the cases (193–197), being more frequent in women (197). This manifests as microalbuminuria progressing to proteinuria, usually associated with hematuria. Proteinuria, which may be intermittent, may present at any age, diagnosed in most of the cases after the second decade of life. Overt nephrotic syndrome is not a common feature and progression to ESRD occurs in 5–14% of the cases, usually many years after proteinuria onset (197, 198). To-date, no recurrence of proteinuria after transplantation has been reported (193, 199). Light microscopy of renal tissue usually reveals no specific changes (200), while glomerular basement membrane exhibits ultrastructural abnormalities that are the most specific histological hallmark of NPS (193, 201–203). Typically, there is irregular thickening and splitting of the GBM glomerular basement membrane, with electron lucent areas, and the presence of clusters of fibrillar type III collagen within the GBM and the mesangial matrix. Finally, primary open angle glaucoma and sensorineural hearing impairment have been recognized as less frequent features of the disease (197, 204).

Chen et al. demonstrated that targeted disruption of the *Lmx1b* gene (LIM homeobox transcription factor 1 β) in mice resulted in distinctive skeletal defects including hypoplastic nails, absence of patellae, joint abnormalities and glomerular basement membrane defects, recapitulating the phenotype of NPS (205). The disease phenotype was observed only in homozygous mutant mice, whereas heterozygous littermates did not exhibit any evident abnormalities. Nevertheless, these results led to the identification of de novo heterozygous mutations in the *LMX1B* gene in patients with NPS (24).

Approximately 85% of families with NPS present mutations in *LMX1B*, which consistently segregate with disease in an autosomal dominant pattern with complete penetrance. The majority of mutations, including nonsense mutations, small intragenic insertions/deletions or splice-site mutations, results in protein truncation. Missense mutations generally involve substitutions in the homeodomain region critical for DNA binding (195–197, 206, 207). Recently, entire-gene deletions were reported by Bongers et al., confirming that haploinsufficiency of the LMX1B transcription factor underlies this disease (208).

The precise role of LMX1B in the kidney remains partially elucidated. Immunohistochemical studies in several patients bearing heterozygous mutations in the *LMX1B* gene revealed that the expression of the α3 and α4 chains of type IV collagen, as well as podocin and CD2AP are no different than normal controls (209). In mouse, *Lmx1b* is expressed exclusively in glomeruli. Podocyte-specific *Lmx1b* inactivation invariably leads to proteinuria, renal insufficiency and death at 2 weeks after birth (210). In addition, LMX1B may be critical for glomerular development, since mice with podocyte-specific inactivation of *Lmx1b* showed severely impaired glomerular development and podocyte differentiation (211). Potential targets of *Lmx1b* in the kidney have been demonstrated in the shared 5′ regulatory regions of *Col4a3* and *Col4a4* genes (212), and in the promoter regions of the *Nphs2* and *Cd2ap* genes (211, 213). In *Lmx1b*-deficient mice, the abundance of α(3)IV and α(4)IV chains of collagen were markedly diminished (212), as were the levels of podocin (211, 213), CD2AP (213), synaptopodin and VEGF (211). Nevertheless, no downregulation in the expression of the α3 and α4 chains of type IV collagen, podocin and CD2AP, were observed in mice in which *Lmx1b* had been inactivated specifically in podocytes (210). Interestingly, immunohistochemical studies in two patients bearing heterozygous mutations in the *LMX1B* gene revealed no downregulation in the expression of the α3 and α4 chains of type IV collagen, and in podocin and CD2AP (209). The latter findings may be explained by the fact that these patients carry one functional and one mutated alleles, whereas mice have two mutant alleles.

Schimke Immuno-Osseus Dysplasia: Mutations in *SMARCAL1*, Encoding the swi/snf-Related Matrix-Associated Actin-Dependent Regulator of Chromatin, Subfamily-A-Like-1

Schimke immuno-osseus dysplasia (SIOD, OMIM 242900) is a rare autosomal recessive disorder characterized by spondyloepiphyseal dysplasia, progressive renal dysfunction due to focal segmental glomerulosclerosis and T-cell immunodeficiency (214). Other additional, although inconstant features include cerebral ischemia, migraine-like headaches, deficiency of other blood cell lineages, hyperpigmented macules, corneal opacities, microdontia, intellectual delay, recurrent infections, premature atherosclerosis, hypothyroidism, cerebellar atrophy and testicular hypoplasia with atrophy and azospermia (215–218). Kidney disease in patients with SIOD manifests typically with proteinuria evolving to overt nephrotic syndrome, which is diagnosed between the first year of life and 14 years of age (215, 216). No response to steroids has been documented in patients who have been

treated; nevertheless, transient reductions in proteinuria using ACE inhibitors, NSAID or even cyclosporin A have been observed (216). Patients who survive infectious complications progress to ESRD between 5 and 15 years of age. Numerous cases have been transplanted without evidence of relapse in the allograft (216); however, the evolution of cerebrovascular and infectious complications do not seem to improve after transplantation.

A genome-wide scan, performed in four families, detected significant linkage at chromosome 2q35, and mutations were identified in the *SMARCAL1* gene (26). This gene encodes a member of an SNF2 subfamily of proteins that mediate DNA-nucleosome restructuring during gene regulation and DNA replication, recombination, methylation and gene repair (swi/snf-related matrix-associated actin-dependent regulator of chromatin, subfamily-a-like-1 gene). The gene consists of 18 exons and encodes a 106-kDa protein with 954 amino acid residues. The majority of mutations identified involve nonsense and frameshifting mutations, likely leading to loss-of function (26). Recently, Clewing et al. showed that *SMARCAL1* biallelic mutations accounted for the phenotype in 38 of 72 independent cases with SIOD (219), revealing the genetic heterogeneity of this syndrome. Patients with two missense mutations tended to have a milder course of disease, surviving beyond 15 years of age. The functional targets of SMARCAL1 remain unidentified.

Action Myoclonus-Renal Failure Syndrome: Mutations in *SCARB2* Encoding the Lysosome Membrane Protein 2

Action myoclonus-renal failure syndrome (AMRF, MIM 254900) is a rare autosomal recessive disease characterized by progressive myoclonic epilepsy associated with renal failure. It typically presents at 15–25 years of age with neurological symptoms including tremor, action myoclonus, seizures and later ataxia, while cognitive function is preserved. Proteinuria is usually diagnosed concomitantly with the onset of neurologic symptoms at a median age of 19 years (220), although proteinuria may also be the first symptom. Progression of renal impairment to ESRD occurs generally within 5 years after onset of proteinuria (220, 221). The renal pathology is characterized by focal glomerulosclerosis, sometimes with features of glomerular collapse (220). In three unrelated families, Berkovic et al. identified a region on chromosome 4q13-21 linked to AMRF. Subsequently, using gene expression profiling to prioritize gene sequencing within the region, they identified homozygous truncating mutations in the *SCARB2* gene (encoding LIMP-2). These mutations led to a downregulation of SCARB2 mRNA and undetectable protein levels in western blots of cell lysates from lymphoblastoid B cell lines from the two affected subjects (29).

LIMP-2 is a transmembrane protein of the CD36 superfamily, which is ubiquitously expressed and is mainly found in lysosomes and late endosomes, where it is required for their biogenesis and maintenance (222–224). It has been shown that LIMP-2 acts as a trafficking receptor for β-glucocerebrosidase (β-GCase) (225), a lysosomal enzyme deficient in most cases of Gaucher disease. Interestingly, a nonsense mutation involving the interaction domain of LIMP-2/β-GCase was recently identified in two patients with ARMF in which a severe β-GCase deficiency was detected in cultured skin fibroblast (221). The pathophysiologic events leading to NS and FSGS in patients with *SCARB2* mutations remain to be elucidated.

In mouse, the *Limp2* gene is expressed in a range of tissues including brain and kidney. Interestingly, a *Limp2*-deficient mouse model presents with hearing impairment, demyelinating neuropathy, cerebral and cerebellar cytoplasmic inclusions, hydronephrosis caused by ureteropelvic junction obstruction, mesangial proliferation and foot-process effacement, but does not recapitulate the glomerular lesions seen in humans (29, 223). Proteinuria is present, but occurs only with aged mice (29).

Mitochondropathies Manifesting with Nephrotic Syndrome

The mitochondropathies are a diverse group of disorders due to structural, biochemical, or genetic derangements of mitochondria (226). Renal dysfunction is a rare event, and may result from mutations in the mitochondrial or nuclear genomes. The mitochondrial genome encodes for 13 essential subunits of the mitochondrial respiratory chain, as well as the 22 transfer RNA (tRNA) and 2 ribosomal RNA (rRNA) genes (227). The c.3243A>G point mutation in the $tRNA^{Leu(UUR)}$ gene is associated with MELAS syndrome (myopathy, encephalopathy, lactic acidosis, and stroke-like episodes) (27). Some patients carrying $tRNA^{Leu(UUR)}$ gene mutations may present with diabetes and deafness (228, 229), cardiomyopathy (230), progressive external ophthalmoplegia and FSGS (231–236), with or without the nephrotic syndrome (234–240). Although most of the affected cases are diagnosed in adulthood and have glomerular disease associated with other manifestations of mitochondrial disease, some patients present with isolated nephropathy or may have an earlier onset during the adolescence (235, 238, 240, 241).

Nephrotic syndrome has, likewise, been described in coenzyme Q_{10} (CoQ_{10}) deficiency (MIM 607426), in association with encephalomyopathy and multisystemic involvement (30, 242, 243). The *COQ2* gene is part of the coenzyme Q_{10} pathway, a component of the mitochondrial respiratory chain vital for the transport of electrons from complexes I and II to complex III. Mutations in the *COQ2* gene were identified in several patients presenting with early-onset nephrotic syndrome, with or without neuromuscular symptoms (244). The clinical presentation varied from severe oliguric renal failure due to crescentic GN on the fifth day of life to development of SRNS at 18 months in association with collapsing glomerulopathy (244). In all renal biopsies dysmorphic mitochondria were characteristic (244).

Lopez et al. recently described an infant with fatal Leigh syndrome, CoQ10 deficiency in muscle and fibroblasts, nephrotic syndrome, and compound heterozygous mutations in the *PDSS2* gene (245). The *PDSS2* gene encodes a subunit of decaprenyl diphosphate synthase, the first enzyme of the CoQ10 biosynthetic pathway (245). Similarly, the *kd/kd* mouse, which develops collapsing glomerulopathy, carries mutations in the murine orthologue of the human *PDSS2* gene (246). Moreover, mice in which the *Pdss2* gene has been conditionally inactivated in podocytes exhibit proteinuria and foot process effacement (247). Recently, Saiki et al. showed that coenzyme Q10 supplementation rescues renal disease in *Pdss2 kd/kd* mice (248).

It is likely that mutations in other genes involved in the CoQ10 biosynthetic pathway are responsible for cases of NS with or without neuromuscular manifestations. In cases with CoQ10 deficiency, early ubiquinone supplementation may be crucial for the resolution of renal symptoms and for preventing neurologic damage, as demonstrated in patients and animal models (248, 249).

Finally, a deletion in the mitochondrial DNA has been associated with FSGS in a cohort of Japanese patients and in a Turkish patient (239, 250).

Hereditary Multisystemic Disorders of Unknown Cause Associated with Steroid-Resistant Nephrotic Syndrome

Galloway-Mowat syndrome

Galloway-Mowat syndrome is a rare disorder, of autosomal recessive inheritance, characterized by SRNS, microcephaly and severe neurological impairment (251). Disease frequency is unknown; however more than 70 cases have been reported since the original description in 1968 (251). Inconstant morphological defects include hiatus hernia, micrognathia, arachnodactyly and floppy ears. Proteinuria is usually discovered within the first year of life; although congenital onset is not unusual as are cases in which the onset of NS is close to the third year of life (251–259). Kidney histology may reveal either FSGS or DMS, the later more frequent in early onset forms (254, 260–263). In addition, a single patient with collapsing FSGS has been recently reported (264). The great majority of patients reach ESRD between 36 and 72 months after birth, although there are rarer cases with preserved renal function after this age (259, 263).

The distinctive neurological feature is marked microcephaly, which might be congenital (primary) or may develop after birth (secondary). Structural brain abnormalities include cortical and cerebellar atrophy, severe myelination deficiency and gyral defects (257, 263, 265–268). Profound mental retardation, hypotonia and seizures are the most recurrent neurological symptoms. In addition, choreoathetosis may develop later in the course of the disease (Antignac, personal observation). Sensorineural blindness and deafness have also been described. Patients may occasionally be able to walk, interact with their families and eventually develop a rudimentary monosyllabic language (Antignac, personal observation). The association with microphthalmia and corneal defects has been occasionally reported (252, 260, 261, 269–271). Undeniably, ocular malformations are a common feature of Pierson syndrome, in which microcephaly might be occasionally observed (272). Due to the overlapping phenotype with GMS, screening of mutations in laminin-β2 and several related proteins was performed by Dietrich et al. in 18 unrelated patients with GMS (273). Unfortunately they failed to find pathogenic mutations. Indeed, GMS represents a heterogeneous group of diseases and so far, the underlying genetic abnormalities have not been identified.

SRNS and Deafness

The association of SRNS and deafness has been described in patients with familial forms of SRNS with both autosomal dominant and recessive inheritance, revealing the genetic heterogeneity of this clinical association. Excluding patients carrying mutations in genes involved in the mitochondrial respiratory chain, in which deafness and nephrotic syndrome may be present in addition to neuromuscular symptoms, two loci have been identified to-date (274, 275).

Ruf et al. mapped the first locus on chr 14q24.2 in a consanguineous Palestinian family (275). Congenital

sensorineural deafness was diagnosed in the four affected cases. The onset of NS ranged from 0.3 to 6.4 years and all the patients progressed to ESRD before 10 years of age. Kidney histology was compatible with FSGS. Three cases were transplanted, with no relapse of proteinuria. Prakash et al. described a 39-member kindred from India, consisting of 7 affected members, showing male-to-male transmission with an AD pattern of inheritance (274). Age at presentation varied between 8 and 44 years of age. Five of the affected cases also had sensorineural deafness. Renal biopsies revealed FSGS with irregular GBM. A genome-wide scan identified a novel locus on chr 11q24, after exclusion of linkage to currently known loci for Alport syndrome.

Epidermolysis Bullosa and FSGS

Nephrotic syndrome and renal failure may occur in some patients with epidermolysis bullosa (276). The most common histological finding is secondary amyloidosis (277–283). The association of FSGS and epidermolysis bullosa has been reported in a male infant with pyloric atresia, junctional epidermolysis bullosa and nephrotic range proteinuria diagnosed 6 weeks after birth (25). Renal biopsy revealed immature glomeruli, segmental sclerosis in the absence of microcystic tubular dilatation, atrophy and interstitial fibrosis. Ultrastructural changes included a thin glomerular basement membrane, extensive foot process effacement and microvillous transformation of podocytes. Mutation analysis of the β_4- and α_6-integrin genes *ITGB4* and *ITGA6* revealed a homozygous missense mutation in exon 31 of the *ITGB4* gene, resulting in a substitution of tryptophan for arginine at codon 1281. This mutation affects the second fibronectin type III domain which is involved in the interaction with bullous pemphigoid antigen 1 (BPAG1) and plectin. Moreover, in one patient with pyloric atresia, epidermolysis bullosa and nephrotic proteinuria diagnosed at 5 months of age, mutation screening of the *ITGB4* gene revealed a c.4851delCA truncating mutation (Dr. Françoise Broux, Rouen, France; personal communication). The mechanisms by which mutations in the *ITGB4* gene induce glomerular disease remain unknown.

Familial Forms of Steroid-Sensitive Nephrotic Syndrome

The incidence of SSNS in pediatric population ranges between 2 and 7/100,000. Although most of the cases are sporadic, several reports have confirmed the existence of hereditary forms of this disease (1, 284–289). The exact incidence of familial forms of SSNS is unknown, but according to a single survey, it may represent up to 3% of the cases (1). Based on six cases reports describing 58 patients from 21 families, the most common pattern of inheritance was autosomal recessive. There was a male-to-female preponderance of 3 to 1 and the average age of onset was 4 years. Kidney histology revealed minimal change disease. Most of the cases presented with multiple episodes of relapse and achieved complete remission at the end of adolescence (284–289). Analysis of our own cohort consisting of 46 affected cases from 23 families, revealed similar results (Antignac, unpublished data).

At least two attempts to identify a putative disease locus have been performed (288, 289). Ruf et al. performed a genome-wide scan in a consanguineous SSNS kindred allowing the identification of a locus on chr 2p12-p13.2 between markers *D2S292* and *D2S289* (288). More recently, Landau et al. studied an extended SSNS Bedouin family with a high rate of consanguinity (289). A whole genome scan was performed, using 382 microsatellite markers; however, the index family was not linked to any of the presently known loci associated with nephrotic syndrome. It remains unanswered whether the primary defect in hereditary SSNS lies in a gene that plays a central role in the function of the immune system, or in a gene expressed in podocytes.

Conclusion

Hereditary forms of NS are far more common than previously thought 10 years ago, since the discovery of mutations in causative genes in cases with Mendelian inheritance, as well as in patients with sporadic disease. Most of the cases with hereditary forms of NS have a disease onset within early childhood, are resistant to immunosuppressive therapy, and do not relapse after kidney transplantation. The highest rates of mutation detection are in patients presenting with proteinuria in the first year of life and subsequently decrease among older patients.

It is plausible that more complex patterns of inheritance, as has been described in patients bearing bi- or triallelic variants, may be associated with an increased risk of developing NS. Indeed, disease predisposing mutations may lead to variable disease expression and penetrance depending upon unidentified environmental and genetic factors. Moreover, common variants in genes expressed in podocytes may account for an increased risk of FSGS and

ESRD observed in selected ethnic groups, as has been described recently with the *MYH9* gene, in which several haplotypes conferred a major-risk effect for FSGS in individuals of African ancestry (290–292).

The accessibility to custom genotyping chips and deep-sequencing techniques will facilitate the screening of mutations in a broader approach, including clusters of podocyte-specific genes. To-date, several fascinating disorders, such as Galloway-Mowat syndrome, involving brain and kidney development, and familial forms of SSNS, connecting podocyte physiology and the immune system, remain unsolved.

Non-syndromic forms of NS are frequently restricted to mutation in genes exclusively expressed in podocytes at the slit-diaphragm, while the association with extrarenal manifestations is observed in cases carrying mutations in ubiquitously expressed genes, mostly transcription factors or components of the mitochondrial respiratory chain. Nevertheless, individuals with mutations in genes associated with syndromic SRNS may present with a milder phenotype and only with SRNS; thus, making directed mutation screening a difficult task.

A promising therapy, still explored at a basic level, include protein chaperones. These drugs redirect the trafficking of missense mutant proteins to the plasma membrane when abnormally retained in subcellular organelles. Additional encouraging results have been obtained with drugs, which stabilize the podocyte actin cytoskeleton.

It is now clear that genetic diagnosis of cases with SRNS or familial NS is necessary to avoid ineffective therapies, to allow for accurate genetic counseling and, in the future, to offer specific mutation-based therapies.

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28 Idiopathic Nephrotic Syndrome in Children: Clinical Aspects

Patrick Niaudet · Olivia Boyer

In children, the most common cause of nephrotic syndrome is idiopathic nephrotic syndrome (INS), also called nephrosis (1). INS is defined by the combination of a nephrotic syndrome (proteinuria, hypoalbuminemia, hyperlipidemia, and edema) and non-specific histological abnormalities of the kidney including minimal changes, focal and segmental glomerular sclerosis (FSGS), and diffuse mesangial proliferation. Glomeruli show a fusion of epithelial cell foot processes on electron microscopy and no significant deposits of immunoglobulins or complement on immunofluorescence.

Many authors consider minimal change disease, diffuse mesangial proliferation and FSGS as separate diseases because of differences in response to corticosteroids and subsequent clinical course. Indeed, these various pathologic features carry prognostic significance. Patients with FSGS and those with diffuse mesangial proliferation have more frequently hematuria, are often resistant to corticosteroid treatment, progress more often to renal failure and may have a recurrence of the nephrotic syndrome soon after renal transplantation. Recent data have comforted the belief that minimal change disease and FSGS are different entities. FSGS appears to be a podocyte disease (2). The notion of "podocyte dysregulation" (3, 4), the different expression of cyclin-dependent kinase inhibitors in minimal change disease and in FSGS, the role of these cell cycle disturbances leading to podocyte proliferation and maturation (5) and the identification of parvovirus B 19 in glomeruli of patients with FSGS (6, 7) are in favor of distinct entities. Moreover, Streulau et al. found TGF-β1 gene expression in 18 of 20 patients with steroid resistant FSGS and in only 3 of 14 steroid-sensitive patients (8). These data support a sequence of immunologically mediated events that contribute to progressive renal damage in children with FSGS.

In the early stages, FSGS and minimal change disease are indistinguishable (9). A significant number of patients with FSGS respond to corticosteroids, whereas some steroid resistant patients have no sclerotic changes on biopsy specimens containing adequate tissue (10, 11). Therefore, some authors believe that, although histological variants of the INS carry prognostic significance, they cannot at present be considered as separate entities (12).

The term minimal change disease has become synonymous with steroid sensitive INS although renal biopsy is usually not performed in patients who respond to steroid therapy. Indeed, in many centers, renal biopsy is recommended only for those patients who fail to respond to steroids. Consequently, renal biopsy findings in recent published series are not representative of the true incidence of various histopathological categories seen in INS. It is therefore more appropriate to classify the patients according to their response to steroid therapy. Response to steroid therapy carries a greater prognostic weight than the histological features seen on initial renal biopsy. Thus, two types of INS can be defined: steroid-responsive nephrotic syndrome, in which the proteinuria rapidly resolves and steroid-resistant nephrotic syndrome, in which steroids do not induce remission.

Epidemiology

The incidence of INS varies with age, race, and geography. The annual incidence in children in the USA and in Europe has been estimated to 1–3 per 100,000 children below age of 16 (13–15), with a cumulative prevalence of 16 per 100,000 children. Similar figures were recently reported from New Zealand (16). Geographical and/or ethnic differences are well known. In the United Kingdom for example, the incidence of INS is sixfold greater in Asian than in European children (17); this is also true for Indians (18), for Japanese and SouthWest Asians. INS is less frequent in Africa (19–21). Such differences underline the role of genetic as well as environmental factors in the pathogenesis of the disease.

Whereas INS accounts for only 25% of adult cases (22), it is by far the most common cause of nephrotic syndrome in children. Almost all nephrotic children between 1 and 6 years of age in Western countries suffer from INS. The International Study of Kidney Disease in Children found minimal change disease in 76.6% of children with primary nephrotic syndrome (13).

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There is a male preponderance in children, with a male:female ratio of 2:1 (13, 23), but both sexes are similarly affected in adolescents.

The familial occurrence of INS is well known (see chapter "Genetic").

Associated Disorders

INS is, by definition, a primary disease. Nevertheless, in a number of cases, an upper respiratory tract infection, an allergic reaction, or another factor may immediately precede the development or relapse of the disease.

Many agents or conditions have been reported to be associated with INS such as infectious diseases, drugs, allergy, vaccinations, and malignancies (*Table 28-1*). The question remains whether these factors are real causes, a simple coincidences, or precipitating agents.

Table 28-1

Conditions associated with idiopathic nephrotic syndrome

Allergy
Pollen
Fungi
Cow's milk
House dust
Bee stings
Cat fur
Poison ivy
Drugs
Nonsteroidal antiinflammatory drugs
Ampicillin
Gold
Lithium
Mercury
Trimethadione
Malignancies
Hodgkin disease
Non-Hodgkin lymphoma
Colon carcinoma
Bronchogenic carcinoma
Others
Viral infection
Kimura's disease
Diabetes mellitus
Myathenia gravis
Immunization

Allergy is associated with up to 30% of cases (24–26). Among a list of anecdotal cases, the allergens reported include fungi, poison ivy, ragweed pollen, house dust, jellyfish stings, bee stings and cat fur. A food allergen may be responsible for relapses of steroid-sensitive nephrotic syndrome, such as cow's milk and egg. Laurent et al. evaluated the effect of an oligoantigenic diet given for 10–15 days to 13 patients. This diet coincided with improvement of proteinuria in nine, including complete remission in five (27).

The association between minimal change disease and malignancies mainly concerns lymphomatous disorders: Hodgkin's disease and non-Hodgkin's lymphomas (28, 29). The nephrotic syndrome may be the presenting feature of the disease. It usually disappears after successful treatment of the malignancy. Other types of neoplasia may also be associated with INS as colon carcinoma, bronchogenic, small-cell carcinoma (30).

Eosinophilic lymphoid granuloma in orientals (Kimura's disease) has also been reported in association with INS (31–33).

Several cases of minimal change disease have been reported in association with the onset of insulin-dependent diabetes mellitus. The disease is usually responsive to corticosteroids and follows a relapsing course.

Clinical and Biological Features

The disease may occur during the first year of life, but it usually starts between the ages of 2 and 7 years, with a male to female ratio of 2/1. The onset is often preceded by an upper respiratory tract infection. The disease is characterized by a sudden onset, edema being the major presenting symptom. It becomes clinically detectable when fluid retention exceeds 3–5% of body weight. Periorbital edema frequently misdiagnosed as allergy, is often the initial symptom. Edema is gravity dependent, localized to the lower extremities in the upright position, and to the dorsal part of the body in reclining position. This edema is white, soft, and pitting, keeping the marks of clothes or finger pressure. Anasarca may develop with ascites, and pleural and pericardial effusions. Although there may also be abdominal distension, dyspnoea is rare. Periorbital edema may limit eye opening and edema of the scrotum and penis, or labiae, may be seen. A rapid formation of ascites is often associated with abdominal pain and malaise: these symptoms may also be related to concomitant hypovolemia. Abdominal pain is occasionally due to a complication such as peritonitis, thrombosis or, rarely, pancreatitis. Cardiovascular shock is not unusual,

secondary to the sudden fall of plasma albumin, with abdominal pain and symptoms of peripheral circulatory failure with cold extremities and hypotension. Emergency symptomatic treatment is needed. Blood pressure is usually normal but sometimes elevated (13, 34).

The nephrotic syndrome is occasionally discovered during a routine urine analysis. Macroscopic hematuria is observed in a few cases (34). The disease may also be revealed by a complication. Peritonitis due to *Streptococcus pneumoniae* is a classical mode of onset (35). Deep-vein or arterial thromboses and pulmonary embolism may also occur during the first attack or during a relapse.

Urinalysis

Proteinuria is detected by dipstick testing 3 or 4+. Quantitative evaluation gives figures ranging from less than 1 g to more than 10 g/day. The nephrotic range proteinuria is defined as >50 mg/kg/day or 40 mg/h/m^2 but the mean value during the first days may be higher as the urinary concentration of proteins also depends on the plasma albumin concentration. In young children it may be difficult to perform 24-h urine collection and urinary protein/creatinine ratio or U albumin/U creatinine ratio in untimed urine specimens are useful. For these two indices the nephrotic range is 200–400 mg/mmol (36).

In most cases, proteinuria is highly selective, consisting of albumin and lower molecular weight proteins. The selectivity of proteinuria may be appreciated by polyacrilamide gel electrophoresis or by the evaluation of the Cameron index which is the ratio of IgG (MW 150 kDa) to transferrin (80 kDa) clearances. A favorable index would be below 0.10, or better below 0.05; a poor index is above 0.15 or 0.20. Such poor index is more often associated with FSGS. However, there is a considerable overlap in results and the test has limited value. The amount of protein excreted in the urine does not reflect the quantity of protein crossing the glomerular basement membrane since a significant amount is reabsorbed in the proximal tubule. Some children with severe steroid resistant nephrotic syndrome and tubulo-interstitial lesions have both glomerular and tubular proteinuria with an increased excretion of β-2 microglobulin, retinol binding protein and lysozyme due to an impaired protein reabsorption in the proximal tubule.

The urine sediment of patients with INS often contains fat bodies. Hyaline casts are also usually found in patients with massive proteinuria, but granular casts are not present unless there is associated acute renal failure and acute tubular necrosis. Macroscopic hematuria is rare, occurring in 3% of patients. Microscopic hematuria is present in 20% of cases and has no influence on the response to steroid therapy.

Urinary sodium excretion is low (<5 mmol/24 h), associated with sodium retention and edema. Kaliuresis is usually higher than natriuresis, but it may be reduced in oliguric patients.

Blood Chemistry

Serum proteins are markedly reduced and serum lipid usually increased. Proteinemia is below 50 g/l in 80% of patients, and below 40 g/l in 40%. Albumin concentration usually falls below 20 g/l and may be less than 10 g/l. Electrophoresis shows not only low albumin levels but also increased α-2 globulins and, to a lesser extent, β-globulins, while γ-globulins are decreased. IgG is markedly decreased, IgA slightly reduced, IgM is increased, while IgE is normal or increased. Among other proteins, fibrinogen and β-lipoproteins are increased and antithrombin III is decreased.

Hyperlipidemia is a consequence of (I) an increased hepatic synthesis of cholesterol, triglycerides and lipoproteins, (II) a decreased catabolism of lipoproteins due to a decreased activity of lipoprotein lipase which normally transforms VLDL to LDL via IDL and (III) a decreased LDL receptor activity and an increased urinary loss of HDL (37, 38). Total cholesterol and LDL cholesterol are elevated while HDL cholesterol remains unchanged or low, particularly HDL2, leading to an increased LDL/HDL cholesterol ratio (39). Patients with severe hypoalbuminemia have increased triglycerides and VLDL. Apoproteins, apo B, apo CII, apo CIII are also elevated. The levels of lipoprotein Lp(a) are elevated in nephrotic patients which further contribute to an increased risk of cardiovascular and thrombotic complications.

Serum electrolytes are usually within the normal range. A low sodium level may be related to dilution from inappropriate renal retention of water due to hypovolemia and inappropriate antidiuretic hormone secretion. The mild reduction of plasma sodium concentration is often an artifact related to hyperlipidemia. Serum potassium may be high in oliguric patients. Serum calcium is consistently low as a result of hypoproteinemia. Ionized calcium is usually normal but may be decreased due to urinary loss of 25-hydroxyvitamin D3 (40) and normal but inappropriate levels of calcitriol (41). Blood urea nitrogen and creatinine concentrations are usually within the normal range, or slightly increased in relation to a modest reduction in the glomerular filtration rate (GFR).

A few patients with FSGS and a poor subsequent outcome present with a Fanconi syndrome: glycosuria, aminoaciduria, urinary bicarbonate loss, and hypokalemia (42). A defect in urinary acidification has also been reported (43).

Hematology

Hemoglobin levels and hematocrit are increased in patients with plasma volume contraction. Anemia with microcytosis may be observed, probably related to urinary loss of siderophilin. The urinary loss of erythropoietin may also contribute to anemia (44). Thrombocytosis is common and may reach 5.10^8 or 10^9/l.

Complications

Hypovolemia

A few children are severely hypovolemic and this complication is observed typically early during a relapse (*Table 28-2*). Sepsis, diarrhea or diuretics may precipitate hypovolemia. These children often complain from abdominal pain, have low blood pressure and cold extremities. Hemoconcentration with a raised hematocrit accompanies hypovolemia.

Acute Renal Failure

Renal function is usually within normal limits at presentation. A reduction of the GFR, secondary to hypovolemia, infection or thrombosis is frequent (45, 46). A reduced GFR may be found in patients with normal effective plasma flow. Bohman et al. showed a close relationship between the degree of foot process fusion and both GFR and filtration fraction, suggesting that fusion of foot processes could lead to a reduction of glomerular filtering area and/or of permeability to water and small solutes (47). This reduction is transitory, with a rapid return to normal after remission. Van de Walle et al. found that changes in glomerular permeability may have a major role in acute renal failure (46).

Marked oliguria may occur in children (48). Oliguric renal failure may be the presenting symptom. Renal failure may be secondary to bilateral renal vein thrombosis, which is recognized by sonography or to interstitial nephritis which has been reported, especially with furosemide. Skin rash and eosinophilia are suggestive of this diagnosis.

Acute renal failure is usually reversible, often with high dose furosemide induced diuresis, especially with intravenous infusion of albumin (49). In some cases, where glomerular structure is normal on initial histology, renal failure may last for as long as a year (50) and sometimes be irreversible (51).

Table 28-2
Nephrotic hypovolemia

Clinical features	Precipitating factors
Abdominal pain	Severe relapse
Hypotension	Infection
Sluggish circulation	Diuretics
Relative polycythemia	Paracenthesis
Acute tubular necrosis	Diarrhea
Thrombosis	

Chronic Renal Failure

The main difference between responders and nonresponders is the tendency of the latter to develop end-stage renal failure, which is seen in less than 3% of responders, even in the highly-selected series. This complication occurs in 50% or more of the steroid resistant patients after a follow-up of 10 years. The only "benefit" of the decrease of GFR is the improvement of the nephrotic syndrome due to a decrease of proteinuria.

We have retrospectively analyzed in the Enfants Malades series the outcome of 181 children with steroid resistant INS who have been followed for at least 5 years. Eighty-five percent were primary nonresponders and 15% late nonresponders. Initial renal biopsy had shown minimal changes in 62 cases and FSGS in 119 cases. Renal survival rates were 65% at 5 years, 50% at 10 years and 34% at 15 years. Interestingly, the rate of progression to end stage renal failure was similar in patients with minimal changes or FSGS on initial biopsy.

The data reported in other series are difficult to compare as most of them deal with patients with FSGS. The Southwest Pediatric Nephrology Study group reported 75 children with FSGS followed for periods of 7–217 months (52). Twenty-one percent had progressed to end stage renal failure, 23% had decreased glomerular filtration rate, 37% had a persistent nephrotic syndrome and 11% were in remission. Paik et al. retrospectively analyzed 92 children with steroid resistant FSGS and found renal

survival rates at 5, 10 and 15 years of 84, 64 and 53% respectively (53). Poor pronostic factors of chronic renal failure were asymptomatic proteinuria at presentation, initial renal failure and higher proportion of glomeruli with segmental sclerosis.

Progression to end stage renal failure has been reported to be more rapid in patients of African or Hispanic descent when compared with Caucasians. Ingulli and Tejani found that among 57 African American and Hispanic children, 50% of them had reached end stage renal failure within 3 years and 95% had reached this stage after 6 years (54). In addition, among the children with INS, the proportion of those with steroid resistant FSGS tends to be more important in African American and Hispanic children.

Growth

Growth may be severely affected in children with persistent nephrotic syndrome. Depletion of hormones due to urinary losses is a possible cause of stunting. Hypothyroidism related to urinary loss of iodinated proteins has been observed and may be corrected (55). A low plasma IgF1 and IgF2 level associated with a urinary loss of the carrier proteins has also been reported (56).

Infections

Bacterial infections are frequent in nephrotic children (*Table 28-3*). Sepsis may occur at the onset of the disease. The most common infection is peritonitis, often with *Streptococcus pneumoniae* (57). Other organisms may be responsible: *E. coli*, streptococcus B, *Haemophilus influenzae* and other gram negative organisms. Apart from peritonitis, children may develop meningitis, pneumonitis and cellulitis. Several factors may explain the propensity of nephrotic children to develop bacterial infections: low IgG levels due to an impaired synthesis, urinary loss of factor B and impaired T lymphocyte function. Factor B is a cofactor of C3b of the alternative pathway of complement which has an important role in opsonization of bacteria such as *Streptococcus pneumoniae*.

Viral infections may be observed in patients receiving corticosteroids or immunosuppressive agents. Chickenpox is often observed in these young children and may be life-threatening if acyclovir is not started rapidly. Interestingly, measles infection may induce long-lasting remissions.

Thrombosis

Nephrotic patients are at risk of developing thromboembolic complications. Arterial thrombosis is less frequent (19–27%) compared to venous thrombosis (73–81%) (58, 59). Several factors contribute the increased risk of thrombosis, including hypercoagulability state, hypovolemia, immobilization and infection (*Table 28-4*). A number of hemostatic abnormalities have been described in nephrotic patients: increase in platelet aggregability, increase in fibrinogen, factors V, VII, VIII, X and XIII while the levels of anti-thrombin III, heparin cofactor, protein C, protein S, factors XI and XII are decreased, increase in the fibrinolytic system components such as tPA, PAI-1 (58). The incidence of thromboembolic complications in nephrotic children is close to 3%. However this percentage may be underestimated as shown by systematic ventilation-perfusion scans showing defects consistent with pulmonary embolism in 28% of patients with steroid dependent INS (60). Pulmonary embolism should be suspected in cases of pulmonary or cardiovascular symptoms; this may be confirmed by angiography or angioscintigraphy. Renal vein thrombosis should be suspected in cases with sudden macroscopic hematuria or acute renal failure. Doppler ultrasonography shows an increase in kidney size and the absence of blood flow in

Table 28-3

Infections in nephrotic syndrome

Clinical syndrome	Risk factors
Pneumococcal peritonitis	Low IgG
Haemophilus infection	Low factor B
Gram negative sepsis	Edematous tissue
Staphylococcus cellulitis	Impaired lymphocyte function
	Corticosteroids
	Immunosuppressive drugs

Table 28-4

Thrombosis in nephrotic syndrome

Clinical syndrome	Risk factor
Pulmonary emboli	Hypovolemia
Pulmonary artery thrombosis	Hyperviscosity
Cerebral venous thrombosis	Low anti-thrombin III
Renal vein thrombosis	High fibrinogen
Peripheral venous	Platelet hyperaggregability
Artery thrombosis	Hyperlipemia

the renal vein. Thrombosis may affect the arteries such as pulmonary arteries or other deep veins.

Renal Biopsy

Renal biopsy is usually not indicated before starting corticosteroid therapy. It is only indicated in children less than 1 year of age, when the child has macroscopic hematuria or hypertension or low C3 levels or persistent renal failure and when the child fails to respond to corticosteroid therapy. A renal biopsy may be indicated in patients who relapse before considering alternative therapy, namely anticalcineurin agents.

Light microscopy shows three morphological patterns: minimal changes, diffuse mesangial proliferation, and FSGS.

Minimal Change Nephropathy

On light microscopy, glomeruli may be normal with normal capillary walls and normal cellularity (*Fig. 28-1*). Swelling and vacuolation of epithelial cells and a slight increase in mesangial matrix are often observed. A mild mesangial hypercellularity may be noted (61) as well as scattered foci of tubular lesions and interstitial fibrosis.

Ultrastructural changes are always present, involving podocytes and mesangial stalks. Podocyte foot process fusion is generalized and constant (*Figs. 28-2* and *28-3*); its extent is closely related to the degree of proteinuria (62). Other epithelial changes consist of microvillus formation and the presence of numerous protein reabsorption droplets. The glomerular basement membranes are normal with no parietal deposits.

Figure 28-1

Minimal change disease. Glomeruli appear normal by light microscopy with no tubulointerstitial lesions.

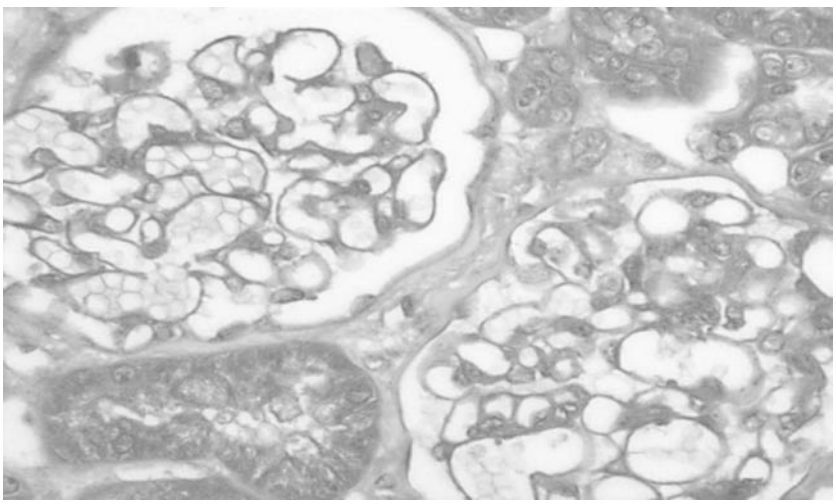
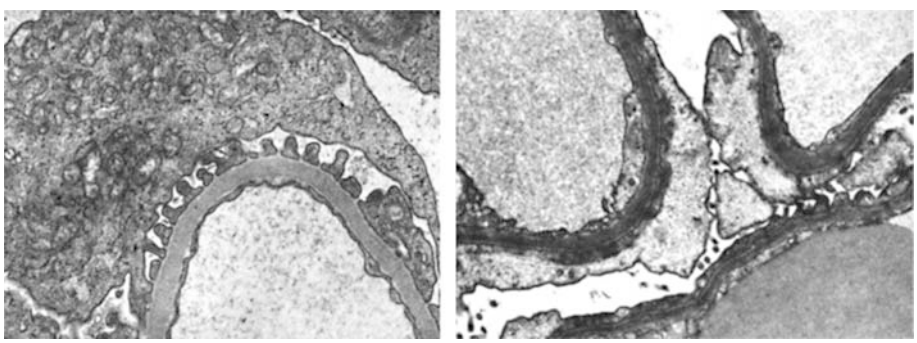


Figure 28-2

Electron microscopy. On the *left*, normal aspect with the podocyte foot processes attached to the glomerular basement membrane. On the *right*, minimal change disease with effacement of foot processes.



The endothelial cells are often swollen (63). Mesangial alterations include mesangial cell hyperactivity, increased mesangial matrix, and occasionally finely granular, osmiophilic deposits located along the internal side of the basement membrane. These ultrastructural alterations are nonspecific and are probably related to massive proteinuria.

Diffuse Mesangial Proliferation

Some patients with steroid resistant INS show a marked increase in mesangial matrix associated with hypercellularity (*Fig. 28-4*) (61, 64, 65). However, peripheral capillary walls are normal, and immunofluorescence microscopy is negative. Electron microscopy shows foot process fusion similar to the changes observed in minimal change disease. The presence of mesangial hypercellularity has been found to have prognostic significance with a higher rate of progression to renal failure (64) but these findings were not confirmed by other authors (52, 66).

Focal and Segmental Glomerular Sclerosis

The glomerular lesions affect a variable proportion of glomeruli (10, 61). The focal changes are limited to a part of the tuft, the other capillary loops showing no modification. The lesions always predominate at the corticomedullary junction (67). The segmental lesion affects a few capillary loops which stick together either at the

Figure 28-3

Scanning electron microscopy showing the normal aspect of podocytes with their foot processes on the *left* and their effacement in minimal change disease on the *right*.

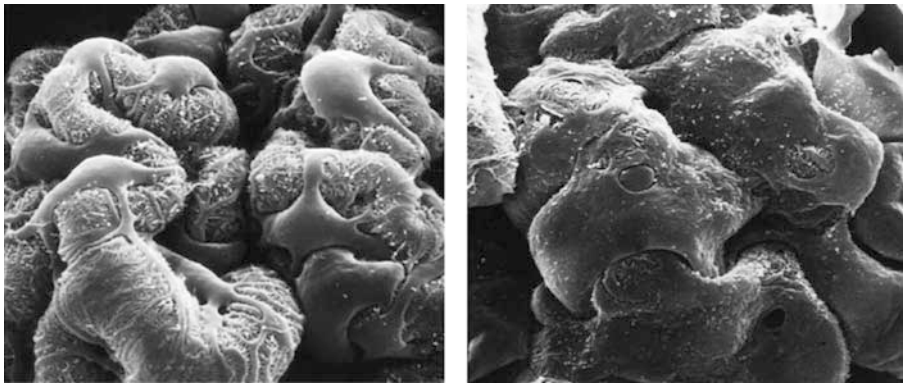
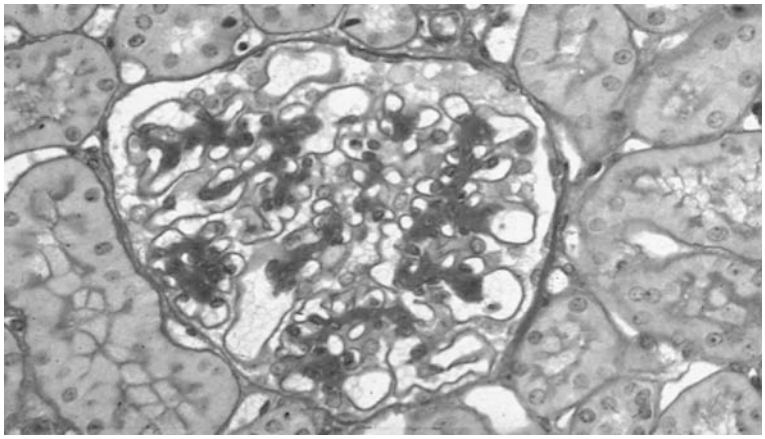


Figure 28-4

Diffuse mesangial proliferation with an increased number of mesangial cells and mesangial matrix.



hilum or at the periphery of the tuft, or at both (Fig. 28-5) (68, 69). The clinical course has been found to be more benign when the location of these sclerotic lesions is peripheral (the tip-lesion), although such findings have not been confirmed by other authors (67, 70–72). Hyaline material is often present within the sclerotic lesions. A clear "halo" zone is observed at the periphery of the sclerotic segments (Fig. 28-6). The segmental lesion has a different aspect depending on whether it affects a group of capillary loops free in Bowman's space or is adherent to Bowman's capsule. The "free" sclerotic segments are always surrounded by a "crown" of flat or hypertrophied podocytes. The podocytes form a continuous layer overlying the damaged areas of the tuft and in close apposition to the clear "halo." When the sclerotic lesion is adherent to Bowman's capsule, there is a direct synechia between the collapsed capillary loops and Bowman's basement membrane. The rest of the tuft and the nonsclerotic glomeruli show either "minimal changes" or "diffuse mesangial proliferation"

Figure 28-5

FSGS. In early stage, there is a segmental collapse of glomerular capillaries surrounded by hypertrophic podocytes containing intra-cytoplasmic vacuoles.

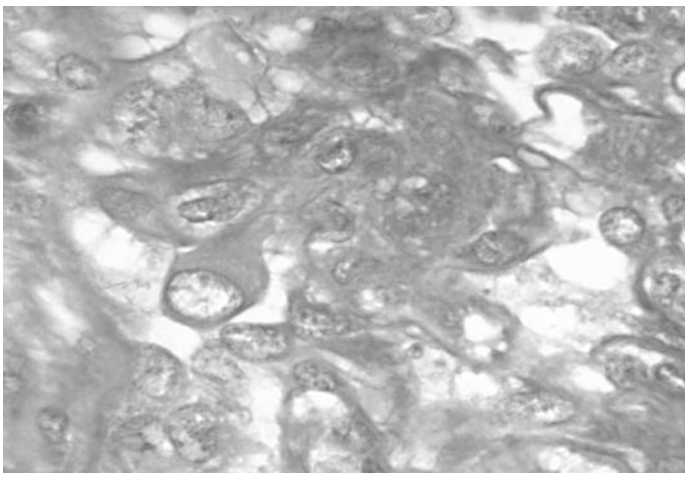
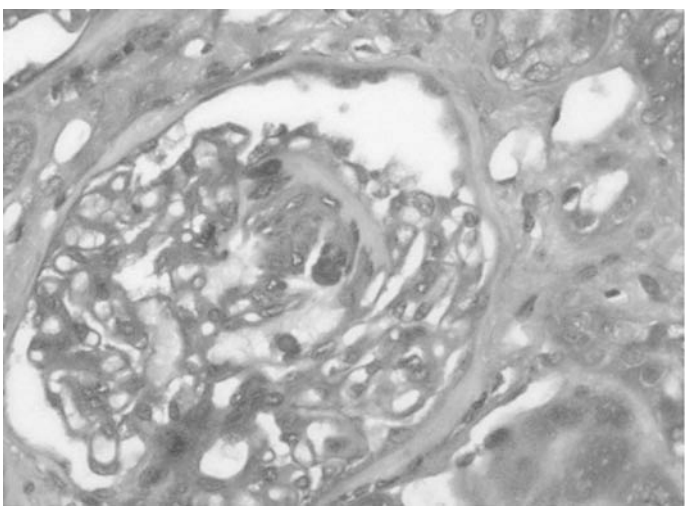


Figure 28-6

FSGS. Segmental lesion of the tuft characterized by the deposition of hyaline material at the inner side of the glomerular basement membrane with a ring of podocytes separated from the glomerular basement membrane by a clear ''halo.''



both with foot process fusion. Glomerular hypertrophy is common in FSGS, and when such hypertrophy is found in minimal change disease, it is somewhat predictive of further development to FSGS (73, 74).

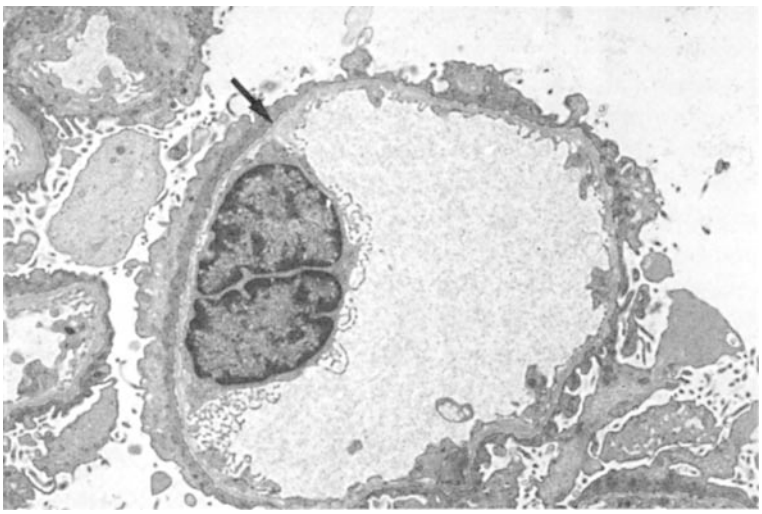
Tubular atrophy and interstitial fibrosis are often present and apparently proportional to the glomerular damage (10, 75). Focal glomerular lesions should therefore be suspected when focal tubular and interstitial changes are found associated with minimal glomerular changes. Erkan et al. found apoptosis in proximal and distal tubular cells of children with idiopathic FSGS (76). There was a correlation between the degree of proteinuria and the number of apoptotic cells. An elevated tubule cell apoptosis rate at the time of initial biopsy was found to be an independent predictor of progression to end stage renal disease.

On electron microscopy, the lesion is characterized by the presence of paramesangial and subendothelial, finely granular, osmiophilic deposits (75, 77, 78) with either disappearance or swelling of endothelial cells, and an increase in mesangial matrix material (*Fig. 28-3*). Fatty vacuoles may be seen, either in the middle of the abnormal deposit or in the cytoplasm of endothelial and mesangial cells. The peripheral synechia, located between podocytes and basement membrane, is formed by the apposition of acellular material in which thin and irregular layers of newly formed basement membranes are visible. Modifications of the podocytes consist of focal cytoplasmic degeneration, breakdown of cell membranes, and detachment of epithelial cells from basement membranes, with filling of the resulting space by cell debris and new membranes (79).

A pathologic classification of FSGS has been proposed with five histologic variants: FSGS not otherwise specified (NOS), perihilar variant, cellular variant, tip variant, and collapsing variant (80). All variants share podocyte alterations. This classification may have clinical implications in terms of response to therapy and risk of progression to renal failure. For example, glomerular tip lesions have been associated with better outcomes and collapsing variant with worse outcomes (71, 72). The glomerular tip lesion was found to be a predictor of a favorable response to therapy in a pediatric series (81). At present, this classification has no implication for treatment options.

A subgroup of patients have collapsing focal segmental glomerular sclerosis characterized by a global collapse of the glomerular capillaries with marked hypertrophy of epithelial cells (*Fig. 28-7*) (82). These patients have a severe nephrotic syndrome and rapidly progress to renal failure. The rate of response to steroids is poor. The incidence of collapsing glomerulopathy seems to have increased in the recent years. The main cause of secondary collapsing glomerulopathy is HIV associated nephropathy (83). Many authors consider that "collapsing glomerulopathy" is a distinct form of FSGS which may be "idiopathic," also observed in patients with recurrent nephrotic syndrome after renal transplantation, associated with HIV, parvovirus B19 infection or CMV infection (84, 85). Idiopathic

Figure 28-7
Collapsing glomerulopathy.



collapsing glomerulopathy predominates in blacks and has a poor prognosis (86).

FSGS is characterized by important changes in the podocytes, with major cell cycle derangement (3, 4). The normal mature podocyte does not divide and does not express proliferative markers such as PCNA and Ki-67. The podocyte express several cell surface proteins such as WT-1, C3b receptor, glomerular epithelial protein-1 (GLEPP-1), podocalyxin, synaptopodin and vimentin. The first stages of FSGS are characterized by the loss of the cell surface proteins (de-differentiation) and the expression of macrophage markers and cytokeratin (trans-differentiation). Proliferations markers (PCNA and Ki-67) are expressed, indicating a mitotic activity. This "podocyte dysregulation" is accompanied by podocyte detachment from the glomerular basement membrane.

FSGS is an irreversible scarring process in the glomeruli, as shown by the analysis of repeat biopsies (77, 78, 87). Studies in experimental animals (88) as well as in nephrotic patients have shown that proteinuria precedes the development of focal sclerotic lesions. The same sequence was reported in patients with recurrence of the disease after transplantation. Within weeks following recurrence of proteinuria podocytes observed by electron microscopy appear swollen and vacuolated. The podocytes exhibit strong mitotic activity, with multinucleation and expression of the PCNA and Ki-67 proliferation markers.

FSGS is not a specific histopathological lesion: similar alterations may be seen in persistent idiopathic proteinuria, heroin-associated nephropathy and, independently, in association with HIV infection, Alport's syndrome, hypertension, pyelonephritis, and obesity. It has also been reported in renal hypoplasia with oligomeganephronia, after partial nephrectomy and in other conditions with a reduction in nephron number, including reflux nephropathy or obstructive uropathy.

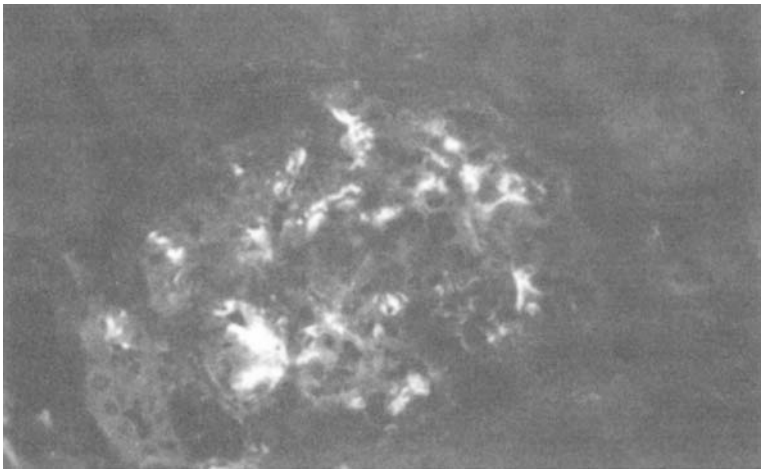
IgM Associated Nephropathy

Immunofluorescence microscopy is usually negative (89, 90). However, mesangial deposits of IgM, IgG, C3 and more rarely IgA have been reported. The most commonly found is IgM (*Fig. 28-8*) and Cohen et al. found that patients with IgM deposits in the mesangium had a poor response to corticosteroids (91). Other studies did not confirm these findings. Habib et al. reported on immunofluorescence studies in a series of 222 children with INS (92). Although IgM was the immunoglobulin most frequently present in the glomeruli (54 of 222), there was no correlation between IgM deposits, the initial response to steroid therapy or the final outcome. IgM deposits have also been described in association with diffuse mesangial proliferation and with FSGS.

A number of patients with INS display mesangial deposits of IgA. They are classified by some as IgA nephropathy. Others consider that mesangial IgA in patients with nephrotic syndrome and minimal changes, that is, without cellular proliferation is coincidental (92–94). This applies to INS associated with mesangial IgA deposits and explains a rapid response to steroids, which would not be the case in true IgA nephropathy.

Figure 28-8

INS with minimal changes. Diffuse mesangial deposits of immunoglobulin M.



Relationship Between the Different Histological Patterns

Repeat renal biopsies in patients with INS have shown morphological transition between the three main histological patterns. Some patients with clear evidence of minimal changes on initial biopsy have FSGS on a second biopsy. A high proportion of these patients are steroid-resistant. Tejani found that FSGS lesions had developed in 60% of 48 patients with steroid resistant minimal change disease, in association with aggravation of symptoms (95). The progression to sclerosis may occur from minimal change or from mesangial proliferation (64, 96). Conversely, some patients who show diffuse mesangial proliferation, whether or not associated with focal sclerosis on initial biopsy, may loose hypercellularity with time and show minimal change or FSGS on repeat biopsy (52).

In conclusion it may be considered that, at least in children, minimal change disease, FSGS, and diffuse mesangial proliferation represent histological variations of INS which may be found alone or in any combination on sequential biopsies in the same patient.

Clinicopathologic Correlations

The relative frequencies of the three histologic patterns differ in steroid sensitive and steroid resistant patients. A report of the International Study of Kidney Disease in Children showed that among 354 patients with INS who had an initial response to prednisone, 95.5% had minimal change disease, 3% had FSGS and 1.5% had diffuse mesangial proliferation (97). Conversely, among 55 patients who had failed to respond to prednisone, 45.5% had minimal change disease, 47.5% had FSGS and 7% had diffuse mesangial proliferation. This study also analyzed the numbers of responders and nonresponders within each histologic category. Among the 363 patients with minimal changes, 91.8% were responders and 6.9% nonresponders whereas among the 37 patients with FSGS, 29.7% responded to prednisone and 70.3% did not. Waldherr et al. found that only 2 out of 36 patients with diffuse mesangial proliferation responded to corticosteroids (64). An increase in steroid resistance has recently been reported in patients with FSGS (98).

Pathophysiology

Mechanisms of Proteinuria

In normal individuals, the clearance of albumin is about 1% of that of neutral proteins with similar molecular weight, such as polyvinylpyrrolidone or dextran. Similarly, the clearance of neutral dextran is higher than that of anionic sulfate dextran of similar molecular weight. These data indicate that the permeability of the glomerular basement membrane (GBM) is determined not only by the size but also by the charge of the protein. It is believed that the anionic charge of the glomerular basement membrane is responsible for the charge selectivity of filtration. The anionic (negative) charges of the glomerular basement membrane repulse the negatively charged albumin molecules, whose isoelectric point is 4.6.

The mechanism of proteinuria in the absence of histological alterations on light microscopy has suggested an electrochemical disorder of the GBM. Indeed it was shown that the glomerular K_f is diminished despite increased permeability to serum albumin. Using polyvinylpyrrolidone (99) or dextrans (100) with Einstein–Stokes radii between 2.0 and 4.8 nm as test macromolecules, the pore-size of the GBM was shown to be reduced contrasting with massive albuminuria. This suggested a loss of glomerular negative charges. Kitano et al., using polyethylamine as a cationic probe, reported a decrease in the anionic charges of the GBM in minimal change disease (101). Carrie et al. studied renal biopsy sections stained by colloidal iron and showed that its glomerular uptake was markedly reduced (100). A reduced sialic acid content in the GBM, as sialic acid residues may be responsible for glomerular negative charges (102).

Van den Born et al. produced a mouse monoclonal antibody to partially purified heparan sulfate proteoglycan isolated from rat glomeruli (103). By indirect immunofluorescence, the monoclonal antibody bound to the GBM on rat kidney sections. By electron microscopy a diffuse staining of the GBM was observed. After intravenous injection, the monoclonal antibody was localized along the GBM with a granular staining, and 1 day later in the mesangium with a concomitant decrease in staining along the GBM. By electron microscopy, 1 h after injection, the antibody was bound mainly to the inner side of the GBM. Intravenous injection of this antibody in rats resulted in selective proteinuria. This model shows that neutralization of heparan sulfate anionic charges may contribute to albuminuria.

Levin et al. and Boulton-Jones et al. presented data indicating that loss of negative charges was not restricted to the glomeruli but was also found on erythrocyte and platelet membranes, as shown by reduced binding of Alcian blue, a cathionic dye (104, 105). A cathionic protein is found in the plasma and the urine of patients in relapse.

The Immune System in Steroid-Responsive INS

In 1974, Shalhoub postulated that INS might be secondary to a disorder of T-lymphocyte function (106). He hypothesized that the expansion of a T-lymphocyte clone might results in the production of a lymphokine, which increases the permeability of the glomerular filtration barrier to proteins. The arguments supporting this hypothesis were the response of the disease to corticosteroids and to alkylating agents, the remission occurring in association with measles, which depresses cell-mediated immunity, the susceptibility of patients to pneumococcal infections and the occurrence of minimal change nephrotic syndrome in patients with Hodgkin's disease. There are other arguments for the role of a circulating permeability factor produced by mononuclear cells in the pathogenesis of the disease (107–109). The immediate recurrence of proteinuria after renal transplantation in some patients (110), the disappearance within few weeks of proteinuria when a kidney from a patient with MCD has been transplanted in a patient without nephrotic syndrome (111), the development of transient neonatal proteinuria and hypoalbuminemia in two children born to a woman with steroid resistant FSGS that disappeared within 2 and 3 weeks respectively (112), the onset of proteinuria in rats following the injection of serum taken from patients with recurrence after renal transplantation (113). The Buffalo/Mna strain of rats spontaneously develops proteinuria with FSGS at 2 months of age. Le Berre et al. found that the nephrotic syndrome recurs when Buffalo/Mna rats receive a kidney from a healthy LEW.1W rat (114). Conversely, proteinuria and renal lesions regress when kidneys from a Buffalo/Mna rat are transplanted into normal LEW.1W rats. Although recurrence of proteinuria is not immediate after transplantation, this model may be helpful to clarify the pathogenesis of the disease and the mechanisms of recurrence after transplantation in man. However, this putative factor has not yet been identified, and it is currently unclear whether INS may be caused by several different factors, and whether this or these factors are identical in MCD and FSGS (115).

Lagrue et al. first described the vascular permeability factor (VPF), a lymphokine found in the supernatant of concanavalin A-activated lymphocytes from patients with MCD which enhances vascular permeability when injected intradermally in the guinea pig (116). Heslan et al. showed that VPF was produced by T lymphocytes and was distinct from interleukin-2 (117, 118). Maruyama et al. showed that cyclosporine at concentrations ranging from 100 to 250 ng/ml was able to suppress the in vitro production of VPF by mononuclear cells from patients with MCD (119). VPF was also found in other diseases such as IgA nephropathy. Tanaka et al. found that the supernatants of concanavalin A-activated lymphocytes from patients with MCD or FSGS induced a marked proteinuria when injected in the renal artery of rats together with a reduction of the anionic charges of the GBM (120). Koyama et al. described a glomerular permeability factor (GPF) in the supernatant of T cell hybridoma derived from the fusion between peripheral T lymphocytes of a patient with MCD and a T cell line, CCRF-HSB2 (121). The GPF was identified by the ability of the supernatant to induce a proteinuria when injected intravenously in the rat. In addition, rats injected with the supernatants show a partial fusion of foot processes of glomerular epithelial cells and no immune deposits. GPF is different from the other known lymphokines. Its molecular weight is between 60 and 160 kDa.

Savin et al. found in some patients with FSGS the presence of a serum factor which increases the albumin permeability of isolated rat glomeruli. The presence of the factor was strongly predictive of the recurrence of proteinuria after renal transplantation (109). A vascular permeability factor, which induces proteinuria when injected into the renal artery of rats, was found in the serum from a transplanted patient who had recurrence of the nephrotic syndrome (113), but many similar attempts to isolate such a factor have failed. Dantal et al. treated patients who had recurrent nephrotic syndrome with plasma protein-A adsorption (122). The administration to rats of material eluted from the protein A columns increased urinary albumin excretion. The active fraction had a molecular weight below 100,000 Da. The factor or factors that may be responsible for recurrent nephrotic syndrome after transplantation seem to be bound to an immunoglobulin (123).

Proteomic analysis of the fractionated serum from children with FSGS identified ten proteins that maintain increased glomerular permeability to albumin including: fibulin, clusterin (apo J), vitronectin, albumin isoforms, γ chain of fibrinogen, and mannan-binding lectin-associated serine protease (124).

Several lymphokines may play a pathogenic role (*Table 28-5*) (125). Increased interleukin-2 levels have been found in lymphocyte culture supernatants from patients with INS and interleukin-2 can induce proteinuria and a reduction of the anionic sites of the GBM when injected into the rat kidney (126). A nephrotic syndrome has been described in several patients treated with

Table 28-5

The role of lymphokines in idiopathic nephritic syndrome (reviewed in (133))

	IL-1	IL-2	IL-2R	INF-γ	IL-4	IL-6	IL-8	IL-10	IL-12	IL-13	IL-18	TNF-α	TGF-β	VPGF
Cytokine levels in serum of INS patients in relapse	N (134–136)	N (136–138) ↑ (135)	N (137, 139) ↑ (135, 140–142)	N (136, 138) ↑ (135, 137)	N (137, 138) ↓ (135)		N (143) ↑ (137, 144) ↓ 2 (135)	N (135, 138)				N (135, 136, 138) ↑ (134)		
Cytokine levels in serum of INS patients in remission	N (134, 135)	N (135, 137, 138)	N (135, 137, 139, 140, 142, 145)	N (135, 138) ↑ 4 (137)	N (135, 138) ↑ 4 (137)		N (135, 138, 144)	N (135, 138)				N (134, 135, 138)		
Cytokine levels in culture supernatants		N (136, 146) ↑ (137, 139, 147) ↓ (148, 149)	↑ (139) ↓ (142)	N (136, 137, 150) ↑ (139) ↓ (149, 150)	N (149, 151) ↑ (137, 139, 151, 152)	N (134)	N (137)	↑ (139, 149) ↓ (153)	↑ (154)		N (150) ↑ (155)	↑ (134, 136, 139, 156)		
Cytokine levels in culture supernatants from INS patients in remission		N (137, 139, 146) ↑ (149) ↓ (148)	N (139, 142)	N (137, 139, 150) ↓ (149)	N (137, 139) ↓ (149)	N (134)	N (137)	N (139, 153) ↓ (149)	N (150, 154)			N (134, 156) ↑ (139)		
Cytokine mRNA expression in INS patients in relapse		N (127) ↑ (138)		N (127, 138)	N (127, 138) ↑ (151)		↑ (151, 157)	↑ (138)		↑ (127)		N (138, 157) ↑ (134)	N (157)	N (157)
Cytokine mRNA expression in INS patients in remission		N (127, 138)		N (127, 138)	N (127, 138)		N (151, 157)	N (138)		N (127)		N (134, 138, 157)	N (157)	N (157)

Table 28-5 (Continued)

	IL-1	IL-2	IL-2R	INF-γ	IL-4	IL-6	IL-8	IL-10	IL-12	IL-13	IL-18	TNF-α	TGF-β	VPGF
Intracellular cytokine production in INS patients in relapse		↑ (158)		N (158–160)	N (159, 160) ↓ (158)	↓ (158)				↑ (127, 159)				
Intracellular cytokine production in INS patients in remission		N (158)		N (158–160)	N (158–160)	↓ (158)				N (127, 159)				

recombinant IL-2 and alpha-interferon. However, Heslan showed that the complete removal of IL-2 from concentrated supernatants by immunoadsorption experiments did not affect the VPF activity. More convincing is the upregulated IL-13 gene expression in both CD4+ and CD8+ T cells in children with steroid-sensitive INS during relapse (127). IL-13 is one of the cytokines secreted by T helper 2 (Th2) T cells, and it has been shown that activated T cell activation early evolves toward Th2 phenotype in minimal change disease (128). In addition, genetic polymorphisms in the *IL-13* gene correlate with long-term outcome of minimal change disease (129). Indeed, the frequency of the AAT haplotype was higher in children with persistent relapses after 5 years from onset, whereas the haplotype GCC was associated with long-term remission. Receptors for IL-13 have been found in podocytes, with direct effects of IL-13 on podocytes and their signaling pathways (130). The IL-13 promoter contains two NFkB responsive elements and high and sustained plasma levels of NFkB have been detected during relapse (131), suggesting a potential role of this pathway in INS. NFkB is down-regulated by IkBa. Sahali et al. demonstrated low levels of IkBa and down-regulation of its mRNA during relapse (131). This may lead to an explanation of the additive effects of steroids and cyclosporine treatment in INS since steroids are thought to induce a transactivation of the IkBa gene while cyclosporine blocks the degradation of the active form of IkBa (132).

The type of cell producing the circulating factor also remains unclear. Sellier-Leclerc et al. developed a humanized mouse model of INS by injecting CD34(+) stem cells or CD34(−) peripheral blood mononuclear cells from affected patients into immunocompromised NOD/SCID mice (161). Only the injection of CD34(+) stem cells induced albuminuria and effacement of podocyte foot processes. These data suggest that the cells involved in the pathogenesis of INS are more likely to be immature differentiating cells rather than mature peripheral T cells.

It was hypothesized that the increased glomerular permeability to albumin may be caused not only by the production of permeability factors but also by the lack of their inhibitors such as apolipoproteins. Sharma et al. showed in several vertebrate species that normal human serum prevents the increased permeability to albumin induced by FSGS serum (162). Inhibitors of the plasma factors may be lost in urine in patients with INS, and their presence in urine has been documented. Urine from patients but not normal urine can block the increased albumin permeability induced by serum from patients with INS in isolated rat glomeruli (163). Candiao et al. demonstrated that components of high-density lipoproteins prevent glomerular albumin permeability induced by serum from patients with FSGS (164). Decreased plasma levels and glomerular expression of clusterin (ApoJ) was demonstrated in FSGS; this suggests a possible role of this protein as the inhibitor of plasma PF (165). In conclusion, the potential of FSGS serum to increase glomerular albumin permeability may result from an imbalance between permeability factors and their natural inhibitors.

Treatment

Symptomatic Treatment

Diet

Dietary therapy should include a protein intake of around 130–140% of the recommended daily allowance according to statural age. Salt restriction is advised for the prevention and the treatment of edema. A very low salt diet is only necessary in cases of massive edema. Fluid restriction is recommended for moderate to severe hyponatremia (plasma sodium concentration less than 125 meq/l). A reduction of saturated fat is recommended. Carbohydrates should be given preferentially as starch or

dextrin-maltose, avoiding sucrose which increases lipid abnormalities.

Hypovolemia

Hypovolemia occurs as a consequence of rapid loss of protein, and is sometimes aggravated by the use of diuretics. This complication needs emergency treatment by rapid infusion of plasma (20 ml/kg) or albumin 20% (1 g/kg) administrated with control of heart rate, respiratory rate and blood pressure.

Diuretics

Diuretics should only be used in cases of severe edema, after hypovolemia has been corrected. Furosemide is administered at a dose of 1–2 mg/kg. If not effective, spironolactone (5–10 mg/kg) or amiloride (0.2–0.5 mg/kg) may be prescribed if the plasma creatinine concentration is normal (166). Patients with severe edema may be treated with furosemide or, if necessary, furosemide plus albumin to increase the rate of diuretic delivery to the kidney. This approach is immediately effective but not long-lasting. Moreover, respiratory distress with congestive heart failure have been observed in some patients (167).

Refractory edema with serious effusions may require drainage of ascites and/or pleural effusions. Immersion of the body up to the neck in a bath may be helpful in these cases (168).

Thromboemboli

Nephrotic patients with severe hypoalbuminemia are at risk for thromboembolic complications. Prevention of this complication includes mobilization, avoidance of hemoconcentration due to hypovolemia and early treatment of sepsis or volume depletion. Prophylactic warfarin therapy may be given to high risk patients with a plasma albumin concentration below 20 g/l, a fibrinogen level over 6 g/l, or an antithrombin III level below 70% of normal. Patients at risk may also be treated with low-dose aspirin and dipyridamole.

Heparin is given initially if thrombi do occur, alone or with thrombolytic agents. The heparin dose necessary to obtain a therapeutic effect is often greater than normal due to decreased the antithrombin III level.

Antihypertensive Drugs

Any arterial hypertension has to be carefully controlled, using preferably a β-blocker or a calcium channel blocker during acute episodes. In cases of permanent hypertension, an angiotensin converting enzyme inhibitor is preferred.

Infections and Immunisations

Prophylaxis of S pneumoniæ with oral penicillin is often applied in patients during the initial treatment with corticosteroids. Pneumococcal vaccine may be performed and is not associated with an increased risk of relapse (169). In cases of peritonitis, antibiotics against both S pneumoniæ and gram-negative organisms are started after peritoneal liquid sampling. Varicella is a serious disease in patients receiving immunosuppressive treatment or daily corticosteroids. Varicella immunity status should be checked in these patients. In cases of exposure, early preventive treatment by acyclovir must be instituted. Varicella vaccination is safe and effective if the child is in remission even if he is on low-dose alternate day steroids (170, 171).

Hyperlipidemia

Persistent hyperlipidemia is a risk factor for atherosclerosis and may play a role in the progression of chronic renal failure. Experience with hypolipidemic drugs in nephrotic patients is still limited but it seems that statins are effective and able to decrease hypercholesterolemia (172, 173). Although long-term side effects of these drugs are not known, it is reasonable to consider a lipid-lowering regimen in children with a persistent nephrotic syndrome (174).

Miscellaneous

Calcium metabolism may be altered by the urinary loss of 25-hydroxycholecalciferol and its carrier protein. Preventive treatment with vitamin D supplements is therefore useful but does not completely prevent bone loss (175). Thyroxine substitution may be indicated, but only in patients with documented hypothyroidism due to urinary loss of iodinated proteins.

Specific Treatment

Steroid therapy is applied in all cases of INS whatever the histopathology, even in patients with FSGS. The majority of patients are steroid-responsive (*Table 28-6*). Urine protein profile (proteome) may in the future help to predict the response to steroid therapy (176). Steroid responders may relapse, but the majority still responds to steroids over the subsequent course. Only 1–3% of patients initially steroid-sensitive subsequently become steroid-resistant and are defined as "late non-responders" (177).

Initial Treatment

Although steroid therapy is often started immediately following the diagnosis of nephrotic syndrome, it should be stressed that spontaneous remission occurs in 5% of cases within 1 or 2 weeks. Therefore, initiation of steroid therapy may be delayed for a few days (178). Some of these early spontaneous remissions are definitive. Infection must be treated before starting steroids, not only to prevent the risk of overwhelming sepsis during treatment, but also because occult infection may be responsible for steroid resistance (17).

Steroid therapy is started when the diagnosis of INS is most likely in a child older than 1 year and younger than 11 years of age, without hypertension, gross hematuria or extra-renal symptoms and normal complement levels. In some cases, the treatment is started after a renal biopsy has been performed. Prednisone remains the reference drug. Prednisolone has the advantage of being soluble in water, making treatment easier in young children, but it may fail to induce remission in some patients who respond quickly to the same dosage of prednisone. The differences in intestinal absorption and drug interactions, for instance with aluminum gels, may explain lesser efficacy in some children.

The ISKDC regimen consists of prednisone, 60 mg/m^2/day with a maximum of 80 mg/day, in divided doses for 4 weeks followed by 40 mg/m^2/day with a maximum of 60 mg/day in divided doses, on three consecutive days per week for 4 weeks (97). The Arbeitsgemeinschaft für pädiatrische Nephrologie showed that an alternate day regimen (40 mg/m^2 every other day for 4 weeks) resulted in a significantly lower number of patients with relapses and fewer relapses per patient (179). It also showed that on alternate days prednisone could be given in a single dose rather than in divided doses.

A response occurs in most cases within 10–15 days (median 11 days) (*Table 28-6*). According to the International Study of Kidney Disease in Children, approximately 90% of responders enter in remission within 4 weeks after starting steroids whereas less than 10% go into remission after 2–4 more weeks of a daily regimen (97). A few more patients go into remission after 8–12 weeks of daily steroids (13, 15), but prolongation of daily steroid treatment beyond 4 or 5 weeks increases the risk of side-effects. An alternative for patients who are not in remission after 4 weeks is to administer three to four pulses of methylprednisolone (1 g/1.73 m^2). This additional regimen seems to be associated with fewer side effects than prolongation of daily high-dose steroids and probably produces remission more rapidly in the few patients who would have entered into remission during the second month of daily therapy (180).

The duration of initial steroid therapy influences the risk of relapse.

The APN compared a standard regimen of 4-week daily prednisone and 4 weeks of alternate day prednisone with a longer initial course of 6 weeks of daily prednisone at a dose of 60 mg/m^2/day followed by 6 weeks alternate day

Table 28-6

Definitions

Nephrotic syndrome: proteinuria >40 mg/h/m^2 or >50 mg/kg/day or protein/creatinin ratio >0.2 g/mmol (>2 g/g) and hypoalbuminemia <25 g/l with or without edema
Remission: proteinuria <4 mg/h/m^2 or 0-trace on Albustix for 3 consecutive days
Steroid responsive: complete remission achieved with steroid therapy
Steroid resistant: failure to achieve remission following 4 week' prednisone 60 mg/m^2 followed by 3 methylprednisolone pulses
Relapse: proteinuria > >40 mg/h/m^2 or >50 mg/kg/day or Albustix +++ for 3 consecutive days after having been in remission
Frequent relapser: 2 or more relapses within 6 months of initial response or 4 or more relapses within a period of 1 year
Steroid dependence: 2 consecutive relapses during corticosteroid therapy or within 14 days after cessation of therapy
Early nonresponder: steroid resistance during the first episode
Late nonresponder: steroid resistance in a patient who had previously responded to corticosteroid therapy

prednisone at a dose of 40 mg/m^2/day (179). The subsequent relapse rate within 12 months following discontinuation of therapy was lower with the prolonged course of therapy compared to the standard course (36 vs. 61%).

Following an 8-week steroid regimen, 50–70% of children experience relapses. Several controlled studies have compared the 8-week regimen with longer duration of steroid regimen (3–7 months) including 4–8 weeks of daily prednisone followed by alternate day prednisone (181–185). With a follow-up of 2 years, a significant reduction of 25–30% in the relapse rate was observed with a prednisone regimen of 3 months or more.

The number of children with frequent relapses is also decreased with a longer course of prednisone. A longer duration is more important than the cumulative dose of prednisone in reducing the risk of relapse. This relative risk decreases by 0.133 (13%) for every additional month of treatment up to 7 months (97). There are no data showing that treating for more than 7 months is beneficial. However, an alternate-day regimen over a year did not reduce the rate of relapse compared to a 5-month alternate day regimen (186, 187). Although the studies were not designed to analyze the side effects of glucocorticoids, the authors did not report increased toxicity with longer duration of treatment.

A slow tapering phase to avoid adrenal suppression may maintain long-term remission as a study showed that moderate to severe adrenal suppression was associated with an increased risk of relapse (188). Some authors have suggested a possible prevention by low-dose maintenance hydrocortisone (189, 190). Another study also concluded that adrenocortical suppression increases the risk of relapse in children on long-term alternate day steroid therapy (191).

Increasing initial immunosuppression by adding cyclosporine to steroid therapy does not change the 2-year relapse rate (192).

Treatment of Relapses

About 30% of children experience only one attack and are definitively cured after a single course of steroids. Persistent remission for 18–24 months after stopping treatment is likely to reflect definitive cure, and the risk of later relapses is low. Ten to 20% of patients relapse several months after stopping treatment and are most often cured after three or four episodes, which respond to a standard course of steroid therapy. The remaining 50–60% experience relapses as soon as steroid therapy is stopped or when dosage is decreased. In some cases, exacerbation of proteinuria is only transient, and spontaneous remissions are observed (193). The risk of relapse is greater in children aged less than 5 years at onset and in males. These steroid dependent patients often raise difficult therapeutic problems.

Steroid-dependent patients may be treated with repeated courses of prednisone, 60 mg/m^2/day, continued 3 days after the urine has become protein free, followed by alternate day prednisone, 40 mg/m^2, for 4 weeks as proposed by the International Study of Kidney Disease in Children (97). Another option consists of treating relapses with daily prednisone, 40–60 mg/m^2, until proteinuria has disappeared for 4–5 days. Thereafter, prednisone is switched to alternate days and the dosage is tapered to 15–20 mg/m^2 every other day, according to the steroid threshold, that is, the dosage at which the relapse has occurred. Treatment is then continued for 12–18 months. The first approach allows better definition in terms of number of relapses but is associated with more relapses. The latter regimen is associated with less steroid side effects as the cumulative dosage is lower. Prolonged courses of alternate day steroid therapy are often well tolerated by young children and growth velocity is not affected. However, prednisone dosage must be as low as possible in order to reduce the side effects. In adolescents, steroid therapy is often accompanied by decreased growth velocity.

A controlled trial has shown that deflazacort reduces the risk of relapse in comparison with equivalent doses of prednisone, without additional side effects (194). Unfortunately, deflazacort is not available in many countries.

The role of upper respiratory tract infections in exacerbating nephrotic syndrome has been highlighted in all series: 71% of relapses were preceded by such an event in a prospective study, although only 45% of respiratory infections were followed by an exacerbation of proteinuria (195). The risk of relapse is decreased during upper respiratory tract infections when steroid therapy is given daily for 5–7 days rather than on alternate days (196, 197).

Steroid Side Effects

Side effects of prolonged steroid therapy are well known and are observed in children with a steroid dependent course. Growth retardation is observed with prolonged daily steroid therapy and alternate day therapy may preserve growth (198). However, when the dose needed to maintain remission is too high, growth may be impaired (199, 200). Osteoporosis has been reported in adults who had suffered from nephrotic syndrome during childhood (201). However, a study in children and adolescent with

steroid dependent nephrotic syndrome failed to find a deleterious effect of alternate day steroid therapy on bone mineral content (202). Biyikli et al. reported that steroid treatment causes a dose dependent decrease in bone formation, as shown by the changes in osteocalcin and alkaline phosphatase levels and low 25-hydroxyvitamin D levels (203). A randomized controlled trial compared vitamin D and calcium supplements with no prophylaxis in children receiving high dose steroid therapy during a relapse. The authors found a decrease in bone mineral content in both groups but less pronounced in the treated group ($4.6 \pm 2.1\%$ vs. $13.0 \pm 4.0\%$, respectivelly; $P<0.001$) (175). Another controlled study showed that biphosphonates are effective in preventing steroid-induced osteoprosis in children receiving long-term steroid therapy (204). The other side effects include weight gain, cataracts, behavior disturbances, and hypertension.

Alternative Treatments (*Fig. 28-9*)

An alternative treatment is indicated in children who develop severe side effects of steroid therapy, in children at risk of toxicity (diabetes or during puberty), in children with severe relapses accompanied by thrombotic complications or severe hypovolemia and in those with poor compliance.

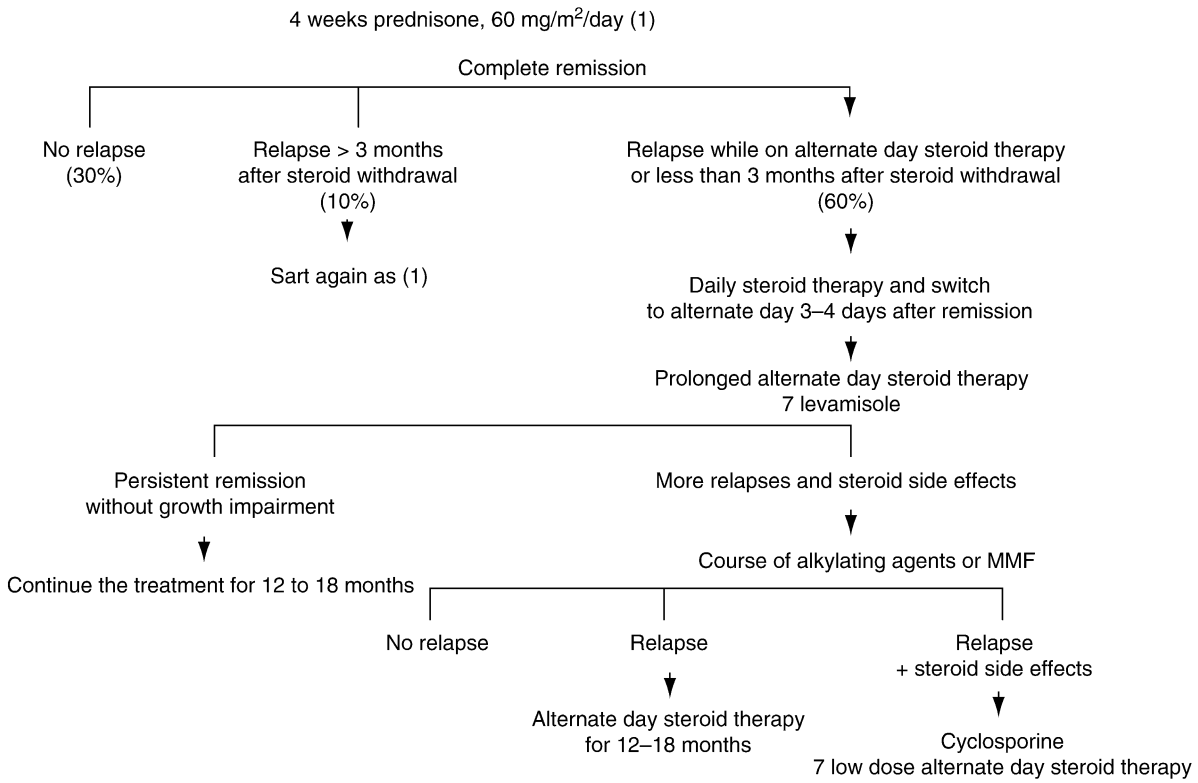
Alternative treatments include levamisole which has a steroid sparing effect, alkylating agents such as cyclophosphamide or chlorambucil, cyclosporine, mycophenolate mofetil and, more recently, rituximab.

Levamisole

The beneficial effect of levamisole was first described by Tanphaichitr et al. (205). Levamisole was subsequently reported to reduce the risk of relapse in steroid dependent patients (206–210). A significant steroid-sparing effect at a dose of 2.5 mg/kg every other day was demonstrated in a prospective controlled trial of the British Association for Paediatric Nephrology (211). Another controlled study confirmed the efficacy of levamisole for preventing relapses (212). However, the beneficial effect of levamisole is not sustained after stopping treatment.

Levamisole given for 6 months was compared with cyclophosphamide given for 8–12 weeks in a retrospective

Figure 28-9



study involving 51 children with steroid dependent nephrotic syndrome (213). The relapse rate and the cumulative dose of prednisone were reduced to the same extent with both drugs.

Levamisole is well tolerated in most children. Side effects occasionally include neutropenia, agranulocytosis, vomiting, cutaneous rash, vasculitis, neurological symptoms including insomnia, hyperactivity and seizures (214, 215). However, levamisole is not widely used due in part to the difficulty in obtaining the drug.

Alkylating Agents

Alkylating agents have been used for more than 40 years to achieve long-lasting remission.

Cyclophosphamide

The efficacy of cyclophosphamide for preventing relapses of INS was reported more than 40 years ago (216), and was proven in a prospective study by Barratt and Soothill who compared an 8-week course of cyclophosphamide to prednisone alone in children with frequent relapses (217). An International Study of Kidney Disease in Children trial found a 48% relapse rate after a mean follow-up of 22 months in children treated with a combination of cyclophosphamide and prednisone compared to a 88% relapse rate in patients on prednisone alone (218). Pennesi et al. showed that the duration of cyclophosphamide treatment had an influence on the duration of remission (219). Several other studies have addressed the relation between dose and duration of treatment and therapeutic efficacy. Treatment for 12 weeks at a daily dose of 2 mg/kg was found more effective than an 8-week course, with 67% of patients as compared to 22% remaining in remission after 2 years (220, 221). However, a randomized trial showed that prolonging the course of cyclophosphamide from 8 to 12 weeks did not further reduce the proportion of children experiencing relapses.

The duration of remission is higher when cyclophosphamide is given in association with steroids compared to cyclophosphamide given alone (218, 222, 223). Cyclophosphamide is less effective in patients with steroid dependency compared to patients with frequent relapses (224). The incidence of relapse after cyclophosphamide is significantly higher in patients with FSGS (73%) or mesangial proliferation, compared to children with minimal change (22%) (225).

More recent data show disappointing results of cyclophosphamide treatment for steroid dependent patients. Kemper et al. reported that only 6 out of 20 children had a sustained remission following a 12-week course of cyclophosphamide. In a retrospective study (226), Vester et al. reported that 24% of 106 children who had received a course of cyclophosphamide were still in remission after 10 years (227, 228). A younger age than 3 years at onset is associated with lower response rate (229).

Cyclophosphamide has also been administered as monthly boluses at 500 mg/m^2 for 6 months. Gulati et al. reported a remission rate of 38% at 5 years among the 29 steroid dependent patients (230). However, Donia et al. reported a remission rate of only 5% at 2 years in a group of 20 steroid dependent patients (231). Prasad et al. compared cyclophosphamide given orally or as boluses in 47 children (232). The remission rate 6 months after the end of treatment was higher following the cyclophosphamide boluses (73% vs. 38%) but was similar with a follow-up of 2 years.

Chlorambucil

Beneficial results have also been achieved with chlorambucil in steroid responsive INS. Grupe et al. reported on the efficacy of chlorambucil given for 2.5–12 weeks, with a relapse rate of only 13% (233). Two trials showed that chlorambucil reduces the risk of relapse at 6 and 12 months compared with placebo or prednisone alone (233, 234). Baluarte et al. obtained similar results in relapsing, steroid-responsive patients (235). Williams et al. showed that low daily doses are preferable: 91% of patients on a dose of 0.3 mg/kg and 80% of those on 3 mg/kg were still in remission 4 years later (236).

A review of 26 controlled trials and cohort studies found that the 2- and 5-year relapse rates following treatment with cyclophosphamide or chlorambucil were 72 and 36% in frequently relapsing nephrotic syndrome compared with 40 and 24% in steroid dependent nephrotic syndrome (237).

Side Effects

Cyclophosphamide toxicity includes bone marrow depression, hemorrhagic cystitis, gastrointestinal disturbances, alopecia, and infection. Leucopenia is frequently observed, but weekly hematological monitoring may limit its severity and concomitant steroids help blunt marrow depression. Hemorrhagic cystitis rarely occurs. Alopecia, which is variably pronounced, remits a few weeks after stopping treatment. Viral infections can be overwhelming if cyclophosphamide is not stopped in due time.

Long-term toxicity includes malignancy, pulmonary fibrosis, ovarian fibrosis, and sterility. Gonadal toxicity is well established and the risk of sterility is greater in boys than in girls. The cumulative threshold dose above which oligo/azoospermia may be feared is between 150

and 250 mg/kg (238–240). Azoospermia is reversible in some patients (241). In females the cumulative dose associated with sterility is greater, but not well defined. Pregnancies have been reported after treatments longer than 18 months (242).

Acute toxic effects are less frequent with chlorambucil than with cyclophosphamide. Leucopenia and thrombocytopenia may occur, and are reversible within 1–3 weeks. Severe microbial and viral infections have been reported, including malignant hepatitis and measles encephalitis.

Long-term toxic effects include the risk of developing cancer or leukemia, which has only been reported in patients who had prolonged courses of treatment. Gonadal toxicity, as with cyclophosphamide, essentially affects boys. Azoospermia is total and probably irreversible at cumulative doses above 10–20 mg/kg. No case of azoospermia was reported in patients given less than 8 mg/kg.

Cyclosporine

Cyclosporine have been shown in a number of uncontrolled studies to reduce the incidence of relapses in 75–90% of patients with steroid dependent INS (243). However, most patients experience relapses when the dosage is tapered or when cyclosporine is withdrawn. The patients thus behave with cyclosporine as they did with steroids; that is, they become cyclosporine dependent. The relapse rate usually returns to the pretreatment rate. Hulton et al. found that patients in whom cyclosporine had been discontinued and later restarted had more relapses, requiring steroids in addition to cyclosporine in order to maintain remission (244).

The effects of cyclosporine have been evaluated in two comparative trials in steroid sensitive patients. Cyclosporine at a dosage of 6 mg/kg/day for 3 months, then tapered over 3 months was compared with chlorambucil given for 2 months. At 12 months, 30% of patients who had received chlorambucil and only 5% of those who were still in remission on cyclosporine (245). A multicenter randomized controlled trial compared cyclosporine for 9 months then tapered over 3 months, with oral cyclophosphamide for 2 months (246). After 2 years, 25% of the patients (50% of adults and 20% of children) who had received cyclosporine had not relapsed, whilst 63% of those treated with cyclophosphamide (40% of adults and 68% of children) were still in remission. During the year following treatment, the relapse rate (1.8 vs. 0.7) and the steroid dosage required (109 vs. 23 mg/kg/year) were significantly higher in children who had received cyclosporine.

Tejani et al. performed a randomized controlled trial comparing low dose prednisone and cyclosporine versus high dose prednisone for 8 weeks as first line treatment in 28 children (247). Thirteen of the 14 children receiving the combined treatment went into remission compared to only 8/14 receiving prednisone alone ($p < 0.05$). The duration of remission after ending treatment was comparable in both groups. Severe hypercholesterolemia may inhibit cyclosporine efficacy and require higher dosages for similar results (248, 249).

A prospective, open multicenter trial from Japan compared the efficacy and safety of two cyclosporine regimen, a dose adjusted to maintain trough level between 60 and 80 ng/ml (group A) and a fixed dose of 2.5 mg/kg (group B) in children who had initially received cyclosporine for 6 months with a trough level of 80–100 ng/ml (250). After 2 years, the rate of sustained remission was significantly higher in group A.

Considering cyclosporine dependency, this treatment must be pursued to prevent new relapses. Indeed, cyclosporine exposes to nephrotoxicity. In case of decreased renal function, it is advisable to reduce dosage or even stop the treatment. Renal function improvement of is in favor of functional renal insufficiency or drug nephrotoxicity. Nevertheless, lesions of chronic nephrotoxicity can develop without any appreciable decline of the glomerular filtration rate (251–253). As it is often necessary to continue treatment for a long time, repeat renal biopsies are highly advisable to detect these lesions. They most often consist of tubulointerstitial injury, characterized by stripes of interstitial fibrosis containing clusters of atrophic tubules and by vascular lesions.

Other side-effects are of less concern: hypertension, hyperkalemia, hypertrichosis, gum hypertrophy, and hypomagnesemia are common but easily manageable. Cyclosporine treatment has no deleterious effect on bone mineral content (254).

Tacrolimus

In a retrospective study of ten children, it was observed that tacrolimus was not better than cyclosporine for the management of severe steroid dependent nephrotic syndrome (255). In a series of five children treated with tacrolimus, two developed insulin dependent diabetes mellitus which resolved after stopping tacrolimus therapy (256).

Azathioprine

Two controlled trials in children showed that azathioprine does not reduce significantly the number of children who relapse at 6 months compared to steroids alone or placebo (257–259).

Mycophenolate Mofetil

During the past 10 years, several reports have shown that mycophenolate mofetil (MMF) treatment may have a beneficial effect in children with steroid dependent INS

(260–272) These studies have shown that MMF allowed to decrease or stop steroid therapy in 40–75% of children. However, relapses were nearly constant after cessation of treatment. MMF was shown to have a significant cyclosporine and/or steroid sparing effect in children with cyclosporine dependent INS with a beneficial effect on renal function (263, 266). These studies confirm the efficacy and safety MMF in patients with steroid dependent nephrotic syndrome and supports its use for a longer duration than 12 months. Doses of 450–600 mg/m^2/day in two divided doses are usually given. Side effects including gastrointestinal disturbances (abdominal pain, diarrhea) and hematologic abnormalities are rare. Many authors now recommend the use of MMF rather than alkylating agents in children with steroid dependent nephrotic syndrome who suffer from side effects of steroid therapy. However, randomized trials should be performed before such recommendations can be made.

Rituximab

During the past 3 years, there have been several case reports of successful treatment of patients with severe steroid dependent nephritic syndrome (273–277). Most of the patients received 1–4 injections of rituximab at a dose of 375 mg/m^2. The duration of remission lasted 9–28 months after the treatment. The safety and efficacy of rituximab were assessed in a multicenter series of 22 patients aged 6.3–22 years with severe steroid-dependent nephrotic syndrome or steroid-resistant but cyclosporin-sensitive INS (278). Patients were treated with two to four infusions of rituximab. Seven patients were nephrotic at the time of treatment. Remission was induced in three of the seven proteinuric patients. One or more immunosuppressive treatments could be withdrawn in 19 patients (85%), with no relapse. Rituximab was effective in all patients when administered during a proteinuria-free period in association with other immunosuppressive agents. When relapses occurred, they were always associated with an increase in CD19 cell count. Adverse effects were observed in 45% of cases, but most of them were mild and transient.

Long-Term Outcome of Children with Steroid Sensitive Nephrosis

About one third of patients have only one attack and are definitively cured after the course of corticosteroids. Ten to 20% of patients experience relapses several months after stopping the treatment and a cure takes place after three or four episodes which respond to a standard course of corticosteroids. The remaining 40–50% of patients experience frequent relapses either as soon as steroid therapy is stopped (frequent relapsers) or when the dose of steroids is decreased (steroid dependent). These steroid dependent patients may have a prolonged course. However, if the patient continues to respond to steroids, the risk of progression to chronic renal failure is minimal.

Schärer and Minges found that 22% of patients had only one attack and 35% of the relapsing patients continued to relapse after 10 years (279). Trompeter et al. reported the late outcome of 152 children steroid-responsive nephrotic syndrome after a follow up of 14–19 years: 127 (83%) were in remission, four had hypertension, 10 were still relapsing, and 11 had died (280). The duration of the disease was longer in children who had started before the age of 6 years. Wynn et al. found that 15% of 132 patients had a persistent relapsing course with a mean follow-up of 27.5 years (281). Lewis et al. reported on 26 patients over the age of 20 years, of whom 5 were still relapsing in adulthood (282).

Koskimies et al. reported on the follow-up of children with INS observed in Finland from 1967 to 1976: 94 of 114 cases had responded to corticosteroids. Twenty-four percent of steroid responders had no relapse, 22% had infrequent relapses and 54% frequent relapses. More than two-thirds were in remission at time of report (283). None of these patients developed renal insufficiency and none died from the disease. Lahdenkari et al. reported a 30-year follow-up of the patients reported previously by Koskimies et al. (284). Of 104 patients, 10% had further relapses in adulthood.

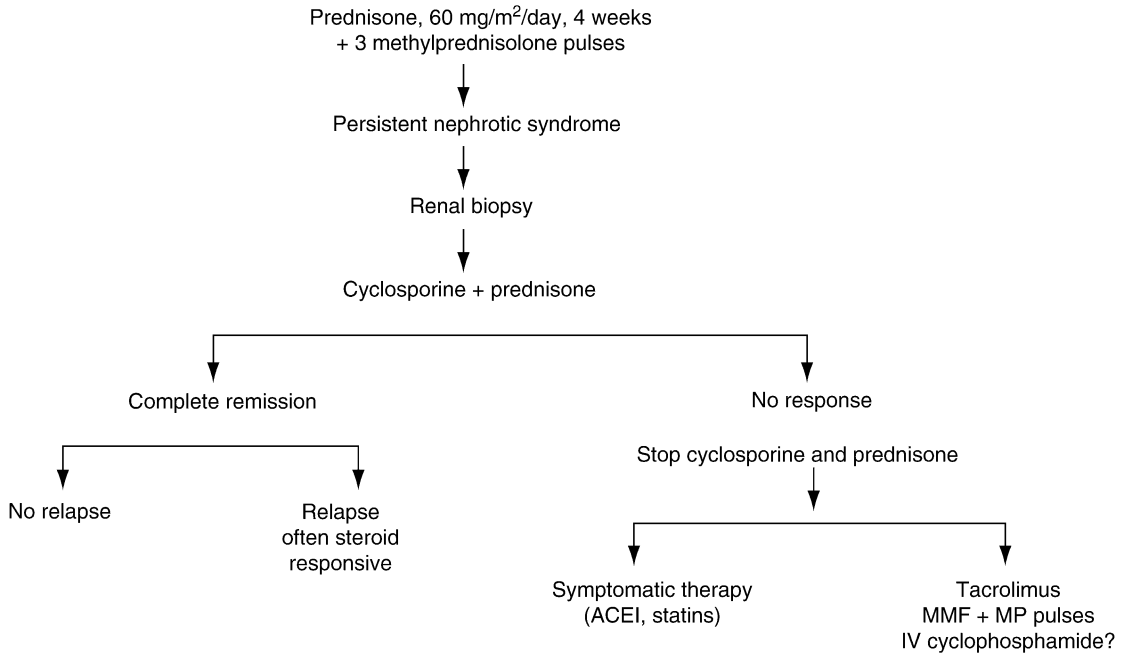
Fakhouri et al. reported on the outcome in adulthood of 102 patients born between 1970 and 1975 (201). Fourty-two percent presented at least one relapse in adulthood. A young age at onset and a high number of relapses during childhood were associated with a high risk of relapse in adulthood. Similarly, Ruth et al. in a study of 42 patients found that 14 (33%) relapsed in adulthood (285). The higher relapse rates in these two reports probably reflect patient selection with more steroid dependent cases compared to Koskimies' series.

This generally good long-term prognosis is also observed in patients with steroid-responsive focal and segmental glomerulosclerosis: the 19 children reported by Arbus et al. remained responders, and none had renal insufficiency after a mean follow-up of 10 years (286).

Treatment of Steroid Resistant INS (◉ *Fig. 28-10*)

Resistance to steroid therapy is defined by the absence of remission after 1 month of daily prednisone therapy at a dose of 60 mg/m^2/day (97). Some authors continue the

Figure 28-10



treatment for 1 or 2 weeks while others favor a series of three methylprednisolone pulses (1,000 mg/1.73 m^2) every other day (287). Indeed, the side effects of this regimen are less than those induced by an increase in the daily prednisone dose. Persistence of proteinuria 1 week after this treatment defines steroid-resistance.

Overall, the prognosis of steroid resistant idiopathic nephrotic syndrome is poor, with a high proportion of children progressing to end stage renal failure. This explains that intensive treatment regimens have been tried. The results of immunosuppressive treatments should take into account the fact that children with genetic forms of INS most often fail to respond to any therapy. However, many published trials include patients who had not been tested for mutations in the different genes involved in steroid resistant INS. Moreover, most studies are nonrandomized and include a small number of patients.

The interpretation of treatment is also complicated by the fact that some studies include patients with minimal change disease, diffuse mesangial proliferation and FSGS while other only include patients with FSGS.

Pulse Methylprenisolone

Methylprednisolone pulse therapy has been advocated for steroid resistant patients. The protocol proposed by Mendoca et al. consists of methylprednisolone (30 mg/kg intravenously), administered every other day for 2 weeks, weekly for 8 weeks, every other week for 8 weeks, monthly for 9 months, and then every other month for 6 months in association with oral prednisone and, if necessary, cyclophosphamide or chlorambucil (288). At an average of over 6 years of follow-up, 21 of 32 children were in complete remission and the 5-year incidence of end-stage renal disease was approximately 5% versus 40% in historical controls (289). Side effects included nausea during the infusion of methylprednisolone in almost all, slowed growth in four, small cataracts that did not interfere with vision in five, and infections in two. There were no cases of abdominal striae, diabetes mellitus, or aseptic necrosis of bone.

A retrospective study of 11 children with steroid resistant INS found pulse methylprednisolone therapy to be safe and effective in inducing remission (290). Similarly, Pena et al. reported that 22 out of 30 children with idiopathic steroid resistant INS entered into complete remission following methylprednisolone pulses and oral cyclophosphamide (291). Although these results are better than those seen in any other study, other reports described less favorable results. Waldo et al. found that pulse methylprednisolone pulse therapy was not effective in inducing remission in black patients (292), whilst Hari et al. in India reported a 65% response rate (293).

Alkylating Agents

Although alkylating agents have little therapeutic effect in steroid resistant patients, they are still widely used either alone or in combination with corticosteroids. Cyclophosphamide has been more often been used than chlorambucil. The rate of full or partial remission is higher in patients with partial steroid resistance, those with late steroid resistance, or those in whom initial renal biopsy has shown minimal changes, by comparison with those showing initial resistance to corticosteroids and/or FSGS. The International Study of Kidney Disease in Children recently reported on 60 children with steroid resistant FSGS who were randomly allocated to receive either prednisone 40 mg/m^2 on alternate days for 12 months (control group) or cyclophosphamide, 2.5 mg/kg BW for 3 months plus prednisone 40 mg/m^2 on alternate days for 12 months (294). Complete remissions were observed in 28% of children in the control group and in 25% of children who received cyclophosphamide. The authors concluded that there was no beneficial effect of cyclophosphamide in these patients. Geary et al. reported full or partial response to cyclophosphamide in 12 of 29 steroid-resistant patients with FSGS (295). Renal failure developed less frequently in partial responders (one of nine) than in those who did not respond at all (seven of eight). Siegel et al. observed complete remissions in six steroid-resistant patients with minimal changes, three of whom relapsed but became steroid responsive (11). Similarly, Bergstrand et al. reported that some patients with steroid-resistant nephrosis treated with cyclophosphamide had become steroid-responsive (296). Conversely, White and Glasgow observed no improvement after cyclophosphamide treatment in 15 steroid-resistant children with focal sclerosis (297). Cameron et al. reported only one responder out of 13 children with steroid-resistant nephrosis and FSGS who received cyclophosphamide (222). Similarly, Tejani et al. reported no remission with cyclophosphamide in ten steroid-resistant children (298). In a controlled trial involving 13 children with steroid resistant minimal change nephrotic syndrome, intravenous pulse cyclophosphamide was shown to be beneficial when compared to oral cyclophosphamide (299). Another report concerning five patients with steroid resistant minimal change disease found no benefit from pulse cyclophosphamide therapy (300). Rennert et al. treated ten children with steroid resistant FSGS with cyclophosphamide pulses. Only two of the five patients who were initial nonresponders went into remission whereas all five late nonresponders achieved complete remission (301). In a prospective study of 24 patients, Bajpai et al. found that therapy with intravenous cyclophosphamide had limited efficacy in patients with initial corticosteroid resistance while Sustained remission was likely to occur in patients with late resistance and those with absence of significant tubulointerstitial changes on renal histology (302).

Chlorambucil may be effective. Williams et al. treated six children who all went into remission with a follow up of 1.3–9.4 years (236). We treated 74 steroid-resistant children with chlorambucil, 0.2 mg/kg, for 2–6 months, and only 14 of them went into complete or partial remission during or shortly after the treatment.

Azathioprine

Azathioprine was considered to be ineffective in steroid-resistant patients after the report of Abramowicz et al. who found no difference between a 3-month course of azathioprine and a placebo (257). However, Cade et al. reported complete remission in 13 adult patients with steroid-resistant INS (303).

Cyclosporine

Cyclosporine has been given to steroid resistant patients and the first reports showed that only 20% of 60 steroid-resistant children achieved complete remission (304–309). A partial response was observed in 13% of cases, but it was usually transient.

The Collaborative Study of Sandimmun in Nephrotic Syndrome analyzed the data from different clinical studies, including 226 steroid resistant patients, adults and children (310). The study showed that the rate of complete remission was significantly higher when cyclosporine was given in combination with steroids: 24% compared to 14%.

The French Society of Pediatric Nephrology reported the results of a prospective trial including 65 children treated with cyclosporine, 150–200 mg/m^2, and prednisone, 30 mg/m^2/day for 1 month and on alternate days for 5 months thereafter (287). Twenty-seven patients (42%) went into complete remission and four (6%) partial remission whereas 34 (52%) failed to respond to the combined treatment. Complete remission occurred in more than half of the patients within the first 2 months of this treatment, which makes it likely that the treatment was responsible for the remission, although spontaneous remission cannot be excluded. Interestingly, eight patients who relapsed after cyclosporine treatment responded to steroid and experienced a steroid dependent course. Progression to renal failure was observed only in patients who

had not responded (12 patients) or had only a partial response (1 patient) to the combined treatment.

Ingulli et al. reported that prolonged cyclosporine treatment in children with steroid resistant FSGS reduces proteinuria and blunts the progression to end stage renal failure (311). The dose of cyclosporine (4–20 mg/kg/day) was titrated to the serum cholesterol level to achieve a remission. In this study, only 5 of the 21 treated patients (24%) progressed to ESRF compared to 42 of 54 patients from an historical group who had not received this treatment. Cattran et al. compared the effects of a 26-week regimen with cyclosporine and prednisone or prednisone alone in 49 adults with steroid resistant FSGS (312). Seventy percent patients of the treatment group versus 4% of the placebo group had a partial or complete remission. Although the rate of relapse was high, preservation of renal function was observed in a significant proportion of treated patients.

Ponticelli et al. compared cyclosporine to supportive therapy in a randomized trial (313). Seven of the 22 treated patients went into complete remission, 6 in partial remission and 9 failed to respond. Only 38% of the patients who responded had sustained remissions. In the control group, only 3 of the 19 patients achieved partial remission. Liberman and Tejani compared cyclosporine and placebo in 25 children with steroid resistant FSGS (314). All 12 patients who were treated achieved a decrease of proteinuria compared to only two in the placebo-treated patients, without a significant decrease in glomerular filtration rate in the cyclosporine treated group.

Gregory et al. treated 15 children with steroid resistant INS with an association of moderate doses of cyclosporine and prednisone. They observed a remission in 13 children after a mean duration of treatment of 2 months (315). Singh et al. reported the effect of cyclosporine in 42 children with steroid resistant FSGS (316). The mean proteinuria decreased from 7.1 g/day to 1.8 g/day while the serum albumin increased from 2.1 g/dl to 3.5 g/dl. The mean serum creatinine increased from 0.85 mg/dl to 1.26 mg/dl. Twenty five patients achieved complete remission. Ehrich et al. reported a retrospective study including 25 children with steroid resistant FSGS who received prolonged and intensified treatment with combined cyclosporine and steroids including methylprednisolone pulses. This treatment resulted in sustained remission in 84% of children with non-genetic forms of steroid resistant INS (317).

Tacrolimus

There is evidence that tacrolimus is effective in a significant proportion of patients. Loeffler et al. found tacrolimus to be effective and well-tolerated for children with steroid resistant INS, with a complete remission rate of 81% and a partial remission rate of 13% (318). Bhimma et al. treated 20 children with tacrolimus and observed a complete remission in 40% of them and a partial remission in 45% of them (319). Similarly, Gulati et al. reported the results of tacrolimus treatment in 22 children, 16 of whom achieved complete remission and 2 partial remission whereas therapy had to be stopped in 3 because of side effects (320).

Mycophenolate Mofetil

There is no convincing data for the beneficial effect of mycophenolate mofetil in these patients. Menzibal et al. treated five patients with steroid resistant INS and only one achieved complete remission (268). Mycophenolate mofetil in association with methylprednisolone pulses and angiotensine converting enzyme inhibitors was reported to significantly reduce proteinuria (321). Cattran et al. reported on a 6-month trial in 18 adults. A reduction of proteinuria was observed in eight patients but no complete remission occurred (322). A prospective trial of the NIH comparing cyclosporine and mycophenolate mofetil in combination with pulse steroids is in progress.

Sirolimus

Tumlin et al. performed a prospective, open trial with oral sirolimus given for 6 months to 21 patients with steroid resistant FSGS. Complete remission was observed in 4 patients (19%) and partial remission in 8 (38%) (323). Glomerular filtration rate in responding patients was maintained whereas it tended to decrease in nonresponders.

Rituximab

Nakayama et al. reported two patients who were successfully treated with rituximab for steroid resistant FSGS (324). Bagga et al. treated five children including three with initial resistance to steroids and two with late resistance to steroids (325). All children had received several other therapies with partial or complete response. Following 4 weekly doses of rituximab, four patients achieved complete remission and one a partial remission. Complete remission persisted in three patients. However, there are several reports of patients who failed to respond to

rituximab and it is too early to recommend such therapy in steroid resistant INS.

Non-steroidal Anti-inflammatory Drugs

These drugs may decrease proteinuria. Several authors have used indomethacin in the treatment of INS with variable results. Donker et al. found a reduction of proteinuria in patients with FSGS but with a simultaneous reduction of glomerular filtration rate (326). Velosa et al. also found a clear reduction in proteinuria in patients treated with meclofenamate (327).

The detrimental effect of non-steroidal anti-inflammatory agents on renal function is well known, and patients with renal disease seem more vulnerable (328, 329). Positive sodium balance, increased edema and risk of arterial hypertension are recognized complications. The decrease of glomerular filtration rate observed with non-steroidal anti-inflammatory drugs is usually reversible in salt-sodium depleted patients. However, irreversible renal failure has been reported with a high incidence in a prospective study in children with steroid resistant FSGS (330).

Angiotensin Converting Enzyme Inhibitors

Numerous studies in adults have demonstrated that angiotensin converting enzyme (ACE) inhibitors and angiotensin II receptor blockers (ARBs) slow the rate of progression of proteinuric chronic renal disease. Captopril was reported to decrease dramatically nephrotic range proteinuria due to renovascular hypertension and secondary FSGS (331). A decrease in proteinuria, and even complete remission, have also been observed in patients with chronic glomerulopathies with or without hypertension (332). A 50% decrease of proteinuria without a concomitant decrease in glomerular filtration rate was reported in children with steroid resistant INS (333). Marked benefits with an ACE inhibitor and/or ARB therapy, plus mycophenolate were observed in nine children with steroid-resistant FSGS (321). At 6 months, mycophenolate plus angiotensin blockade resulted in a 72% decrease in proteinuria from baseline values, a benefit that was maintained for a minimum period of 24 months.

Lipid-Lowering Agents

Hyperlipidemia has been shown in experimental animals to accelerate the progression of glomerular sclerosis. Controlled trials in adult patients have shown that lipid-lowering agents can prevent the decline of renal function. No such studies have been performed in children.

Recurrence of INS in Transplanted Kidneys

The major problem of patients with INS who progress to end stage renal failure and who undergo renal transplantation is the risk of recurrence of the nephrotic syndrome in the graft. The overall risk of recurrence is estimated to be between 20 and 30% (334). The risk is different in children and in adult patients. Senggutuvan et al. found that 8 of 16 children had experienced recurrence compared to 3 of 27 adults (335). In children, recurrence is more frequent when the disease has started after the age of 6 years than before (336, 337). Similarly, a rapid progression of the disease to end stage renal failure seems a major factor associated with recurrence: in most series, when the duration of disease has been shorter than 3 years, the nephrotic syndrome recurs in half of the patients (336, 338). Recurrence is less frequent in African-Americans than in whites and Hispanics (339). The histopathological pattern observed on the first biopsy during the course of the disease is also an important predictive factor (335–337, 340, 341). Recurrence occurs in 50–80% of patients in whom initial biopsy showed diffuse mesangial proliferation but in only 25% of patients with minimal changes on first biopsy. Most patients who experience a recurrence in a first graft also show recurrence in a second graft (342–344). Conversely, when the graft has been lost due to rejection without recurrence, a second graft can be performed safely as recurrence is exceptional in this setting. Patients with podocine mutations or other genetic forms of INS have a very low risk of recurrence (345).

In children, recurrence of proteinuria occurs in most cases within the first hours or the first days after transplantation. A high proportion of patients with immediate recurrence show delayed graft function (344, 346). In some patients, proteinuria recurs several months later. Proteinuria is most often associated with a nephrotic syndrome. Transplant biopsy when performed early shows minimal glomerular changes with foot process fusion (347–349). Lesions of FSGS appear after several days or weeks which is a strong argument to consider these lesions as secondary rather than the cause of heavy proteinuria (348).

Graft failure occurs in about 60% of patients with recurrence versus 23% of those without recurrence

(335, 344, 350). Some patients may show good renal function for several years despite persistent nephrotic syndrome.

The beneficial role of cyclosporine in recurrent steroid resistant INS is still debated. There is no evidence that cyclosporine can prevent the recurrence of nephrotic syndrome following transplantation (351–356). Following the introduction of cyclosporine, the incidence of recurrence did not change, but graft survival was improved (351, 352, 355). In patients who have recurrent disease, high doses of cyclosporine may be effective. Mowry et al. reported on 11 children who received 12 renal transplants and who had been treated with high dose cyclosporine, plasma exchanges or a combination both (357). Remission was observed in 10 of the 12 recipients. Ingulli and Tejani reported on two children with recurrent nephrotic syndrome who both achieved remission when the dose of cyclosporine was gradually increased from 15 mg/kg/day to 27 and 35 mg/kg/day (358). A similar experience was reported by Srivastava et al. (359). In our group, seventeen children with recurrence have been treated with intravenous cyclosporine at an initial dose of 3 mg/kg/day which was afterward adapted in order to maintain whole blood levels between 250 and 350 ng/ml. In 14 of the 17 cases (82%), proteinuria completely disappeared after 20.8 $\pm$ 8.4 days (range: 12–40 days). The treatment was ineffective in the remaining three patients. Plasma exchanges were performed in four patients during the first 2 months and proteinuria regressed in three cases and persisted in one. Persistent remission was observed in 11 patients with a follow-up of 3.7 $\pm$ 3 years. Actuarial graft survival was 92 and 70% at 1 and 5 years (360). We advocate the early use of intravenous cyclosporine as a first-line treatment in recurrent INS after renal transplantation. Similarly, Raafat et al. reported that high doses of oral cyclosporine was effective in inducing long-lasting remission of recurrent nephrotic syndrome (361). There is limited experience with the use of tacrolimus (FK 506) in recurrent nephrotic syndrome after transplantation (362).

Plasma exchange has been performed in a number of patients, in some cases combined with increased immunosuppression (350, 363–368), with often partial or transient remissions. Better results are observed when the treatment is started early. Dantal et al. treated eight kidney-transplant recipients with recurrent nephrotic syndrome with plasma protein adsorption (122). The treatment consistently decreased urinary protein excretion by an average of 82% at the end of a cycle. The effect of adsorption was short-lived, with a return of proteinuria to pre-treatment levels within a maximum of 2 months.

Conclusion

Most children with INS respond to corticosteroid therapy. Although about half of them experience relapses requiring corticosteroids and several corticosteroid sparing agents, these children maintain normal renal function and the severity of the disease is mainly related to the complications of the treatments needed to maintain remission.

There is evidence to suggest that minimal changes and FSGS represent a spectrum of diseases with FSGS at the severe end (369). Indeed, it is likely that several disease entities are included in the term "steroid resistant INS." Defects in podocyte proteins have been identified in some cases and these patients do not respond to any therapy. In other patients, circulating factor(s) produced by immune cells increase the glomerular basement membrane permeability to proteins. Cyclosporine may be effective. However, more than 50% of the patients progress to end stage renal failure. Recurrence of the nephrotic syndrome occurs in 30–40% of patients after renal transplantation.

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29 Immune-mediated Glomerular Injury

Michio Nagata

Overview

Glomerular injury is the central basis of renal insufficiency. Accumulated immunohistopathologic evidence from human kidney diseases and experimental glomerular disease models indicates that immunologic mechanisms are often pivotally involved in glomerular injury. Founded on the classical evidence, recent advances in basic immunology, the molecular and cellular biology of intrinsic glomerular cells, and genetic engineering in mice have provided significant clues to understanding the immune mechanisms that target the glomerulus.

The pathogenesis of immune-mediated glomerular injury involves two major steps: antibody deposition on glomerular components, and the subsequent activation of secondary inflammatory mediators. Under certain circumstances, the activation of inflammatory process occurs in the absence of tissue antibody deposition (pauci-immune). For instance, membranoproliferative glomerulonephritis (MPGN) type II by the inherited defects in complement regulation and ANCA-related glomerulonephritis represent an immune-mediated, but pauci-immune glomerular injury. Basic immunology has promoted research into the complex immunopathogenesis of glomerular injury mediated by the two axes of the adaptive immune system, i.e., antibody-mediated and cell-mediated immunity.

The glomerular deposition of antibodies is promoted by the humoral immune process of Th2 activation, which leads to antibody production via B-lymphocyte activation. This deposition may be composed of preformed immune complexes from the circulation, which are trapped in the glomerulus, or antibodies (autoantibodies) that bind to intraglomerular antigens or interact with intrinsic glomerular cell surface antigens, as a result of changes to the cell surface.

Among the four mechanisms underlying hypersensitivity, immune-mediated glomerular injury is primarily of the type II and III mechanisms. As seen in type III hypersensitivity reactions, antibody deposition on glomerular capillary walls or the mesangium activates the complement cascade and intrinsic cell-derived chemotactic factors result in inflammatory cell influx, which is a crucial event in the glomerular injury by production/activation of pro-inflammatory cytokines, chemokines, growth factors, reactive oxygen species (ROS), eicosanoids, and nitric oxides (NO). These secondary mediators not only stimulate intrinsic cells to proliferate and/or produce matrix materials, but they also induce the synthesis of similar molecules by intrinsic glomerular cells. The secondary mediators involves severe endothelial injury which potentially result in dysregulation of local coagulation system, accompanied by exudation and the formation of cross-linking fibrins. This end-products of the extrinsic coagulation cascade also damage glomeruli particularly tuft necrosis, the common forerunner of crescentic formation.

An alternative immune mechanism is cell-mediated immunity, with Th1 activation promoting cytotoxic glomerular injury. Proliferative glomerulonephritis in the absence of immune complexes or antibodies suggests a role for cell-mediated immune injury. This is supported by the experiment showing that transfer of sensitized T cells successfully induced glomerular injury in the host. Although the precise mechanism remains unclear, ANCA-related crescentic glomerulonephritis appears to involve T-cell-mediated glomerular injury. It is clear that the antibody- and cell-mediated immune systems are not independent, and often interact in glomerular injury.

Immune mechanisms may also be involved in glomerular diseases that are typically considered to have a non-immunologic basis. For example, although diabetic nephropathy is categorized as a metabolic disease, it involves glomerular macrophages and/or lymphoid cell infiltration, and the expression of adhesion molecules, all of which are mediators of inflammatory processes and significantly involved in this disease. Recent experiments have shown that the inhibition of pro-inflammatory cytokines or macrophages suppresses the progression of diabetic glomerulopathy. Similarly, monocyte/macrophage or foam cell influx is sometimes observed in idiopathic focal segmental glomerulosclerosis, which usually shows no evidence of antibody deposition. Thus, immune mechanisms may be linked to a wide variety of glomerular diseases, even those typically considered to be non-immune-mediated conditions.

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This chapter provides a review of our basic understanding of and up-dated information regarding immune-mediated glomerular injury, with emphasis on the pathogenic mechanism(s). Although numerous scientific advances have come out of animal experiments, it should be kept in mind that experimental models of immune-mediated glomerular injury are largely relevant to the acute phases of anti-GBM antibody nephritis, nephrotoxic serum nephritis or anti-Thy1 nephritis of mesangial proliferation. Furthermore, the molecular profiles of the immune systems of mice and humans are significantly different. This review of our basic knowledge of immune-mediated glomerulonephritis may help to explain some aspects of the mechanism(s) underlying chronic glomerulonephritis in humans.

Glomerular Components

Glomerulonephritis arises from the responses of intrinsic glomerular cells to inflammatory reactions. Glomerular deposition, hypercellularity (intrinsic and inflammatory cells), and capillary destruction are typical features of glomerular injury (*Fig. 29-1*). The biologic characteristics of the intrinsic glomerular cells are implicated in this process. Although the cellular components of the glomerulus are anatomically defined by three cell types, mesangial cells, endothelial cells, and podocytes, parietal cells are also involved in glomerulonephritis, particularly in cellular crescent formation and adhesion (the renal corpuscle comprises these four cell types). In addition, the biochemical profiles of the extracellular matrices, glomerular basement membrane, and mesangial matrices are unique, and may be targets for antibody deposition, leading to effects on the corresponding intrinsic cells. Ultrastructural analyses provide a good opportunity to define the involvement of extracellular matrices in glomerular injury.

The Glomerular Basement Membrane (GBM)

The GBM, which is a central component of the filtration barrier based on charge and size, is composed of extracellular matrix materials, including collagen IV, nidogen (entactin), heparan sulfate proteoglycans (HSPG), laminin, and perlican. GBM is synthesized by podocytes and endothelial cells; however, the details of the formation of the membrane from the individually synthesized proteins remain unknown. Nevertheless, this unique permeability system traps macromolecules and antibodies and is responsible for their glomerular deposition.

The GBM also contains potentially harmful antigens. Pathogenic autoantibodies directed against the C-terminal globular non-collagenous domain 1 (NC1) of the α3-chain of type IV collagen, which has a distinct GBM molecular composition, promote severe glomerulonephritis in patients with Goodpasture syndrome by epitope-specific autoantibody binding and effector T cell reaction (*Fig. 29-2*) (1). Similar epitopes are targeted by alloantibodies in a subset of patients with Alport nephritis who have received allografts. GBM is also the target of bioactive substances released from inflammatory cells and intrinsic cells during inflammation. GBM changes may directly influence the physiologic stability of filtration, thereby reducing the glomerular filtration rate and causing proteinuria. Electron microscopy has revealed GBM alterations in association with immune deposits, and inflammatory cell influx can directly alter the morphology of the GBM (*Fig. 29-3*). Proteolytic enzymes derived from neutrophils may directly alter the GBM, resulting in membranolysis, lamination, gaps, and edema (typical features of GBM injury), which may lead to urinary abnormalities. Immune deposition on the GBM, as seen in membranous glomerulonephritis and lupus nephritis, can also alter the GBM by changing its barrier of charge and size properties.

Mesangial Cells

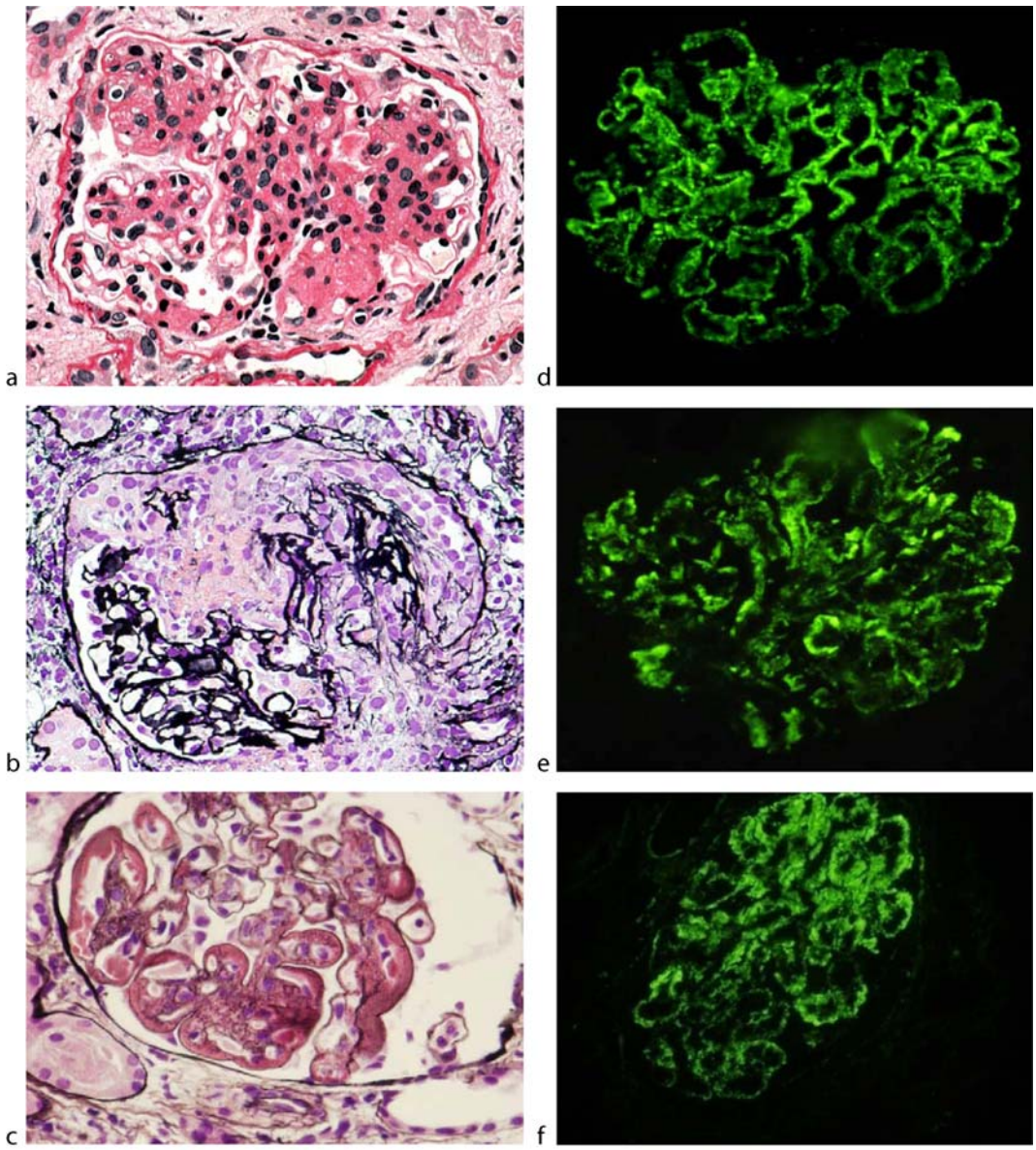
The mesangium is the axis of the glomerulus, holding the capillaries in a developed dense network of matrix, which includes microfibrils and fibronectin. Mesangial cells are derived from the metanephric mesenchyme and there are one or two cells within each mesangial area. The biological properties of mesangial cells have some similarities with vascular smooth muscle cells. Both cell types show contraction and cell proliferation in response to vasoactive substances and cytokines In vitro.

The lack of intact GBM between endothelial cells and the mesangium enables bone marrow-derived inflammatory cells and macromolecules to access the mesangium (*Fig. 29-4*). Early studies showed that resident cells in the mesangium expressed the phenotype of bone marrow cells (Ia-positive) in the normal state, and that their numbers were increased in glomerulonephritis. GFP-tagged bone marrow cell transplantation in rat anti-Thy1 nephritis, a model of mesangial proliferative glomerulonephritis, have provided evidence that bone marrow-derived cells are responsible for mesangial hypercellularity.

The mesangial fluid pathway was discovered in an experiment involving the ligation of lymph vessels around

Figure 29-1

Typical features of immune mediated glomerular injury seen in human biopsy samples by LM and IF. (a) Mesangial proliferation in IgA glomerulonephritis (PAS stain), (b) crescentic formation with tuft necrosis and fibrin deposition in ANCA-related glomerulonephritis (PAM stain), (c) subendothelial immune deposition in lupus nephritis (PAM stain), (d) granular and peripheral pattern of IgG in membranous glomerulonephritis, (e) fringe pattern of C3 deposition in MPGN, (f) subendothelial IgG deposits in lupus nephritis.

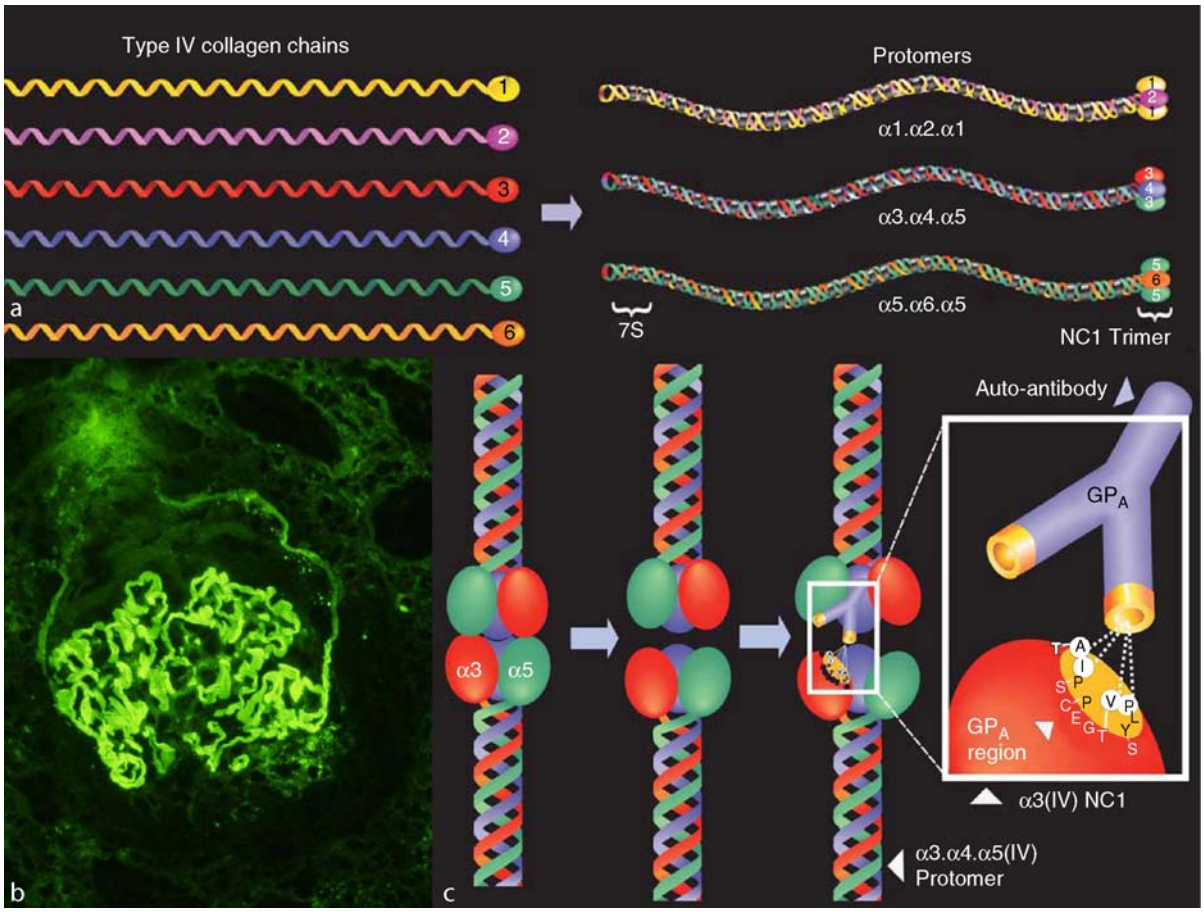


the renal artery. This leaky barrier and fluid pathway allow circulatory macromolecules, including antibodies, to be trapped in the mesangium as mesangial deposits. The phenotypes and behaviors of cells in the mesangium are also modulated by the glomerular scaffold of self-produced extracellular matrix materials.

In glomerular injury, deposition, cell proliferation, matrix production, and cell death occur in the mesangium. Mesangial proliferation is a typical feature of glomerular injury. Activation of cell cycle molecules, as revealed by immunohistochemical detection of proliferating cell nuclear antigen or Ki-67, is detected in the

Figure 29-2

Structure of Goodpasture antigen epitopes in collagen Type IV. a. c. GP epitope is localized in the NC1 domain of α3(IV) chain of type IV collagen. In the GBM, the α3(IV) chain is associated with the α4(IV) and α5(IV) chains, forming protomers and networks. The GP epitopes are cryptic within the α3·α4·α5(IV) NC1 hexamer and inaccessible for autoantibody binding unless the NC1 hexamer dissociates. Several hydrophobic amino acids were found critical for the epitope of the immunodominant GPA autoantibodies (inset); which appears to be sequestered by an interaction with an α5 (IV) chain in the same protomer b. Linear staining pattern of IgG in glomerulus from patient with GP syndrome. (GP Goodpasture, GBM glomerular basement membrane.) (Modified figure from 124 with permission.)

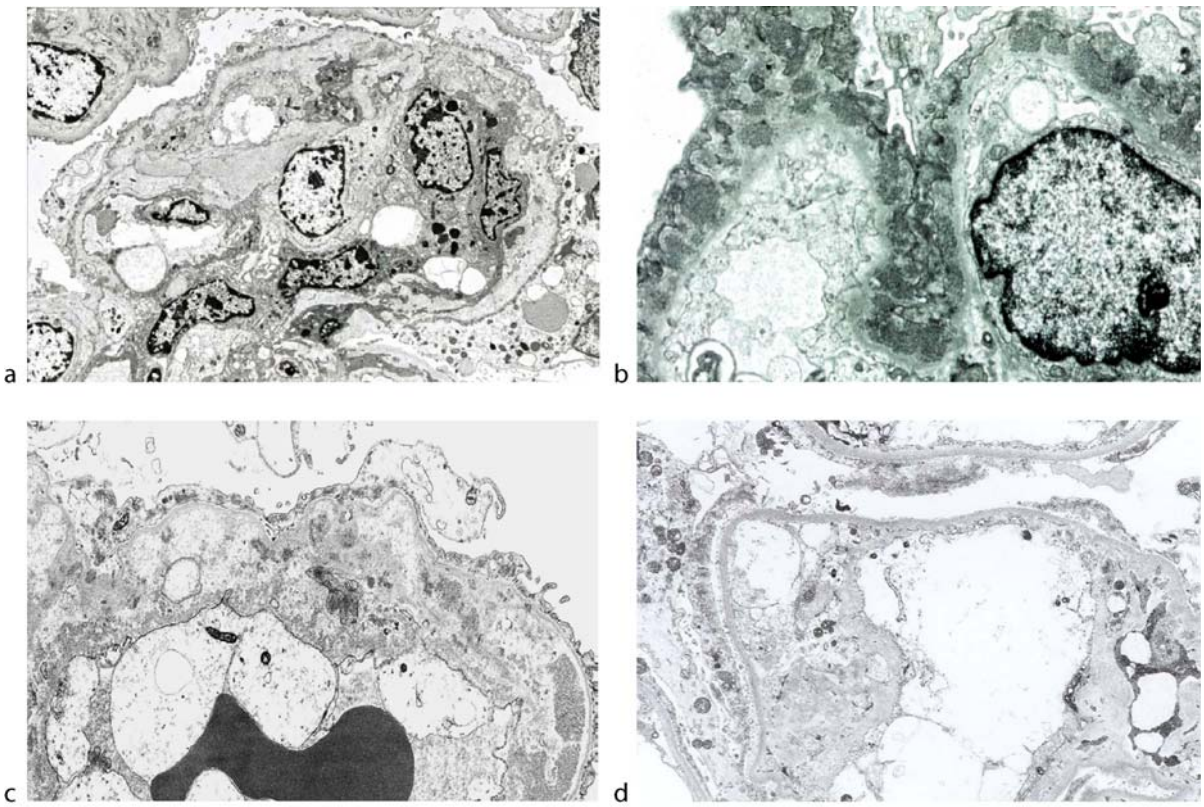


mesangial cells in glomerulonephritis, indicating mesangial cell proliferation In situ. Cell cycle is ultimately regulated by the balance of cell cycle promoter (cyclins) and inhibitors (cyclin dependent kinase inhibitors) (*Fig. 29-5*). Cyclin D1, coupled with cyclin-dependent kinases (CDK4 and CDK6), promotes the re-entry of quiescent mesangial cells in G0 to G1. The normal mesangium expresses the cyclin-dependent kinase inhibitor (CKI) p27 and $p27^{-/-}$ mutant mice with glomerulonephritis show higher levels of mesangial cell proliferation and matrix deposition. The mechanism of PDGF-stimulated mesangial cell proliferation is apparently through the down-regulation of p27. Together, cell cycle quiescence in mesangial cells is regulated by CKIs, p27. A study showed that activation of T-type calcium channels in smooth muscle cells promoted cell cycle progression in mesangial cells. (2)

Mesangial activation occurs though receptor-mediated mechanism. Mesangial cell proliferation is stimulated by various cytokines and growth factors, including interleukins (ILs), PDGF, and fibroblast growth factor (FGF). In addition, Fc fragments of IgG and IgA binds the receptor in mesangium and stimulate the cells. For example, PDGF binds to a tyrosine kinase receptor, which activates a number of downstream effectors, including the Ras/MAPK

Figure 29-3

Changes of filtration barrier in glomerulonephritis by EM. (a) Lytic change in GBM is accompanied with endothelial swelling with loss of fenestra on the background of inflammatory cells in the capillary. Note foot process effacement in podocytes with actin accumulation in podocyte, (b) subepithelial dense deposits with thickening and irregularity in GBM and foot process effacement, (c) thickening of GBM due to membranolysis containing cellular debris, deposits and cellular infoldings, (d) Endothelial cell swelling and podocyte detachment from GBM.



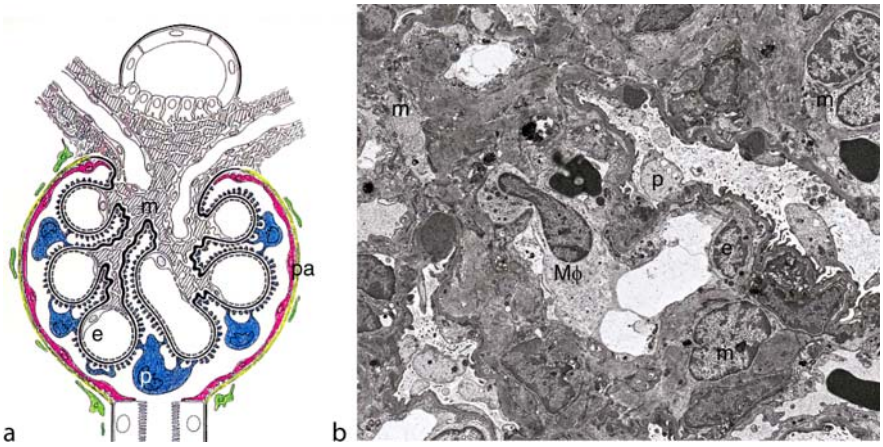
pathways, to activate various transcription factors (c-fos, c-myc, c-jun) and proto-oncogenes. Activated mesangial cells synthesize cytokines and growth factors similar to those produced by inflammatory cells, as well as various vasoactive substances, such as angiotensin, endothelin, and vasopressin. As soluble cytokines and growth factors act in autocrine, paracrine, and juxtacrine manners, mesangial inflammatory cells effectively activate resident cells, and both cell types accelerate cell proliferation and matrix production In situ. During active glomerulonephritis with mesangial proliferation, mesangial cells frequently express α-smooth muscle actin (α-SMA), desmin, calponin, and matrix-binding integrin receptors. This may not only be an example of phenotypic changes in mesangial cells reflecting cell activation, but may also represent involvement in further glomerular injury. Mesangial proliferation is recognized as a forerunner of glomerulosclerosis, and several In vitro experiments have revealed that mesangial cells synthesize matrix components in response to various inflammatory cytokines and growth factors. Hyperplastic mesangial cells reduce cell numbers by apoptosis mediated via a variety of signaling mechanisms.

Podocytes

Podocytes have many intrinsic properties sustained by actin cytoskeleton and some unique molecules that participate in the glomerular pathophysiology. Podocytes are located outside of glomerular capillaries and do not affect directly the bloodstream; instead, they face the primary ultrafiltrate, which contains various biochemical substances. Podocytes are an important component of the filtration barrier, in that they have slit membranes and

Figure 29-4

Structure of glomerulus and monocyte influx. (a) Schematic presentation of the glomerular profile. Glomerulus is composed of four cell types; mesangium (m), endothelial cell (e) podocyte (p) and parietal cell (pa). Note lack of GBM between endothelial cell and mesangium Original illustration is courtesy from Dr. W. Kriz with permission, (b) invasion of macrophages (Mϕ) into mesangium in the biopsy of in MPGN.



synthesize GBM components. Podocytes are highly specialized post-mitotic cells that are derived from the metanephric mesenchyme. Indeed, podocytes have a negligible capacity to proliferate, in that they undergo nuclear division but cytokinesis is not evident In vivo. Up-regulation of the p27 and p57 is closely associated with the podocyte differentiation phenotype at the capillary loop stage. Metanephric organ cultures of mice that lack p27 and p57 have revealed enlarged glomeruli, with increased podocyte numbers, and the expression of WT1 and synaptopodin, indicating that cell cycle inhibitor per se control podocyte number, but not cell differentiation in podocytes. Actin dynamics in podocytes has been intensively investigated and its role for cell integrity and function emerged. Podocytes are rich in actin filaments, and the cytoskeletons of their intermediate filaments are linked to foot process maintenance and slit membrane function. Several podocyte markers, such as WT1, CALLA, CD10, synaptopodin, podocalyxin, podoplanin and nestin have been identified. The slit membrane-related molecules nephrin and podocin are also sometimes used as specific markers. In addition, the podocyte is a major source of growth factors, such as VEGF, TGF-β and CTGF. Podocyte also expresses angiotensin II type 1 receptor which mediates local renin-angiotensin system for podocyte function. Podocyte injury is the base of proteinuria and podocyte loss from GBM (podocytopenia) may leads to glomerulosclerosis.

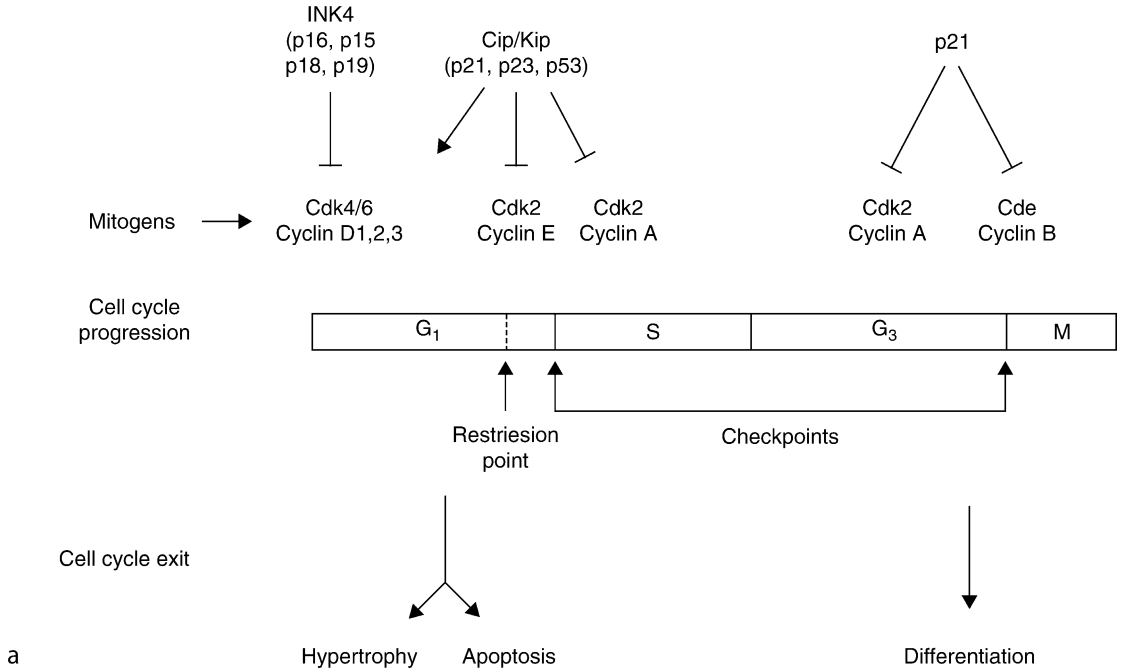
Immunologic mechanisms including GBM deposition, complement activation, and inflammatory mediators involve podocyte damage, and alteration of podocyte molecules results in the proteinuria and sclerosis. Podocyte injury is visible by morphology. Electron microscopy reveals cytoplasmic vacuolation, pseudocyst formation, foot process effacement with actin accumulation and detachment. In addition, phenotypic changes, namely, expression of desmin also represents podocyte injury in the rodents. These changes are induced not only by hemodynamic effects, but also by immune-mediated properties. Membranous glomerulonephritis is an example of immune-mediated podocyte injury, which results from complement activation. The antigen responsible for experimental membranous glomerulonephritis (Heyman nephritis) is the podocyte membrane protein megalin. Similarly, the recently identified neural endopeptidase (NEP), which is located in podocytes, is potentially an intrinsic antigen that forms In situ the immune complexes that cause membranous glomerulonephritis in humans (3). Podocytes express B7-1, which is a costimulatory molecule for T cells that is up-regulated in injured podocytes and causes dysregulation of the actin cytoskeleton, leading to proteinuria (4).

Endothelial Cells

The glomerular endothelial cell is the only intrinsic glomerular cell type that directly faces the circulation. These endothelial cells are different from those in other organs because of their extraordinary flatness and highly

Figure 29-5

Cell cycle cascade and its changes in the glomerular diseases. (a). Cell cycle progression is processed by four phases, G1, S, G2 and M. Cell cycle is controlled phase-corresponding molecules and check points. Cell cycle exit in G1 phase results in cell hypertrophy or apoptosis and exit in G2, (b) cellular response in mesangial cells and podocytes to injury is associated with the changes in cyclin A/CDK2 and p21 family (p21, p27 and p57). (After Griffin SV et al. (2) with permission.)



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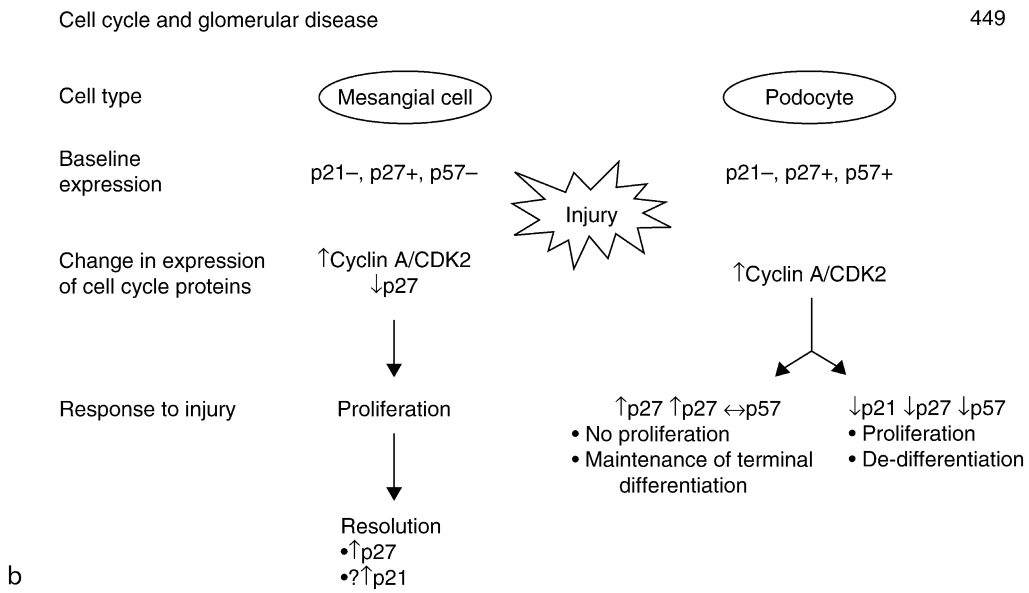


b

Table 29-1

Neutrophils endothelial cells express various adhesion molecules for interaction

Endothelial molecules	Leukocyte molecules	Major function
P-selectin	Sialyl-Lewis X-modified protein	Rolling (neutrophils, monocytes, lymphocytes)
E-selectin	Sialyl-Lewis X-modified protein	Rolling and adhesion PMNs, monocytes, T cells)
GlyCam-1, CD34	L-selectin	Rolling (neutrophils, monocytes)
ICAM-1 (immunoglobulin family)	CD11/CD18 integrins (LFA-1, Mac-1)	Adhesion, arrest, transmigration (neutrophils, monocytes, lymphocytes)
VCAM-1(immunoglobulin family)	VLS-4 integrin	Adhesion (eosinophils, monocytes, lymphocytes)
CD31	CD31	Transmigration (all leukocytes)

ICAM-1, intercellular adhesion molecule 1; LFA-1, leukocyte function-associated antigen 1; VCAM-1, vascular cell adhesion molecule 1; VLA-4, very late antigen 4

fenestrated features, allowing effective filtration. The fenestration (70–100 nm in size) is maintained by vascular endothelial growth factor A (VEGF-A), which is derived from the podocytes. The endothelial cell surface layer provides a negatively charged mesh of polysaccharide moieties of glycoproteins, glycosaminoglycans (heparan sulfate, sialic acid, chondroitin sulfate, hyaluronan) which determine vascular rheology and are probably involved in permselectivity. The charge and various adhesion molecules on the cell surface permit interactions between endothelial cells and blood-borne factors, including leukocytes, platelets, the third component of complement, antigens, and immune complexes (*Table 29-1*, *Fig. 29-6*). Endothelial cells physiologically synthesize several coagulation proteins, growth factors, extracellular matrix components, and various substances, including nitric oxide, prostaglandins, and endothelin, to maintain cell integrity and the vascular environment. Angiotensin converting enzyme (ACE) and endothelin converting enzyme participate in the local formation of bioactive angiotensin II and mature endothelin, respectively.

Endothelial cells are involved in immune-mediated glomerular injury by many aspects, including characteristic cell surface which is a feasible target of inflammatory reaction, expression of MHC proteins, and changes of variety of bioactive/physiologic substances.

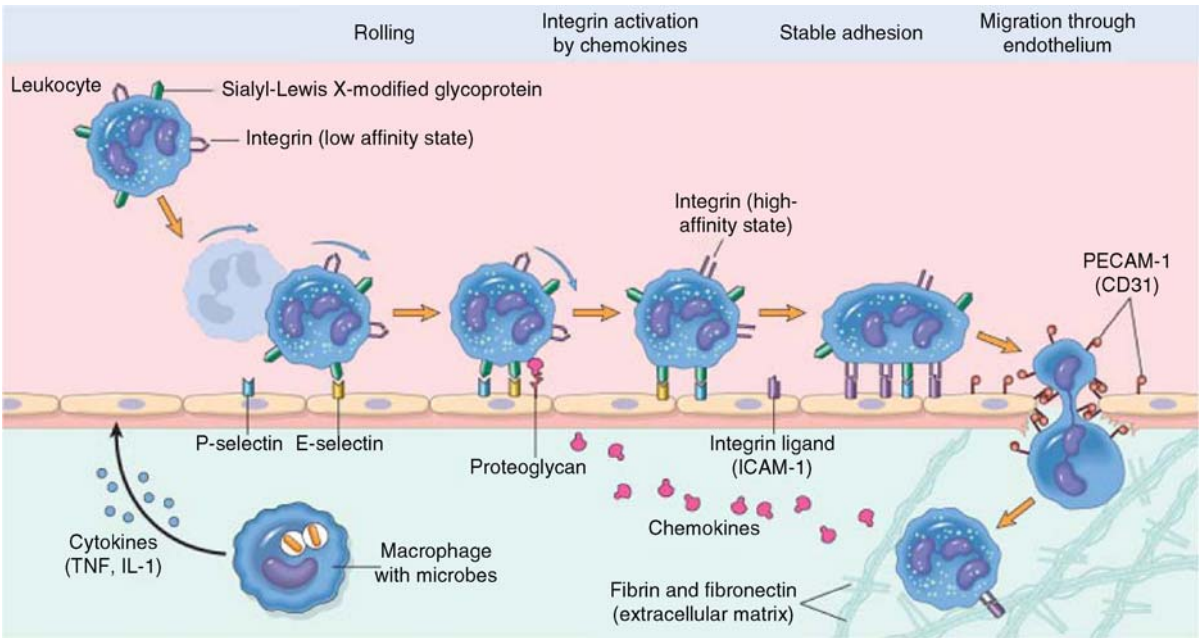
Endothelial cell proliferation, typically observed in endocapillary proliferative glomerulonephritis and preeclampsia (also known as endotheliosis) is considered as post-injury regenerative responses. Since endothelial cells actively regenerate and cell loss is replaced either by neighboring cells or bone marrow-derived cells, endothelial cell injury may be usually reversible. Endothelial cell injury is also evidenced by fibrin deposition, as seen in thrombotic microangiopathy. An additional feature of endothelial injury is the double contour of the GBM, as typically seen in transplant glomerulopathy. The mechanism of this humoral-mediated rejection is unclear, but MHC class II antigens on the endothelial surface interact with anti-donor antigens and form an In situ immune complex that persistently activates or injures endothelial cells, resulting in matrix production and subendothelial widening. These features have also been noted in MPGN, in which persistent complement activation is the trigger for endothelial cell injury.

Parietal Epithelial Cells (PECs)

Parietal cells, also known as Bowman's epithelium, are located in the continuous epithelial lining between the podocytes and proximal tubular cells. PECs and podocytes share a common phenotype during glomerular differentiation until the S-shaped body stage. Although PECs seem to play a role in glomerular injury, little is known about their biologic and physiologic properties. PECs express receptors for PDGF and FGF, and their ligands derived from inflammatory or intrinsic cells can stimulate cell proliferation and matrix synthesis. PECs are the major constituent of the cellular crescent, which expresses connective tissue growth factor (CTGF) mRNA, suggesting a growth factor-mediated mechanism for glomerular scar formation. An In vitro study revealed that FGF-2 stimulated PECs proliferation and PDGF stimulated matrix production in PECs in culture. In addition, tissue factor or cross-linked fibrin stimulates proliferation in PECs In vitro. The experimental model of crescentic glomerulonephritis has shown that PECs undergo an epithelial mesenchymal transition, which is involved in scar formation in glomerular crescents (5) PEC proliferation has also been

Figure 29-6

Endothelial cell inflammatory cell interaction and migration. The complex process of leukocyte migration through blood vessels is shown. The leukocyte first roll, then become activated and adhere to endothelium, then transmigrate across the endothelium, pierce the basement membrane, and migrate towards chemoattractants emanating from the source of injury. Different molecules, namely chemokines and adhesion molecules, play pivotal roles for stepwise processes of this inflammatory action. Similar mechanism may work in the glomerular injury. (The figure was published in Kumar et al. Robbins Basic Pathology, 8 edn. 2007, pp. 36, Copyright Elsevier.)



demonstrated in non-immune-mediated glomerulonephritis, particularly in focal segmental glomerulosclerosis and other immune-/non-immune-mediated glomerulosclerosis conditions. Cell cycle stability in PECs is controlled by p21, and mice with glomerulonephritis that lack p21 exhibit PEC hyperplasia, as shown by genetic tagging experiments in nephrin-Cre mice. In addition, a recent investigation has proposed the multipotency of PEC differentiation (6). Cell proliferation, matrices production and distortion of functional architecture in PECs involve in the glomeruloeclerosis by immune or non-immune mechanisms.

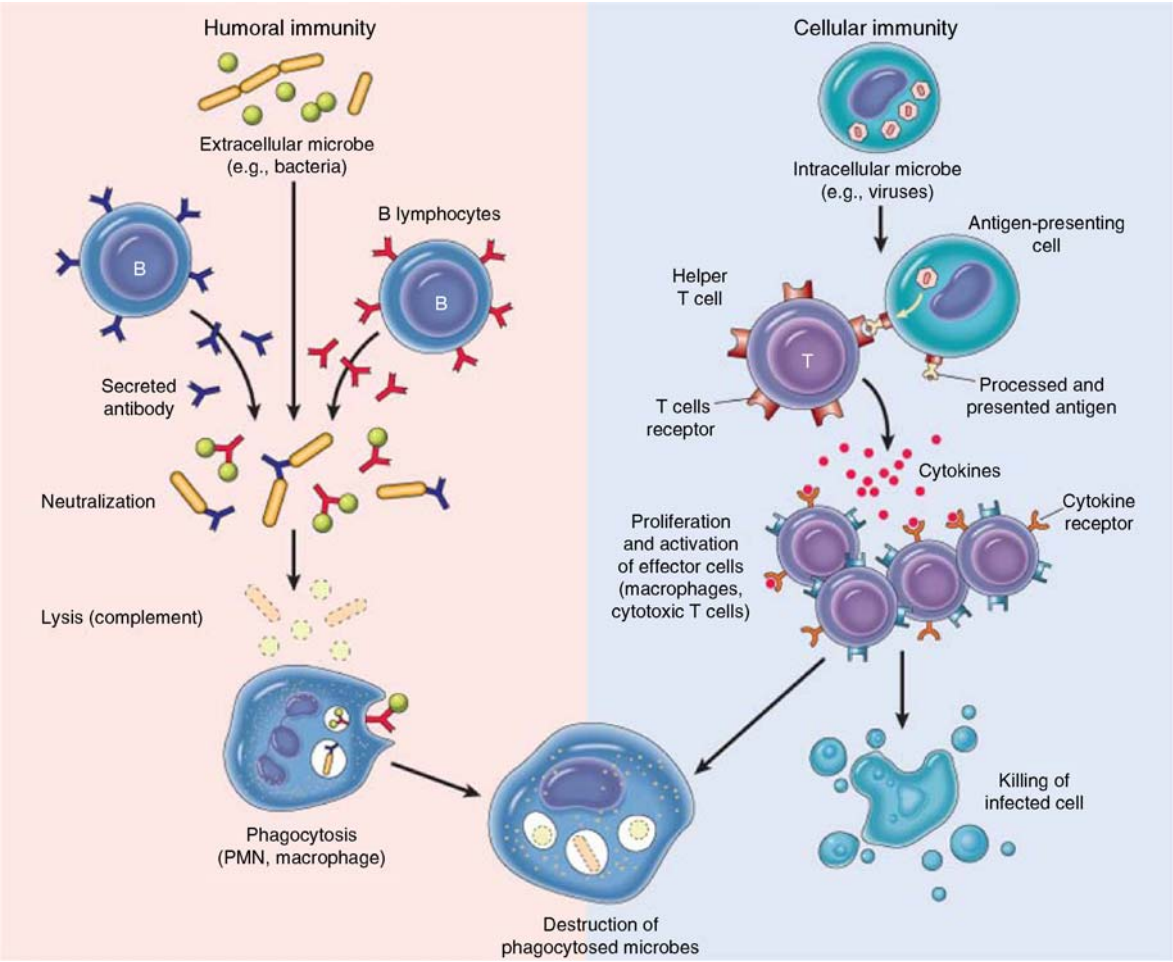
Immune System and Animal Models

Innate and adaptive immune systems are the pathogenic background of immune-mediated glomerular injury. Although molecular and cellular mechanism of immune system is substantially complex, it may be convenient to follow the mechanism of glomerular injury by two steps, initiation and promotion. Lymphocytes mainly drive an initiation and neutrophils and monocyte/macrophages largely participate in promotion as secondary mediators.

Lymphocyte mediated injury is processed by antibody-mediated (B cells driven) or cell-mediated (T cells driven) mechanisms and they usually interact and synergistically involved in the tissue damage (*Fig. 29-7*). B cell system promotes production and In situ deposition of antibodies (majority forms antigen-antibody complexes) in the glomerulus. This initiation induces the secondary inflammatory mediators, including cytokines and chemical mediators, derived from activated leukocytes. T cell mediated glomerular injury may include rather wide ranged processes, since T cell regulates B cell system. However when we concern about T cell-dependent glomerular injury as a narrow meaning, it is less common. One example of this mechanism is cytotoxic action of T cells which may damage the intrinsic glomerular cells or alter glomerular filtration barrier, causing proteinuria. This complex immune system profiled by Th1/Th2 subsets may determine the type of glomerular injury.

Figure 29-7

Antibody-mediated immunity and cell mediated immunity. In antibody-mediated immunity, B lymphocytes secrete antibodies that eliminate extracellular microbes or antigens. In cell-mediated immunity, T lymphocytes injure intrinsic cells via activation of macrophages to destroy phagocytosed microbes or kill infected cells. Cytotoxic T cells may directly damage intrinsic cells. (The figure was published in Kumar et al. Robbins Basic Pathology, 8 edn. 2007, pp. 109, Copyright Elsevier.)



There are varieties of experimental models of immune-mediated glomerulonephritis that have been used to analyze the nephritogenic mechanisms. Nephrotoxic serum nephritis, a classical model initially established by Masugi in 1933, is induced by injection of duck anti-rabbit kidney anti-sera to rabbits resulting in crescentic glomerulonephritis. Anti-GBM antibody nephritis is induced by administration of anti-GBM IgG showing proliferative (usually crescentic) glomerulonephritis. Both are the basic and widely examined models of glomerulonephritis. There are two phases of immune reaction in these models, heterologus and autologus phase. Former is provoked by the binding of injected allo-antibody to GBM, whereas the latter is caused by host-produced autoantibody binds to the GBM. Heterologus phase alone cause mild and self limiting nephritis, but prolongation or acceleration of autologus phase by pre-immunization with IgG of immunized animals provokes severe glomerulonephritis. Autoantibody may be important to induce severer nephritis. Several mice models of lupus, including (NZBXNZW) F1, BXSB, and MRL/lpr mice, reveal spontaneous and proliferative glomerulonephritis with immune complex deposition, similar to the human lupus nephritis. Heyman nephritis is a model of

membranous glomerulonephritis and responsible antigen is identified as megalin. In this model, complement activation plays significant roles for proteinuria and proliferative nephritis is not usually seen. All these models are immune complex-mediated glomerulonephritis as revealed by immunoglobulin or complement deposition, regardless of whether circulating or In situ immune complex formation. On the contrary, widely used model of anti-Thy1 nephritis reveals mesangial proliferative glomerulonephritis but pauci-immune. In this model, antibody against thymus immediately binds to receptor on mesangial cells resulting cell lysis (mesangiolysis) associated with transient activation of complements, however immune complex and complements are lost immediately. Mesangial proliferation in this model may reflect repair process from mesangiolysis to reconstruct glomerular architecture mimicking glomerulogenesis, rather than the result of mitogenic stimuli of inflammatory mediators (unlike immune-mediated glomerulonephritis). Since majority of experimental works using these models investigated an acute phase of the diseases, it is necessary to understand the pathogenetic difference in each model and recognize the limitation of the results to translate human chronic glomeurlonephritis.

Antibody-Mediated Immunity

Antibody (humoral) -mediated immune glomerular injury is initiated by antigen-antibody complex formation within the glomerulus. B-cell activation by interleukins (IL-4, IL-5), which are synthesized by antigen-specific $CD4^+$ T cells, is a prerequisite for antigen-specific antibody production. Many glomerular lesions result from the glomerular binding of antibodies. Immune-mediated glomerular injury is seen in a wide spectrum of glomerular diseases, with various histologic patterns, and the clinical outcomes may be related to the nature of the antigen(s) and their glomerular binding site(s).

Circulating Immune Complexes

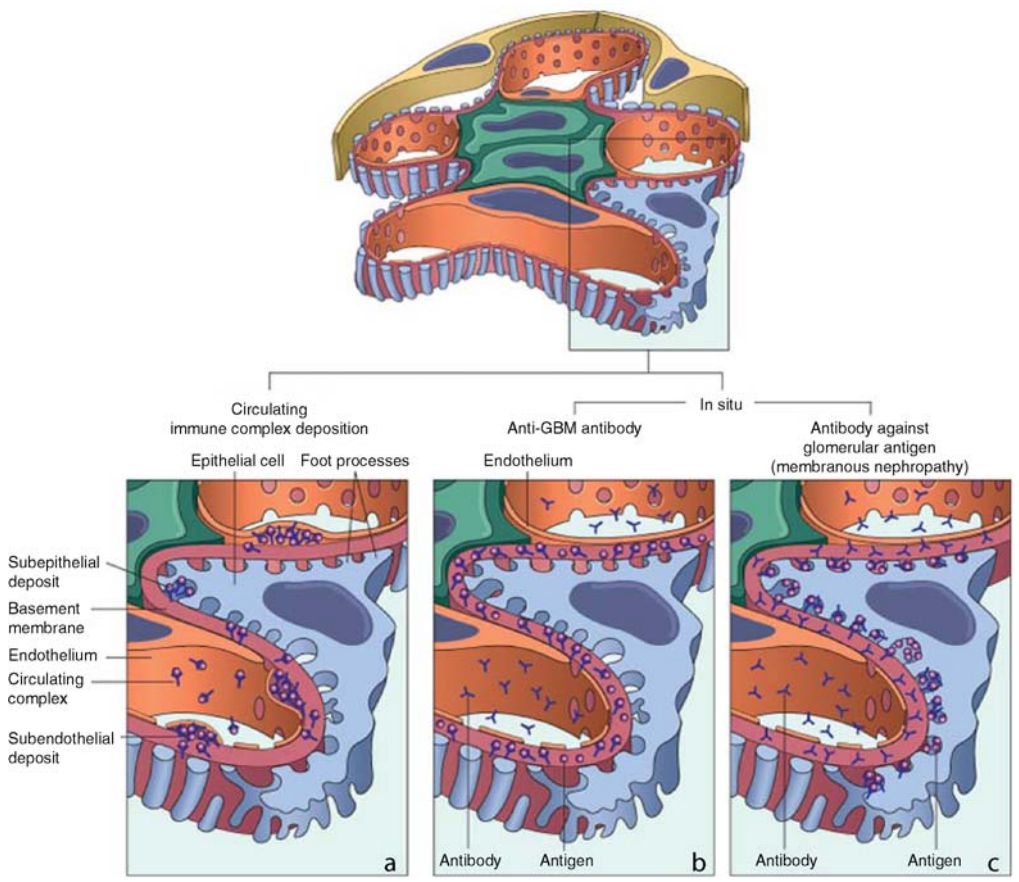
If the immune system produces autoantibodies, immune complexes can be formed. Circulating immune complex-mediated glomerulonephritis is primarily seen as a type III hypersensitivity reaction (*Fig. 29-8*). As known in the serum sickness disease model, high amount of foreign serum injection resulted in the appearance of immune complex in the circulation which accumulates in the glomerulus and induces acute glomerulonephritis and vasculitis. Regardless of whether they are endogenous or exogenous, the antigens are not glomerular in origin. Exogenous antigens can be bacterial (*Streptococcus* and *Staphylococcus spp.*), viral (hepatitis B), parasitic (*Plasmodium spp.*) or spirochete (*Treponema spp.*) in origin. In contrast, DNA and histones are endogenous antigens that are typically seen in lupus nephritis.

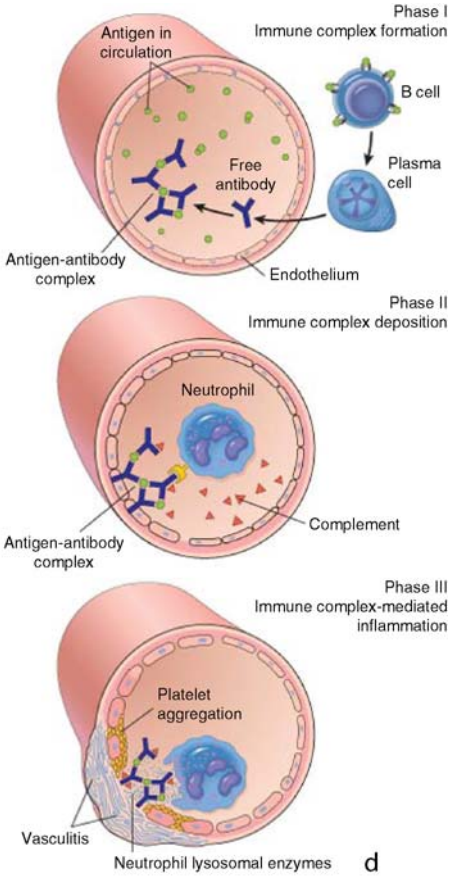
The highly permeabilized glomerular filter is susceptible to passive trapping of preformed immune complexes from the circulation. The highly negative charge of the capillary wall also attracts circulating macromolecules with positive charges.

The biological activities of circulating immune complexes depend largely on the biochemical and quantitative features of the circulating antigens. The size of an immune complex is determined by the ratio of antigen to antibody. When the antibody is present in excess, cross-linking of immune complexes occurs and the complex becomes large. The primary site of such a large immune deposition is either the subendothelium or the mesangium and efficiently activate the complement cascade by binding to Fc receptors (the Arthus reaction), thereby further stimulating the inflammatory processes. In contrast, when an antigen bears few epitopes or when there is an excess of antigen relative to antibody, the immune complexes formed tend to be smaller in size and soluble. These small immune complexes may be deposited within the GBM or subepithelial areas, but they often fail to induce inflammation, because the deposition and complements are isolated from circulation. When the antigen to antibody ratio nears equivalence, the immune complexes formed tend to be of maximal size and insoluble. The net charge of the complex is an additional determinant of immune complex deposition, based on the interactions with the negatively charged GBM and endothelial surface. The levels of circulating immune complexes have been shown to correlate with glomerular injury in lupus glomerulonephritis. The size and biochemical characteristics of immune complexes may change with disease activity or with therapeutic modulation. Indeed, immune complex deposition in subendothelial or mesangial cells occasionally shifts to the subepithelial cells in lupus nephritis, such as Class IV to Class V transformation. Acceleration of glomerular capillary permeability in the process of glomerulonephritis may be an alternative factor to change the site of immune deposition. Although experimental models suggest induction of glomerulonephritis by glomerular immune complex deposition, it remains unclear as to whether passive trapping of preformed immune complexes alone is sufficient to induce glomerulonephritis in humans.

Figure 29-8

Mechanism of immune deposition and inflammatory reaction in the glomerulus. (a) Circulating immune complex interaction with endothelium results in deposition in GBM, (b) in case of anti-GBM antibody glomerulonephritis (Goodpasture syndrome), fixed antigen is a component of GBM (NC1 domain of α3 (IV) chain of type IV collagen). Thus antibody binds GBM and show linear distribution, (c) in case of membranous glomerulonephritis (Heyman nephritis), antigen is podocyte component (megalin). Antigen-antibody complex is formed in situ and complex shift to accumulate at subepithelial area (d), antigen-antibody complex in the circulation deposits on the endothelial surface is an initiation of immune-mediated glomerular injury. Local activation of complement system chamoattracts inflammatory cells followed by secretion of secondary messengers which injure glomerular component. (The figure was published in Kumar et al. Robbins Basic Pathology, 8 edn. 2007, pp. 545 and 127, Copyright Elsevier.)





In situ immune complexes

In situ immune complex formation is basically the result of the combination of unbound circulating soluble antibody and antigen within the tissue. In situ tissue antigens are either fixed (components of the glomerulus) or planted (exogenous) (*Fig. 29-8*). The best example of an In situ immune complex disease is anti-GBM antibody-induced glomerulonephritis (anti-GBM antibody nephritis). In this condition, antibodies are directed against a fixed antigen of a component of the GBM, i.e., the non-collagenous domain of the α3 chain of collagen type IV. These antibodies occasionally cross-react with the lung alveolar basement membrane, and the resulting simultaneous inflammation of the kidneys and lungs represents a severe clinical phenotype, known as Goodpasture syndrome. Another example of an In situ immune complex disease is Heyman nephritis, which serves as a model of membranous glomerulonephritis in humans. Combine this model with isolated perfused kidney system provided first evidence for In situ immune complex induced glomerulonephritis (7). Auto-antigenic target in this model is 516kD protein megalin that binds receptor associated protein (RAP); a podocyte-membrane glycoprotein (8). However megalin is not expressed in humans and may not be the antigen in human membranous glomerulonephritis.

In clinical practice, various antibodies are detected in the blood, while circulating immune complexes are generally undetectable. It seems that In situ immune complex formation is the major process for immune deposition in various glomerular diseases in humans.

Cell-Mediated Immunity

Cell-mediated immunity can be summarized as the T-cell-dependent interaction of antigen-presenting cells and CD4 helper T cells through the T-cell receptor (TCR), which promotes cytokine release and stimulates the proliferation and activation of effector cells, macrophages, and $CD8^+$ cytotoxic T cells (9).

T-cell-mediated glomerular injury includes several mechanisms, such as T-cell-mediated B-cell activation, cytotoxic effects, and the direct actions of lymphokines on the glomerular filtration barrier. The first process is T-cell-driven, antibody-mediated immunity, as described above, and the latter two processes are narrowly defined, basically because of the paucity of antibody deposition In situ.

Inappropriate T-cell responses may cause hypersensitive immune reactions and provoke glomerulonephritis. In humans, T-cell infiltration is observed in the glomeruli in pauci-immune glomerular diseases, such as ANCA-related glomerulonephritis, and therapeutic interventions suppress glomerular inflammation and T-cell infiltration. Anti-GBM antibody nephritis in Wistar Kyoto rats revealed infiltration of $CD8^+$ T cells in the glomeruli and suppression of circulating $CD8^+$ T cells resulted in decreased glomerular inflammation. However in mice model of anti-GBM antibody nephritis, glomerular injury is $CD8^+$ T cell independent. These observations provide circumstantial evidence to establish the basis of a T-cell-dependent glomerular injury mechanism. Transfer of rat $CD4^+$ cell line specific for Col4α3NC1 successfully induced severe nephritis without glomerular IgG and C3 deposition, which may be the direct evidence for T cell mediated glomerular injury (10). Conversely, T-cell-mediated injury cannot be excluded, even when antibodies (reflecting a B-cell response) are present.

Regulatory T cells (Treg) suppress effector T cells through receptor-mediated mechanism and thus, failure of Treg to control T-cell activity can provoke autoimmunity. Transfer of $CD4^+$CD25 Treg inhibited murine anti-GBM antibody nephritis via T-cell and macrophage suppression, with significant reductions in the levels of INF-γ, TNF-α and TGF-β mRNA (11). This may be the mechanism of the protective roles of T reg in autoimmune-mediated glomerulonephritis. However Treg transfer to Lupus-prone mice suppressed autoantibody production but not glomerulonephritis (12).

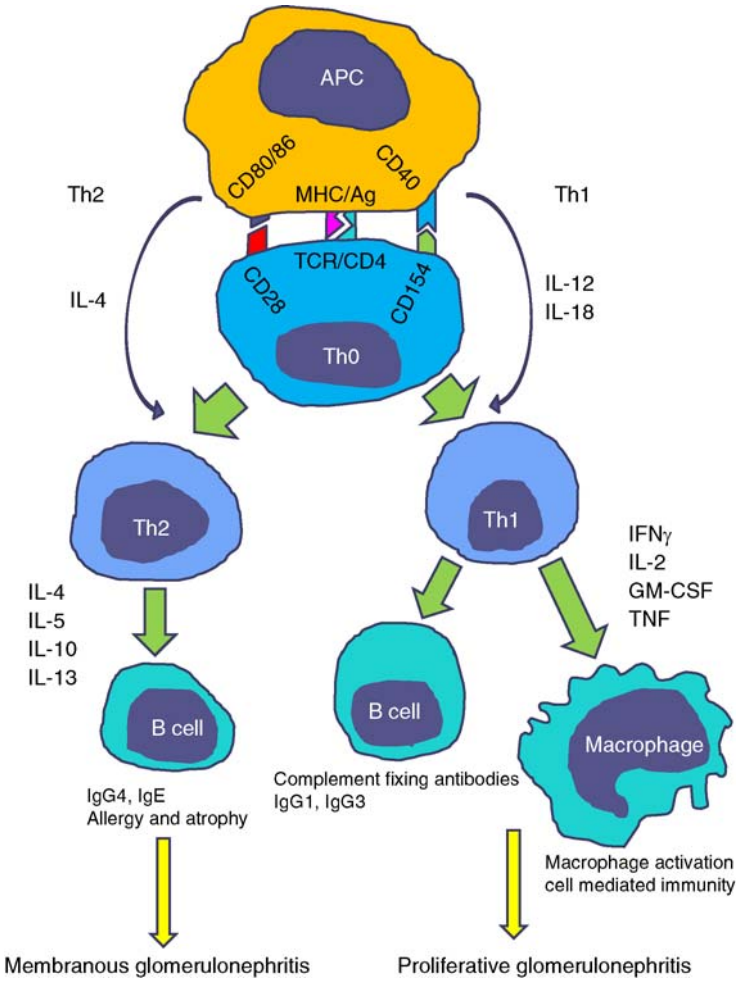
$CD4^+$ T cells can license dendritic cells to stimulate cytotoxicity in auto-reactive $CD8^+$ T cells. Dendritic cells act on uptake, transport, and processing and antigen presentation to T cells (13). Intrinsic dendritic cells in the kidney stimulate IL-10 production, which attenuates nephrotoxic serum nephritis in mice, and depletion of dendritic cells aggravates nephrotoxic serum nephritis (14). Thus, Tregs and renal dendritic cells may provide clues to renal protection in immune-mediated glomerular injury. The T-cell-mediated immune mechanisms of glomerulonephritis have been analyzed in an acute nephritis model, and the role of T cells in chronic glomerulonephritis, particularly in humans, remains largely unknown.

Th1 and Th2 Subsets

The complex immunologic network-driven immune-mediated glomerular injury involves the Th1 and Th2 subsets (*Fig. 29-9*). Although both pathways interact, the predominance of a particular subset may explain the different patterns of glomerular histology and disease activity, which are related to disease outcome (15).

Figure 29-9

Th1/Th2 subset in cytokines. Th1 and Th2 cells are subsets of primed CD4$^+$ T-helper cells, which can be distinguished based on their cytokine, chemokine, and antibody isotype profiles and which generate different immune effector responses. Antigen presenting cells (APC) interact with naïve precursors of Th0 cells by specific antigen stimulation via their α/β T-cell receptors. Th1 cells differentiate macrophages and B cells by several cytokines indicated. Th1 subset predominancy tends to resulted in proliferative glomerulonephritis. Th2 subset differentiate B cells by specific cytokines and tend to result in membranous glomerulonephritis.



Th1 and Th2 cells are subsets of primed CD4$^+$ T-helper cells, which can be distinguished based on their cytokine, chemokine, and antibody isotype profiles and which generate different immune effector responses. Both cell types differentiate from naïve precursors, "Th0" cells, following specific antigen stimulation via their α/β T-cell receptors. Antigen-presenting dendritic cells in humans are derived from monocytes or plasmacytoid cells, with a bias towards the stimulation of either INF-γ or IL-4, IL-5, and IL-10 production by naïve T cells, as demonstrated In vitro (*Table 29-2*).

Th1 development is promoted primarily by INF-γ and IL-12, and partially by IL-18, IL-27, and IL-23, indicating complex and overlapping mechanisms for Th1 immunologic activities. Th2 responses are driven by IL-4, IL-10 and INF-γ and they inhibit Th1 responses. IL-10 appears to be more active in inhibiting Th1 differentiation than in promoting Th2 responses. Differences in chemokine receptor expression between Th1 and Th2 cells also influence the activation patterns of these cells by different chemokines. CXCR3, the receptor for INF-γ-inducible chemokines, is expressed at high levels on INF-γ-producing

Table 29-2

Characteristics of T helper subsets

Characteristics	Th1 cells	Th2 cells
Inducing stimuli	IL-12, INF-γ, IL-18, IL-27, PRR signaling	IL-4
Transcription factors	STAT4, STAT 1, T-bet, NFκB	STAT6, GATA3
Cytokine produced	INF-γ, IL-2, TNF, lymphotoxin-β	IL-4, IL-5, IL-13
Chemokine receptor expression	CXCR3, CCR5, CCR1	CCR3, CCR4, CCR8
Antibody isotypes	Human: IgG1, IgG2, IgG3	IgG4, IgE
	Mouse: IgG2a, IgG2b, IgG3	IgG1, IgE
Effector response	Cell mediated immunity, macrophage activation, antibody-mediated cellular cytotoxicity	Eosinophils activation, allergy

(Modified from Scholz J, Lukacs-Kornek V, Engel DR et al. Renal dendritic cells stimulate IL-10 production and attenuate nephrotoxic nephritis. *J Am Soc Nephrol* 2008;19:527–537.)

Th1 cells and at low levels on Th2 cells. The CCR3 and CCR4 chemokine receptors are expressed by Th2 cells that produce IL-4.

The roles of Th1 and Th2 cells in glomerular injury have been studied using different strain for Th1 (mice: C57BL/6, rats: Lewis) and Th2 (mice: BALB/c, rats: Brown Norway) dominance. Induction of nephrotoxic serum nephritis in Th1-predominant C57BL/6 mice results in severe glomerular injury. Anti-GBM antibody nephritis in Lewis (Th1) and Brown Norway (Th2) rats resulted in more severe glomerulonephritis, with monocytic and T-cell influx and Th1-profile cytokine production, in Lewis rats than in Brown Norway rats. Mice deficient in Th2 cytokines show more pronounced crescentic glomerulonephritis, while the administration of Th2 cytokines ameliorates this disease. Mercuric chloride-induced autoimmune glomerulonephritis in congenic strains of Brown Norway rats resulted in a membranous-like form of glomerulonephritis. These experiments indicate that Th1 predominance is related to crescentic glomerulonephritis, and that Th2 predominance tends to be involved in membranous glomerulonephritis.

In human anti-GBM antibody nephritis, there is an INF-γ-predominant, antigen-specific effector cell response in the active stage and IL-10 predominance in remission. In humans, $CD4^+$ cells and macrophages are present in the cellular crescents of crescentic glomerulonephritis. In ANCA-related glomerulonephritis, a T-cell response with Th1 predominance is involved. In the renal tissues of ANCA-related glomerulonephritis, the level of INF-γ mRNA is high and the level of IL-4 mRNA is low. Peripheral blood T cells show a high INF-γ:IL-4 ratio in patients with ANCA-associated glomerulonephritis, as compared with patients with non-proliferative glomerulonephritis. Corticosteroids diminish this ratio, which suggests that Th1 predominance is linked to ANCA-associated glomerulonephritis in humans.

For full activation of $CD4^+$ cells, engagement of T cell receptor with the antigenic peptide-MHC molecule complex in antigen presenting cells need a secondary costimulatory signals for full activation. T cell costimulatory molecules include CD28-B7 family/CD80 (B7-1) + CD86 (B7-2), and CD40 (TNF-receptor on B cells)/CD154 (ligand of CD40 on T cells) that determines Th1 and Th2 polarity. Protection of anti-GBM nephritis in CD28 deficient mice is due to inhibition of autologous antibody production (16). Selective blockade of B7-1 prevented the development of crescentic glomerulonephritis in anti-GBM nephritis in Wistar Kyoto (WKY) rats (17). In the same nephritis in mice, administration of either CD80 or CD86 revealed no effect on glomerulonephritis, however administration of anti-CD80/CD86 antibody attenuated glomerulonephritis (18). Anti-GBM antibody nephritis in CD80 (B7-1) deficient mice reveals attenuation of crescentic glomerulonephritis in concert with reduction of glomerular $CD4^+$ cells accumulation. By contrast, same nephritis in CD86 (B7-2) deficient mice show disease exacerbation (19). Thus CD80 is pathogenic in crescentic glomerulonephritis by enhancing survival and proliferation of $CD4^+$ cells, whereas CD86 is protective by enhancing Th2 and attenuating Th1 responses. Inducible costimulatory molecule (ICOS) and ICOS ligand (ICOSL) are a receptor-ligand pair that belongs to the CD28/B7 family. Administration of antibody against inducible costimulatory molecule ligand in mice with anti-GBM nephritis increased glomerular accumulation of T cells and macrophages without affecting systemic

immune response. This suggests that ICOSL plays protective roles during induction of anti-GBM nephritis by locally reducing accumulation of T cells and macrophages (20). Blockade of CD154-CD40 costimulatory signals in anti-GBM antibody nephritis in WKY rats prevented glomerulonephritis (21). In addition, antiGBM nephritis in CD40 chimeric mice (CD40 is absent in glomeruli but intact in bone marrow) revealed suppression of glomerulonephritis accompanied by reduced level of MCP-1, IL-10 mRNA and T cell and macrophages influx. This indicates that CD40 expression in intrinsic glomerular cells promote Th1 effector cell response in glomerulonephritis (22).

Inflammatory Cells

Leukocytes

Leukocytes are bone marrow-derived cells that include granulocytes, lymphocytes, and monocytes/macrophages. Granulocytes include neutrophils, eosinophils, and basophils. Neutrophils (polymorphonuclear cells, PMNs) and macrophages play central roles in the acute inflammation process. Neutrophils and macrophages have common effects in terms of tissue injury, but macrophages more actively involved in glomerular injury by their immunologic functions. Usually, PMNs predominate in the acute phase of glomerulonephritis, e.g., in acute post streptococcal glomerulonephritis and ANCA-related glomerulonephritis. Macrophages appear in acute and chronic glomerulonephritis and both cell types are sometimes co-presented in the two phases of glomerulonephritis, particularly acute phase on chronic glomerulonephritis.

In the variety of pathogenesis of glomerulonephritis, leukocytes are involved in the loss of immune tolerance, T cell directed adaptive immune responses, cellular effectors inducing injury in delayed type hypersensitivity-like reactions, and macrophage/neutrophil recruitment via the deposition of circulating or In situ immune complexes through receptor-mediated fashion (23). Leukocytes express Fc-γ receptor on cell surface and it binds Fc portion of IgG and lack of Fc-γ R protects immune complex mediated glomerulonephritis (24). In addition, ANCA have been postulated to activate neutrophils, leading to glomerular capillaritis.

Polymorphonuclear Cells

PMNs are implicated in glomerular injury owing to their numerous bioactive/toxic products. PMNs are chemoattracted by anaphylatoxin (C5a and C3a), CXC chemokines, the immune adherence of complement receptors, and Fc receptor-dependent binding to antibodies. The corresponding receptors include FcR for IgG, CR1, CR2, and CR3 for complement fragments, and C1q for the collectins. Locally activated PMNs express sialyl-Lewis X-modified proteins and interact with endothelial cells, through selectin or integrin ligands on the endothelial surface. Various cytokines stimulate endothelial cells to express these adhesion molecules (*Table 29-1*, *Fig. 29-6*). Occasionally, PMNs on endothelial cells migrate to the mesangium and stimulate cells there, through bioactive or toxic products. Among the PMN-derived substances, lysosomal enzymes and reactive oxygen species (ROS) are the most harmful for glomerulus. Lysosomal enzymes include metalloproteases and other proteases, which along with cathepsin G exert proteolytic activities and damage endothelial cells or promote lysis of the GBM and mesangium. Microbes, cytokines, immune complexes, and various inflammatory stimuli promote ROS synthesis in PMNs through the NADPH oxidase pathway. ROS ordinarily function to destroy phagocytosed microbes and bring about cell death. Low-level secretion of ROS accelerates the inflammatory cascade by up-regulating the expression of cytokines, chemokines, and adhesion molecules. At high levels, ROS damage endothelial cells, activating the coagulation cascade and increasing glomerular permeability, with consequent breakdown of the ECM, which links mesangiolysis and GBM changes, and injuring the intrinsic cells. Activation of antioxidant mechanisms that involve catalase, superoxide dismutase, and glutathione peroxidase ordinarily protects tissues from oxidant stress. The role of ROS in glomerular injury is separately discussed.

A good example of neutrophil-mediated glomerular injury is ANCA-related glomerulonephritis. ANCA activates cytokine-primed neutrophils and monocytes through Fab'2 binding and Fc receptor engagement In vitro (25). These PMNs adhere to endothelial cells and release ROS, proteolytic enzymes, and inflammatory cytokines, which promote cytotoxic tissue damage.

Intravenous injection of mouse antibodies or splenocytes specific for mouse MPO alone induces necrotizing crescentic glomerulonephritis in $Rag2^{-}$ mice (lack functioning B cells and T cells). In this model, it appeared that PMNs infiltration was conspicuously associated with glomerular necrosis and crescent formation with macrophages, whereas lymphocytes were not prominent. Mice depleted of circulating PMNs with rat monoclonal anti-neutrophil antibody (NIMP-R14) were completely protected from anti-MPO IgG-induced necrotizing crescentic

glomerulonephritis (26). Local pretreatment of cremaster muscle with TNF-α followed by systemic administration of anti-MPO IgG in wild type mice leads to enhancement of leukocyte-endothelial cell interaction in the cremasteric microvessels, whereas this effect were not seen in Fc receptor γ chain deficient mice. In addition, co-administration of anti-MPO antibody with anti-CD18 antibody (CD18; integrin β2) has no effect on the local leukocyte-endothelial cell interaction (27). This indicates Fc-γ receptor and integrin β2 are important for processing MPO-induced small vessel vasculitis. Immunization of MPO in mice with bone marrow transplantation of MPO-positive cells to MPO deficient recipient (chimera) developed crescentic glomerulonephritis, whereas chimeric mice of MPO-negative bone marrow cells into wild type mice did not, (28) indicating that bone marrow cells are the necessary target to mediate MPO antibody induced crescentic glomerulonephritis. The local inflammation induced by cytokines (e.g., TNF-α) primed PMNs and anti-MPO antibody via FcR-γ effectively promotes cytotoxic endothelial cell damage and activate coagulation cascade. This occasionally results in fibrinoid necrosis followed by breakdown of GBM and subsequent crescentic formation.

Monocytes/Macrophages

Macrophages are derived from circulating blood monocytes. In normal rat mesangial areas, tissue-resident macrophages are occasionally present, but they are uncommitted. When inflammation occurs, monocyte-derived macrophages are recruited, programmed, and activated, largely by INF-γ. Examination of anti-Thy1 nephritis reveals that infiltration per se is insufficient to promote macrophage programming, but this is completed shortly after localization to the appropriate local microenvironment.

Human renal biopsies and experimental models of glomerulonephritis reveal glomerular macrophage accumulations, which may be indicative of disease activity or severity. IgA glomerulonephritis in children represents relatively acute inflammation, and the mesangial influx of macrophages leads to mesangial proliferation, as demonstrated by electron microscopy and immunohistochemistry (*Fig. 29-10*). Local activation of macrophages occurs in various glomerular diseases, including non-immune-mediated conditions, such as diabetic glomerulosclerosis. Macrophages are blood-borne, and it has been suggested that they proliferate In situ. Macrophages may change phenotype to one that resembles fibroblasts, and some intrinsic glomerular cells can acquire macrophage-like phenotypes.

Macrophages produce a wide range of potentially cytotoxic products, including pro-inflammatory cytokines and chemokines, proteolytic enzymes, ROS, eicosanoids, and growth factors (*Table 29-3*, *Fig. 29-11*). Experiments on the suppression or depletion of macrophages in acute and chronic glomerulonephritis models have demonstrated reduced glomerular injury (29, 30). In crescentic glomerulonephritis in rats, liposome-MDP (dichrolomethylene diphosphonate) attenuated glomerular macrophage influx accompanied by suppression of CD8 and expression of adhesion molecule (31). These findings circumferentially support the role of macrophages in the glomerulonephritis. In this context adoptive transfer of macrophages in leukopenic rats with

Figure 29-10

Macrophage infiltration in IgA glomerulonephritis. (a) Immunohistochemical detection of macrophages by CD68 in IgA glomerulonephritis. Note many immunolabelled cells (brown) locate predominantly in the mesangial proliferation and less in the capillary. (PAS stain), (b) EM reveals monocytes/macrophages in the mesangium and in the capillary (*allowhead*).

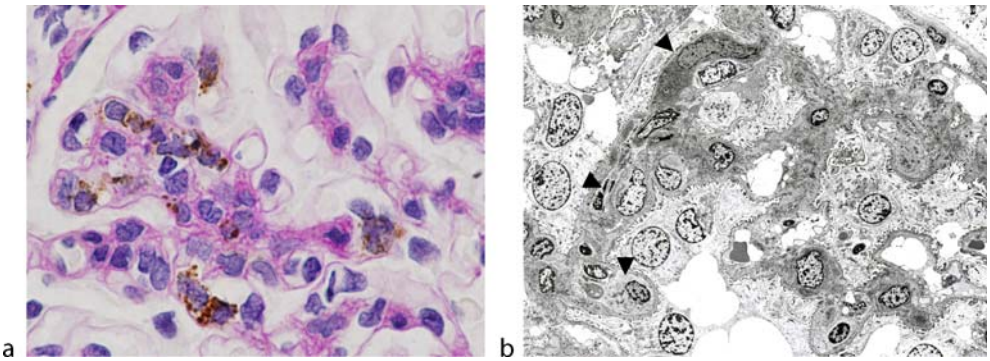


Table 29-3

Macrophage activation status

Status	Alteration	Product
Classically activated (M1)	Up-regulation	iNOS, IL-1β, TNF-α, IL-6, HMGB1, IL-8, MCP-1, MHC class I
Alternatively activated (M2)	Up-regulation	Mannose receptor, MHC class II, arginase, FIZZ1/Ym1, IL-ra
	Down-regulation	iNOS, TNF-α, IL-6
IL10 activated	Up-regulation	Soluble TNF receptors, IL1ra
	Down-regulation	IL-b, TNF-α, IL-12, IL-6, IL-8, iNOS, MHC class I
Type II activated	Up-regulation	IL-10, TNF-α, IL-6, MHC class I
	Down-regulation	IL-12
Uptake of apoptotic cells	Up-regulation	TGF-b, PGE2
	Down-regulation	TNF-α, IL-8, MCP1
Steroid treated	Up-regulation	IL-10, uptake of apoptotic cells
	Down-regulation	IL-12, TNF-α, IL1-β, IL-6

HMGB1, high mobility group B1; IL-1ra, IL-1 receptor antagonist

anti-GBM nephritis revealed proteinuria and glomerular cell proliferation and indicates direct evidence for macrophage to induce glomerulonephritis (32). Given these results, it has long been suggested that the scale of macrophage influx promotes glomerular injury. However, it has recently become clear that it is the functional properties, rather than the total number, of macrophages that determine the severity of inflammation (30). In vitro studies have led to the hypothesis of functional heterogeneity in macrophages. Polarized macrophages can be subdivided into several phenotypes, particularly classically activated (M1) and alternatively activated (M2) macrophages (33, 34). The difference in activators determines the heterogeneous phenotype (*Table 29-4*, *Fig. 29-12*). Innate activation is promoted by Toll-like receptor (TLR) ligands, which induce the production of pro-inflammatory cytokines, NO, and ROS. INF-γ TNF-α and/or LPS primes macrophages to convert classical activation (M1 polarization) and leads to increased production of pro-inflammatory cytokines, iNOS, and ROS, as well as the up-regulation of MHC class II molecule expression and co-stimulatory CD86 molecules, thereby promoting antigen presentation. In contrast, IL-4- or IL-13-primed macrophages undergo alternative activation (M2 polarization), which is associated with increases in endocytosis, arginase, anti-inflammatory cytokines, and the synthesis of extracellular matrix, thereby promoting angiogenesis. M2 polarization also leads to decreased iNOS and NO production, and is involved in anti-inflammatory effects, cell growth, and tissue repair. Although the molecular regulation of such macrophage heterogeneity has been largely demonstrated In vitro, transfer experiments with programmed macrophages (M1 or M2) in adriamycin nephropathy in SCID mice (lack endogenous T or B cells) have provided evidence that M1 macrophage infusion aggravates the disease, whereas M2 macrophages reduce histologic and functional damage (35). This phenotypic heterogeneity of macrophages may be switched during the course of chronic glomerulonephritis, depending on the background pathophysiology.

Macrophage migration inhibition factor (MIF) is a kind of pro-inflammatory cytokines to induce cytokines, chemokins and adhesion molecules in the inflammatory environment. Neutralizing antibody for MIF or MIF deficient mice resulted in prevention of immune mediated glomerulonephritis (36, 37). Activator protein (AP-1) transcription factor JUND is a major determinant of macrophage activity and associated with susceptibility to glomerulonephritis (38).

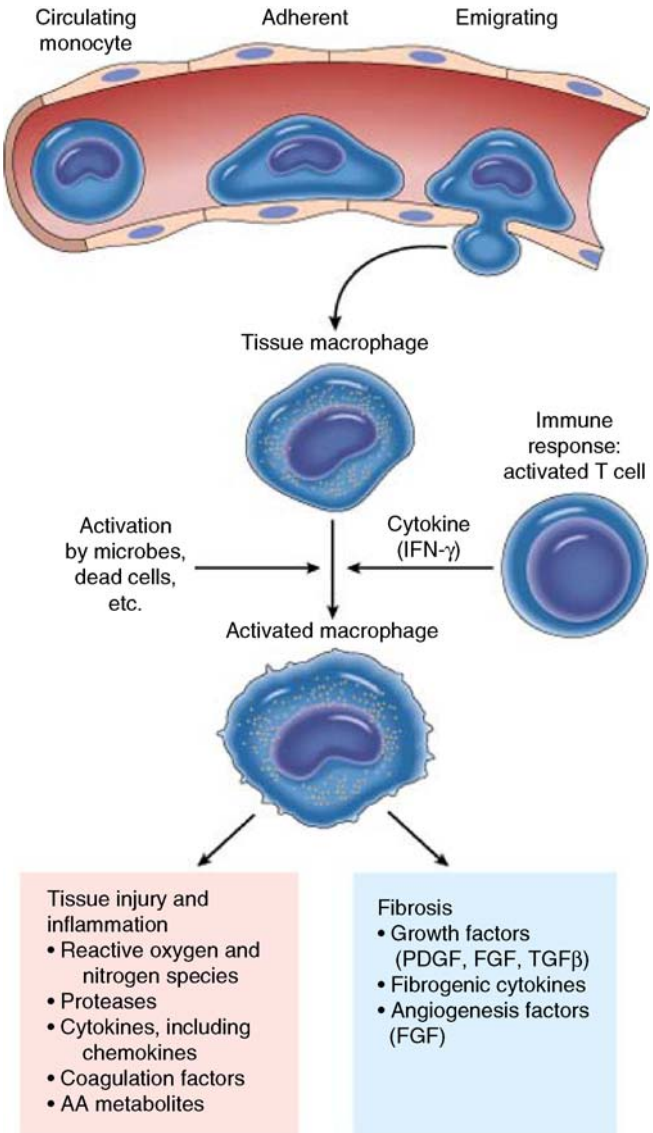
During the process of glomerular injury, macrophages take up apoptotic cells, most of which are infiltrated inflammatory cells. This uptake causes the macrophages to exert anti-inflammatory effects, through increased expression of TGF-β and prostaglandin E2 (PGE2) and decreased production of IL-8, TNF-α, and IL-1β. Suppression of T-cell proliferative responses also results from the anti-inflammatory effects of macrophages that have taken up apoptotic cells.

Mast Cells

Mast cells are bone marrow-derived cells that play significant roles in innate and adaptive immune responses. Mast

Figure 29-11

Activation of macrophages and production of various cytokines. Circulation monocytes transmigrate to the site of inflammation and T cell derived cytokines (INF-γ) stimulate activation in monocytes/macrophage and produce many soluble factors for tissue cell injury/tissue fibrosis. In glomerulus, macrophages are able to migrate into mesangium, subendothelial space or urinary space. (The figure was published in Kumar et al. Robbins Basic Pathology, 8 edn. 2007, p55, Copyright Elsevier.)



cells secrete various inflammatory mediators, including chemical vasomotor mediators, cytokines, growth factors, and eicosanoids. These substances attract inflammatory cells and are involved in mediating tissue remodeling. Mast cell detection in tissues is typically performed by staining with toluidine blue, Alcian blue, and safranin, all of which enable the visualization of cell granules. Immunohistochemistry to detect enzymes specific for mast cells, such as tryptase and chymase, can be used to subdivide mast cells into MCT (containing tryptase) and MCTC (containing tryptase plus chymase) cells. This enzyme visualization method reveals many more mast cells than classic

Table 29-4

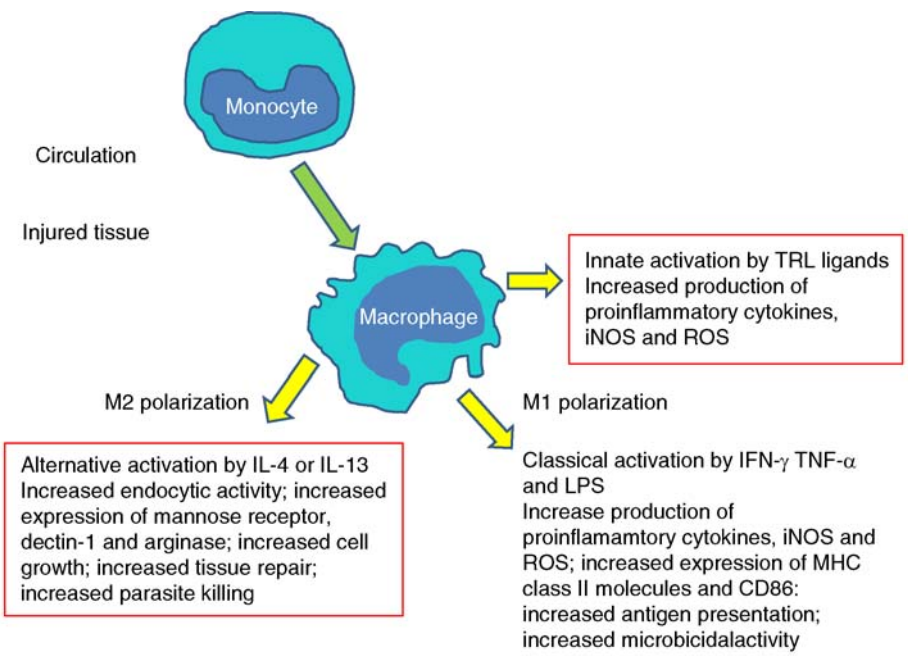
Macrophage polarized activation

Phenotypes	Classical (M1 polalization)	Alternative (M2 polalization)
Activating signal	LPS + INF-γ	IL-4, IL-13 Glucocorticoid TGF-β
Function	Type I inflammation Tissue destruction Killing of microorganism Tumor resistance	Type II inflammation Tissue remodeling and angiogenesis Parasite encapsulation Tumor promotion
Molecular markers and products	IL-12, IL-23, IL-1, TNF MHC class II CD86 M1 chemokine Reactive oxygen intermediate Reactive nitrogen intermediate	IL-1RA, IL-10 CD23 M2 chemokines Mannose receptor Scavenger receptor

(Modified from Montaviani et al., Eur J Immunol 2007; 37:14–16)

Figure 29-12

Macrophage heterogeneity during inflammation. Monocytes attracted to the injured tissue are thought to be able to acquire distinct phenotypes and physiological functions. Largely there are two major phenotypes in macrophages, M1 and M2. In M1 polarization, macrophages are stimulated with INF-γ and reveal high microbicidal activity and produce ROS, and iNOS for proinflammatory action. By contrast M2 polarization is promoted by IL-4, IL-10, IL-13 or TGF-β and it acted on the tissue repair and parasite killing. However, these hypothesis has been drawn by the in vitro experiments and still remain undetermined in vivo. (Modified figures from reference Gordon et al (33).)



toluidine blue staining. Mast cells are present in human glomerulonephritis, primarily in the interstitium, and are probably involved in fibrosis. The role of mast cells in glomerulonephritis has been examined in anti-GBM antibody nephritis in congenitally mast cell-deficient mice (W/Wv). W/Wv mice with nephritis show a higher mortality rate and enhanced disease activity, as compared with wild-type mice (39). This condition can be rescued by mast cell reconstitution, suggesting a protective role for mast cells in immune complex-mediated glomerulonephritis.

Toll-Like Receptors (TLRs)

The recently identified TLR families provide receptor-mediated pathogen recognition and activation of the immune system. Ten human and nine murine TLR proteins related to the original Drosophila Toll gene have been identified to date. Inflammatory cytokines and chemokines are induced through the activation of TLRs by a variety of ligands (40). The biologic activities that result from TLR binding include activation of antigen-presenting cells, dendritic cell maturation, B-cell activation, and different cytokine production profiles. The responsibility of TRL in the innate and adaptive immune response may be a likely background in the immune-mediated glomerular injury. Small nuclear RNA and autoantigen for lupus activate B cells and dendritic cells via TLR-7. TLR-7 overexpression is associated with antinuclear autoantibody production and lupus-like disease in mice and backcrossing TLR-7 deficient mice with MRL lpr/lpr mice attenuated kidney and lung disease. Administration of synthetic oligodeoxynucleotides with immunoregulatory sequences (IRS) which specifically blocks TLR-7 suppressed autoimmune kidney and lung diseases (41). Endogenous or exogenous CpG-DNA activates TLR-9 and administration of CpG DNA to MRL-Fas(lpr) mice resulted in disease progression accompanied by MCP-1 and RANTES expression and leukocyte recruitment (42). In ddY mice of spontaneous IgA glomerulonephritis, genome-wide scan revealed myeloid differentiation factor 88 (MyD88) as a candidate gene for disease progression and TRL-9, the receptor for MyD88, was increased in a conventionally housed condition. Nasal challenge with CpG-oligonucleotide (a ligand for TRL-9) aggravated renal injury with strong Th1 polarization and increased mesangial IgA. TRL-9 polymorphism in the TLR-9 gene associated with disease progression of IgA in humans. TLR-9 pathway may be involved in the pathogenesis and determination of severity of IgA glomerulonephritis (43).

TLR-3 recognizes dsDNA of viral origin and is expressed in mesangial cells. Cytokines stimulate TLR-3 mRNA in mesangial cells In vitro. TLR-3mRNA is significantly upregulated in glomeruli associated with enhanced mRNA of RANTES/CCL5 and MCP-1/CCL2 in hepatitis C-associated glomerulonephritis in humans. This suggests that immune complex containing viral RNA activate mesangial cells via TLR-3 mediated chemokine/cytokine effects (44).

Complement Cascade

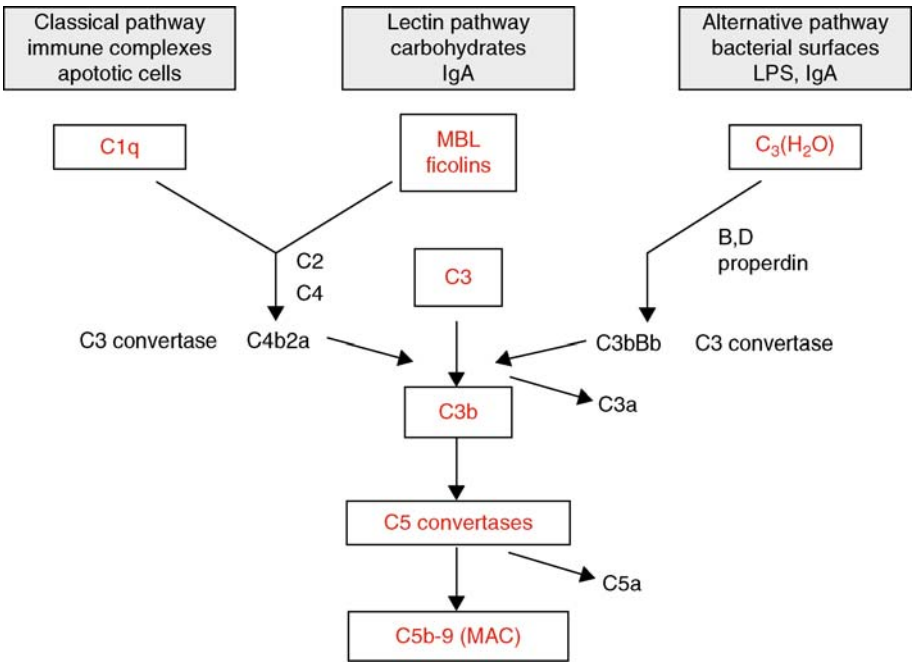
The complement system, which is a cascade of plasma proteins, involves approximately 30 plasma and membrane-bound proteins that play a pivotal function in antimicrobial defense and immune complex clearance. Complement components are largely liver-derived and are present in inactive forms (numbered C1 through C9) in the plasma and local complement production occurs in the inflammatory milieu involved in tissue damage, including leukocyte recruitment, cell necrosis, and apoptosis. Immune complex clearance and proinflammatory functions of complement system provide its complex participation in immune-mediated glomerulonephritis.

The biologic actions of complement ultimately generate a pore-like membrane attack complex (C5-9, MAC), which punches holes in invading microorganisms. Among the several important complement fragments produced in the MAC-generating processes are the cleavage products of C3 and C5 (C3a, C5a), collectively termed anaphylatoxin, which promote vascular permeability (C3a, C5a) and leukocyte chemotaxis and activation (C5a).

The presence of complement components in the tissue of glomerular diseases represents local activation of complement system that involved in the pathogenesis of glomerular injury. Classically, inhibition of the complement cascade by cobra venom factor attenuated various glomerulonephritis models. Complement depletion, by aggregated human IgG, protected against complement/PMN-dependent glomerular injury in anti-GBM antibody nephritis, with marked attenuation of leukocyte influx. In addition, defective complement regulation system, as seen in human and animal models with specific complement component deficiencies have provided evidence of a complement-mediated/-dependent pathway in glomerular injury. This includes certain forms of hemolytic uremic syndrome and MPGN II (45).

Figure 29-13

Three pathways of complement cascade. The early activation steps of each pathway converge in a common terminal pathway towards MAC formation. The classical pathway is initiated by C1 binding to an antigen-antibody complex (IgG or IgM), acute phase proteins (CRP) or apoptotic cells, leading to conformational changes that result in the activation of the associated serine proteases C1r and C1s. C1s cleaves C4 into C4a and C4b. C4b binds to C2, resulting in the C4bC2a complex, which is the classical pathway C3 convertase. Mannan-lectin pathway activation begins with recognition of carbohydrate ligands by mannose-binding lectin (MBL) and ficolin, which are present on various microorganisms. MBL activates MBL-associated serine proteases (MASP-1, MASP-2, MASP-3) and MASP-2 cleaves C4, resulting in the formation of the same C3 convertase as in the classical pathway. Alternative pathway activation is triggered by C3b binding to various activating surfaces, such as microbial endotoxins, aggregated immunoglobulins, and complex polysaccharides (e.g., LPS). This pathway involves a distinct set of serum positive regulator components, called the properdin system (properdin P, factor B, factor D). In this pathway, the C3 molecule is spontaneously activated by hydrolysis of the internal thioester. Hydrolyzed C3 binds to factor B, making it susceptible to cleavage by factor D, resulting in initial C3 convertase C3 (H2OCb) formation. This initial convertase further cleaves C3 into C3a and C3b, the latter interacts with factor B to form the more active alternative pathway C3 convertase, C3bBb. The presence of C3 convertase in the individual complement pathways leads to the formation of C3bBbC3b (alternative pathway) and C4bC2aC3b (classical and lectin pathways), also called C5 convertase. Through this molecule, C5 is split into C5a and C5b. C3a and C5a works as a potent anaphylotoxin to promote inflammatory processes and cytotoxic effects. (After Berger et al. (45) with permission.)



The complement system consists of three distinct pathways: the classical, mannan-lectin, and alternative pathways (*Fig. 29-13*). These pathways sometimes synergistically involved in the glomerulonephritis, such as simultaneous activation of lectin pathway and alternative pathway in IgA glomerulonephritis and purpura nephritis (46).

Of the four classical types of hypersensitivity reactions, complement is thought to be involved in types II and III. Type II hypersensitivity, antibody-mediated hypersensitivity, targets intrinsic glomerular cells or extracellular matrix, provoking tissue damage by a complement-dependent mechanism. This mechanism typically includes Goodpasture Syndrome and Heyman nephritis. In type III hypersensitivity, antigen-antibody complexes within the glomerulus (glomerular capillary endothelium or mesangium) and complement bind the C3b receptor or the Fc receptor of immune-competent cells and activate the production of chemical mediators or cytokines that lead to tissue injury. Lupus nephritis and post streptococcal acute glomerulonephritis are promoted by this mechanism.

The complement activation-involved glomerular injury is thought to be mediated by leukocyte recruitment via C5a, which accelerates the inflammatory process (leukocyte-dependent) or through direct attack by complement fragments on intrinsic glomerular components (leukocyte-independent).

Leukocyte-dependent tissue injury mechanisms are involved in injury to the glomerular capillary. C5a affects the chemotaxis of neutrophils, monocytes/macrophages, and eosinophils, and accelerates the release of inflammatory mediators. C5a also increases the adhesion of leukocytes to endothelium, by activating the leukocytes and increasing the avidity of the surface integrin for the ligands C3b and C3bi deposited on endothelial surface. C5 deficient mice show reduction of glomerular PMNs infiltration in cryoglobulin-induced immune complex glomerulonephritis (47). Complement anaphylatoxins binds several receptors including G-protein-coupled receptors (C3aR and C5aR). C5a binds two receptors, C5aR and C5L2, and most of the functional effects of C5a are mediated by C5aR. Transplantation of C5aR-deficient marrow suppressed glomerulonephritis in MPO deficient mice immunized with MPO, indicating C5a involves leukocyte activation via C5aR (48). These inflammatory process involves GBM breakdown and activation of the coagulation system, due to endothelial injury, lead to proliferative and matrix changes in the mesangium and parietal cells, and accelerate podocyte damage. MAC stimulates NF-κB, which is linked to the activation of the pro-inflammatory cytokines IL-8 and MCP-1. In humans, C3aR is upregulated in the glomerular endothelial region in lupus nephritis, but not other glomerular diseases (49).

Leukocyte-independent mechanism of glomerular injury is seen in Heyman nephritis. Like type II mechanisms, antigen (megalin) on podocyte surface is a target of complement cytotoxicity and results in direct injury for filtration barrier and complement depletion attenuated Heyman nephritis. In addition, sublyric levels of MAC damage the DNA in podocytes (50).

Glomerulonephritis in complement deficiency provides a clue to understand the roles for complement in glomerular injury. C6-deficient/-depleted rats with anti-Thy1 nephritis or Heyman nephritis show attenuated disease activity. By contrast occurrence of IgA glomerulonephritis even in the hereditary C6 or C9 deficiency in humans indicated that MAC per se is not essential in the IgA glomerulonephritis.

Interestingly, C1q or C4 deficiency develops lupus-like disease in mice, and C1q, C2 and C4 deficiency in humans increased risk of developing SLE. Although the phenomenon may be contradict to the many literatures suggesting complement system injures the glomerulus, it may be explained by the scenario that complement deficiency may leads to auto-immune phenomena due to defective clearance of apoptotic cells which are rich source of autoimmunity in SLE.

Glomerular cells are normally protected from complement-mediated injury by cell-surface defense mechanisms and complement regulatory proteins are expressed in intrinsic glomerular cells. CR1 (CD35) preferentially binds to C3b and C4b, which are normally expressed in podocytes and protect against complement-dependent cellular damage. CR2 is also expressed in intrinsic glomerular cells, including podocytes and mesangial and endothelial cells, and is up-regulated in membranous nephropathy. In addition, ER (endoplasmic reticulum) stress protein may have protective role for MAC mediated cell injury as shown in podocytes (51).

In human glomeruli, decay accelerating factor (DAF or CD55) and membrane cofactor protein (MCP or CD 46) regulate C3 and C5 activation. CD59, which is a membrane protein inhibitor of the MAC of complement, inhibits at the level of C8 activation, preventing MAC formation. Glomerular resident cells normally express MCP and CD59, and these molecules are up-regulated in glomerular disease. Nephrotoxic serum nephritis in mice deficient for either DAF1 or CD59a or both DAF1 and CD59a gene reveal that mice lacking DAF1 gene developed severer proteinuria and histology compared to wild type or CD59a deficient mice. Severe disease in DAF1 gene deficiency is associated with glomerular C3 and C9 deposition, whereas immunoglobulin deposition is comparative (52). Crry is a rodent homolog with activities similar to DAF and MCP. Blocking CD59 or Crry accelerates complement-dependent glomerulonephritis, and the soluble forms of these molecules effectively inhibit complement and attenuate glomerular damage. Double knockout mice with DAF and Crry revealed exacerbation of nephrotoxic serum nephritis despite marked reduction of systemic complement activation (53) and transgene of Crry in MRL/lpr mice protect renal disease (54).

Clusterin is a plasma protein that is frequently found within glomerular immune deposits, in association with vitronectin and soluble C5b-9. Clusterin depletion enhances immune complex glomerulonephritis in mice.

Alternative mechanism of complement-dependent glomerular injury is defective complement regulatory proteins. Protective proteins against the complement cascade include factor H, factor H-like protein, factor I, and C4 binding protein, and functional defects in these proteins

are the background. Factor H is a soluble glycoprotein that regulates complement, both in the fluid phase and on the cellular surface. It acts in the amplification loop of the alternative pathway to regulate C3bBb through dissociation of C3bBb into inactive factor Bb and C3bfH, followed by irreversible inactivation by factor I. Factor H has affinity for polyanions on the cell surface and protects the cell from alternative pathway-mediated injury.

MPGN type II is a peculiar disease with persistent complement activation without immunoglobulin deposition, as revealed by immunofluorescence (55). Electron microscopy reveals a characteristic continuous, electron-dense deposition along the lamina densa of the GBM (thus, it is also known as dense deposit disease). The disease has a poor prognosis, with chronic renal failure and frequent relapses after renal transplantation, which suggests that genetic or humoral factors cause the disease. Systemic and persistent alternative pathway activation in MPGN type II is generally caused by either the presence of autoantibodies to C3 convertase (C3bBb), C3NeF, which stabilizes it, or by inherited mutations in or the presence of antibodies against factor H, resulting in the inhibition of convertase inactivation (56). C3NeF mediated convertase stabilization may be dependent on the factor H-mediated inactivation of the convertase.

The genes for the factor H family of proteins localize to the "regulators of complement activation" (RCA) region at 1q32. Few reports have addressed the potential association between human MPGN type II and factor H mutations, although such mutations are important in the pathogenesis of the disease. A 13-month-old Native American boy with MPGN type II, who had a C518R mutation in the factor H short consensus repeat (SCR)9 in trans with a C941Y mutation in factor H SCR16, showed retention of the defective factor H in the endoplasmic reticulum. A relationship between MPGN type II and factor H has also been demonstrated in animal models. Norwegian Yorkshire piglets with the I1166R mutation in SCR20 developed MPGN type II; this mutation prevented extracellular release of factor H, resulting in intracellular accumulation. These findings in humans and animals suggest that mutated factor H is not delivered to the cell surface and/or is unable to bind to surface-bound C3b, resulting in incomplete convertase inactivation. Factor H-deficient mice show significant reductions in C3 and reproducible MPGN type II, with C3 deposition in the GBM, whereas combined factor H and factor I deficiency or factor I deficiency alone did not show MPGN II (57). Spontaneous MPGN in factor H deficient mice was attenuated in additional deficient with C5, but not those with C6. Severe nephritis of antiGBM nephritis in factor H deficient mice is blocked by administration of anti C5 antibody (58). Notably, the site of pathologic lesions is limited to the glomerulus, suggesting a unique requirement in the GBM for factor H-mediated protection against complement attack.

Another type of glomerular injury with persistent complement activation is atypical hemolytic uremic syndrome (aHUS), which is typically seen as a familial condition. A factor H mutation has been implicated in aHUS, and the HUS database (http//:www.FH-HUS.org) lists more than 100 mutations related to factor H: 69 factor H mutations, 12 factor I mutations, and 25 MCP mutations linked to aHUS, as well as 6 factor H mutations linked to MPGN type II, as of December 2008. Factor H mutations apparently generate different glomerular injury phenotypes in MPGN type II and aHUS. In contrast to the factor H mutations in patients with MPGN type II, aHUS patients with factor H mutations are usually heterozygous (few cases with homozygous mutation are reported) for the mutation, and these patients have normal levels of circulating factor H protein (59). In addition, factor H mutations in aHUS patients are typically located in the C-terminal region, which is important for binding to the cell surface. Transgenic mice that lack the exons encoding the C-terminal region of factor H develop aHUS, closely resembling the human disease. These mice show preservation of fluid-phase complement activation, associated with defects in endothelial protection against complement attack, leading to persistent endothelial injury. This corresponds to the pathogenic background of aHUS, unlike MPGN type II. Similarly, heterozygous mutations in MCP have been reported in cases of familial aHUS. These mutations resulted in three amino acid changes (positions 233–235) and the insertion of a premature stop codon, resulting in loss of the transmembrane domain of the protein and severely diminishing the cell-surface expression of MCP (60). Thus, persistent complement activation on the endothelial surface may promote aHUS.

Soluble, Secreted Peptides

Following the initiation of immune-mediated glomerular injury, regardless of whether it is cell-mediated, antibody-mediated or both, soluble and secreted peptides are significantly involved in the promotion of glomerular injury as second messengers. Recent developments in the molecular and biochemical aspects of inflammatory mediators have been applied to glomerulonephritis and In vitro experiments effectively reproduce In vivo tissue injury

mechanisms. Not only do interactions between these molecules occur among inflammatory cells, but their direct actions on intrinsic cells and cellular responses are also important in the pathogenic background of glomerular injury.

Various cytokines, chemokines, and growth factors are soluble, secreted peptides that promote the responses of intrinsic cells, including cell proliferation, cell death, and matrix production/degradation. They are also involved in protection against glomerular tissue damage. These peptides are produced by inflammatory cells, platelets or intrinsic glomerular cells and bind to specific receptors on the cell surface, acting in paracrine, autocrine, and/or juxtacrine fashions. The cellular responses of intrinsic cells and the features of inflammatory cells lead to the glomerular pathology that defines the stage and grade of glomerulonephritis.

Interleukins

Interleukins (ILs), initially identified as cytokines secreted by leukocytes, are important signaling molecules. To date, approximately 35 subtypes have been identified. ILs are known to mediate cellular immune/inflammatory reactions and they play key roles in immune-mediated glomerular injury. Many cells synthesize ILs that act locally, resulting in the proliferation or maturation of T cells, T-cell interactions with B cells, antibody production by B cells, and changes in the Th1 and Th2 subset balance.

In the kidneys, ILs stimulate mesangial cells and endothelial cell proliferation, and stimulate the production of oxygen radicals, collagenase, cytokines, chemokines, adhesion molecules, and extracellular matrix, and they may accelerate cell injury/death. Among the ILs, IL-1, -2, -4, -6, -8, -10, -11, -12, and -13 are known to be involved in many aspects in glomerular injury.

IL-1, IL-6, IL-8, IL-12, and IL-18 primarily act to promote glomerular injury, whereas IL-4, IL-10, IL-11, and IL-13 have anti-inflammatory actions. These molecules act on glomerulus by similar but occasionally different mechanisms and their roles are sometimes disease specific. For example, IL-10, IL-12, and IL-18 are pathogenesis-specific ILs in lupus nephritis and are involved in glomerular injury by mediating leukocyte infiltration.

IL-1 is produced by macrophages, neutrophils, and dendritic cells and accelerates helper T-cell co-stimulation, B-cell maturation, and macrophage and endothelial cell activation, via the specific receptors Cd121a/IL1R1 and CD121b/IL1R2. IL-1β is involved in macrophage-mediated glomerular injury, as is TNF-α. IL-1 is transiently expressed during acute glomerular injury, and correlates with the degree of glomerular inflammation; it is not expressed during chronic glomerular damage. IL-1 accelerates leukocyte adherence to endothelial cell surface, resulting in mesangial proliferation, activation of pro-coagulant activity, and the synthesis of prostaglandins. Indeed, IL-1 mRNA is expressed in acute glomerular injury and the administration of a soluble IL-1 receptor antagonist attenuates the damage. In addition, IL-1 stimulates mesangial VEGF synthesis via the PI3-K/mTOR pathway and stimulates matrix production by epithelial cells (61).

IL-6 is produced by several types of blood-borne cells, glomerular endothelial cells, and mesangial cells. IL-6 is a multifunctional cytokine that is important for B-cell differentiation and maturation, immunoglobulin synthesis, acute-phase protein production, bone marrow progenitor stimulation, mesangial proliferation, and the activation of monocytes/macrophages. IL-6 is detected in the serum and urine in both animals and humans with glomerulonephritis. Overproduction of IL-6 results in lupus and Castleman's disease in mice and IL-6-transgenic mice demonstrate glomerulonephritis. Blockade of IL-6 receptors using a neutralizing antibody ameliorates lupus glomerulonephritis in NZB/W F1 mice. The promotive role of IL-6 for mesangial proliferation or glomerulonephritis may largely mediated by activation of monocyte/macrophages.

IL-8 is synthesized by macrophages, lymphocytes, epithelial cells, and endothelial cells. IL-8 stimulates the activation and chemotaxis of granulocytes via CXCR1/IL8RA. In addition, activated mesangial cells in glomerular injury synthesize IL-8 and neutralization of IL-8 by a specific antibody attenuates immune-complex type glomerulonephritis, concomitant with the suppression of leukocytes. Glomerular podocytes express IL-8 in the normal state, and they express high levels in MCNS (62). In post streptococcal acute glomerulonephritis in children, increased levels of IL-8 were detected in the urine and plasma particularly in the acute phase and up-regulation of glomerular IL-8 is associated with endocapillary proliferative lesions (63). Thus, IL-8 acts to promote the acute and active phases of glomerulonephritis.

IL-12, which is a heterodimeric molecule composed of the p35 and 40 subunits, plays a key role in uncommitted T cells of the Th1 phenotype. Antigen-presenting cells are the dominant source of the IL-12 but intrinsic renal cells are also the effector to produce IL-12. Anti-GBM antibody nephritis induced in IL-12p40−/− mice reveals attenuation of glomerulonephritis associated with suppression of leukocyte infiltration (64).

IL-18 is produced by macrophages and stimulates INF-γ synthesis by Th1 cells and NK cells, resulting in NK-cell activation. IL-18 is overexpressed in patients with lupus nephritis associated with higher INF-γ and lower IL-4 production (65). Anti GBM nephritis in $IL18^{-/-}$ mice reveals reduction of local synthesis of TNF, IL1β, IFN-γ and MIP1 (66). IL-18 deposition in tissues is observed in ANCA-related glomerulonephritis and IL-18 is involved in the priming or recruiting of neutrophils in this disease (67). IL-18-primed neutrophils synthesize MPO and PR3 ANCA via p38 MAP-kinase activation In vitro.

IL-4 acts by promoting Th2 responses and it inhibits the various pro-inflammatory effects of macrophages. Macrophages primed with IL-4 become unresponsive to pro-inflammatory cytokines, including INF-γ and TNF-α. Glomerular-resident cells, i.e., mesangial cells and podocytes, synthesize IL-4. IL-4 ameliorated crescentic glomerulonephritis in Wistar Kyoto rats and this is associated with macrophage suppression (68) and transfection of IL-4 expressing macrophages in rats with nephrotoxic serum nephritis prevented glomerulonephritis with reduction of ED-1 positive macrophage influx (69). The aggravation of glomerulonephritis in IL-4-deficient mice is attenuated by treatment with recombinant IL-4. These findings indicate a renoprotective role for IL-4 in glomerulonephritis.

IL-10, a pluripotent cytokine, is produced by various activated immune cells, including T-helper cells, B cells, monocytes/macrophages, and keratinocytes. IL-10 down-regulates the co-stimulatory molecules that participate in T-cell activation. IL-10 suppresses mesangial cell proliferation in anti-Thy1 nephritis and also attenuates glomerular injury in nephrotoxic serum nephritis. C-reactive protein suppresses nephrotoxic serum nephritis in B6 mice and the effect is abolished in IL-10 deficient mice (70). Renal dendritic cells stimulate $CD4^+$ T cells, inducing IL-10 production. Nephrotoxic serum nephritis in mice depleted renal dendritic cells by diphtheria toxin revealed advanced tubulointerstitial and glomerular lesions, without any effects on the macrophages. Production of IL-10 by renal dendritic cells is mediated by promotion of the IL-10 inducer molecule, inducible costimulatory molecule ligand (ICOS-L). Long-term expression of IL-10 by a recombinant adeno-associated virus modulated glomerulosclerosis in a subtotal nephrectomy model accompanied by suppressing the INF-α and IL-2 mRNA levels. This suggests a renoprotective role for IL-10 in non-immune-mediated chronic models of glomerulosclerosis (71).

IL-11 is a bone marrow-derived, multifunctional, anti-inflammatory cytokine that is involved in acute inflammatory responses mediated by acute-phase protein production. Rat mesangial cells and macrophages express the IL-11 receptor α-chain. IL-11 suppresses the expression of the pro-inflammatory cytokines TNF-α, IL-1β and IL-12 in LPS-stimulated peritoneal macrophages. Recombinant human IL-11 attenuates crescentic glomerulonephritis in WKY rats, showing reduced necrosis, macrophage infiltration, and apoptosis (72). Subcutaneous IL-11 injection in mice with nephrotoxic serum nephritis in mice attenuated glomerular injury via suppression of NF-kappa B activity (73).

Interferons

Interferon (INF) stimulate the immune response, activating NK cells and macrophages, and increasing antigen presentation to lymphocytes and is now known to comprise three subtypes, αβ and γ. INF-γ is a key molecule for Th1 cells and is synthesized by many cell typesunder various conditions, although macrophages are the most important source in inflammation. INF-γ is important in the effector phases of nephritogenic immune responses, including IgG2a generation, T-cell and macrophage recruitment, and macrophage activation. Evidence suggests that INF exerts different effects in various nephritogenic conditions.

In human lupus nephritis, laser microdissection of glomeruli shows up-regulation of glomerular INF-γ in association with IL-10, IL-12 and IL-18 and that was correlated with glomerular leukocyte influx (74). INF-γ is also up-regulated in the mice model of lupus nephritis and blocking INF-γ attenuated the kidney disease. INF-γ enhanced autoimmunity, generating damaging autoantibodies in lupus model. Lymphocyte depletion in the anti-Thy1 nephritis model revealed attenuation of glomerular injury through the suppression of INF-γ. Endogenous INF is pathogenic in anti-GBM antibody nephritis. In contrast, anti-GBM antibody nephritis in INF-γ receptor-deficient mice showed aggravation of the nephritis and progressive crescentic glomerulonephritis (75). Both aggravating and protective effects for INF-γ in glomerulonephritis is probably depending on the mechanistic background of the diseases. INF-γ may be an effector for other cytokines or may modify the reactions of macrophages, rather than having direct actions on glomerular cells.

Role of INF-β in glomerulonephritis is not well known. Recombinant rat INF-βreduces proteinuria in the different models of glomerulonephritis (nephrotoxic nephritis in WKY rats, anti-Thy1 nephritis and puromicyn nephrosis) via direct action for glomerular filtration barrier (76).

Tumor Necrosis Factor (TNF)

TNF is a monocyte/macrophage-derived, pro-inflammatory cytokine and an important mediator of inflammatory tissue damage. There are three family members; TNF-α, TNF-β and lymphotoxin-β, and TNF-α is the best characterized TNF among them. Two receptors for TNF (TNFR1 and TNFR2) have been identified and are involved in mediating local inflammatory injury and systemic immune-regulatory functions in the kidney. Many experimental studies and clinical observations support roles for TNF-α in the pathogenesis of acute and chronic renal disease. TNFR1 attenuates LPS-enhanced nephrotoxic nephritis with down-regulation of IL-1β. Anti-GBM glomerulonephritis is attenuated in TNF-α-deficient mice, and blocking TNF-α in nephrotoxic serum nephritis in Wistar Kyoto rats attenuated glomerulonephritis accompanied by down-regulation of urinary MCP-1 (77). Wild type bone marrow transplantation to TNF deficient recipients (intact bone marrow but absent renal-derived TNF) resulted in attenuation of anti-GBM nephritis, whereas the effect was not seen in mice with TNF deficient leukocytes but intact intrinsic renal cells. This suggests intrinsic renal cells are the major source of TNF and participate in TNF-mediated renal injury (78). Likewise TNF stimulates cytokine production in mesangial cells In vitro. TNF-α probably activates pro-inflammatory cytokines and participates in the progression of glomerulonephritis.

Although TNF-α generally plays a stimulatory role in glomerulonephritis, it can also act as an immune modulator. The fact that blockade of TNF-α in human rheumatoid arthritis or Crohn's disease leads to the development of autoantibodies, a lupus-like syndrome, and glomerulonephritis in some patients suggests a B cell modulator of TNF-α (79).

Given these apparent dual functions of TNFs, balance between the pro-inflammatory and immunosuppressive effects of TNF may participate in immune-mediated glomerulonephritis.

Chemokines and their Receptors

Chemokines (chemotactic cytokines) are small proteins (8–10 kDa) that are now known to constitute a superfamily of leukocyte subset-specific activating and/or chemoattractant cytokines (*Table 29-5*). The four classes of chemokines (CXC, CC, C, and CX3C) are classified

Table 29-5

Intervention of chemokine receptors on experimental glomerulonephritis

	Target	Intervention procedures	Outcome
Immune complex glomerulonephritis	IL-8/CXCL8	Neutralizing antibody	Improved
	RANTES/CCL5	Met-RANTES	Worse
		Amyno-oxyopentane-RANTES	Worse
Crescentic glomerulonephritis	CINC	Neutralizing antibody	Improved
	IL-8/CXCL8	Neutralizing antibody	No effect
	MIP-2/CXCL2	Neutralizing antibody	Improved
	SR-PSOX/CXCL16	Neutralizing antibody	Improved
	MCP-1/CCL2	Neutralizing antibody	Improved
		Gene targeting	No effect
	CCR2	Gene targeting	Worse
	MIP1 alfa/CCL3	Neutralizing antibody	Improved
	RANTES/CCL5	Met-RANTES	Improved
	CCR1	Gene targeting	Worse
	MDC/CCL22	Neutralizing antibody	Improved
Anti-Thy1nephritis	IP-10/CXCL10	Neutralizing antibody	Worse
	MCP-1/CCL2	Neutralizing antibody	Improved
	RANTES/CCL5	Amyno-oxyopentane-RANTES	Improved

(Modified from Huber TB, Reinhardt HC, Exner M et al. Expression of functional CCR and CXCR chemokine receptors in podocytes. *J Immunol* 2002;168:6244–6252.)

based on similarities in their amino acid sequences; specifically, they share patterns of paired cysteine repeats and two internal disulfide bridges.

The CC and CXC chemokines have been extensively investigated in kidney diseases. In humans, urinary CC chemokines are detected in crescentic glomerulonephritis and glomerulosclerosis. Increased levels of urinary CXC chemokines are associated with glomerular deposition in cases of active glomerulonephritis. Important roles for chemokines and their receptors have been established in the progression of glomerulonephritis in experimental glomerulonephritis models and In vitro studies.

The CC chemokines (β-chemokines), comprising to date a family of 27 members, are involved in the recruitment of monocytes/macrophages and T cells. Monocyte chemoattractant protein (MCP-1/CCL2), macrophage inflammatory protein (MIP1/CCL3), and regulated upon activation normal T cells expressed and secreted (RANTES/CCL5) are the major CC chemokines involved in glomerulonephritis. There are five receptors for CC chemokines. CCR1 is expressed on monocytes, CCR3 and CCR5 are expressed on Th1 cells, and CCR3 and CCR4 are expressed on Th2 cells.

MCP-1 accelerates glomerular injury. Expression of MCP-1 has been observed in proliferative glomerulonephritis and crescentic glomerulonephritis. Urinary and serum MCP-1 levels are increased in post streptococcal acute glomerulonephritis (PSAGN) in children and urinary, but not serum MCP-1 levels correlate with proteinuria in acute phase (80). MCP-1 and MIP1-α have been detected in human crescentic glomerulonephritis and correlate with the degree of macrophage influx into the glomeruli (81). MCP-1 deficient MRL-Fas/lpr mice showed decreased disease severity, with suppression of macrophage and T-cell recruitment, resulting in decreased local levels of cytokines and chemokines. Dermal transplantation experiments with MCP-1 antagonist-transfected MRL/N-1 cells resulted in attenuated glomerulonephritis via the suppression of T cells and macrophages in murine lupus nephritis. An MCP-1 antagonist attenuated glomerulonephritis in MRL-Fas/lpr mice (82) and a neutralizing antibody to MCP-1 attenuated the disease in a mouse model of crescentic glomerulonephritis. Gene therapy with NH2-terminal deletion mutant of the MCP-1 gene for MRL/lpr mice attenuates glomerulonephritis with reduction of leukocyte infiltration (83). A Spiegelmer (L-enantiomeric RNA oligonucleotide, mNOX-E36) that binds to murine CCL inhibited its function and improved the survival rate of lupus mice by modulating nephritis (84). In addition, P38MAPK ameliorates crescentic GN by suppressing MCP-1, independently of TNF-α and IL-1β (85). An AT1 receptor antagonist and prostaglandin E1 suppressed MCP-1 expression, suggesting that MCP-1 is the target of such blockade.

RANTES binds CCR1 and CCR5 in monocytes. Inhibition of RANTES leads to protective effects in experimental glomerulonephritis, and CCR5 deficiency aggravates T cell-dependent nephrotoxic serum nephritis via enhanced CCL3/CCL5-CCR1 driven renal T cell recruitment. (86). CCR1 antagonist prevents lupus nephritis by inhibiting interstitial inflammation but not affect on glomerular injury (87), suggesting the role of CCR1 is different in the pathogenic background.

CXC chemokines exert chemoattractant effects on PMNs by intracellular phosphatase activation, via CXCR1 and CXCR2. IL-8 interacts with CXCR1 and CXCR 2, advancing PMN chemotaxis. Immunohistochemistry revealed expression of CXCR1 predominantly in MPGN and lupus nephritis in humans and that CXCR-1 expressing cells are mainly PMNs (88). The INF-γ-inducible protein of 10 kDa (IP-10) binds to CXCR3. Renal microvascular endothelial injury model provided evidence that neutralizing antibody against IP-10 attenuated renal dysfunction through reduced the number of infiltrating tubulointerstitial T cells without affecting monocytes/macrophage migration (89). Fractalkine (CX3CL1), the only CX3C chemokines, binds CX3CR1 on NK cells. Mesangial cells express fractalkine In vitro, and its expression is up-regulated in anti-Thy1 nephritis, in parallel with mesangial proliferation (90).

Cytokines stimulate glomerular resident cells to express chemokines, including IL-8, MCP-1, MIP-1α, and macrophage inflammatory protein (MIL)-1α, which can lead to local inflammation and glomerular injury. Podocytes express CCR2 and CXCR1, 3, 4, 5 and these set of chemokine receptors are upregulated in podocytes in membranous glomerulonephritis (91). IP-10 protects podocytes against proteinuria and progressive glomerular damage (92). CXC chemokine-primed mesangial cells express CC chemokines In vitro. This indicates that a chemokine network is involved in acute and chronic glomerulonephritis. Chemokines are also involved in non-immunologic renal injuries, including ischemia-reperfusion models and interstitial nephritis (93).

Growth Factors

Growth factors are cytokines that function as cell-to-cell signaling molecules, regulating cellular processes, such as maturity, proliferation, differentiation, and polarity. Growth factors bind to specific receptors on cell membranes. Several

growth factors are involved in acute and chronic glomerular injuries, and affect the survival, proliferation, migration, and matrix production in both bone marrow-derived cells and intrinsic glomerular cells. Tissue expression of growth factors has been examined by immunohistochemistry in humans and in animal models of glomerular injury, cell culture experiments, and transgenic models with glomerulonephritis, demonstrating the involvement of growth factors in glomerular injury.

Platelet-Derived Growth Factor (PDGF)

PDGF acts primarily on mesenchymal cells, driving cell migration and mitogenesis, extracellular matrix production, anti-inflammatory mediator production, tissue permeability, and hemodynamic regulation. Intrinsic glomerular cells that are mesenchyme-derived are plausible targets of PDGF. PDGF is released by activated platelets, activated macrophages, endothelial cells, and smooth muscle cells. PDGF exists in five isoforms (A, B, C, D, AB), which bind two receptor chains ($-\alpha$, $-\beta$) on the cell surface (receptor tyrosine kinases). PDGF-B is a ligand for PDGF α-and β -receptor, whereas PDGF-D binds β receptor. PDGF activates intranuclear protooncogenes and immediate-early gene expression, which are effectors of the receptor tyrosine kinase and responsible for particular downstream functions. PDGF-A and -B are secreted as homo- or hetero-dimers, whereas PDGF-C and -D are homodimers. The impressive glomerular structures seen in PDGF-B- and PDGFR-β-deficient mice reveal a lack of mesangium and aberrantly developed glomerular tufts, indicating a key role for PDGF in glomerular angiogenesis and cell migration. The intra-renal distribution of PDGF varies among species. PDGF-A is absent from the glomerulus in humans and rodents, whereas PDGFR is expressed in mesangial cells and endothelial cells. Podocyte expression of PDGF-A and B is limited to humans.

In experimental glomerular diseases, intense up-regulation of PDGF-B and PDGFRβ is observed. PDGFR-β is expressed in normal human glomeruli, and this expression is up-regulated in all resident glomerular cells. Anti-Thy1 nephritis shows expression of PDGF in areas of mesangial proliferation, and blockade of PDGF by neutralizing antibodies or aptamers (PDGF receptor IgG) attenuates mesangial proliferation in the acute and chronic phase. (94) PDGF antagonists also prevent glomerular damage in anti-Thy1 nephritis, nephrotoxic serum nephritis, and lupus nephritis models. PDGF-C is expressed in podocytes and mesangial cells in areas of glomerular injury (95). PDGF-D has a similar action as PDGF-B by β-receptor-mediated activation (96). Treatment with PDGF-D-neutralizing antibody in chronic progressive anti Thy1 nephritis resulted in attenuation of glomerulosclerosis accompanied by suppression of monocytes/macrophage influx and complement activation (97). Stimulation of mesangial cells In vitro with different PDGF ligands leads to cellular proliferation and migration. PDGF induces extracellular matrix production in mesangial cells, parietal cells, and tubular cells. PDGF-AB stimulates the production of TGF-β, CCL2, CXC3CL1, plasminogen activator inhibitor-1 (PAI-1), IL-6, endothelin, and iNOS in mesangial cells. The roles of PDGF in renal disease is extensively reviewed (98).

Transforming Growth Factor (TGF)

TGF is known to be a superfamily of cytokines, consisting of about 40 molecules, including the TGF-β, activin, and BMP subfamilies. Several family members involve many aspects in the kidney, i.e., morphogenesis, cell differentiation, inflammation and remodeling. TGF-β is produced by a variety of cell types in an inactive (latent) form and proteolytic cleavage renders it functional. TGF-β exerts its functions through its type I and type II receptors. An intracellular signaling pathway, involving Smad proteins, transduces the TGF-β signal to the target genes, including PAI-I and collagen type I (α2). TGF-β has pleiotropic functions and the effects differ by cell type and dosage. It acts as a growth inhibitor in epithelial cells, whereas it sometimes stimulates cell proliferation and matrix synthesis in the mesenchyme. The net result of TGF-β action in damaged tissue tends to be the formation of scars.

TGF-mediated glomerular injury includes inhibition of cell growth/proliferation, apoptosis, cell differentiation, angiogenesis, and fibrosis. TGF-β stimulates proteoglycan, biglycan, and decorin production in mesangial cells In vitro. The role of TGF-βin immune-mediated glomerular injury is not well understood. Latent TGF-β transgenic mice have no renal disease, despite high levels of circulating TGF-β. Over-expression of active TGF-β results in severe renal damage. Neutralizing antibodies to TGF-β attenuate anti-Thy1 nephritis. Latent TGF-β over-expression in anti-GBM antibody nephritis showed suppression of inflammatory cells and cytokines, by Smad7-mediated inhibition of NF-κB-dependent inflammation (99). In contrast, blockade of TGF-β by adenovirus-mediated gene transfer of the TGF-β IIR failed to attenuate anti-GBM antibody nephritis. Blockade of TGF-β signaling in the T cells of Smad7 transgenic mice inhibited glomerulonephritis, only with a medium dosage of anti-GBM antibodies. Smad 7 gene therapy ameliorates

autoimmune crescentic glomerulonephritis (100). Furthermore, TGF-β IIR knockout heterozygous mice with anti-GBM antibody nephritis showed attenuated glomerulonephritis, accompanied by suppression of ERK and the transcripts of target genes (101). The pleiotropic and complex functions of TGF-β are thought to be differentially regulated in the phases and pathogenetic background of the renal diseases.

Fibroblast Growth Factor (FGF)

The FGFs are a family of growth factors involved in angiogenesis, wound healing, and embryonic development. The FGFs are heparin-binding proteins that interact with cell surface-associated heparan sulfate proteoglycans, which are a prerequisite for FGF signal transduction. FGFs are key players in the processes of proliferation and differentiation in a wide variety of cells and tissues. Among the 22 FGFs identified to date, FGF-2 (basic-FGF) is the major factor investigated in glomerular injury.

As a potent angiogenic factor, FGF-2 mediates proliferation and matrix synthesis through binding with receptors FGF-R1 to R4. FGF-2 is increased in mesangial proliferative glomerulonephritis. Nephrotic syndrome, induced by the puromycin aminonucleoside, leads to FGF-2 expression in podocytes. Recombinant FGF-2 injection in rats leads to podocyte mitosis, without cytokinesis, resulting in severe nephrosis and focal segmental glomerulosclerosis. Parietal epithelial cells express FGF-R2, and FGF-2 stimulates matrix synthesis (102). FGF-1 and its receptor are expressed in the glomeruli, but precise function is still unknown.

Vascular Endothelial Growth Factor (VEGF)

VEGF is known to regulate vasculogenesis and angiogenesis, through effects on endothelial mitogenesis. It is involved in the chemotaxis of macrophages and granulocytes. Vasomotor activity for VEGF has also been noted.

The VEGF family consists of five members (A, B, C, D, PIGF), and VEGF A is the most intensively investigated. VEGF A has four isoforms, VEGF121, 165, 189, and 206; the former two are soluble and the latter two are cell-bound. In glomeruli, VEGF is constitutively and most abundantly expressed by podocytes, and occasionally by activated mesangial cells. The VEGF receptors, flt and flk-1, are expressed on endothelial cells. Inhibition of VEGF worsens anti-Thy1 nephritis, and treatment with VEGF attenuates thrombotic microangiopathy. Systemic administration of VEGF 165 in anti-GBM antibody nephritis reduced inflammation and advanced glomerular repair (103). Administration of sFlt-1 affected neither the infiltration of macrophages nor the crescents.

Connective Tissue Growth Factor (CTGF)

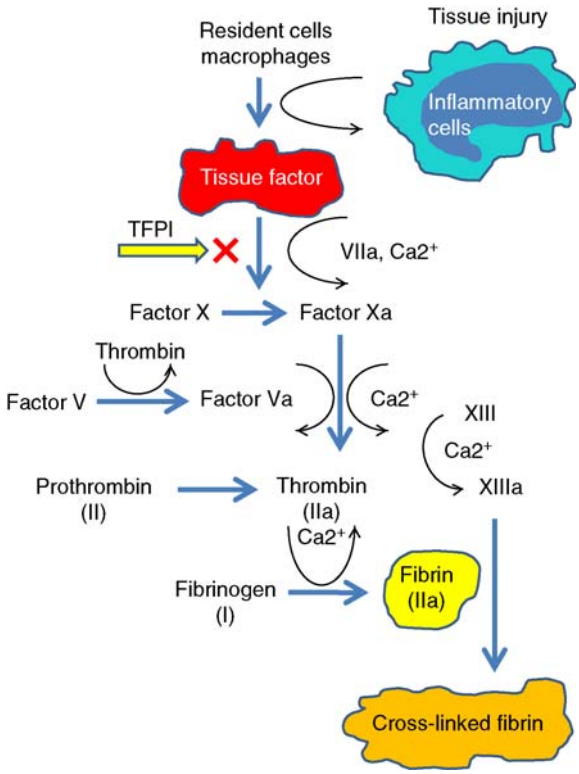
CTGF (CCN2) is a secreted, cysteine-rich, matrix-associated, heparin-binding protein of 38 kDa. A primary function of CTGF is to modulate and coordinate signaling responses involving cell surface proteoglycans. CTGF operates the downstream of TGF-β and directly enhances the TGF-β/Smad signaling pathway or independently acts to promote fibrosis and wound healing (104). CTGF is expressed in the intrinsic glomerular cells and tubular cells, and potentiates to promote renal scar formation (105). TNF-α is an up-regulator of CTGF in mesangial cells. (106) CTGF rapidly activates intracellular signaling and transcription of TGF-β inducible early gene through neurotrophin receptor TrkA in human mesangial cells. (107). CTGF enhances TGF-β action by blocking negative feedback loop of Smad 7 provided TGF-β/Smad signaling pathway in mesangial cells (108). CTGF is expressed in podocytes and PECs in normal glomeruli and binds perlican, a known component of the GBM. Increased CTGF in PECs is associated with matrix production. Up-regulation of CTGF mRNA is found during crescent formation in humans and rats and the expression is diminished when the crescents become scars (102). CTGF promotes TGF-β-dependent mesangial dysfunction and is involved in diabetic nephropathy.

Epidermal Growth Factor (EGF)

EGF is a 53-kDa aminopeptide that was initially identified in mice submandibular glands and human urine. The renal production sites of EGF are the Henlé loop and distal convoluted tubule. EGF is known to be involved in cell survival and the differentiation of tubular cells. Although the urinary levels of EGF are a marker of renal injury and urinary MCP-1/EGF seems to be a prognostic factor in IgA nephropathy, the function(s) of EGF in the glomeruli and its role in glomerular injury are not well understood. EGF induces mesangial cell contraction In vitro. Heparin-binding EGF is a strong mitogen that stimulates mesangial proliferation In vitro. However, immunostaining in human glomerular disease failed to detect glomerular EGF expression.

Figure 29-14

Coagulation is the cascade promoting the transformation of soluble fibrinogen into insoluble fibrin monomers. The cascade is initiated in the injured tissue by exposure of tissue factor (TF) at the surface of the injured endothelium. TF synthesis is stimulated by inflammatory cells, which activated resident cells or macrophages for production. Tissue factor activated conversion of X to Xa, which further converts prothrombin to thrombin. Thrombin participate conversion of fibrinogen to fibrin, which finally forms cross-linked fibrin by XIIIa. TF pathway inhibitor (TFPI) is a potent inhibitor of this cascade.



Platelets and the Coagulation Cascade

As for vascular endothelial cells in general, glomerular endothelial cells normally possess anti-platelet, anticoagulant, and fibrinolytic properties, to maintain the microcirculation in the glomerulus and this is very consistent background that coagulation and the fibrinolysis system are involved in immune-mediated glomerulonephritis. Local activation of the coagulation cascade of the extrinsic pathway eventually produces fibrin or thrombi (*Fig. 29-14*) Glomerular thrombi are occasionally observed in the active phase of glomerulonephritis, reflecting severe endothelial damage. Fibrin deposition is more frequently noted in glomerular injury, with tuft necrosis and thrombotic microangiopathy. The influences of these products in glomerular injury are to accelerate inflammation, rather than ischemia due to glomerular capillary obstruction. In human glomerulonephritis, localization of coagulation system-related molecules has been described, and their functions have been tested in experimental models, using neutralizing antibodies or knockout mice.

Platelets

Platelets are involved in glomerular injury, through leukocyte recruitment, increased vascular permeability, and the vasoactive effects of their intrinsic chemical substances. Platelets contain two types of cytoplasmic granules, α and σ. The α granules express P selectin on their membranes and contain fibrinogen, fibronectin, factors V and VIII, platelet activating factor (PAF), platelet factor 4, PDGF, and TGF-αl. The σ granules contain adenosine diphosphate (ADP), calcium ions, histamine, and serotonin. In local injury in glomerular capillaries, platelets adhere to the extracellular matrices at sites of endothelial injury and become activated. Activated platelets secrete granular products and synthesize TXA2, PDGF, and PAF, which together stimulate clot formation, cell proliferation, and matrix production. PAF is bioactive and increases leukocyte chemotaxis, vascular permeability, and vascular spasms. Platelets include phospholipid complexes that are important in the intrinsic coagulation pathway. Injured or activated endothelial cells trigger aggregation of platelets, forming a clot, and exposing tissue factor, which provokes capillary necrosis and fibrin exudates, leading to GBM breaks and extraglomerular lesions and cellular crescent formation. In addition, platelets activate complement as well as the intrinsic coagulation pathway, and release vasoactive amines.

Fibrin

Fibrin is an insoluble fibrous protein that is the end-product of a locally accelerated coagulation system. Fibrin stabilizes and anchors aggregated platelets. In glomerular diseases, fibrin is occasionally deposited in the capillary or mesangium. In IgA glomerulonephritis soluble fibrin deposition is located in the mesangial proliferation of active stage (109). Fibrin is detected in the capillary necrosis in cellular crescentic formation. Cross-linked fibrin, immunoglobulin deposition, and C3 are occasionally seen in

glomerulonephritis and co-localization of Factor V with fibrin in the mesangium indicates local activation of the coagulation cascade in glomerulonephritis.

Fibrin deposition is mediated largely by the stimulation of macrophage pro-coagulant activity. Most of the fibrin deposition associated with immunologic glomerular injury is mediated by activation of the extrinsic coagulation cascade, commencing with tissue factor pro-coagulants (110). In crescentic glomerulonephritis, fibrin deposition, renal pathology, and dysfunction are synchronous with glomerular macrophage and T-cell influx. In experimental models, glomerulonephritis is attenuated by the administration of anti-Factor V antibody. Defibrinogenation suppresses proteinuria in crescentic glomerulonephritis, although it has no effect on the inflammatory influx, indicating that the coagulation system promotes crescentic glomerulonephritis independently of the inflammatory reaction (111). Activation of the local coagulation system can be initiated by immunologic effects, although secondary events, which include intrinsic glomerular cell proliferation, microcirculation defects, and cell necrosis, are promoted by bioactive molecules of the coagulation system. In the activation of coagulation system, the fibrinolytic cascade also limits clot formation. Fibrinolytic agents have shown relatively consistent efficacy in attenuating immune-mediated glomerular injury in several models.

Tissue Factor

Tissue factor is a cellular lipoprotein that is present at sites of tissue injury, and activates extrinsic coagulation processes. In glomerular injury, inflammatory cell-derived cytokines induce tissue factor secretion by resident cells and macrophages. Endothelial tissue factor release initiates glomerular fibrin deposition, through activation of the extrinsic coagulation pathway, by binding circulating factor VII and facilitating its allosteric conversion to factor VIIa. Tissue factor converts X to Xa and factor V is a cofactor for the Xa, which is responsible prothrombin activation. Factor Va then converts prothrombin to thrombin, which further converts fibrinogen to fibrin (*Fig. 29-14*). Tissue factor also has fibrin-independent pro-inflammatory effects, mediated by MHC class II protein expression and leukocyte accumulation.

Specific Xa inhibitor ameliorates anti-Thy 1 nephritis by inhibiting macrophage infiltration and fibrin accumulation, suggesting that tissue factor promotes mesangial proliferation by function of proinflammatory and procoagulant mechanism of factor X (112). Administration of anti-factor V neutralizing antibody suppressed profibrotic cytokines in glomeruli (113). Inhibition of tissue factor activity by an antibody or tissue factor pathway inhibitor (TFPI) significantly ameliorates the development of crescentic glomerulonephritis, accompanied by decreased glomerular inflammatory cell influx. Infusion of recombinant TFPI ameliorates glomerular fibrin deposition and glomerular pathology in experimental glomerulonephritis.

The Enzymatic Fibrinolysis System

In addition to the progression of coagulation processes in tissues, the clotting cascade simultaneously sets into motion a fibrinolytic cascade that restricts final clot size, which may influence tissue scarring. The fibrinolytic system consists of an inactive proenzyme (plasminogen), which can be converted to an active enzyme (plasmin) that degrades fibrin into soluble fibrin products. This system is under the control of tissue-repair-related molecules, including plasmin. Plasmin is derived from the enzymatic breakdown of its inactive circulating precursor, plasminogen, either by a factor XII-dependent pathway or by plasminogen activators. Plasmin is involved in fibrinolysis. Activation of plasmin significantly involves matrix degradation and fibrinolysis, which influences the fate of tissue injury. Activation of plasmin inhibits fibrosis by enhancing matrix metalloproteinase 2 activation, thereby promoting ECM degradation. Plasminogen activator (PA) is a serine protease that converts plasminogen to plasmin to regulate ECM tissue deposition. Two plasminogen activators, tissue type (t-PA) and urokinase-type (u-PA), are known. t-PA is synthesized primarily by endothelial cells and is active when attached to fibrin, which it dissolves. Plasminogen binds to the plasminogen activator receptor (PAR), which belongs to the G protein-coupled receptor family, and activation of PAR leads to cytoskeletal rearrangements, proliferation of fibroblasts, and glomerular epithelial cell proliferation. Thrombin also interacts with PAR.

PA and plasmin are suppressed in some glomerulonephritis conditions and may be involved in mesangial matrix accumulation via α_1-antitrypsin, α_2-macroglobulin or plasminogen activator inhibitor (PAI). PAI is synthesized by endothelial cells to modulate the coagulation/anti-coagulation balance by blocking fibrinolysis. PAI-1 is a single glycoprotein of about 45 kDa consisting of 379 or 381 amino acids. It is a member of the serpin family with reactive site peptide bound Arg345-Met346. Activation of PAI-1 requires complex processes. PAI-1 promotes fibrosis by inhibiting plasmin generation, through the suppression of plasminogen activator. PAI-1 is also activated by interaction with TGF-β and it can modulate fibrosis and PAI-1, down-regulating TGF-β activity in a

negative feedback loop. PAI-1 deficiency leads to pathogenic over-activation of TGF-β

PAI-1 is increased in glomerulonephritis and plasmin-independent pro-fibrotic action of PAI-1 in glomerular injury involves the recruitment of fibroblasts and macrophages. Experimental cryoglobulin-associated mesangial proliferative glomerulonephritis reveals upregulation of the protease Nexin-1, t-PA, and PAI-1 (114). Induction of crescentic glomerulonephritis in PAI-1-deficient mice resulted in improvements in nephritis, accompanied by depressed inflammatory infiltrates and collagen accumulation.

Protease-activated receptor-2 (PAR-2), receptor of factor Xa, is expressed in epithelial, mesangial, and endothelial cells, and macrophages. PAR-2 is activated by serine proteases, such as trypsin, tryptase, and factors VIIa and Xa, and increases PAI-1 expression and macrophage-derived cytokine induction. PAR-2 is involved in human mesangial cell proliferation (115). Experiments with PAR-2-deficient mice revealed that PAR-2 promoted glomerular fibrin deposition and the progression of crescentic glomerulonephritis, due to stimulation of renal PAI-1 expression and MMP-9 activation (116) Thrombin-activatable fibrinolysis inhibitor (TAFI) is a potent inhibitor of plasmin generation, and immune complex-mediated glomerulonephritis is ameliorated in TAFI-deficient mice.

Although the coagulation cascade/fibrinolysis system is substantially involved in the acute and exudative phases of glomerular injury, by interacting with inflammatory cells, its roles in chronic progressive glomerulonephritis remain unknown. Further studies are needed to investigate the coagulation/fibrinolysis-independent actions of PAI-1 and tissue factor, such as inflammatory responses, cell migration, cell proliferation, angiogenic actions, and matrix remodeling in glomerular injury.

Eicosanoids

Eicosanoids are short-acting, hormone-like substances. They are arachidonic acid (AA) metabolites, and have effects in inflammation and hemostasis. AA is a 20-carbon polyunsaturated fatty acid that is present mainly in cell membrane phospholipids. Mechanical, chemical, and physiologic stimuli release AA from the cell membrane into the cytoplasm and specific enzymes then synthesize the metabolites. The AA cascade branches into two major pathways, the cyclooxygenase and lipoxygenase pathways, both of which are involved in glomerular injury (*Fig. 29-15*).

The cyclooxygenase pathway products include prostaglandins (PG) E_2, PGD_2, PDF_2a, PGI_2 (prostacyclin), and thromboxan (TX) A_2. The lipoxygenase pathway begins with 5-lipoxygenase, a well-characterized AA-metabolizing enzyme. Three major products, 5-hydroxyeicosatetraenoic acid (HETE), the leukotriene (LT) family of compounds, and the lipoxins, are known.

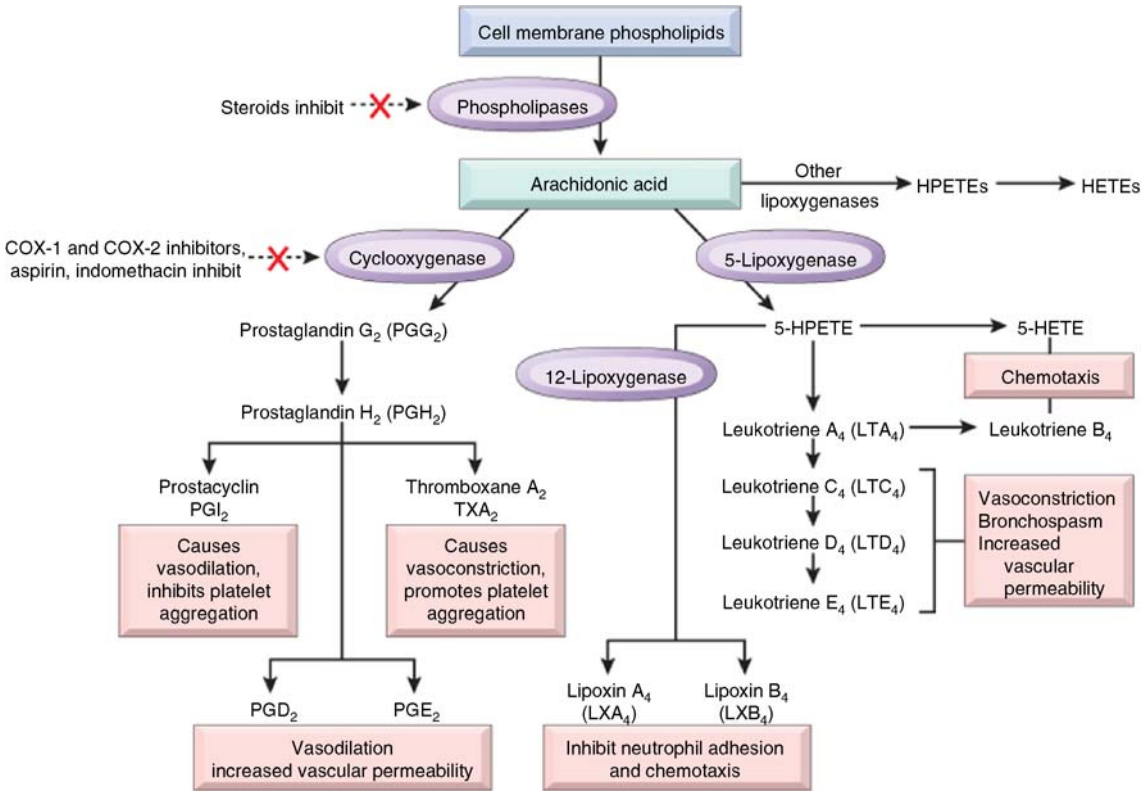
The restricted distribution of enzymes may determine the cell-specific synthesis of certain substances and cell to cell transaction to produce active endoproducts. For example, the AA in neutrophils is converted into LTA4 followed by LTB4, which is the end-product in neutrophils, since neutrophils lack the further converting enzyme of LTC4 synthase, and it is then secreted from the neutrophils. In contrast, platelets lack the 5-lipoxygenase enzyme but have 12-lipoxygenase and LTC4 synthase. Thus, in platelets, the LTB4 derived from neutrophils is converted into lipoxin and LTC4, both of which are final platelet products. This transcellular interaction of AA metabolites promotes inflammation In situ, resulting in biologic responses by the cells. Inflammatory mediators, such as complement components, also accelerate the release of these metabolites. Common functions among the eicosanoids are vasomotor effects; TXA_2 and LTs cause vasoconstriction, whereas PGs, PGI_2, and lipoxin participate in vasodilatation. LTB4 and lipoxins also promote chemotaxis and leukocyte adhesion. LTs affect vascular permeability. All these functions of AA products are involved in the processes of glomerular inflammation and renal function.

Considering wide ranged actions of AA metabolites for inflammation and tissue damage/repair, their roles for glomerular injury are likely important. AA involvement in glomerulonephritis has been extensively investigated before 2000 mainly using cell lines to demonstrate proliferation and macrices production, or administration of inhibitors in experimental models.

There are two forms of cyclooxygenase, COX-1 (constitutive) and COX-2 (inducible). COX-1 is constitutively expressed in the kidney, including in the mesangial cells, arterioles, PECs, and cortical and medullary collecting ducts. COX-2 is an inducible protein; its mRNA is detectable in the macula densa and the cortical thick ascending limb cells immediately adjacent to the macula densa, whereas it is not detected in the glomeruli. COX-2 expression in the glomeruli may appear in glomerulonephritis. In particular, podocyte expression of COX-2 is prominent in renal ablation models and anti-Thy1 nephritis. Furthermore, podocyte-specific COX-2 expression, driven by the nephrin promoter, aggravates proteinuria and upregulates endogenous COX-2. Podocyte COX-2 expression is induced by glomerular inflammation or various stresses and renders podocytes susceptible to further injury (117).

Figure 29-15

The arachidonic acid cascade and metabolites. Generation of arachidonic acid metabolites and their roles in inflammation. Note enzymatic activities whose inhibition through pharmacologic intervention blocks major pathways. (Denoted with COX-1 and COX-2, cycloxygenase 1 and 2; HETE, hydroxyeicosatetraenoic acid; HPETE, hydroperoxyeicosatetraenoic acid. (The figure was published in Kumar et al. Robbins Basic Pathology, 8 edn. 2007, pp. 48, Copyright Elsevier.)



PGE_2 is primarily produced by mesangial cells. In acute glomerular inflammation, PGE_2 is increased to maintain effective renal circulation by relaxing vascular smooth muscle cells. Another important function of PGE_2 is its anti-inflammatory actions in suppressing inflammatory cell recruitment, chemokines synthesis, and NO production. PGI_2 is a vasodilator and a potent inhibitor of platelet aggregation. Glomerular damage with endothelial injury may result in decreased PGI_2 In situ and may promote further glomerular injury. TXA_2 has largely opposite effects to PGI_2 and is abundantly synthesized in nephritic glomeruli. TXA_2 is produced by inflammatory cells and intrinsic glomerular cells and accelerates glomerular injury, through platelet aggregation and vasoconstriction. The LTs, a family of compounds converted by 5-lipoxygenase, include LTA4, LTB4, LTC4, LTD4, and LTE4. LTB4 is a potent chemotactic compound that promotes the recruitment of neutrophils, and is elevated in experimental models with glomerular damage. LTC4, LTD4, and LTE4 participate in vasoconstriction and increase vascular permeability. Receptor antagonists of LTs attenuate glomerular injury.

Lipoxins are the products of a two-step lipoxygenation, i.e., 5-lipoxygenase followed by 12-lipoxygenase. Lipoxins are produced locally at sites of inflammation by the transcellular interaction of neutrophils, platelets, and intrinsic cells. The platelets themselves cannot produce lipoxin, so they take up neutrophil-derived LTA4 and convert it into lipoxin. Lipoxins have both pro- and anti-inflammatory actions. Lipoxin A4 inhibits cytokine-induced mesangial cell proliferation In vitro and has been implicated as anti-inflammatory function in glomerulonephritis. (118, 119) And anti-inflammatory action of blocking LT-mediated neutrophil chemotaxis predominates. The vasodilatory actions of lipoxin may occur partly through the promotion of vasodilatory prostaglandins (prostacyclin) and NO. Inhibition of the AA cascade can be archived using specific inhibitors,

antagonists or even dietary manipulation. Aspirin and non-steroidal anti-inflammatory drugs (NSAIDs) inhibit all the steps upstream of COX activity. Specific inhibitors of TXA_2 and LTs have been shown to attenuate experimental glomerulonephritis. Dietary manipulation, leading to changes in eicosanoid metabolites, has been reported to affect glomerulonephritis in humans. Dietary fish oil that contains omega-3 fatty acids (eicosapentanoic acid and docohexanoic acid) modulates both COX and lipoxygenase metabolism. In addition, dietary restriction of essential fatty acids impairs renal production of a specific lipid-derived macrophage chemoattractant and LTB4, resulting in decreased production of cytokines, ROS, and NO in macrophages.

Reactive Oxygen Species (ROS)

ROS are chemical species with a single unpaired electron in an outer orbital. They are extremely unstable and readily react with various chemicals. Generation of ROS can occur via the rapid activation of NADPH oxidase, which oxidizes NADPH and reduces oxygen to the superoxide anion (O_2^-). ROS are produced within the lysosome and may be released from leukocytes by exposure to chemotactic agents, immune complexes, complement, and other factors. In addition, ROS are occasionally released by intrinsic glomerular cells. ROS are inherently unstable and generally decay spontaneously. The influence of oxygen-derived free radicals in inflammatory reactions depends on the balance between the production and inactivation of the related metabolites.

The biologic actions of ROS include the stimulation of expression of chemokines, cytokines, and endothelial leukocyte adhesion molecules. In addition, ROS stimulate intracellular signaling molecules that are involved in mitogenic pathways. The superoxide anion, hydrogen peroxide, and the hydroxyl radical are major species of ROS. The superoxide anion typically being the most abundant, though its potential for tissue injury is limited. Hydroxyl radicals induced by the Haber Weiss reaction promote considerable tissue damage. Activated leukocytes in the inflammatory milieu synthesize ROS and stimulate endothelial ROS production through xanthine oxidation, resulting in increased vascular permeability. Activation of NADPH oxidase is involved in cell proliferation and extracellular matrix production, mediated by intracellular signaling that includes the mitogen-activated protein kinase (MAPK) and activator protein 1 (AP-1) or TGF-β. Angiotensin II increases superoxide production in mesangial cells, through activation of NADPH oxidase activity. Antioxidants suppress proliferative anti-GBM glomerulonephritis (120). PDGF BB, TNF-α and IL-1 β stimulate ROS production in mesangial cells In vitro. In the acute phase of anti-Thy1 nephritis, ROS and NADPH-dependent oxidase activity are increased. In glomerulonephritis, inhibition of oxidative stress activates ERK and reduces fibrotic growth factors, resulting in attenuation of anti-Thy1 nephritis (121). Direct infusion of H_2O_2 in rat renal arteries causes proteinuria, and H_2O_2 depletion by catalase reduces neutrophil-dependent proteinuria, suggesting that ROS play a particularly significant role in the promotion of neutrophil-dependent glomerular injury. Likewise, ROS are involved in ANCA-associated glomerulonephritis.

Tissue fluids and the target cells of oxygen-derived radicals have antioxidant mechanisms to protect against harmful species. Several enzymatic and non-enzymatic antioxidant systems inactivate free radicals. Superoxide dismutase (SOD) significantly accelerates the spontaneous decay of ROS. Glutathione peroxidase also protects against ROS-mediated cell injury by catalyzing free radical breakdown. Catalase, which is present in cytoplasmic peroxisomes, directs the degradation of hydrogen peroxide. Exogenous antioxidants (vitamins E, A, C, β-carotene) block the formation of free radicals or scavenge such products. The effects of these antioxidants on glomerulonephritis have been reported, although presently there is insufficient evidence regarding their effects.

Nitric Oxide (NO)

NO, which is a pleiotropic mediator of inflammation initially discovered as the endothelial cell-derived factor relaxing vascular smooth muscle cells, produces vasodilatation. NO is a soluble gas that is produced by endothelial cells and macrophages and is a typical cytotoxic substances of macrophages. NO production is stimulated by acute inflammatory molecules, including TNF-α, ILs, and INFs. As the half-life of NO is extremely short, NO action is limited to the immediate proximity of the production site. NO acts on target cells through a paracrine mechanism and induces stable products, such as nitrates, nitrites (NO_2), and cyclic GMP-mediated intracellular alterations.

Three NO synthase isoforms have been distinguished in mammalian species: the constitutive calcium-dependent neuronal NOS (nNOS) and endothelial NOS (eNOS), and the inducible calcium-independent iNOS. In the glomerulus, eNOS is constitutively expressed in endothelial cells and occasionally, iNOS transcripts are detected.

In acute phase glomerulonephritis, macrophages are the major source of NO. iNOS is induced in glomerular capillary inflammation and glomerular NO_2 is increased in anti-GBM antibody nephritis models. NO_2 expression is relatively acute and transient. Early studies showed that decreasing NO production by L-arginine depletion in rat nephrotoxic serum nephritis exacerbated proteinuria. Increased NO synthesis observed in acute models of glomerular injury is not only a promotive factor, but also has protective roles. In fact, iNOS inhibitor in Wistar-Kyoto rats with nephrotoxic serum nephritis showed reduced crescentic formation and eNOS-deficient mice with anti-GBM nephritis reveals attenuation of nephritis indicating protective roles for NOS in glomerular injury. Anti-Thy1 nephritis shows an association between NO synthesis and mesangiolysis and suppression of NO resulted in reduced mesangiolysis. Another experiment using anti-GBM antibody nephritis in iNOS-deficient mice revealed no effect on glomerular histology, macrophage-mediated proteinuria or glomerular tissue damage. In humans, iNOS expression has been observed in proliferative lupus nephritis and IgA glomerulonephritis, although iNOS expression is not associated with the presence of macrophages and its function remains to be investigated more thoroughly (122). Attenuation of diabetic nephropathy in eNOS knock-out mice indicates involvement of eNOS-derived NO in the progression of diabetic nephropathy (123).

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30 Acute Postinfectious Glomerulonephritis

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Introduction

The term acute postinfectious glomerulonephritis includes a large group of glomerulonephritis that result from a variety of infectious agents. In this chapter we will deal only with the most common glomerulonephritis that result from bacterial infections. The term acute glomerulonephritis defines a pathological process that may be manifested clinically as an acute nephritic syndrome, nephrotic syndrome or rapidly progressive glomerulonephritis. Since post-streptococcal glomerulonephritis is the best known of the acute postinfectious glomerulonephritis and its most frequent clinical picture is the acute nephritic syndrome, the terms poststreptococcal glomerulonephritis, acute glomerulonephritis and acute nephritic syndrome are often, and incorrectly, used interchangeably.

In acute postinfectious glomerulonephritis the inflammatory process that takes place in the glomeruli is triggered by antigen-antibody reactivity that results in local activation of the complement system and of the coagulation cascade. Immune complexes causing glomerulonephritis may be formed in circulation or in situ in the glomerular basement membrane (GBM). Classic experimental studies in the acute (one-shot) (1, 2) and chronic serum sickness (3) have demonstrated that glomerular deposition of circulating immune complexes depend on the size of the complexes (300–500 kDa, on antigen load and the intensity of the antibody response (antigen/antibody ratio). The development of the glomerular inflammation and severity, as well as the progression to chronic renal lesions depends, on a large measure, on the duration of antigen exposure and the host's capacity to remove the deposited complexes. In situ formation of immune complexes result from the deposition of the antigen in the GBM and its penetration to the epithelial side of the GBM where is met by the corresponding antibody to form immune complexes that present a characteristic subepithelial electron dense deposits. In situ formation of immune complexes occur in conditions of antigen excess and are typical of cationic antigens that have electrostatic charge-facilitated penetration of the polyanionic GBM (4).

Immune complexes may be predominantly located in the mesangium or in the GBM or in both and by immunofluorescence the imunoglobulin deposition is of the granular (not linear) type. When heavy, these deposits in the GBM have a garland appearance (5) and are usually associated with heavy proteinuria, while mesangial deposits with speckled appearance are usually associated with only mild-to-moderate proteinuria and with features of the acute nephritic syndrome.

Acute Poststeptococcal Glomerulonephritis

Dark urine and suppression of urine output was a feared complication of the convalescent period of scarlet fever recorded in the medical literature more than six centuries ago (6). The findings that acute glomerulonephritis followed streptococcal upper respiratory and skin infections (7–9) clarified the etiology of the disease as acute poststreptococcal glomerulonephritis (APSGN) and the seminal theoretical contribution of Clemens von Pirquet (reviewed in 10), attributing APSGN to antigen-antibody complexes of nephritogenic potential defined the pathogenesis of this condition and opened the era of immune complex-mediated diseases.

Epidemiology

APSGN may occur in sporadic cases or in epidemic outbreaks. Usually the cases occur in communities that are poor, with deficient hygienic conditions and with uneasy access to opportune medical care. Epidemic outbreaks tend to appear periodically in closed populations as the Red Lake Indian Reservation in Minnesota and in specific areas in less developed countries as Port of Spain in Trinidad and in Maracaibo, Venezuela. The risk of nephritis in epidemics range from 5% in throat infections, to as high as 25% in pyoderma caused by M type 49 streptococci.

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In recent years the epidemiology of APSGN has changed along with a decline in its incidence worldwide and particularly in industrialized countries where the disease now is associated with debilitating conditions such as alcoholism or diabetes (11). In under privileged communities the most recent epidemic have been caused by *Streptococcus zooepidemicus* and associated with the consumption of unpasteurized milk from cows with mastitis (12).

The reduction in the incidence of APSGN is attributed to a variety of factors, including easier and earlier access to competent medical treatment of streptococcal infections and the widespread use of fluorination of water since virulence factors in *Streptococcus pyogenes* are reduced with fluoride exposure (13). Despite the lower incidence of the disease, endocapillary glomerulonephritis of demonstrated or assumed poststreptococcal etiology is the most common histological presentation of primary renal disease in most countries in the underdeveloped world. In these countries the global burden of APSGN continues to be significant. Two recent studies (14, 15) have calculated the global burden of APSGN and came to almost similar conclusions, with lower estimates of 9.3–9.8 cases per 100.000 population per year in underdeveloped countries and higher estimates that could be as high as three times these values. Furthermore, clusters of cases are more frequently reported in poor communities in industrialized countries while epidemics of more than 100 cases are reported from countries in the middle range of Human Development Index and a mean annual health expenditure per capita of about 550 $US (15).

Pathogenesis

Streptococcal Antigens and Immune Reactivity

APSGN has an immune complex pathogenesis. The identity of nephritogenic antigens has been the subject of considerable investigation and controversy. Specific M proteins in group A streptococci were initially considered "nephritogenic," a notion that originated from the observation that group A streptococcal infections could cause rheumatic fever (rheumatogenic streptococci) or glomerulonephritis but only exceptionally both (16, 17). Studies in epidemics revealed that APSGN was associated with pyodermitis due to Group A streptococci of M types 47, 49, 55 and 55 and with upper respiratory infections due to streptococci of M types 1, 2, 4 and 12. However, more recently it has been found that glomerulonephritis may result from group C streptococci, as noted earlier making thus evident that antigenic fractions capable of causing nephritis are shared by a large variety of streptococci.

While M proteins were initially investigated extensively, more recent research has focused in two antigenic fractions: the so-called nephritis associated plasmin receptor (NAPlr), identified as glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and the streptococcal pyrogenic exotoxin (erythrotoxin) B (SPEB) and its zymogen precursor (zSPEB). The characteristics of these fractions and their nephritogenic potential have been reviewed recently (15). NAPlr as well as SPEB have been identified in early biopsies and elicit antibody response that may be detected in the vast majority convalescent sera. The frequency of the antibody response to SPEB/zSPEB has resulted in optimum ROC curves and the best antistreptococcal antibody response for the purposes of identifying a potentially nephritogenic infection (18). In a recent study, back-to-back testing of both these fractions in renal biopsies of APSGN patients and convalescent sera indicated that in the population of patients tested, antibody response to and glomerular deposits of SPEB were frequent while NAPlr were unusual (19). The discordant results may be due to the role of host-related factors in the development of APSGN since NAPlr studies were done in a relatively homogenous Japanese population while the SPEB studies were done in populations of patients in Latin America and Europe. In fact, studies in biopsies of Japanese patients did demonstrate the deposits of both putative nephritogens (15).

SPEB is co-localized in the glomeruli with complement and Ig deposits and is the only streptococcal antigen that has been localized within the electron dense subepithelial deposits (humps) (19) that are characteristic of APSGN and the cationic (pk > 8.0) nature of this component favors the penetration of the anionic barrier in the GBM. The nephritogenic potential of the NAPlr may reside in its plasmin binding characteristics since it is not co-localized with either Ig or complement (20, 21) and, in fact this characteristic is shared by both NAPlr and SPEB (15). Increased plasmin binding may promote the local inflammatory reactivity and facilitate the penetration of nephritogenic antigen-antibody complexes.

Finally, the possibility that more than one streptococcal antigen is nephritogenic was recently emphasized by the demonstration that the gene encoding for SPEB was absent in the *S. zooepidemicus* causing the recent APSGN epidemic in Brazil which would indicate that this antigen was not involved in this epidemic (12).

Immune complexes formed against nephritogenic streptococcal antigen(s) may be formed in circulation,

and deposited in the glomeruli, or formed in situ against antigenic fractions planted in the glomeruli. The relevance of the circulating immune complex mechanism derives from the classic experiments of the glomerulonephritis in acute serum sickness; however, glomerular deposition of free antigen and in-situ immune complex formation has been more recently proposed as an important pathogenetic mechanism in APSGN. Such a mechanism would easily explain the formation of subepithelial electron dense deposits (humps) which are extremely difficult to produce by the injection of preformed immune complexes and are, in contrast, the rule with the injection of cationic antigens (4). Since the existence of a large number of humps in biopsies is associated with heavy proteinuria and worse prognosis (see later), the in-situ mechanism may have significant clinical relevance.

APSGN results from the inflammatory reactivity induced by the immune complexes involving complement activation. Complement activation occurs preferentially by the alternate pathway. Ig-binding proteins in streptococcal surface interfere with the classical pathway of complement activation (22, 23). Recent studies suggest that complement regulatory proteins that play a role in protecting streptococci may be removed by SPEB. Recent investigations have shown that the lectin pathway of complement may also be activated in APSGN by the recognition of glucosamine residues (24).

In addition to humoral immunity, cell-mediated mechanisms are also involved in the development of APSGN. Glomerular infiltration of lymphocytes and macrophages has long been recognized as a feature of the disease (25). Intercellular leukocyte adhesion molecules, such as ICAM-1 and LFA are over expressed in glomeruli and tubulointerstitial regions and correlated with the intensity of the inflammatory infiltration (26).

Autoimmune Reactivity

A number of autoimmune findings have been reported in APSGN (reviewed in 15). Among them, anti-IgG reactivity is the best studied. Cryoglobulins are high titers of rheumatoid factor are present in about two-third of the patients in the first week of the disease; furthermore, anti-IgG glomerular deposits are frequently found in biopsies (see later) and anti-IgG reactivity has been documented in the IgG eluted from the kidney in a fatal case of the disease. The anti-IgG reactivity may be the result of autoantigenic modification of Ig that occurs with its desialization caused by streptococcal neuraminidase. This possibility was raised by experimental studies showing that injection of neuraminidase-treated autologus IgG causes the appearance of anti-IgG reactivity and received support from the demonstration of plasma neuraminidase activity and increased free sialic acid levels in patients with APSGN. Another potential cause of anti-Ig reactivity is the binding of IgG to type II receptors in the streptococcal wall. Anti-IgG reactivity is a constant feature of injections of streptococci cultured in a medium with autologous serum.

Additional autoimmune phenomena have been found in APSGN patients. Anti-DNA antibodies, antiCiq antibodies and anti-neutrophil cytoplasmic antibodies (ANCA) are present in some patients. Interestingly, the later has been found in two-third of the patients with azotemia and 70% of the patients with APSGN that develop crescentic glomerulonephritis (27).

Cytokines and Chemokines

The pathogenetic importance of cellular immune mechanisms was suggested by early studies that demonstrated lymphocyte and macrophage infiltration in renal biopsies of patients with the disease (28). The infiltration of immunocompetent cells is facilitated by overexpression of intercellular adhesion molecules ICAM-1 and LFA-1 in APSGN (29). Cytokine and chemokine production is a central process in damage induced by immune cells. IL-6 plays an important role in proliferative glomerulonephritis (30) and one of the putative nephritogenic antigens, SPEB, induces increased production of IL-6 and proliferation on mesangial cells (31). Mezzano et al. (32) have shown that glomerular IL-8 correlates with neutrophil infiltration and transforming growth factor – β with mesangial expansion and Yokohama et al. (33) found a direct correlation between serum TNFα levels and glomerular ICAM-1 expression.

Clinical Characteristics

Children from 4 to 14 years are more frequently affected by APSGN. It is rare below the age of 2 and above the age of 20 and is twice more frequent in males than in females. The usual sites of antecedent infection are the skin and the throat, although any location of streptococcal infection is possible. The latent period between infection and nephritis is longer after skin infections (3–5 weeks) than after upper respiratory infections (7–15 days). During the latent period asymptomatic children may have microscopic hematuria.

ASPGN may have subclinical course or may present with the acute nephritic syndrome, and more rarely with

nephrotic syndrome or, exceptionally, with a rapidly progressive (crescentic) glomerulonephritis (34). Subclinical disease is characterized by a reduction of serum complement, microscopic hematuria and normal or increased blood pressure in asymptomatic patients. In epidemic conditions occurs 1.5 times more frequently than clinically apparent disease (35). Prospective studies in household members of index cases have shown that in non-epidemic situations the patients without symptoms are 4–5 times more frequent than symptomatic patients (36, 37).

The most typical clinical picture in APSGN is the acute nephritic syndrome (hematuria, edema, hypertension and moderate proteinuria). *Glomerular hematuria* is an almost universal finding and gross hematuria is present in one-third of the patients. The absence of red cells in the urine is usually due to delays in the examination of the urinary sediment since red cells are rapidly destroyed, especially in alkaline urine. As a rule, red cell casts are present in association with dysmorphic of red cells, frequently presenting doughnut shape with one or more blebs. Macroscopic hematuria usually disappears after a few days but microscopic hematuria may persist for a year and occasionally exacerbates during febrile episodes and more rarely after strenuous exercise. *Edema* is the chief complaint more frequently in children (90%) than in adult patients (75%). Younger children tend to have more often anasarca than adolescents or adult patients but ascities is uncommon, except in the cases with nephrotic syndrome. *Hypertension* is present in 60–80% of the children and is severe enough to require specific antihypertensive treatment in about half of the cases. Edema and hypertension typically disappear in 5–10 days. *Oliguria* is referred on admission by the patient or their family in less than half of the patients. Other nonspecific symptoms include general malaise, weakness, headache, dull lumbar pain and nausea.

Massive proteinuria with or without other features of the nephrotic syndrome are found in about 2–4% of the cases and its persistence is a risk factor for progression to chronic renal disease. *Azotemia* occurs in 25–30% of the patients but the need of dialysis is infrequent. A rapidly progressive azotemia occurs in less than 0.5% of the cases and when present is due to the development of crescentic glomerulonephritis.

Pathophysiology of the Acute Nephritic Syndrome

The acute nephritic syndrome results from the reduction of the glomerular filtration rate caused by the inflammatory reaction in the glomeruli. The renal blood flow it is usually normal and therefore the filtration fraction is depressed, frequently below 1%. The reabsorption is appropriately reduced in the proximal tubule and assumed to be maintained at the distal areas of the kidney inaccessible to micropuncture. While the reduction in glomerular filtration has long been assigned a central role in the fluid retention in acute glomerulonephritis, the participation of additional more distal factors that remain undefined is required. It is obvious that the reduction of glomerular filtration is a feature of renal conditions that are not necessarily associated with fluid retention and, conversely, severe fluid retention may some times occur in association with mild reduction in glomerular filtration rate. Potential influences that may modulate sodium and fluid retention at more distal levels are endothelial or mesangial factors released by glomerular injury (38), overexpression of the epithelial sodium channel (39) and interstitial inflammatory cells capable of maintaining an increased intrarenal angiotensin activity (40). At any rate, the net result of the hemodynamic changes in glomeruli and tubules is a tendency to sodium and water retention that results in expansion of the extracellular volume, edema and hypertension. The potential additional effects of hypoalbuminemia are not present in the acute nephritc syndrome and, accordingly, the plasma levels of hormones that regulate the extracellular volume (renin-angiotensin, aldosterone and atrial natriuretic peptide) show an appropriate response for an expanded extracellular volume. The suppression of plasma renin activity and aldosterone and the stimulation of atrial natriuretic peptide are correlated with the severity of edema. Renal prostaglandin and kallikrein activities are suppressed (reviewed in 41). In accordance with this pathophysiology, the improvement of volume retention with the development of spontaneous or induced diuresis results in a prompt reduction of the edema and correction of the hypertension.

Serological Findings

The most constant serological finding is the reduction in serum complement levels that occurs in more than 90% of the cases. The activation of the complement system is usually via the alternative complement pathway and reduced C3 with normal C1 and C4, but, as mentioned earlier, the classic pathway and the lectin pathways of complement activation may also be engaged. There are no clinical characteristics associated to a specific complement activation modality and complement levels return to normal usually within one month of the development

of acute glomerulonephritis. IgG and IgM serum levels are elevated 80–90% of the patients with APSGN.

Rising antistreptococcal antibody titers are the usual clinical indication of a preceding streptococcal infection since positive cultures are obtained in only 20–25% of the cases, except during epidemics. Anti-zSPEB/SPEB serum titers have been found to be the best markers of nefritogenic streptococcal infections in multicentric studies in LatinAmerica (18), but these tests are not generally available. Antistreptolysin O titers and anti-DNAse B titers are the most frequently elevated antibody titers after streptococcal throat infections and after streptococcal impetigo, respectively. Two-thirds of the patients with APSGN in the first week of the disease present cryoglobulins and elevated IgG-antiIgG rheumatoid factor titers (41).

Pathology

Renal biopsy is usually not done in patients with APSGN with well defined clinical picture. Biopsy is indicated when specific features raise the possibility of a different diagnosis. For instance, if the serum complement is normal a biopsy may define if the acute nephritic syndrome has a different etiology, such as IgA nephropathy and if the serum complement remains low after one month the biopsy may help to rule out hypocomplementemic mesangiocapillary glomerulonephritis or lupus nephritis. In patients with proteinuria in the nephrotic range a biopsy may serve to confirm the diagnosis of APSGN and to raise awareness of a worse long-term prognosis. Finally, if the patient has a rising serum creatinine, the diagnosis of crescentic glomerulonephritis needs biopsy confirmation. Biopsy findings in APSGN are those of endocapillary proliferative glomerulonephritis. Glomerular basement membrane is normal. Macrophages and lymphocytes can be found in increased numbers in the glomeruli and in tubulointerstitial areas but the more remarkable histologic characteristics are found in the glomeruli that present a diffuse increment in cellularity. Occasionally, the glomeruli presents abundant infiltration of polymorphopnuclear neutrophils, a characteristic that has been named exudative glomerulonephritis.

Glomerular immune deposits C3, IgG and IgM and less frequently anti-IgG are usually present and they may be demonstrated in the mesangium and in the glomerular basement membrane. The immunofluorescent appearance of this deposits have been described by Sorger et al. (5) as resembling a starry sky (discrete mesangial and basement membrane deposits, usually of complement) or in more thick deposits in he mesangium (mesangial form) or forming heavy, garland-like deposits in the basement membrane. The garland form is clinically important because it associates with heavy proteinuria, numerous subepithelial electron-dense deposits and worse long-term prognosis (36, 42). Recent studies have co-localized SPEB deposits with complement and IgG and NAPlr with plasmin activity in the glomeruli. Electron microscopic studies reveal the hallmark lesion of APSGN, the subepithelial "humps" that represent subepithelial immune complex deposition. Immune electron microscopy has permitted to demonstrate IgG, C3 and SPEB within the subepithelial humps (19). It should be noted that this characteristic lesion is not pathognomonic of APSGN since it may also be found in other immune complex glomerulonephritis, notably lupus nephritis.

The resolution of the proliferative changes is by apoptosis and the number of apoptotic cells has a direct relationship with the number of proliferating cells (43). Prolonged oliguria or oligoanuria has been associated with mesangiolysis (44).

Follow-up studies performed several years after the initial episode of acute poststreptococcal glomerulonephritis, have revealed immune deposits and a variable degree of mesangial sclerosis and obliteration, even in the absence of clinical manifestations of renal disease (45).

Figure 30-1 shows the typical histopathologic, immunohistologic and electron microscopic characteristics of APSGN.

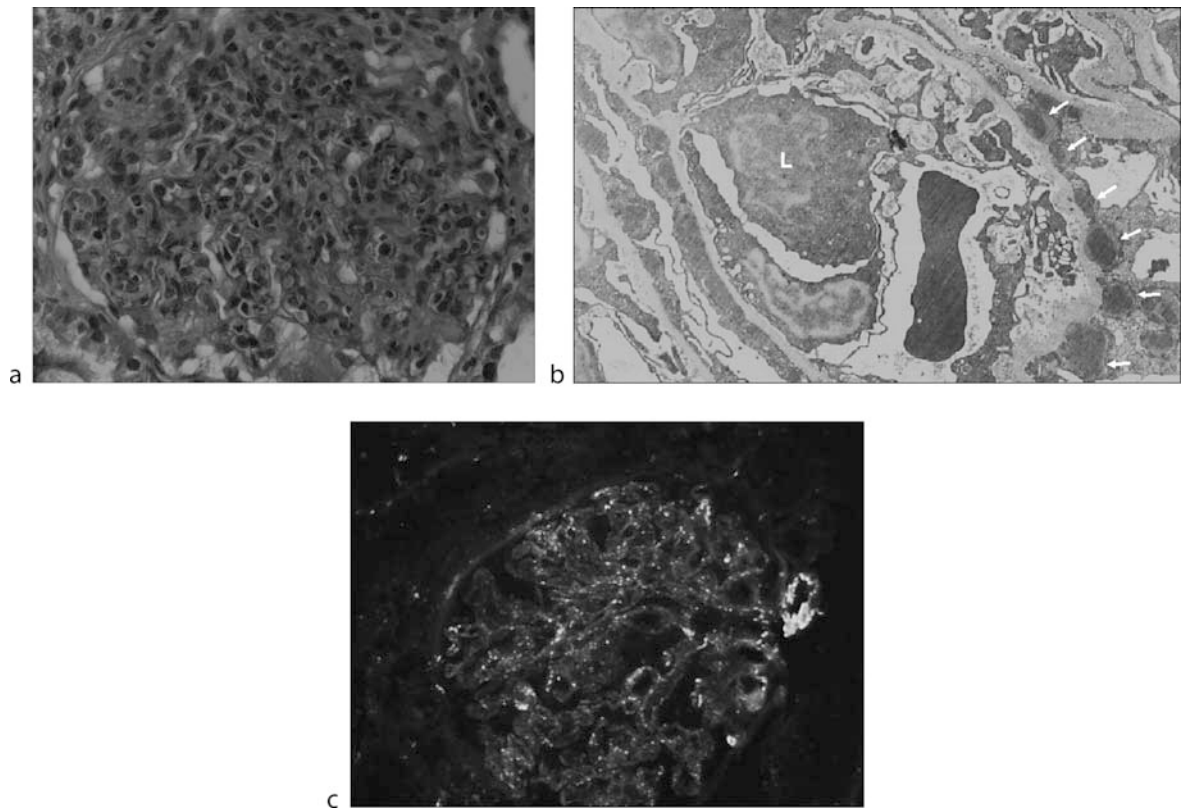
Diagnosis

The clinical presentation is that of a child of 4–15 years who suddenly develops dark and scanty urine and swelling of the face and legs. History and physical examination determines a high blood pressure and the absence of a systemic illness.

The diagnosis of glomerulonephritis should prompt to establish an etiological diagnosis. A history of a precedent upper respiratory infection and/or skin infections suggests a poststreptococcal etiology that maybe confirmed by a positive culture or rising anti-streptococcal antibody titers. Positive streptococcal cultures are frequent in epidemic conditions but in less than 25% of the sporadic cases. Consequently, the evidence of previous streptococcal infection usually resides in the demonstration of a rising titer of serum antistreptococcal antibodies. Anti-NAPlr and antiSPEB-zSPEB titers that have a greater specificity for nephritogenic infections (18) but are not generally available. Antistreptolysin O titers and

Figure 30-1

(a) Light micrograph of a glomerulus with acute poststreptococcal glomerulonephritis demonstrating endocapillary hypercellularity with PMNs (HE, magnification 630 ×). (b) Inmunofluorescence micrograph of a glomerulus from a patient with acute poststreptococcal glomerulonephritis showing coarsely granular capillary wall staining for C3. (FITC anti-C3, × 200) (c) Electron micrograph of a portion of a glomerular capillary from a patient with acute poststreptococcal glomerulonephritis showing subepithelial dense deposits, so called "humps" (*arrows*), and a luminal leukocyte (L) (magnification × 4,000). (See color plate 11)



anti-DNAse B titers are the most frequently elevated in upper respiratory infections and pyodermitis, respectively. Streptozyme test which includes four antigens (DNAse B, Streptolysin O, hyaluronidase and streptokinase) is reported to be positive in more than 80% of the cases.

The initial diagnostic approach should attempt to define if the acute nephritic syndrome is associated with a systemic condition or if is resulting from a primary renal disease. The complement is a first line diagnostic test that permits consideration of acute glomerulonephritides that present low complement (APSGN, Lupus nephritis, shunt nephritis, endocarditis, cryoglobulinemia, hipocomplementemic membranoproliferative glomerulonephritis) and those that present normal complement (IgA nephropathy, Mesangioproliferative GN, hemolytic uremic syndrome, Henoch-Schoenlein purpura, vasculitis, anti gomerular basement disease). The finding of profoundly depressed C4 levels in association with normal or mildly reduced C3, suggests the diagnosis of cryoglobulinemia type II and the reduction of C1, C4 and C3 levels, indicating activation of the classic pathway of complement, is characteristic of lupus erythematosus. Serum complement levels return to normal in less than 1 month and persistence of low complement levels should raise suspicion of a diagnosis different from APSGN.

Physical findings suggestive of nephrotic, rather than nephritic, are a soft, paper-like consistency of the ear cartilage and the existence of ascites, since both these signs are associated with long standing hypoalbuminemia.

Inasmuch as the nephrotic syndrome is rare in APSGN the demonstration of a massive proteinuria favors the diagnosis of lupus nephritis, nephritis associated with visceral abscesses and membranoproliferative glomerulonephritis.

Treatment

Antibiotic treatment. The first question to be considered is when to give antibiotic treatment to a suspected nephritogenic streptococcal infection. From a clinical view point, the diagnosis of active skin infection is usually straight forward. However, clinical judgment may miss half of the streptococal pharyngitis and may incorrectly diagnose a sore throat as due to streptococcal infection in 20–40% of the cases (46). Several clinical scores have been proposed to increase the accuracy of this diagnosis and among the most popular of them is the one proposed by McIsaac et al. (47). This score has a range from 0 to 4 and incorporates age as one of the criteria. The score gives a +1 to each one of the following: temperature >38°C, cervical adenopathy, no cough, tonsillar exudate and age between 3 and 14 years. Age > 44 years is assigned −1 point. Sensitivity and specificity of the score is 85 and 95%, respectively. Antibiotic treatment is recommended (without culture confirmation) when the score is 4, and antibiotic treatment is not indicated (and cultures unnecessary) when scores are 0–1.

Rapid, high sensitivity streptococcal test are good guide to treat if they are positive but a negative test requires confirmation (48). However, a recent report indicates that a decision to treat or not to treat based on the results of these tests is not associated with a higher incidence of poststreptococcal complication (49). The diagnosis of PSGN carries with it the indication of treatment with penicillin or, in allergic individuals, erythromycin. If infection is present at the time of diagnosis, it requires treatment. Early administration of penicillin is reported to prevent or ameliorate the severity of acute glomerulonephritis and at least one report suggests that APSGN patients that receive antibiotic treatment have a milder clinical course (50). If infection is not apparent at the time of diagnosis, antibiotic treatment should be given anyway because positive cultures are sometimes obtained in apparently healthy patients and cross infection of household members and siblings of index cases is very high (37). 1.2 million units of units of benzathine penicillin in adults or half this dose in small children, or alternatively, oral phenoxymethyl or phenoxyethyl penicillin G 125 mg, every 6 h for 7–10 days, are adequate treatment. Erithromycin (250 mg every 6 h in adults, and 40 mg/kg in children, for 7–10 days) is the treatment of choice in patients allergic to penicillin. It should be noted that the existence of erythromycin-resistant strains may be increasing in developing countries. The incidence of resistance to erythromycin in isolates of Streptococcus pyogenes is 9.7% in Japan (51), 21.7% in Spain (52) and ranges from 6.8% in the United States (53) to 11.6% in bacterial isolates from several European countries (54).

Preventive antibiotic treatment is indicated in epidemic situations and to household members of index cases in non-epidemic conditions since most of them present evidence of recent infection and about one-third of them develop nephritis (35, 41). In high risk communities the strategy of treating household contacts has resulted in a decrease in the number of cases of PSGN (55).

Treatment of the Acute Nephritic Syndrome

Patients with subclinical disease maybe followed as outpatients but patients with the acute nephritic syndrome require hospitalization. Bed rest is difficult to enforce and is of unproven value, yet most children keep it on their own while they are in the acute phase. Restrictions of fluid and sodium intake are the cornerstones of the treatment of patients with he acute nephritic syndrome. Cases that present significant edema, hypertension and circulatory congestion benefit from the administration of loop diuretics (40 mg IV or orally every 12 h). This therapy facilitates the resolution of edema and ameliorates the hypertension that is driven by extracellular volume expansion. Diuretic therapy seldom if ever is required for longer than 48 h. Other diuretics are without effect (thiazide diuretics) or dangerous because of the possibility of hyperkalemia (aldosterone antagonists).

Patients who present severe hypertension may require antihypertensive treatment and Nifedipine (5 mg in children, every 4–6 h) is usually effective. Parenteral hydralazine may be required but the possibility of tachycardia requires close observation. Angiotensin converting enzyme inhibitors and type 1 receptor blockers carry the risk of hyperkalemia. Exceptionally, nitroprusside is required to control hypertensive encephalopathy.

Another potential complication, frequently associated with hypertension is the posterior reversible leukoencephalopathy that has recently been reported in acute PSGN (56). This complication is manifested clinically with mental disturbances, visual hallucinations, headache and convulsions and may be confused with hypertensive

encephalopathy. The diagnosis requires the use of nuclear magnetic resonance image studies.

Pulmonary edema is rare and should be treated with oxygen therapy, rotating tourniquets and loop diuretics Digitalis is not indicated because is ineffective and may result in intoxication. Rarely overt heart failure and pulmonary edema may complicate the clinical course. Hemodialysis or peritoneal dialysis may be required occasionally in children for the treatment of hiperkalemia, uremia or severe circulatory congestion.

A rapidly progressive azotemia is usually associated with crescentic APSGN. While of unproven efficacy, there are anecdotal reports of beneficial effects of pulse methyprednisolone therapy.

Prognosis of APSGN

The short term prognosis of APSGN is excellent in children. Fatalities may occur as a result of hyperkalemia or pulmonary edema, but they are exceedingly rare. The long term prognosis of APSGN has been the subject of many reports since the initial studies in the first half of the twentieth century reported essentially a complete recovery, but the follow-up periods were short and the patients were only children in the majority of the studies. Subsequent observations gave widely different results and abnormal urinary findings in patients followed for extended periods of time ranged from 3.5% (57) to 60% (58). As recently summarized (15), APSGN followed for 10–20 years present frequently abnormal urine analysis (20%) but azotemia occurs in less than 1% of the patients. In our own follow-up studies (28) of 110 children followed for 15–18 years after the acute attack, non-nephrotic proteinuria was found in 7.2%, microhematuria in 5.4%. hypertension in 3.0% and azotemia in 0.9%. Studies in Australian aboriginal communities indicate that patients who had APSGN have an increased risk for albuminuria (adjusted odds ratio 6.1, 95% CI 2.2–16.9) and hematuria (OR 3.7, CI 1.8–8.0) in relation to controls that did not have APSGN (15).

The long-term prognosis of children of APSGN may be significantly worse in communities were other risk factors of chronic renal failure are prevalent. The superimposition of APSGN on other conditions such as diabetes and metabolic syndrome have been postulated to play a role on the high incidence of end stage renal disease in some aboriginal communities in Northern Australia (59).

The long term prognosis of APSGN is substantially worse in adults, particularly those with persistent proteinuria in the nephrotic range: 77% of these patients may develop chronic renal failure. Unfavorable course was also observed in the patients in the Nova Serrana epidemic, most of whom were adults. Chronic renal failure developed in 8% of these patients after 5 years (60).

Renal Disease Associated with Infective Endocarditis

Almost a century ago Max Friedererich Lohlein and George Baehr described focal, embolic, non suppurative glomerulonephritis caused by bacterial endocarditis. Subsequent descriptions two decades ago emphasized the immune complex pathogenesis causing diffuse glomerulonephritis (61, 62) and more recent studies have disclosed that the most common renal lesions associated with bacterial endocarditis are localized infarcts and vasculitic glomerulonephritis (without demonstrable immune complex deposits) and interstitial nephritis (63). Approximately 15,000 new cases of IE occur in the United States each year (64). In the United States and Western Europe the incidence of community acquired native-valve endocarditis is essentially unchanged at 1.7–6.2 cases per 100,000 person years (65) but there has been a decline in the proportion of cases associated with rheumatic heart disease and an increase cases associated with valve surgery (66). The most important epidemiological change in recent years has been the increment in cases associated with health care interventions and, particularly, the cases associated with hemodialysis and those caused by staphylococcus. Bacterial endocarditis is 20–60 times more common in hemodialysis patients than in the general population (67).

Etiology and Pathogenesis

The most common infecting bacteria are *Staphylococcus aureus,* and *S. epidermidis*, *Streptococcus viridans and pyogenes*, *Enterococcus fecalis* and less commonly *E. Coli, Proteus*, microorganisms of the *Bartonella* and *Candida* species. When an immune complex pathogenesis is involved, bacterial antigens may be demonstrated in the glomeruli (68) and the nephritogenic mechanisms are similar to those operating in APSGN. Polyclonal (type III) cryoglobulinemia may be found in roughly half of he patients. In addition, supernatigen-driven T cell activation with polyclonal gammopathy may occur in infections caused by methicillin-resistant *Staphylococcus aureus* (69, 70).

Microembolization is responsible for the localized infarcts and tubulointerstitial nephritis is usually secondary to antibiotic treatment. In the patients in hemodialysis

programs, the original infection is usually related to the vascular access. Calcifications of heart valves are significant risk factors and treatment directed to reduce the risk of ectopic calcification are important preventive measures of IE (71).

Clinical Characteristics

The classic clinical picture of subacute bacterial endocarditis include Osler's nodules, Janeway lesions and splinter hemorrhages; however, these findings are rare at the present time. Fever may be the only manifestation, accompanied or not with arthralgias, leukocytosis, increased sedimentation rate and purpura. Patients in hemodialysis who develop infective endocarditis usually have synthetic arteriovenous grafts or venous dialysis catheters and had the vascular access in place for more than 1 year (72, 73).

The cardiac lesion is best demonstrated by transesophageal echocardiography. Factors usually associated with higher mortality are a vegetation size ≥ 20 mm, age lower than 1 year, the existence of heart failure and *Staphylococcus aureus* as a causative organism (74). Embolization to the central nervous system with stroke may occur in 6% of the children; mortality is rare but recovery is variable (75). Systemic embolism maybe microscopic or large; the latter usually correspond to endocarditis caused by fungus or *Haemophilus*.

Microscopic hematuria and proteinuria are indicative of renal lesion. Azotemia may develop in one-third of the patients (76) but is usually mild except in cases that develop crescentic glomerulonephritis. Eosinophilia and eosinophiluria should rise the suspicion of antibiotic induced interstitial nephritis.

Serological findings include reduced C3 and C4 (except in superantigen mediated nephritis), high titers of rheumatoid factor, cryoglobulinemia (66, 77) and, on occasion positive anti-PR3 ANCA antibodies in the serum (78). The later finding in a patient suspected of having vasculitis has been considered an indication to rule out infective endocarditis (79).

Treatment

Antibiotic treatment needs to be given for 4–6 weeks (80). Urinary findings usually persist for weeks or months after eradication of the infection. The complement levels return to normal after the infection has been eradicated and this finding is associated with good prognosis of the renal lesion.

In patients who present crescentic glomerulonephritis and rapidly progressive course, pulse steroid therapy and plasmapheresis have been used with success but their overall value needs to be confirmed in more definite studies.

Pathology

In a retrospective study of 62 patients with infective endocarditis in whom renal tissue was available for evaluation (20 patients with renal biopsy and 42 autopsy specimens), the most common lesion were localized infarcts in 30% and glomerulonephritis was found in 26%. More than half of the infarcts were due to septic emboli, especially in patients with *S. aureus* endocarditis (63). Rather than focal or diffuse glomerulonephritis the authors (63) emphasize that lesions are of the vasculitis type without immunoglobulin deposition and proliferative or type I membranoproliferative glomerulonephritis with immunoglobulin (usually IgM) and complement deposition.

Glomerulonephritis may be present with subendothelial and subepithelial deposits. Crescent formation maybe found in about half of the proliferative glomerulonephritis. Electron dense deposits in subendothelial, mesangial and subepithelial locations are frequent in subacute forms of infective endocarditis.

Tubulointerstitial lesions are frequent in renal biopsies and they may be part of the glomerulonephritis, vasculitis or the microemboli in the nephropathy of infective endocarditis or result from the treatment with nephrotoxic agents. "Pure" interstitial nephritis may be found in 10% of the renal biopsies in recent retrospective studies and is characterized by inflammatory infiltrate with variable degrees of tubular dilatation and atrophy. If these findings are associated with eosinophilic infiltration, the interstitial nephritis is likely related to antibiotic therapy Cortical necrosis was present in 10% of the cases and all of them in autopsy material (63).

Shunt Nephritis

Infection develops in about one-third of the patients with vetriculoatrial (VA) and ventriculoperitoneal (VP) shunts used for amelioration of intracranial pressure in congenital or acquired hydrocephalus (81). The development of nephrotic syndrome in a patient with infected ventriculoatrial (VA) shunt used for the treatment of hydrocephalus was first described by Black et al. in 1965 (82) and is now recognized that this complication develops on 0.7–2% of the infected VA shunts.

The infecting microorganisms are *S. epidermidis* in about 75% of the cases in most series (70) and less frequently *Staphilococcus aureus*. More rarely *Propionibacterium acne*, diphtheroids, *Pseudomonas* and *Serratia* species are the infecting organisms. The renal complication occurs in a period of time that ranges from 2 months to many years after the shunt insertion (81). In contrast with VA shunts, VP shunts are rarely complicated with glomerulonephritis.

The clinical manifestations are recurrent episodes of fever, anemia, hepatosplenomegaly, skin rash and cerebral symptoms related to increased intracraneal pressure. Renal involvement is indicated by proteinuria, in most cases in the nephrotic range, and at times with a full nephrotic syndrome, hematuria, and hypertension. The combination of skin and renal manifestation may lead to the incorrect diagnosis of Henoch-Schönlein purpura and the possible association of a urinary tract infection frequently accompanying a neurogenic bladder may complicate the interpretation of the urinary findings. Serological findings include reduced C3 and C4 levels, indicating activation of he classic complement pathway, cryoglobulinemia, anemia, elevated sedimentation rate and high rheumatoid factor titers. Serum P3 ANCA titers are sometimes present.

The renal lesion demonstrated at biopsy is usually membranoproliferative glomerulonephritis. Deposits of complement and IgM are almost always present and IgG in about two-thirds of the cases (81). Electron microscopy reveals electron dense deposits in the subendothelial and mesangial regions (83). Bacterial antigens have been demonstrated in the glomeruli of patients with shunt nephritis (84, 85).

Treatment of shunt nephritis requires intravenous antibiotic therapy and removal of the infected shunt and, if possible, substituting it for a VP shunt. Rarely, if ever, are antibiotics alone capable of eliminating the infection. The long-term prognosis of the renal function is good if the shunt is removed within a few weeks of detecting the infection (81). In cases were the removal of the shunt is delayed the renal damage may progress to irreversible end stage renal failure.

Hemodialysis is the preferred modality in patients with end stage renal disease because peritonitis associated with peritoniteal dialysis carries the risk of meningitis if a VP shunt is in place. Given the high rate of complications in the VA shunts, the VP shunt is the preferred initial procedure but VA shunts may be the selected method in children with chronic renal failure in whom renal transplantation is considered. There are no reports of recurrence of shunt nephritis in the transplanted kidney.

Glomerulonephritis Associated with Other Bacterial Infections

Congenital and acquired syphilis cause glomerulonephritis. Congenital syphilis is usually manifested by rash, rhinitis and osteochondritis, but 8–10% of the patients may develop a full blown nephrotic syndrome that presents as anasarca 4–12 weeks after birth. In acquired syphilis the nephrotic syndrome occurs in less than 1% of the cases and the acute nephritis syndrome is very rare (86). The most common histologic appearance is that of membranous nephropathy and electron microscopy shows electron dense deposits along the glomerular basement membrane (87). Other types of glomerulonephritis, including mesangioproliferative, membranoproliferative and diffuse proliferative glomerulonephritis have been reported, especially in adult patients. Treponema antigens have been demonstrated in the glomerular immune deposits (88). Serological tests for syphilis are positive and complement levels are normal. Treatment of the syphilis is associated with improvement of the renal disease but recovery usually takes several months.

Acute typhoid fever may be associated with overt glomerulonephritis in about 2% of the cases but proteinuria and hematuria may develop in 25% of the patients (89).

Lobar pneumonia caused by *Strep. pneumoniae* has been reported to cause glomerulonephritis usually manifested by proteinuria and hematuria. Pneumococcal antigen type 14 has been localized in the glomeruli and the capsular antigen is capable of activating the alternative complement pathway (90).

Visceral abscesses and osteomielitis may be associated with glomerulonephritis that is usually manifested by the nephrotic syndrome. Complement levels are normal. The histological picture reveals membranoproliferative, diffuse proliferative or mesangial proliferative glomerulonephritis with or without crescent formation. Treatment of the infection, if started early, improves the renal disease (91).

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31 Immunoglobulin A Nephropathy

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Introduction

Immunoglobulin A (IgA) nephropathy was first described in 1968 by Berger and Hinglais (1) and is now recognized as a distinct clinicopathologic entity with a higher frequency worldwide than any other primary glomerulopathy (2). It was initially considered a benign condition, but extended follow-up of patients indicated that 20–50% of adults would ultimately progress to end-stage renal failure (2, 3). Likewise, the favorable prognosis initially attributed to children with IgA nephropathy must be questioned in the light of studies (4–8).

Epidemiology

IgA nephropathy has been diagnosed all over the world, but its prevalence varies widely from one country to another. In the Pacific Rim (e.g., Japan, Singapore, Australia and New Zealand), IgA nephropathy accounts for as many as one-half of cases of primary glomerulonephritis. In Europe, it accounts for between 20 and 30% of all primary glomerulonephritis, whereas in North America, it is responsible for only 2–10%. The explanation for this apparent variability in incidence is uncertain, but it may be due to a racial difference in the incidence of IgA nephropathy or to differences in biopsy selection practices (9).

Genetic factors and environmental influences could contribute to geographic differences in prevalence. A lower prevalence among blacks than whites has been reported in the United States. However, in American children, similar incidences of IgA nephropathy in Caucasian and African-American children from Shelby County, Tennessee have been reported (10). In Australia (11), where the population is heterogeneous and includes many immigrants from Third World countries, all racial groups seem to be affected equally.

The high incidence of IgA nephropathy in certain countries may reflect the practice of routine urinalysis. In Japan, all children between the ages of 6 and 18 years are screened annually, and those found to have urinary abnormalities are referred for further investigation. Thus, IgA nephropathy is the most common primary glomerulonephritis in children seen in Kobe University and Wakayama Medical University Hospitals, detected in approximately 30% of biopsy specimens obtained. This periodic screening of urine of healthy populations and subsequent investigation of those found to have abnormalities is also an accepted routine amongst adults in Japan.

Etiology

IgA nephropathy is generally considered to be an immune complex-mediated or aggregated (polymerized) IgA-mediated glomerulonephritis. Because IgA is the main immunoglobulin directed against antigens (viral and bacterial) in the exocrine system, and because of the frequent association between upper respiratory tract or gastrointestinal infection and the onset of macroscopic hematuria, it has been suggested that certain viral or bacterial infections may lead to IgA nephropathy. Considerable effort has been directed towards the search for antigens and for the antibody specificity of the mesangial IgA, but it has met with limited success. Many antigens, including herpes simplex virus, cytomegalovirus, Epstein-Barr virus nuclear antigen, adenovirus and milk antigen, have been identified. The observation of numerous antigenic substances in the glomeruli indicates that the antigenic materials in IgA nephropathy may be heterogeneous. The presence of *Hemophilus parainfluenza* antigens in a diffuse and global distribution in the glomerular mesangium and the presence of IgA antibody against *H. parainfluenza* in sera of Japanese patients with IgA nephropathy have been demonstrated (12, 13). Recently, it has been reported that *Staphylococcus aureus* cell envelope antigen was localized in the glomeruli in 68.1% (79/116) of renal biopsy specimens from patients with IgA nephropathy and that the data confirmed that the antigen was colocalized with IgA antibody in the glomeruli. *Staphylococcus aureus* cell envelope antigen may be a new candidate for the induction of IgA nephropathy (14). These findings remain unconfirmed in children with IgA nephropathy yet.

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Predisposing Genetic Factors

Predisposing genetic factors have been suggested as important in the development of IgA nephropathy (15). Moreover, it has been suggested that genetic factors may not only determine susceptibility to glomerulonephritis, but also influence the pathological severity and natural course of IgA nephropathy (16, 17).

Evidence for genetic factors being important in IgA nephropathy is provided by family studies (16–20). Rambausek et al. reported that 9.6% of patients with mesangial IgA nephropathy in Germany had one or more siblings with glomerulonephritis (16). Julian et al. described kindred from eastern Kentucky in which six patients with IgA nephropathy descended from one ancestor and eight other patients belonged to potentially related pedigrees (18). Moreover, they indicated that at least 48 (60%) of 80 IgA nephropathy patients who were born in same region were related to at least one other patient (19). Scolarim et al. reported that 26 (14%) of 185 patients with IgA nephropathy investigated in Italy were related to at least one other patient with the disease (20). These family studies suggest that familial predisposition is a very common finding and genetic factors are influenced in the pathogenesis of IgA nephropathy. Gharavi et al. (21) demonstrated linkage of IgA nephropathy to 6q22–23 under a dominant model of transmission with incomplete penetrance. Further linkage-based approaches to the study of familial forms of the disease have identified significant or suggestive loci on chromosomes 4q26–31, 17q12–22 (22) and 2q36 (23), but no causal gene has yet been identified.

Schena et al. (24) reported that familial IgA nephropathy had a poorer outcome than sporadic IgA nephropathy. This fact means that genetic factors are implicated in both disease susceptibility and disease progression (15). Polymorphisms of Ig heavy-chain switch region gene (25), Iα1 germ-line transcript regulatory region gene (26), genes of the renin angiotensin system (27–29), and platelet activating factor acetylhydrolase gene (30) have been reported. Associations among IgA nephropathy and genetic polymorphisms of other molecules, in which relation to disease susceptibility and disease progression are suggested, have been reported in sequence (31). Although some associations have emerged, they have been inconsistent (32). Some of the discrepancies may be caused by different sample sizes and different geographical regions of the patients included in the studies.

Recently in Japan, single-nucleotide polymorphisms have been screened on a genomewide scale to clarify various complex diseases, including IgA nephropathy. As a part of the benefit of this project, case-control association studies were designed, using single-nucleotide polymorphisms found in the selectin gene (33), the class II region of the major histocompatibility complex (34) and the polymeric immunoglobulin receptor gene (35), and haplotypes of these genes that might serve to identify regions containing loci responsible for IgA nephropathy phenotypes were estimated. Although these studies were conducted in adult patients with IgA nephropathy, these data may be beneficial even to children with IgA nephropathy. Further investigations for these genes in a large-scale number of children with IgA nephropathy are required.

Pathogenesis

Although the pathogenesis of IgA nephropathy remains uncertain, there is substantial evidence that it is an immune complex disease (2, 36). Granular electron-dense deposits are observed in the glomerular mesangial areas by electron microscopy, and confirmed as containing IgA and C3 by immunofluorescence microscopy. Circulating IgA immune complexes have been detected by several different specific assays often associated with IgG immune complex. Many immunological abnormalities that may lead to the formation of IgA immune complex have been reported in patients with IgA nephropathy. Recurrences of IgA nephropathy frequently occur in allografts (37), and a rapid disappearance of glomerular IgA deposits is observed when kidneys with mesangial IgA deposits are transplanted to patients without IgA nephropathy. These clinical observations have provided strong support for the notion that IgA nephropathy is a systemic disease. Although much of this work was performed in adults, there is no evidence to suggest that the findings cannot be extrapolated to children. Moreover, glomerular IgA deposits associated with histological lesions similar to those of human IgA nephropathy can be induced in laboratory animals by passive administration of preformed IgA immune complex or by active immunization (38–42).

Nature of Mesangial Immunoglobulin A Deposits

IgA is the second most common immunoglobulin and contributes to immunity at the level of the external secretory system. IgA exists in monomeric and polymeric forms. Monomeric IgA represents approximately 90% of the serum IgA and is produced mainly by the circulating lymphocytes and plasma cells in the spleen and bone

marrow. Polymeric IgA is produced mostly by lymphocytes and plasma cells in the gastrointestinal and respiratory tracts, where it is synthesized as monomers and then secreted as dimers linked by the J-chain, which is also produced within the plasma cells. During the passage of dimeric IgA molecules through the mucosal epithelium toward the external lumen, the secretory component is attached through specific noncovalent interactions; this component appears to protect the dimeric IgA from the proteolytic enzymes present in the external secretions. IgA has two subclasses, IgA1 and IgA2. Approximately 90% of serum IgA is composed of IgA1 mostly produced in the bone marrow, whereas IgA2 is mostly derived from the local mucosa of the gastrointestinal and respiratory tracts. Both IgA1 and IgA2 are produced in the mucosae.

The most prominent finding in the glomeruli of renal biopsy specimens from patients with IgA nephropathy is mesangial IgA deposition. The majority of investigators has indicated that IgA1 is the predominant subclass present in the glomeruli (43). The J-chain has also been identified in the mesangium in patients with IgA nephropathy (44). Secretory component is not present in the mesangial deposits, but immunofluorescence studies of renal biopsy sections from patients with IgA nephropathy have indicated that it binds to the mesangial areas in vitro (45). These observations suggest that the mesangial IgA deposits are polymeric, a hypothesis further supported by the immunochemical characterization of IgA eluted from renal biopsy sections (46). The onset of IgA nephropathy may be associated with infections in the upper respiratory tract. It has therefore been proposed that IgA nephropathy results from hyperactivity of the mucosal immune system. However, assessment of polymeric IgA1 production by in situ hybridization for J-chain messenger RNA in IgA plasma cells shows downregulation in the mucosa (47) and upregulation in the bone marrow (48). Impaired mucosal IgA responses allowing enhanced antigen challenge to the marrow shown by de Fijter et al. (49) could be the primary abnormality in IgA nephropathy, although this remains unproven (50).

A number of studies have suggested that the alternative complement pathway has a pathogenetic role in IgA nephropathy. This hypothesis is consistent with the typical immunohistologic demonstration of C3 and properdin in a pattern and distribution similar to that of IgA in the glomeruli, in the absence of C1q and C4. The detection of the membrane attack complex of complement further supports the pathogenetic role of complement activation in this disease (51). Certain types of IgA aggregates or IgA from patients with myeloma have been shown to activate complement in vitro (52). IgA has been reported to activate the complement system via the mannan-binding lectin pathway (53). However, there is no direct evidence that IgA deposits in the glomeruli mediate complement activation. Activation of C3 is observed in the majority of adult and pediatric patients with IgA nephropathy, but the mediator as well as the pathophysiologic significance of this complement activation remains to be determined. C3 is deposited in the kidney but is also produced by mesangial cells in IgA nephropathy (54).

With regard to antibody specificity, IgA eluted from cryostat sections of IgA nephropathy biopsies has been reported to react with mesangial areas of its own and other IgA nephropathy patients' biopsies, but not with normal kidney (55). Such eluates have also been shown to contain antibodies that react with tonsillar cells and cultured fibroblasts obtained from patients with IgA nephropathy (56).

Despite intensive investigation, the mechanism underlying glomerular IgA deposition in IgA nephropathy has not been defined. Proposed potential mechanisms on glomerular IgA deposition are as follows. Decreased IgA response to mucosal antigens may promote increased production of polymeric IgA1 by the bone marrow, leading to increased serum levels of IgA1. Defective galactosylation of IgA1 (later described in detail) may decrease hepatic clearance of IgA1 and promote binding of IgA1 complexes to glomerular mesangial cells. Aberrant IgA1 deposits in the kidney trigger the production of a variety of cytokines and growth factors by renal cells and by circulating inflammatory cells, leading to the characteristic histopathological features of mesangial-cell proliferation and extracellular-matrix deposition (57). Recently it has been proposed a model whereby two types of IgA receptors participate in sequential steps to promote the development of IgA nephropathy, with soluble IgA Fc receptor I (FcαRI/CD89) being initially involved in the formation and/or amplification of the size of circulating IgA-immune complexes and, subsequently, mesangial transferrin receptor (CD71), in mediating mesangial deposition of nephritogenic IgA-immune complexes (58–62).

In summary, the immunochemical nature of the mesangial deposits in IgA nephropathy is consistent with antigen-polymeric IgA complexes predominantly of A1 subclass, and perhaps, multispecific for ubiquitous mucosally derived antigens.

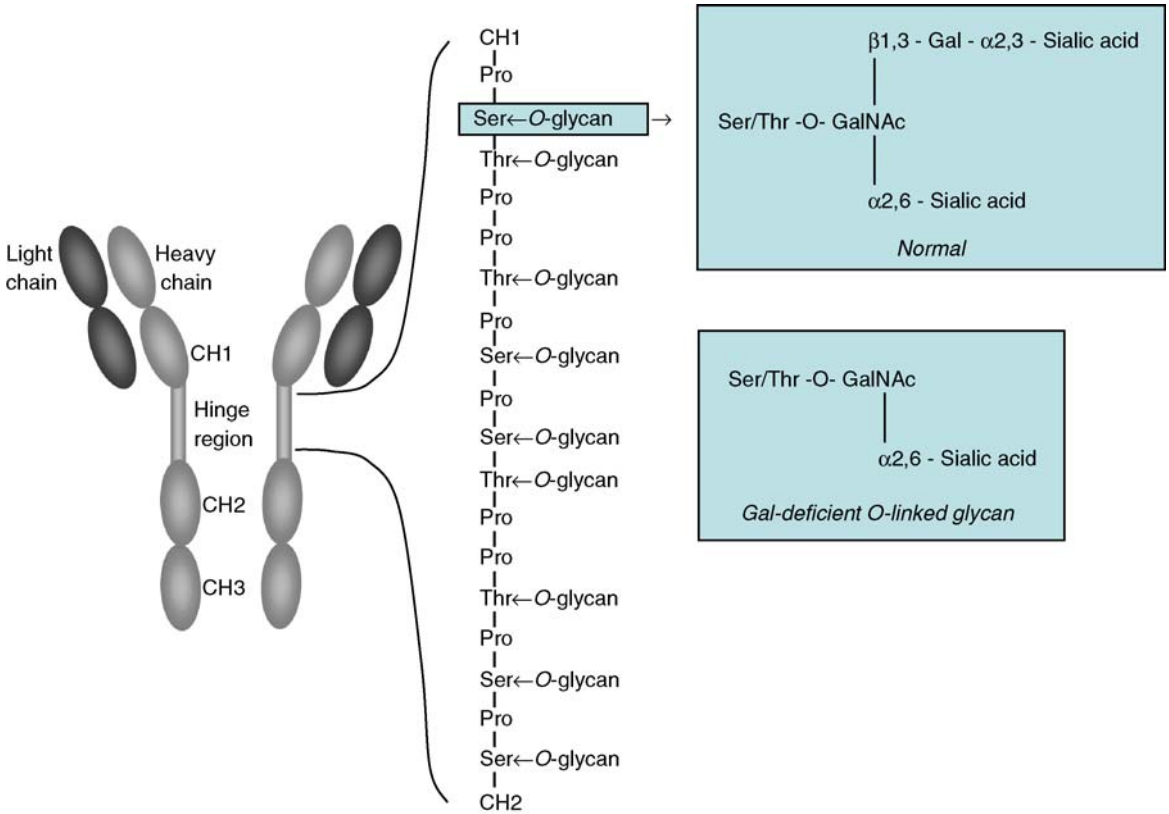
Immunoglobulin A Glycosylation

IgA glycosylation has received recent attention as a putative nonimmune feature of IgA, which may explain its

abnormal behavior and glomerular deposition in IgA nephropathy (63). IgA1 subclass is prominent in IgA nephropathy. IgA1 is unique among all Igs in its possession of a hinge region rich in proline, serine and threonine and characterized by *O*-glycosylation sites (*Fig. 31-1*). These *O*-glycosylation sites consist of *N*-acetylgalactosamine *O*-linked to the serine or threonine residues of hinge region. In patients with IgA nephropathy, mesangial IgA1 and a small fraction of circulating IgA1 contain incompletely galactosylated *O*-linked glycans in the hinge region (64–68). This glycosylation abnormality is absent in other glycoproteins with *O*-linked glycans, such as complement C1 inhibitor and IgD, indicating a defect specific for IgA1 (66, 69). Abnormal galactosylated IgA1 increases affinity for glomerular fibronectin, laminin and collagen IV (70) and may lead to accumulation of IgA in the mesangium (67). Preliminary data indicate that deficient galactosylation of hinge region glycans may be detected even in family members of patients with IgA nephropathy (64). Altered amino-acid sequence of the IgA1 hinge region is a possible mechanism to consider for abnormal galactosylation of IgA1. However, the hinge region is a highly conserved region of IgA1 molecule. There is no evidence for any nucleotide sequence alteration or transcriptional abnormality of the hinge region in IgA nephropathy (71). It has also been postulated that altered galactosylated IgA1 in IgA nephropathy may be due to a deficiency of structural modification of β1,3-galactosyltransferase, the enzyme responsible for the terminal galactosylation of GalNAc on *O*-linked

Figure 31-1

IgA1 molecules with hinge region *O*-glycosylation sites. IgA1 molecules have two heavy chains with three constant region domains CH1 to CH3 and a hinge region between CH1 and CH2. Each serine (Ser) and threonine (Thr) residue is a potential site for an *O*-glycan side chain. Although there are nine potential glycosylation sites, IgA1 contains up to six *O*-glycans per hinge region. *O*-glycosylation sites consist of *N*-acetylgalactosamine (GalNAc) *O*-linked to Ser or Thr residues of hinge region. The largest *O*-linked saccharide of IgA1 is a tetrasaccharide with GalNAc, galactose (Gal) and two sialic acid residues. Aberrant IgA1 molecules with Gal-deficient *O*-linked glycans can contain terminal GalNAc or sialylated GalNAc at one or more sites. Pro, proline.



glycans (72). This structural or functional deficiency may be genetically determined. The recent sequence of β1, 3-galactosyltransferase may help us to understand the genetic basis of these abnormalities (73, 74). Circulating IgA1 has reduced terminal galactose on *O*-linked hinge-region sugars in IgA nephropathy (66), apparently because of a B-cell defect in β1,3-galactosyltransferase, the enzyme responsible for placing terminal galactose on *O*-linked sugars (72). The IgA1 *O*-glycan chains are truncated in IgA nephropathy (75). Circulating immune complexes in IgA nephropathy consist of IgA1 with galactose-deficient hinge region, and the deficiency of galactose may result in the generation of antigenic determinants that are recognized by naturally occurring IgG and IgA1 antibodies (65). Sano et al. demonstrated that enzymatically deglycosylated human IgA1 molecules accumulate and induced inflammatory cell reaction (76); Amore et al. showed glycosylation of circulating IgA modulated mesangial proliferation in IgA nephropathy (77). Recently it has been showed that EBV-immortalized IgA1-producing cells from peripheral blood cells in IgA nephropathy patients secreted mostly polymeric IgA1 with galactose-deficient *O*-linked glycans (78). Until recently, although it has not been known whether the aberrant glycosylation is the result of an acquired or inherited defect, or whether the presence of aberrant IgA1 glycoforms alone can produce IgA nephropathy, recent studies have demonstrated that abnormal IgA1 glycosylation is an inherited rather than acquired trait and that abnormal IgA1 glycosylation clusters in most but not all families with IgA nephropathy suggests the possibility of IgA nephropathy patients with different pathogenic mechanisms of disease (79).

Immunoglobulin A Immune System

There is a general agreement that serum levels of IgA are increased in 50–70% of patients with IgA nephropathy, with elevations in both monomeric and polymeric IgA. There is an increase in polymeric IgA1-producing plasma cells in the bone marrow (48) and in the tonsils (80) of patients with IgA nephropathy. The proportion of IgA-λ in serum IgA is also increased. Serum IgA is more anionic, owing to the increased anionicity of λ- compared with κ-light chain (80). The binding of IgA to mesangial cells is charge dependent and anionic charge may play an important role for in IgA1 deposition in the mesangium (81). In addition to the increased levels of serum IgA, various types of autoantibodies of the IgA class have been recognized. These IgA autoantibodies include rheumatoid factor (82), antinuclear antibodies (83), and anticollagen antibody (84). However the IgA may be polyspecific, indicating a polyclonal increase rather than true antigen-specific autoantibodies (85). IgA immune complexes are also frequently detected (86). Cultured peripheral blood lymphocytes from patients with the disease produce more IgA than those of normal individuals, either spontaneously or after polyclonal stimulation in vitro. We also demonstrated an increased spontaneous and pokeweed mitogen-stimulated IgA production by peripheral blood lymphocytes in children with IgA nephropathy (87). This increased IgA production remained stable during the follow-up period in patients with persistent urinary abnormalities, but decreased toward normal in patients with clinical remission.

IgA production is T cell-dependent, and the increased production in IgA nephropathy may indicate altered T cell function. An increased circulating OKT4–OKT8 cell ratio, due to increased OKT4 helper T lymphocytes and decreased OKT8 cytotoxic-suppressor T lymphocytes, has been reported in patients (88). Increased IgA-specific helper T cell activity and decreased IgA-specific suppressor T cell activity have also been reported (89, 90).

Defective clearance of immune complexes from the circulation may also be important (91), but this seems more likely to be a consequence rather than the cause of the increased immune complex load.

Progression Mechanism

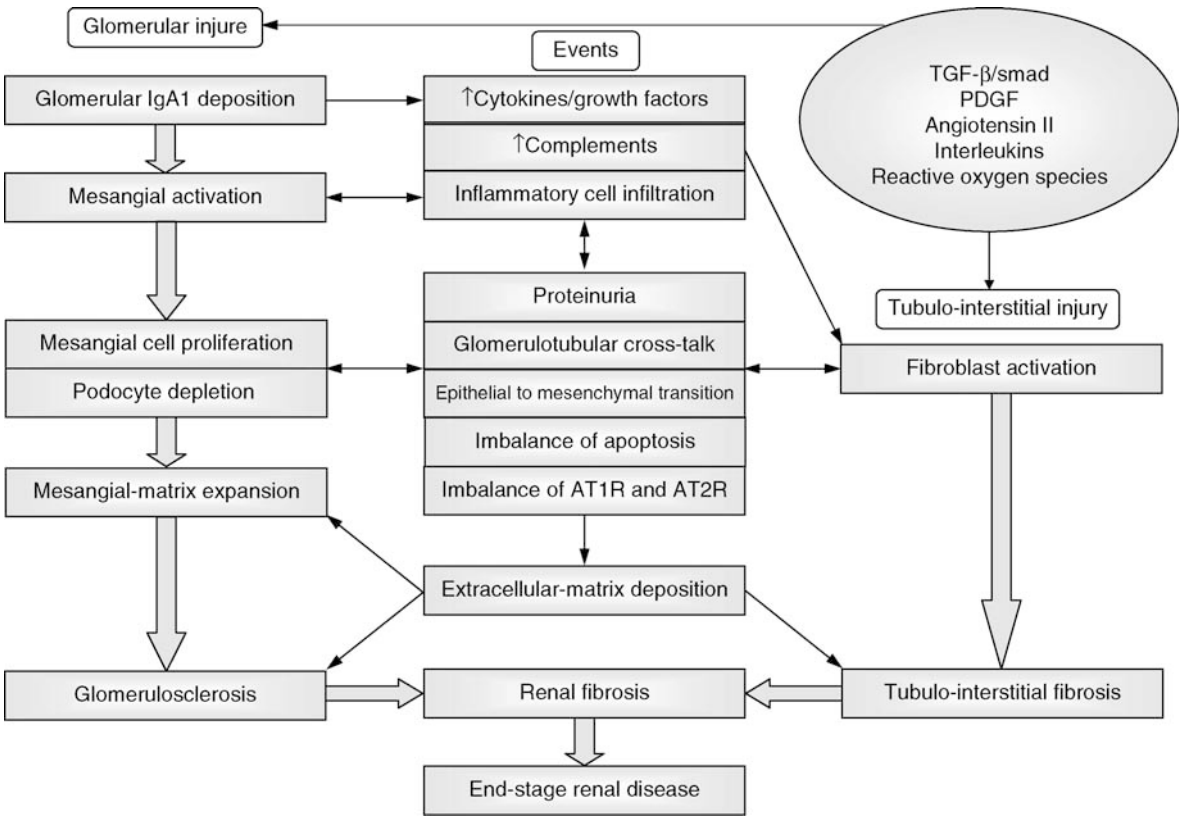
Renal fibrosis is the final common manifestation of chronic kidney diseases (92). There is little to suggest that the mechanisms of mesangial proliferative glomerulonephritis, progression, and scarring are distinct in IgA nephropathy compared with other types of chronic glomerulonephritis. A simplified scheme of progression mechanism in IgA nephropathy is shown (*Fig. 31-2*).

Initiation and Progression Mechanism of Glomerular Injure

As described above, deposition of polymeric IgA, but not of monomeric IgA, can induce the production and local release of a variety of cytokines, growth factors, complements, and angiotensin II by renal resident cells and by circulating inflammatory cells leading to inflammatory injury, characteristic histopathological features of mesangial cell proliferation and extracellular-matrix deposition (93–96). IgA alone also appears to be sufficient to provoke injury in susceptible individuals (97). Studies in vitro

Figure 31-2

A simplified scheme of progression mechanism in IgA nephropathy.



and in animal models of mesangial proliferative glomerulonephritis have shown the key role of cytokines and growth factors, particularly platelet-derived growth factor (PDGF) and transforming growth factor-β (TGF-β), in the induction and progression of mesangial injury, and there is evidence that these are also involved in IgA nephropathy (98–100). Although the role of infiltrating activated monocyte/macrophage into glomeruli are also thought to be important in glomerular injure (101), it has been showed that resident glomerular cells (mesangial and endothelial cells) are predominantly the major source of up-regulated these growth factor production in IgA nephropathy (98).

Studies in children with IgA nephropathy suggest that mesangial proliferation may in part be the result of local production of cytokines, interleukin-1, interleukin-6, tumor necrosis factor (TNF), PDGF, TGF-β and vascular permeability factor/endothelial growth factor (VPF/VEGF) (102–104). C3 deposits in mesangium and activation of complement proteins in mesangium (105), excessive oxidant stress (106) may mediate glomerular injury in IgA nephropathy.

Glomerular injury and proteinuria in IgA nephropathy is related to the degree of podocyte depletion in humans (107). Recent study has supported for the concept that podocyte depletion could be a major mechanism driving glomerulosclerosis in human glomerular diseases (108). Dysregulation of apoptosis may have the important role in podocyte depletion. It has been shown that down-regulation of Bcl-2 by podocytes is associated with progressive glomerular injury and clinical indices of poor renal prognosis in human IgA nephropathy (109). Recently, it has been reported that IgA1 from IgA nephropathy patients may induce apoptosis of podocytes through direct and indirect pathways and that IgA1 may accelerate progression of IgA nephropathy by inducing apoptosis of podocytes (110).

It has been showed that enhanced gene expression for the renin-angiotensin system is detected in glomerular mesangial cells in IgA nephropathy (111). Recent studies

have demonstrated an altered angiotensin II subtype 1 receptor expression in human mesangial cells in response to raised intrarenal angiotensin II in IgA nephropathy. In vitro studies have also support that an imbalance of angiotensin II subtype 1 and 2 receptor activity in human mesangial cells following exposure to polymeric IgA plays a significant pathogenetic role in the inflammatory injury in IgA nephropathy (112).

Progression Mechanisms from Glomerular Injure to Tubulointerstitial Injury

It remains in part unclear how mesangial IgA deposition leads to tubulointerstitial injury in IgA nephropathy. Several mechanisms of tubulointerstitial injury may operate independently or synergistically (96). Recently, a glomerulotubular cross-talk has been proposed in addition to monocytic/macrophage infiltration, proteinuria, complement activation and direct inflammatory effect of IgA (113). The importance of infiltrating inflammatory cells in the tubulointerstitium in mediating tubular injury and renal fibrosis in IgA nephropathy has been demonstrated (96, 114). Especially, recent attention has been focused on the role of proximal tubular epithelial cells in organization inflammatory cell infiltration and renal fibrosis via production of inflammatory mediators upon activation (96).

Proteinuria is the major stimulus of proximal tubular epithelial cell activation and subsequent chemotaxis of infiltrating immunocompetent cells in most glomerular diseases (115). Endothelin-1 synthesis was enhanced in culture proximal tubular cells exposed to high concentrations of albumin, IgG, or transferrin. Similarly, monocyte chemoattractant protein (MCP-1) gene was up-regulated by albumin and transferrin. Very similar findings were reported for albumin-induced up-regulation of RANTES (Regulated upon Activation, Normal T cell Expressed and Secreted), an immunoregulatory cytokine with chemotactic properties for monocytes and memory T cells in culture proximal tubular cells (116). Albumin is also a strong stimulus for tubular interleukin-8 expression, which occurs with nuclear factor-kappaB-dependent pathways (115). Consistent with these in vitro studies, vasoactive and proinflammatory molecules was enhanced in renal tissue from rat models with proteinuric renal disease, particularly at proximal tubular level (117, 118).

Activation of complement proteins in the proximal tubule has major proinflammatory potential in interstitial damage with proteinuric conditions. Intracellular C3 staining was detected in proximal tubules of proteinuric rats with remnant kidneys early after 5/6 nephrectomy, and preceded the appearance of inflammation. C3 colocalized with IgG to the same tubular cells (119). Induction of tubular C3 during protein overload has also been reported (120).

Excessive protein reabsorption by proximal tubular cells promotes fibrogenesis by release of chemoattractants, which leads to local recruitment of mononuclear cells. Interstitial accumulation of inflammatory cells by release of TGF-β, PDGF, and other cytokines leads to interstitial cell transformation into myofibroblasts. In addition, proximal tubular epithelial cells interact with surrounding interstitial fibroblasts to promote fibrogenesis by paracrine release of profibrogenic molecules such as TGF-β, PDGF, and endothelin-1 (121).

The other possible contributory factor is the direct toxic effect following tubular binding of IgA. IgA nephropathy patients have increased urinary IgA concentration that correlates with serum creatinine concentration, as well as the urinary protein excretion (122).

Recently, a glomerulotubular cross-talk, a new mechanism in which mesangial IgA deposition may lead to tubulointerstitial injury in IgA nephropathy, has been proposed. It has been documented that inflammatory cytokines, including angiotensin II, are released from mesangial cells following binding to IgA from patients with IgA nephropathy. These mediators may alter the glomerular barrier pore size that allows the passage of these inflammatory mediators to the tubular lumen. These mediators then activate proximal tubular epithelial cells, which may amplify the inflammatory cascade by local production of chemotactic mediators, which attract more inflammatory cells. This glomerulotubular cross-talk will generate a positive feedback loop of activation in the renal tubules that leads to the overproduction of extracellular matrix components, resulting in fibrosis. This hypothesis was tested by conducting an experiment in which proximal tubular epithelial cells were cultured with medium prepared from mesangial cells incubated with IgA from IgA nephropathy patients (113). Contrary to the absent stimulatory effect on proximal tubular epithelial cells upon direct incubation with IgA, increased proliferation and enhanced expression of inflammatory mediators (including interleukin-6, tumor necrosis factor-α, soluble intercellular adhesion molecule-1 (ICAM-1), and angiotensin II) in proximal tubular epithelial cells cultured with medium prepared from mesangial cells incubated with IgA from IgA nephropathy patients were observed. Tubular and interstitial ICAM-1-positive cells may participate in adhesive interactions with interstitial leukocytes (123, 124). Upregulation of renal interleukin-6 expression correlates

well to the degree of tubulointerstitial damage in IgA nephropathy (125).

Renal Fibrosis

Glomerulosclerosis, tubulointerstitial fibrosis, inflammatory infiltration, and loss of renal parenchyma characterized by tubular atrophy, capillary loss, and podocyte depletion are constituent pathologic findings of renal fibrosis (92). The cellular events leading to these histologic findings include mesangial and fibroblast activation, tubular epithelial to mesenchymal transition (EMT), monocyte/macrophage/T-cell infiltration, and apoptosis (92). At present, renal fibrogenesis process is thought to be similar to wound-healing response to injury (92, 126, 127). The reason why the difference between a healthy wound-healing and fibrotic response occurs remains unknown. The duration of the injury may affect the course. Following the initial injury, affected kidney tissues attempt to repair and recover from damage. This process includes activation of resident kidney cells, which produce and secrete proinflammatory cytokines. Chemotactic cytokines generate a signal for monocytes/macrophages/T-cell infiltration to the inflammatory regions. Glomerular and interstitial infiltrated inflammatory cells become activated, and produce injurious molecules such as reactive oxygen species, as well as fibrogenic and inflammatory cytokines. These stimulate mesangial cells, fibroblasts, and tubular epithelial cells, which show activation or EMT. These cells produce a large amount of extra cellular matrix components. Extra cellular matrix proteins are in turn deposited in the extracellular compartment. Since they are often crosslinked, they are resistant to degradation. Continuous deposition of extra cellular matrix results in fibrosis and destroys the normal architecture of kidney tissues, leading to renal function loss (92).

Molecular Pathway in Renal Fibrosis

It is widely accepted that TGF-β and its downstream Smad signaling play an essential role in tissue fibrosis in general, and renal fibrosis in particular (92, 128, 129). Upregulation of TGF-β is a universal finding in every type of chronic kidney diseases, both in animal models and in humans. TGF-β induction also appears to be a main pathway that integrates, directly or indirectly, the effects of many other fibrogenic factors. Some of these, such as angiotensin II and high glucose, act as an upstream TGF-β inducer, whereas others such as connective tissue growth factor (CTGF) work as its downstream effector.

The TGF-β signal is transduced through its cell membrane type I and type II serine/threonine kinase receptors (128). Receptor activation triggers the phosphorylation and activation of its downstream signaling mediators, Smad2 and Smad3. Phosphorylated Smad2/3 bind to common partner Smad4, and then translocate into the nuclei, where they control the transcription of TGF-β-responsive genes.

Smad transcriptional corepressors including SnoN, Ski, and TGIF restrain Smad signaling tightly in normal kidney. These Smad antagonists effectively restrict Smad-mediated gene transcription with various mechanisms. It has recently demonstrated that SnoN and Ski are progressively diminished in the fibrotic kidney, suggesting that the loss of Smad antagonists is an important mechanism that amplifies the TGF-β signal (130). It is thought that the TGF-β/Smad signal in renal fibrosis is changed into such a way without control, much similar to the loss of tumor suppressor genes during tumorigenesis (92).

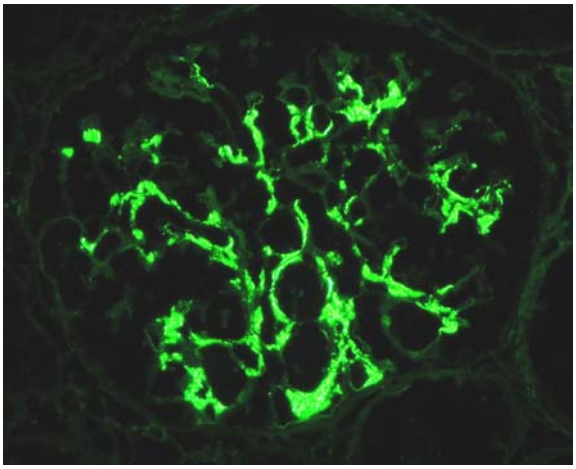
Pathology

Immunohistologic Findings

The diagnostic immunopathological pattern of IgA nephropathy is the presence of IgA in the glomerular mesangium as the sole or predominant Ig. IgA deposits often extend just beyond the mesangiocapillary junctions into the adjacent capillary walls (*Fig. 31-3*). There are also deposits of IgG and/or IgM with the same staining pattern as IgA but with lesser intensity and frequency. In our series, mesangial IgA deposits were associated with IgG in 32% of patients, IgM in 8%, and both IgG and IgM in 11% (131). C3 deposits were observed in a similar distribution pattern in 64% of cases. The early components of the classical complement pathway, C4 or C1q, are absent. Fibrin- or fibrinogen-related antigens are found in a diffuse mesangial distribution in 25–70% of patients and are believed to be one of the injurious agents in the glomeruli (132). Although, in most patients, IgA is present only in the mesangial regions, in approximately 10% of patients it is also observed in the peripheral capillary walls. Such peripheral capillary wall deposits, whether documented by immunofluoresence or electron microscopy, have been associated with more severe clinical manifestations and a poor renal outcome (133–136).

Figure 31-3

Immunofluorescence micrograph showing mesangial IgA deposits in a patients with IgA nephropathy. (See color plate 12)



Light Microscopic Findings

Various glomerular changes are observed. The most characteristic abnormality is mesangial enlargement, caused by various combinations of hypercellularity and increase in matrix (*Fig. 31-4*). Occasionally, small eosinophilic and PAS-positive fibrinoid mesangial deposits are also seen. Biopsies can be graded according to the amount of mesangial cell proliferation on the basis of the World Health Organization criteria (137).

1. Minimal glomerular lesions. The majority of glomeruli appear optically normal, although a few may show a slight increase of mesangial matrix, with or without accompanying hypercellularity. The number of mesangial cells per peripheral mesangial area does not exceed three. There are also small foci of tubular atrophy and interstitial lymphocyte infiltration in some patients.
2. Focal mesangial proliferation. Up to 80% of glomeruli show moderate or severe mesangial cell proliferation, (i.e., more than three cells per peripheral mesangial area). The degree of mesangial cell proliferation varies considerably among glomeruli as well as segmentally within individual glomeruli. The proliferation is usually associated with increased matrix. Small cellular or fibrocellular crescents are frequently found but rarely affect more than 20% of the glomeruli. Capsular adhesions are frequently seen overlying lobules showing mesangial proliferation. Segmental capillary collapse is often observed in association with crescents. A small number of glomeruli showing global sclerosis is often present. Tubular atrophy, interstitial fibrosis, and interstitial lymphocyte infiltration are frequently present but are not extensive.
3. Diffuse mesangial proliferation. More than 80% of glomeruli show moderate or severe mesangial cell proliferation, which varies in intensity in different regions of the mesangium in a given glomerulus as well as from one glomerulus to another. Mesangial cell proliferation is always accompanied by increased mesangial matrix. Cellular and fibrocellular crescents are often found, usually affecting less than 50% of the glomeruli, although in approximately 10% of patients, more than 50% are involved. Capsular adhesions are frequently seen in the absence of crescents. A small number of globally sclerosed glomeruli are often present. Tubular atrophy, interstitial fibrosis, and interstitial lymphocyte infiltration are frequently present and are extensive in 10% of patients.

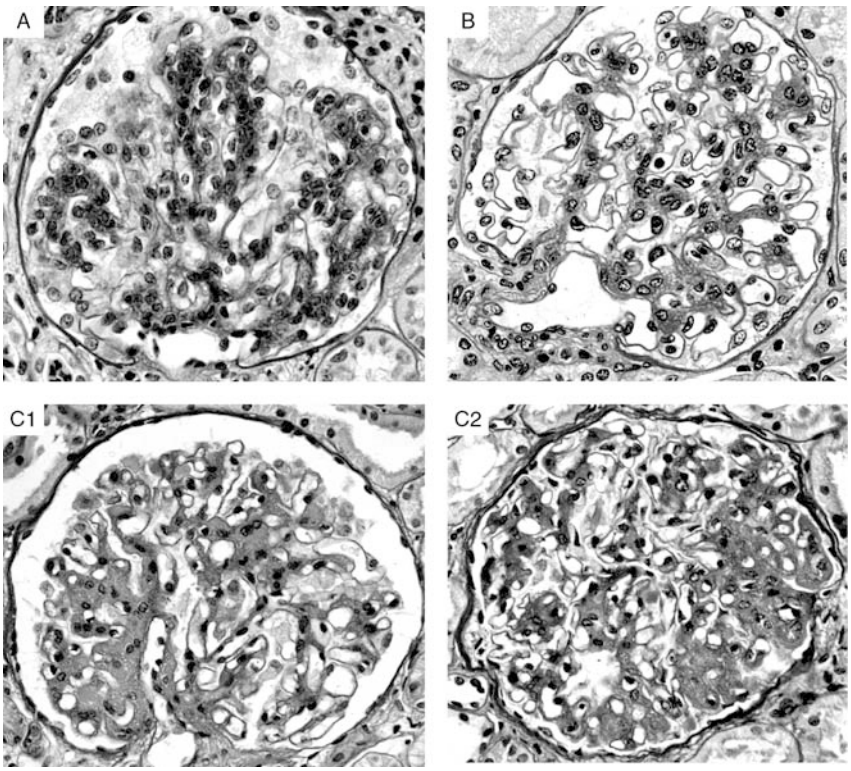
Three types of mesangial change are identified in children with IgA nephropathy (5) (*Fig. 31-4*): (1) Mesangial hypercellularity is more prominent than the increase in matrix, (2) the degrees of mesangial hypercellularity and matrix increase are similar, and (3) the increase in matrix is more prominent than the mesangial cellularity.

The first type of lesion is seen in biopsies in which the interval between onset of disease and biopsy is short. Serial pathologic observations reveal that prominent mesangial hypercellularity is almost exclusively seen in initial biopsies and disappears in follow-up biopsies. These observations suggest that predominant mesangial hypercellularity is characteristic of the early lesion of childhood IgA nephropathy and may disappear within a matter of months. An increase in mesangial cells, although sometimes present, is seldom striking in adult patients (138). In contrast, biopsies with a predominant matrix increase show a long interval between onset of disease and biopsy and a high percentage of glomerular sclerosis. Serial pathologic observations reveal that this type of change is usually seen in follow-up biopsies. An increase in the amount of mesangial matrix with duration of the disease has also been noted in adult patients (139). These findings suggest that progression of IgA nephropathy leads to gradual resolution of mesangial hypercellularity and an increase of matrix associated with the development of sclerosis (140).

The severity of tubulointerstitial changes usually reflects the severity of glomerular damage. Vascular lesions, such as arterial or arteriolar sclerosis, are reported to be common in adults (141) but are very unusual in

Figure 31-4

Light micrograph showing mesangial proliferation in patients with IgA nephropathy. Three types of mesangial change are identified: (a) mesangial hypercellularity is more prominent than the increase in matrix (b) the degrees of mesangial hypercellularity and matrix increase are similar (c) the increase in matrix is more prominent than the mesangial cellularity.



children with IgA nephropathy (135). This difference may be related to the age at biopsy and the duration of disease before biopsy.

Electron microscopy

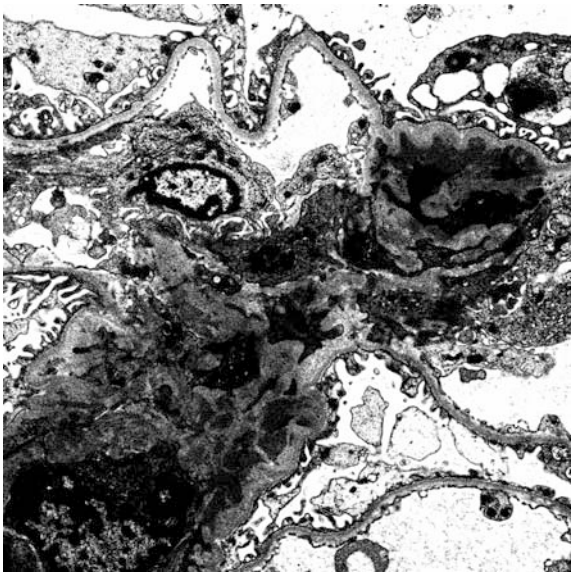
Electron microscopic abnormalities are mainly observed in the mesangium, which is variably enlarged by a combination of increased cytoplasm and matrix. Electron-dense deposits in the mesangium are the most constant and prominent feature and are seen in almost all patients (*Fig. 31-5*). They are granular masses situated immediately beneath the lamina densa in the perimesangial region and expanded mesangium. The size and extent of mesangial deposits varies from patient to patient; in some patients, they are large and produce localized protrusions. Peripheral glomerular capillary wall deposits are also found in the subendothelial and subepithelial regions. Subendothelial deposits occur most frequently in the capillary wall adjacent to the mesangium, although they are also observed in the peripheral part of the loop. Subepithelial deposits are reported to be unusual in adult patients but are frequently found in children with IgA nephropathy. They are generally small and flat and localized to a few capillary loops; the humps typical of acute poststreptococcal glomerulonephritis are never observed. Lysis of the glomerular basement membrane is also seen quite frequently in children (142). In affected areas of the glomerular capillary walls, the lamina densa is thin and irregular, and the epithelial aspect of the glomerular basement membrane shows irregular segments of low electron density with an expanded, washed-out appearance. The epithelial foot processes are generally well preserved, but diffuse foot process effacement may be seen in patients with the nephrotic syndrome.

Repeat Renal Biopsy Findings

There have been only a few reports on the results of repeat renal biopsies (143, 144). We previously reported our

Figure 31-5

Electron micrograph showing numerous electron-dense deposits in the mesangium in a patient with IgA nephropathy.



results in children with IgA nephropathy (6). At the time of the second biopsy, 23 patients had showed clinical remission, defined as complete disappearance of proteinuria and hematuria with normal renal function, whereas 38 had persistent urinary abnormalities with normal renal function. There were no differences between the two groups with regard to the initial clinical findings and the pathologic findings in the initial biopsy. The second biopsy of patients who were in clinical remission showed improvement of the glomerular changes on light microscopy, a disappearance of or a decrease in mesangial IgA deposits, and a decreased amount of electron-dense deposits. Conversely, light microscopy showed a progression of histologic lesions and the persistence of both mesangial IgA deposits and electron-dense deposits in patients with persistent urinary abnormalities. Clinical remission and histologic regression have been reported in adults with IgA nephropathy (145).

Differences between Childhood and Adult Patients with IgA Nephropathy

Significant differences in the early glomerular lesions of IgA nephropathy between children and adults have been demonstrated (146, 147). Glomerular hypercellularity in mesangial area is prominent in children and significantly greater than in adults. In contrast, glomerular matrix expansion, crescent formation and interstitial damage are more severe in adults compared to children. Glomerular hypercellularity correlated with proteinuria in children but not in adults, whereas glomerular matrix correlated with proteinuria and renal function in adults but not in children (146).

Clinical Features

IgA nephropathy occurs at all ages but is most common during the second and third decades of life; it affects boys more often than girls, with the reported male to female ratio varying from less than 2:1 to 6:1 (2). In a study of Japanese children (131), the mean age at presentation was 9.3 years in boys and l0.3 years in girls, and the male to female ratio was 3:2. The clinical presentation of IgA nephropathy varies. Some patients have asymptomatic microscopic hematuria with or without proteinuria. Other patients have recurrent epithodes of macroscopic hematuria. Some patients present with acute nephritic syndrome and, more rarely, with acute renal failure.

Sixty-two percent of our 258 Japanese children were found to have microscopic hematuria with or without asymptomatic proteinuria (131). Twenty-six percent presented with macroscopic hematuria and 12% with an acute nephritic syndrome or nephrotic syndrome. Several studies from Europe and the United States reported that more than 80% of the patients have episodes of macroscopic hematuria, and recurrent macroscopic hematuria is traditionally regarded as the hallmark of childhood IgA nephropathy (148–151). However, it was the initial feature in only 26% of our series, presumably because of the school screening program that detected a high prevalence of asymptomatic urinary abnormalities rather than regional variation in the expression of IgA nephropathy. During the observation period, 60% of patients had one or more episodes of macroscopic hematuria, whereas the other 40% remained asymptomatic.

Macroscopic hematuria often occurs in association with upper respiratory tract infections; less frequently, it occurs in association with other infections involving the mucosal system (e.g., diarrhea and sinusitis). Episodes of macroscopic hematuria are sometimes associated with loin pain. The interval between the precipitating infection and the appearance of hematuria ranges from 1 to 2 days compared with 1 or 2 weeks in acute postinfectious glomerulonephritis. Many patients have recurrent episodes of macroscopic hematuria, each often associated with the

same type of infection. The number of recurrences and the intervals between different episodes are variable. The incidence of macroscopic hematuria is lower in adults (151, 152). Emancipator et al. (3) summarized the previous reports of IgA nephropathy, in which most the patients were adults, and reported that 43% had macroscopic hematuria. However, in Japan, only 18–32% adult patients have been reported to have macroscopic hematuria (152, 153). The reason for the age-related differences in the incidence of macroscopic hematuria has yet to be elucidated.

In asymptomatic patients, microscopic hematuria is almost always present and persistent. Proteinuria is common. The blood pressure and renal function at onset are normal.

Patients with a nephritic or nephrotic onset have the most severe glomerular damage. The most common presenting symptom is macroscopic hematuria. Hypertension is infrequent and usually mild to moderate. Malignant hypertension is not a presenting feature in childhood. Nephrotic edema is reported in approximately 10% of patients. Acute renal failure is occasionally associated with episodes of macroscopic hematuria and is usually reversible. However, a number of investigators have documented a subset of patients with IgA nephropathy that is characterized by extensive crescents and a rapidly progressive course (153–155). A review of published cases of crescentic IgA nephropathy revealed that 41% of patients with this rapidly progressive form of disease were 16 years of age or younger (155).

Laboratory Investigation

Serum IgA levels are increased in 30–50% of adult patients but in only 8–16% in children with IgA nephropathy (131). For this reason, it is seldom of diagnostic significance. Serum complement component concentrations are usually normal, but the C3 level should be measured routinely if the patient has been referred for investigation after the first attack of hematuria to eliminate a diagnosis of postinfectious glomerulonephritis or membranoproliferative glomerulonephritis. Likewise, the antistreptococcal antibody titers should be determined after initial hematuria. These investigations are of little value when hematuria is known to have been present for more than 3 months. The serum creatinine should be measured routinely to estimate renal function; if necessary, the glomerular filtration rate should be determined. If present, proteinuria should be quantified, as proteinuria is associated with histologic lesions and a risk of progression. The plasma proteins should be measured routinely in the presence of heavy proteinuria.

Differential Diagnosis

The diagnosis of IgA nephropathy is based on the presence of IgA as the sole or predominant Ig in the glomerular mesangium. Because diffuse mesangial IgA deposits are observed in a variety of other disorders (*Table 31-1*), the diagnosis of IgA nephropathy can be made only by exclusion. The IgA deposits are often incidental findings and the pathogenesis and clinical significance is unclear.

Table 31-1

Diseases associated with diffuse mesangial IgA deposits

Primary
IgA nephropathy
Secondary
Multisystem disease
Henoch
Henoch-Schönlein purpura
Systemic lupus erythematosus
Cystic fibrosis
Celiac disease
Crohn's disease
Dermatitis herpetiformis
Ankylosing spondylitis
Neoplasms
Carcinomas of the lung and colon
Monoclonal IgA gammopathy
Mucosis fungoides
Non-Hodgkin's lymphoma
Infectious diseases
Mycoplasma infections
Leprosy
Toxoplasmosis
Others
Chronic liver disease
Thrombocytopenia
Pulmonary hemosiderosis
Mixed cryoglobulinemia
Polycythemia
Scleritis

Relationship between Immunoglobulin A Nephropathy and Henoch-Schönlein Purpura

There is a close relationship between IgA nephropathy and Henoch-Schönlein purpura (156). The morphologic and immunopathologic features are similar in the two conditions (156, 157), which are characterized by various degrees of focal or diffuse mesangial proliferation, the diffuse deposition of IgA in the mesangium, and electron-dense deposits in the mesangium. Elevated serum IgA levels are found in both IgA nephropathy and Henoch-Schönlein purpura nephritis, and IgA-containing circulating immune complexes have been demonstrated in both conditions. Infective episodes precede Henoch-Schönlein purpura nephritis in 30–50% of patients, and the presence of *H. parainfluenza* antigens in a diffuse and global distribution in the glomerular mesangium and the presence of IgA antibody against *H. parainfluenza* in sera of Japanese children with Henoch-Schönlein purpura nephritis have also been demonstrated (13). The two disorders have been reported to coexist in different members of the same family, including a pair of monozygotic twins who developed the disorders simultaneously after a well-documented adenovirus infection (158–161). Moreover, the evolution of IgA nephropathy into Henoch-Schönlein purpura nephritis in the same patient is described in both adults and children (162–164). It has been suggested that the two conditions are variants of the same process and that IgA nephropathy is Henoch-Schönlein purpura nephritis without the rash. Although there are similarities in their pathologic and immunologic features, the two conditions are clinically different, and the pathogenesis is not clear. Our study suggests that Henoch-Schönlein purpura nephritis is an acute disease, with glomerular lesions nonprogressive after the onset (165). Therefore, in most patients, the prognosis is associated with the severity of glomerular change at the onset. In contrast, IgA nephropathy is a chronic, slowly progressive glomerular lesion, which may eventually lead to chronic renal failure, whatever the presentation. A few patients with Henoch-Schönlein purpura nephritis have recurrent episodes of macroscopic hematuria and a progressive renal disease on repeat renal biopsies. Finally, Henoch-Schönlein purpura nephritis occurs mostly in young children and is rare in adulthood, whereas IgA nephropathy affects mainly older children and young adults. It is therefore reasonable that IgA nephropathy and Henoch-Schönlein purpura nephritis are treated as different clinicopathologic entities until pathogeneses of the two conditions are better understood.

Chronic Liver Disease

Glomerular IgA deposits may be observed in patients with various types of chronic liver diseases (166). The presence of glomerular IgA deposits in patients with hepatic cirrhosis was first demonstrated in 1970. Thereafter, many investigators have documented the presence of glomerular abnormalities in patients with various types of chronic liver disease (166). Mesangial proliferation and IgA deposits are the most common findings. The light, electron, and immunofluorescence microscopic features in patients with liver disease are similar to those in patients with primary IgA nephropathy. Most patients with chronic liver disease have clinically asymptomatic renal disease. Microscopic hematuria and mild proteinuria are commonest findings and macroscopic hematuria or a nephrotic syndrome is rare. Renal functional impairment is also rare. Glomerular lesions in children with chronic liver disease are very unusual. The pathogenetic mechanisms that contribute to mesangial IgA deposition in chronic liver diseases remain unknown. Significant elevations of the serum monomeric and polymeric IgA levels have been reported in patients with chronic liver disease (167, 168). Impaired hepatic clearance and increased synthesis of polymeric IgA, abnormalities of IgA metabolism, and portosystemic shunting of antigens and immune complexes have been suggested as possible causes of mesangial IgA deposition in chronic liver disease (168).

Idiopathic Nephrotic Syndrome

A few patients with steroid-sensitive nephrotic syndrome show mesangial deposits of IgA on renal biopsy. They are classified by some as IgA nephropathy, whereas others consider that mesangial IgA in patients with minimal changes (i.e., without cellular proliferation) is coincidental. This probably applies to idiopathic nephrotic syndrome associated with mesangial IgA deposits occurring in Asians and explains a favorable response to steroids, which is not the case in true IgA nephropathy (169).

Natural History and Prognosis

In adult series, the incidence of renal insufficiency varies from less than 10% to as high as 45% in patients followed up for more than 1 year. In long-term follow-up of adult patients, 30–35% have been found to develop progressive renal insufficiency 20 years after the initial discovery of disease (2, 170–172). It can be estimated that 1–2%

of adult patients will enter end-stage renal failure each year from time of diagnosis (173). The long-term prognosis of the 169 Japanese children with IgA nephropathy followed more than 10 years indicates that 9% of the patients had developed chronic renal failure by 15 years (*Fig. 31-6*).

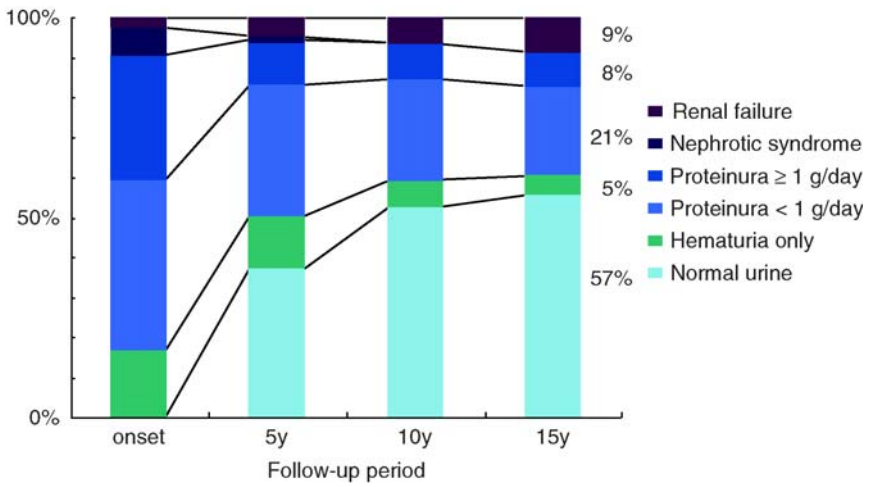
Because of the variable rate of progression to chronic renal failure, there have been attempts to identify features present at the time of diagnosis that would predict the ultimate outcome. The following clinical findings are regarded as poor prognostic indicators in adult patients (2): persistent hypertension, persistent heavy proteinuria, and reduced glomerular filtration rate at presentation (170–172, 174–179). In children, several studies have shown that the degree of proteinuria correlates with the severity of morphologic glomerular lesion (135, 150, 180), and heavy proteinuria at the time of biopsy predicts a poor outcome (136, 181). In contrast, slight proteinuria or its absence at the time of biopsy predicts a favorable outcome. There is general agreement that hypertension and low glomerular filtration rate at presentation are significant factors in determining the outcome of adult patients with IgA nephropathy. In children, acute renal failure at onset is usually transient and associated with macroscopic hematuria and reversible tubular lesions. Male gender has also been considered an unfavorable prognostic feature by some investigators (171), but we (136) and others (134) could not confirm it in large cohort of adult and pediatric patients. Schena et al. reported an increased risk of end-stage renal disease in familial IgA nephropathy (24).

Several pathologic features are associated with a poor outcome: diffuse mesangial proliferation; a high proportion of glomeruli showing sclerosis, crescents or capsular adhesions; the presence of moderate or severe tubulointerstitial changes; the presence of subepithelial electron-dense deposits; and lysis of the glomerular basement membrane by electron microscopy (136). Patients with diffuse mesangial proliferation have been reported to have a significantly worse prognosis than those with focal proliferation or minimal lesions by light microscopy in adults (170, 175, 182). In many adult studies (134, 141, 170, 178), glomerular sclerosis and crescents have also been associated with poor renal outcome. Levy and associates found that mesangial proliferative glomerulonephritis with crescents was associated with poor prognosis in children (150). Because the severity of the tubulointerstitial changes usually corresponds with the severity of the glomerular changes, tubulointerstitial changes in IgA nephropathy are believed to be secondary to the glomerular injury. Vascular lesions, such as arterial or arteriolar sclerosis, have been reported to play an important role in the progression of IgA nephropathy in adults. However, vascular changes are very unusual in children (5, 151, 154). This difference may be related to the age at biopsy and the duration of disease before biopsy.

Recurrence of mesangial IgA deposits is often observed in transplant recipients whose original disease was IgA nephropathy (37, 183). Clinically such recurrences are mild or even asymptomatic: despite this risk of recurrent glomerulonephritis, graft survival in patients with IgA nephropathy is considered good (37).

Figure 31-6

Long-term prognosis of the 169 Japanese children with IgA nephropathy followed more than 10 years.



At the beginning of the 1990s, use of angiotensin-converting enzyme inhibitors for focal mesangial proliferation and combined therapies including corticosteroids for diffuse mesangial proliferation increased dramatically in Japan. Our recent retrospective cohort study of 500 children with IgA nephropathy has clarified an improved renal survival in Japanese children with IgA nephropathy (184). Among all patients, the actuarial renal survival was 96.4% at 10 years, 84.5% at 15 years and 73.9% at 20 years. Diagnosed in 1976–1989, the renal survival was 94.0% at 10 years, 80.1% at 15 years and 70.1% at 20 years. Diagnosed in 1990–2004, the renal survival was 98.8% at 10 years, 98.8% at 15 years (p = 0.008). With diffuse mesangial proliferation, both the 10- and 13-year renal survivals were 97.8% in 1990–2004, compared with 78.5% and 68.6%, respectively, in 1976–1989 (p = 0.0003). In the same study, prognostic factors for end-stage renal disease-free survival were analyzed (184). Mesangial proliferation degree and initial renal biopsy year were significant in both the univariate and the multivariate analysis. For children with IgA nephropathy, the most influential prognostic variable was mesangial proliferation degree. Proteinuria at diagnosis was significant in the univariate, but not in the multivariate analysis. Multivariate analysis showed that initial renal biopsy year was a significant factor for renal survival independently of mesangial proliferation degree, proteinuria at diagnosis and estimate creatinine clearance at diagnosis (hazard ratio = 0.08, 95%CI 0.004–0.43).

Treatment

IgA nephropathy is a leading cause of chronic renal disease and end-stage renal disease in adult patients, and recent long-term studies assessing the prognosis in children have challenged earlier views that the condition represents a benign disorder. Thus, IgA nephropathy presents a therapeutic challenge in both adults and children. The most appropriate treatment for patients with IgA nephropathy is still a matter of controversy (185). At present, there is no curative therapy for IgA nephropathy (186). Because of the variable rate of progression to renal failure, and because of the probable multifactorial pathogenesis of the disease, the effectiveness of any treatment can only be properly evaluated by means of a randomized controlled trial (185). When considering treatment protocols, an issue of great importance is the selection of appropriate patients in whom the treatment is to be evaluated. Patients with heavy proteinuria at biopsy and the most severe glomerular lesions on renal biopsy appear to be at greatest risk of progressive renal deterioration and, therefore, the most appropriate candidates for specific therapeutic interventions. Patients with long-standing disease and extensive, irreversible glomerular damage are unsuitable for such treatments.

In a randomized controlled trial, another important thing is to select adequate endpoints. Although in a clinical trial of progressive IgA nephropathy the ultimate endpoint is development of chronic renal insufficiency, most pediatric patients do not develop it during the study period. Thus, studies of pediatric patients with IgA nephropathy may differ markedly from studies of adults with regard to the apparent risk of progressive disease (185). Therefore, due to the long time period from clinical onset of disease until progression to end-stage renal disease, surrogate markers of outcome must be used to evaluate efficacy of therapy for IgA nephropathy in clinical trials. It is a noteworthy fact that validation of these surrogate markers may be lacking, resulting in the potential for inappropriate conclusion with regards to therapeutic efficacy. Therefore, the careful investigation in detailed long-term outcome of previous randomized controlled trials is important.

With regard to treatment of IgA nephropathy, it is important to consider the differences in the nature of IgA nephropathy between children and adults. An "evidence-based" therapy is important in both chidren and adults. However, available evidence for treatment is partially, clearly, different between chidren and adults. Generally and roughly speaking, the evidence for treatments of IgA nephropathy in adults supports relatively passive treatments, whereas that in children supports relatively active treatments. The reason of this difference is unknown. Differences in the diagnosis timing and histological differences may be associated. Although we should refrain from radical treatments that are not based on evidence, however appropriate active treatments that are based on evidence are important in treatments for children with IgA nephropathy.

Fish oil/omega-3 Fatty Acids

Controlled double-blind trial in adult patients with IgA nephropathy (187–189) showed that treatment with fish oil for 2 years retarded the rate at which renal function was lost, but a meta-analysis showed that there was only a 75% probability that fish oil was beneficial (190). A recent randomized, placebo-controlled, double-blind trial by the Southwest Pediatric Nephrology Study Group in USA and Canada evaluated the role of omega-3 fatty

acids in children and young adults with IgA nephropathy, and the treatment group did not showed benefit over the placebo group with respect to time to failure, defined as estimated GFR < 60% of baseline (191). Furthermore, from analysis of these clinical trials, it was reported that efficacy of omega-3 fatty acids in children and adults with IgA nephropathy is dosage- and size-dependent (192).

Coagulation Modifying Agents

Warfarin, urokinase, and anti-platelet agents have all been assessed for the treatment of IgA nephropathy. At present there is no sufficient evidence to support the use of coagulation modifying agents (193, 194). However, coagulation modifying agents may have a role in combination therapy. To investigate this question, a randomized controlled trial is currently in progress in Japan to compare the effects of prednisolone, immunosuppressive agents, warfarin, and dipyridamole with those of prednisolone and immunosuppressive agents in children with severe IgA nephropathy.

Angiotensin-Converting Enzyme Inhibitors and Angiotensin II Receptor Blockers

As already demonstrated in non-diabetic chronic nephropathies (195–197), studies including randomized controlled trials have indicated that angiotensin-converting enzyme inhibitors reduces urinary protein excretion (198, 199) and preserves renal function (200) in adult patients with IgA nephropathy. However, there is no randomized controlled study only in children with IgA nephropathy demonstrating that angiotensin-converting enzyme inhibitors preserve renal function. Recently, a randomized controlled trial has proved a significant benefit of angiotensin-converting enzyme inhibitor on the progression of IgA nephropathy in children and young people when it was assessed as a composite end point of 30% reduction in GFR or an increase of proteinuria over the nephrotic range. It has also showed the benefit of angiotensin-converting enzyme on remission of proteinuria below what is generally considered a harmful level (201). In adult setting a randomized controlled trial has showed that combination therapy with angiotensin-converting enzyme inhibitor and angiotensin II receptor blocker has an additive dose-dependent antiproteinuric effect compared to monotherapies in patients with IgA nephropathy (202). The *Cooperate* trial, a large randomized study of 336 patients with non-diabetic renal disease, in which 50% of subjects had IgA nephropathy, has demonstrated that combination treatment safely retards disease progression compared with monotherapy (203). A randomized controlled trial in 109 adults with IgA nephropathy showed that valsartan significantly slowed renal deterioration compared with placebo (204). Although there is no randomized controlled trial for angiotensin II receptor blockers in children with IgA nephropathy, some studies have demonstrated their antiproteinuric effect in combination therapies with angiotensin-converting enzyme inhibitor and angiotensin II receptor blocker (205, 206).

Corticosteroids

Corticosteroids have been widely used to treat moderate to severe IgA nephropathy, particularly in pediatric patients. To date, information concerning not only the effectiveness but also safety of corticosteroid therapy over a long time course has been largely defective. It has been difficult to assess the results of treatment trials with these agents in terms of preservation of renal function, due in part to wide variations in the length of therapy and the dosing regimens employed, and also to the use of corticosteroids in combination with other drugs (185). At present, however, some evidence has been obtained for the role of corticosteroids in the treatment of IgA nephropathy (207–209). In adults with IgA nephropathy, an Italian prospective randomized controlled trial demonstrated that a six-month course of steroid treatment protected against deterioration of renal function with no notable adverse effects during follow-up (207). Recently, the long-term follow-up data of the trial showed that corticosteroids significantly reduced proteinuria and protected against renal function deterioration (208). An English single-center randomized controlled trial has demonstrated that the value of combined immunosuppressive treatment with prednisolone and cytotoxic agents in reducing renal failure in IgA nephropathy (209). A homogeneous cohort of 38 patients who had mean blood pressure within 10% of current targets and estimated GFR 50% normal but losing 10% estimated GFR/year were randomly assigned; no patient had crescentic disease. Renal survival improved 12-fold at 5 year, with remission of nephritis by urinalysis.

With regard to children, our previous studies (193, 210) are the only randomized controlled trials so far to have demonstrated that treatment including corticosteroid for two years early in the course of disease reduces

immunologic renal injury and prevent any further increase of glomerular sclerosis. Up to now, however, it has been unclear whether corticosteroid alone is sufficient for treatment of IgA nephropathy in children, and there has been no reliable evidence for its effectiveness in this group of patients (185). High-dose intravenous methylprednisolone were shown to delay development of renal failure in a randomized controlled trial in adult patients (207). However, no convincing evidence has been published to date to support the use of high-dose intravenous methylprednisolone for the treatment of children with IgA nephropathy.

Immunosuppressants

The use of immunosuppressants other than corticosteroids for treatment of IgA nephropathy has still not been sufficiently evaluated, even in adult patients. Although the results of prospective trials of cyclophosphamide (209) and mycophenolate mofetil (211–213) for IgA nephropathy have been published, their efficacy is controversial. It has been difficult to assess the results of treatment trials with these agents in terms of preservation of renal function, due in part to wide variations in the length of therapy and the dosing regimens employed, and also to the use of corticosteroids in combination with other drugs (185). There is currently insufficient evidence to support the use of immunosuppressants alone for treatment of children with IgA nephropathy. Recently a meta-analysis of immunosuppressive treatments for IgA nephropathy suggested a benefit of corticosteroids and immunosuppressants (214).

Clinical Trials for Combination Therapy Including Corticosteroids and Immunosuppressant in Japan

There has been an even lower number of prospective studies for evaluation of immunosuppressants in children with IgA nephropathy. In this situation, prospective trials by The Japanese Pediatric IgA Nephropathy Treatment Study Group have provided some useful data regarding the treatment of children with IgA nephropathy (193, 210). In the trials, treatment was started early in the course of disease because the duration of the disease before treatment was short and the extent of glomerulosclerosis was low as a result of the Japanese school screening program. Also in the trials, the majority of patients presented with asymptomatic proteinuria and microscopic hematuria detected by this school screening program, which is one of the important factors considered in our prospective trials.

A randomized controlled trial by The Japanese Pediatric IgA Nephropathy Treatment Study Group demonstrated that treatment of children with severe IgA nephropathy showing diffuse mesangial proliferation with prednisolone, azathioprine, heparin-warfarin, and dipyridamole for 2 years early in the course of disease prevents immunologic renal injury and progression of the disease (193).

In a randomized controlled trial carried out sequentially by The Japanese Pediatric IgA Nephropathy Treatment Study Group, the effects of prednisolone, azathioprine, warfarin, and dipyridamole (combination) with those of prednisolone alone was compared in 80 children with newly diagnosed IgA nephropathy showing diffuse mesangial proliferation (210). Patients were randomly assigned to receive either the combination or prednisolone alone for 2 years. It has been conclude that the combination treatment including prednisolone, azathioprine, warfarin, and dipyridamole may be better for severe IgA nephropathy than treatment with prednisolone alone (210).

Based on the two previous randomized controlled trials described above, the immunosuppressant is considered to be important for the treatment in children with IgA nephropathy. Often, however, it was unable to complete azathioprine regimen due to toxicity. Therefore, a different but effective immunosuppressant may be worth trying. Mizoribine, like azathioprine, is an antimetabolite that exerts its immunosuppressant effect by inhibiting lymphocyte proliferation. In a recent prospective pilot study by The Japanese Pediatric IgA Nephropathy Treatment Study Group, mizoribine was administered instead of azathioprine as part of the combination therapy for treatment of 23 children with severe IgA nephropathy, and the efficacy and safety was evaluated (215). In conclusions, the efficacy and safety of the mizoribine combination seems to be acceptable for treatment of children with severe IgA nephropathy (215).

The detailed long-term outcome of previous randomized controlled trials is being investigated at present. One patient reached end-stage renal disease at 10-year after the start of treatment with the azathioprine combination. At the most recent follow-up, none of the 23 patients with the mizoribine combination in the current study had renal insufficiency.

Chinese Herbal Medicine (Sairei-to)

To determine the effect of the Chinese herbal medicine, Sairei-to (TJ-114) in children with newly diagnosed IgA nephropathy showing focal/minimal mesangial proliferation, a randomized controlled trial was undertaken by The Japanese Pediatric IgA Nephropathy Treatment Study Group (216). One hundred and one patients were randomly assigned to receive Sairei-to for 2 years or no drug for 2 years. The trial has demonstrated that 2-year Sairei-to treatment early in the course of disease is effective in children with IgA nephropathy showing focal/minimal mesangial proliferation (216).

Tonsillectomy

Several retrospective studies have analyzed the role of tonsillectomy in the treatment of IgA nephropathy mainly in adult (217, 218). Treatment was not, however, homogeneous between the study groups; patients who underwent tonsillectomy were also treated with steroid pulses, cyclophosphamide, antiplatelets and warfarin. At present studies provide conflicting data. Up to today there is no randomized controlled study demonstrating that tonsillectomy is beneficial even in adult patients with IgA nephropathy. Therefore, it cannot be recommended for widespread use for treatment of IgA nephropathy patients, especially for children with IgA nephropathy (219, 220). The results of an ongoing randomized controlled trial in Japan, which compares the combination therapy of tonsillectomy combined with steroid pulses versus steroid pulses monotherapy, are awaited (219).

Japanese Guidelines for the Treatment of Childhood IgA Nephropathy

Recently, the Japanese Society for Pediatric Nephrology has developed "Guidelines for the treatment of childhood IgA nephropathy." In these guidelines, the disease severity has been divided into two categories, i.e., mild and severe IgA nephropathy, and according to the severity treatments were proposed. A summary of the guidelines is shown in *Table 31-2*.

Concluding Remarks

Progression of IgA nephropathy leads to gradual resolution of mesangial hypercellularity and an increase of matrix and is associated with the development of sclerosis. The majority of children with IgA nephropathy in Japan are diagnosed early in the course of the disease, and the asymptomatic period before the discovery of urinary abnormalities is short. In our controlled trials, the average interval between onset and discovery of disease and start of treatment was 11 months, and no patient showed predominant matrix increase or extensive glomerulosclerosis. Adequate management seems to have improved

Table 31-2

Summary of guidelines for the treatment of childhood IgA nephropathy (ver. 1.0, by the Japanese Society for Pediatric Nephrology)

Mild cases
Definition: Patients who meet both clinical findings AND histological findings as follows
Clinical findings: Slight proteinuria (early morning urinary protein to creatinine ratio <1.0)
Histological findings: $<80\%$ of glomeruli showing moderate or severe mesangial cell proliferation, crescent formation, adhesion, or sclerosis AND $<30\%$ of glomeruli showing crescent formation
Treatments: Either lisinopril or saireito should be given for more than 2 years
Severe cases
Definition: Patients who meet either Clinical findings OR Histological findings
Clinical manifestations: Heavy proteinuria (early morning urinary protein to creatinine ratio ≥ 1.0)
Histological findings: $\geq 80\%$ of glomeruli showing moderate or severe mesangial cell proliferation, crescent formation, adhesion, or sclerosis OR $\geq 30\%$ of glomeruli showing crescent formation
Treatments: The combination therapy of prednisolone, azathioprine or mizoribine, warfarin, and dipyridamole should be given for 2 years

renal survival in Japanese children with IgA nephropathy. Patients with long-standing disease and extensive glomerulosclerosis are unsuitable for treatment. Early diagnosis including exact evaluation disease severity on renal histology and early treatment according to disease severity are very important in IgA nephropathy.

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32 Membranoproliferative Glomerulonephritis

Michael C. Braun · Christoph Licht · C. Frederic Strife

Introduction

Membranoproliferative glomerulonephritis (MPGN) is characterized on histology by glomerular hypercellulatity, increased mesangial matrix, thickening of the peripheral capillary walls, and a splitting of the glomerular basement membranes (GBMs) due to mesangial interposition in the capillary walls. The term MPGN was originally used by Habib et al. (1) to describe the glomeruli in a group of patients with chronic nephritis. In 1965, this appearance was associated with hypocomplementemia (2, 3). In 1963, a second chronic nephritis, originally called *dense deposit disease* by Berger and Galle (4), was classified as a variant of MPGN (5) and later designated *MPGN type II*. The more common type, in which subendothelial deposits predominate, was designated *type I*. The terms *lobular* and *mesangiocapillary glomerulonephritis* have been used interchangeably with MPGN. A nephritis morphologically similar to type I found in patients with chronic antigenemia or malignancies lead to the additional classification of secondary as opposed to idiopathic MPGN (*Table 32-1*). In the 1970s, several observers (6–9) described a third type of MPGN. The defining lesion of type III as described by Burkholder et al. (6) was the presence of abundant segmental subepithelial deposits, which shared many features seen in membranous nephropathy. In the type III lesion described by Strife et al. (7) and Anders et al. (9) the subepithelial deposits were much less abundant. Using silver impregnated electron micrographs, they described complex disruptions or laminations of the GBM, together with subendothelial, subepithelial, and intramembranous deposits. This dichotomy within the classification has lead some to suggest there are two "variants" of type III. Although some aspects of MPGN types I and III are similar, the clinical and morphologic differences between the two make their separation feasible. Since this distinction is not universally accepted, this chapter will consider MPGN types I and III together, whereas type II, which is now accepted as a distinct disorder and called Dense Deposit Disease (DDD), will be discussed separately.

Complement Abnormalities in Membranoproliferative Glomerulonephritis

The distinctive feature of MPGN types I, III and DDD is hypocomplementemia (*Fig. 32-1*), caused in many by the presence of autoantibodies, called *nephritic factors* (NFs), directed at epitopes on complement proteins (*Fig. 32-2*). At presentation, the serum C3 concentration is low in 80–95% of patients with types I and III or DDD. In all, four distinct mechanisms are thought to be responsible for the hypocomplementemia: (1) circulating immune complexes which activate the classical complement pathway, (2) NFs which interfere with the normal control of the complement cascade, (3) inhibition of C3 synthesis mediated by circulating C3 breakdown products, and (4) genetic deficiencies or mutations in complement regulatory proteins(10–12).

Of patients with MPGN type I about 40% of those with a low serum C3 level will have a concurrent low serum level of C4, suggesting classical complement pathway activation (*Fig. 32-2*) (13). Reduced serum levels of other early classical complement proteins, such as C1q or C2, may be seen on occasion (14). A NF which acts to stabilize the activity of the classical pathway convertase (NF_c), C4bC2a, is also present in some patients (15).

The complement perturbation in MPGN type III is distinct from that in both type I and DDD; C4 levels are typically normal and severely hypocomplementemic patients (C3 $\leq$ 30 mg/dl) commonly have depressed levels of C5 and properdin and of one or more of the other terminal components, C6, C7, and C9 (16–18). Depression of the latter components is seen in only 10% of those with type I and is not seen in DDD. The NF in MPGN type III is presumably responsible for the low levels of

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Table 32-1
Classification of MPGN

MPGN type I
Primary/idiopathic
Familial
Secondary
Malignancy
B-cell lymphoma
Chronic lymphocytic leukemia
Non-Hodgkin's lymphoma
Immunologic
Cryoglobulinemia
Sjogren's syndrome
Complement deficiencies
SLE
Infectious
Hepatitis C
Hepatitis B
Schistosoma mansoni
HIV
Malaria
Other
Heroin abuse
Partial lipodystrophy
MPGN type III
Primary/idiopathic
Familial
Dense Deposit disease (MPGN type II)
Primary/idiopathic
Familial
Complement deficiencies
Partial lipodystrophy

terminal components (NF_t) (*Fig. 32-2*). NF_t differs from that found in type I (NF_c) and DDD (NF_a) in that in vitro it converts C3 slowly with maximum conversion in 4 h rather than 30 min. The conversion is properdin dependent and is thought to stabilize a convertase with the composition, ($C3bBb_n$), PNF_t, and serves to cleave C5 preferentially (18).

The complement profile and the frequent presence of NF_a are distinctive in DDD (*Fig. 32-2*). The profile is characterized by levels of C3, which may be markedly depressed with normal or near normal levels of other components (16). Thus, the depressed properdin, C4, or C5 levels seen in the other types of MPGN, in acute post-infectious glomerulonephritis and in the nephritis of systemic lupus are not present in DDD. The depressed C3 is produced by activation of native C3 by the alternative pathway convertase, C3bBb, which has been stabilized by the NF of the amplification loop, NF_a (*Fig. 32-2*) or by functional abnormalities in complement Factor H, a key regulator of the alternative complement pathway. The NF_a stabilized alternative pathway C3 convertase, C3bBb, NF_a, has a half life more than 15 times that of the native convertase (19). The activated C3 it forms is quickly inactivated by Factors H and I and is subsequently degraded to the ultimate C3 breakdown products, C3c and C3dg. All three of these derivatives may be found in the circulation (20–22). Recent results obtained from in vivo studies in Factor H and I knock out mice (*Cfh*−/− and *Cfi*−/−) mice suggest a crucial role for these breakdown products for the formation of glomerular complement (C3) deposition (23). However, the relevance of this finding for the understanding of DDD in humans has still to be defined. Finally, heavily contributing to hypocomplementemia is an inhibition of C3 synthesis attributed to a negative feedback produced by the circulating C3 breakdown products (24, 25).

MPGN Types I and III

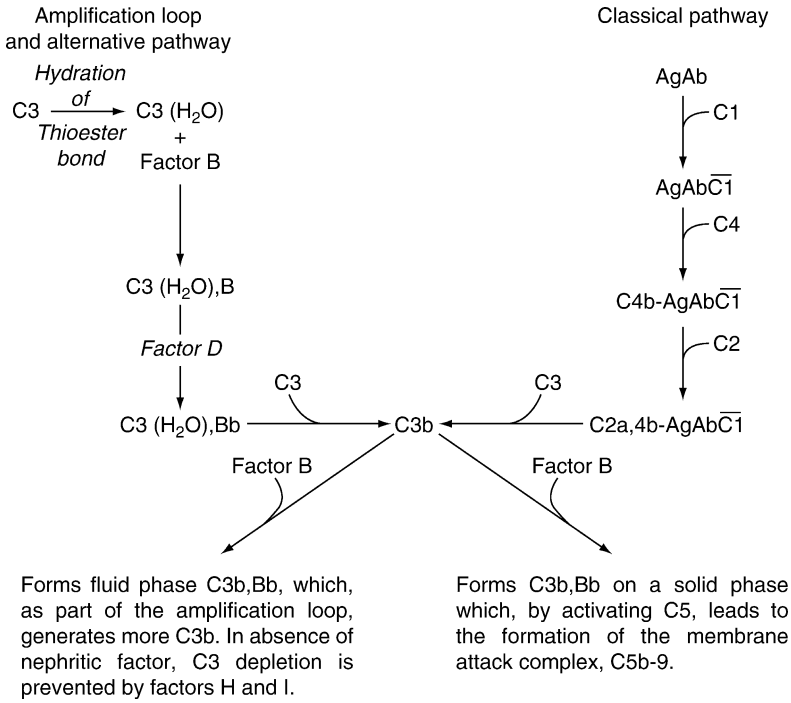
Epidemiology and Genetics

The incidence of MPGN is difficult to ascertain, however it is roughly estimated that there are 1–2 cases per 10^6 pediatric patients (26). There is considerable geographic variation in the frequency of MPGN worldwide. In the US, MPGN I accounts for approximately 1.5% of pediatric patients with ESRD (NAPTRCS Annual report 2007), while reports from India and Nigeria suggest that MPGN is diagnosed in 15 and 52%, respectively, of children with nephrotic syndrome (27, 28). This wide variation in incidence is likely due to secondary MPGN and not idiopathic disease. In most series, MPGN type I has been found to be the most common form (*Table 32-2*). In those studies which distinguished MPGN type I and III, the relative frequency of type III ranges from 14 to 37% (29–34) (*Table 32-2*). In our experience, type III is at least as common as type I (31). MPGN types I and III usually present in older children or adolescents. Occasionally they have been observed in patients older than 30 years or younger than 5 years.

Support for a genetic susceptibility for both MPGN types I and III was provided by the observation that an extended haplotype, HLA-B8, DR3, SCO1, GLO2, is

Figure 32-1

Activation of either the classical or alternative complement pathways produces activated C3, C3b, which can form fluid phase or solid phase C3b, Bb (convertase). Normally both fluid phase and solid phase C3b, Bb are rapidly inactivated by complement control proteins. Both can be stabilized by NF (*Fig. 32-2*). C3b, Bb stabilized on a solid phase can activate C5 and form the membrane attack complex, C5–C9 (Courtesy of John Bissler, M. D.).



significantly more frequent in patients with these two types than in the general white population (35). Also, patients with MPGN types I and III have a similarly high frequency of inherited complement deficiencies (36). Finally, there are a number of reports of familial clustering of MPGN type I (37–41). Reports of familial type III are rare (42). However, an Irish family with eight affected members in four generations was reported. Significant evidence for linkage was observed on chromosome 1q31–32 (43). Interestingly, linkage analysis identified a 22 cM region on chromosome 1 in this cohort which contains the regulators of complement activation (RCA) locus.

Presentation and Clinical Manifestation

The presentation of MPGN generally falls into three categories: nephrotic syndrome, acute nephritic syndrome or asymptomatic hematuria and proteinuria discovered by chance (*Table 32-3*). Due to extensive over-lap, specific MPGN types can not be distinguished solely by presenting clinical features (13).

Asymptomatic micro-hematuria and proteinuria: Almost 50% of patients with MPGN present with isolated hematuria and proteinuria in an otherwise healthy child (*Table 32-3*). This presentation is most common in MPGN type III patients (13). Renal function and serum albumin level are usually normal.

Nephrotic syndrome. Edema as the presenting symptom is present in about one third of patients with either MPGN type I or III. These patients have significant proteinuria usually accompanied by microscopic hematuria with red cell and granular casts. Serum concentrations of albumin and IgG are typically low and about 70–80% will have a low serum C3 level. In the absence of effective treatment, a nephrotic syndrome is strongly associated with a poor prognosis.

Acute nephritic syndrome: Gross hematuria with an acute nephritic syndrome is the presenting feature in about 25% of patients with MPGN. Mild hypoalbuminemia is common. Renal function is usually normal. However, rare patients may develop rapidly progressive glomerulonephritis. Such patients usually have a low serum level of C3, making distinction from acute

Figure 32-2

Fluid-phase activation of C3 in MPGN. In MPGN type II (DDD), the convertase stabilized by NF_a activates only C3. The convertase produced in type I by immune complexes and the nephritic factor complex produced in type III can form a C3, C5 convertase that activates both C3 and terminal components. This results in low C3 levels in DDD, low C3 and C5 levels in type I and low C3, C5 and terminal component levels in type III. On a solid phase such as the renal glomerulus, it is possible that in type I, a C3,C5 convertase is deposited and nephritogenesis is the result. Solid phase events which are nephritogenic in DDD and MPGN type III are not clear. NF_t, nephritic factor of the terminal pathway. P, properdin (Courtesy of John Bissler, M. D.).

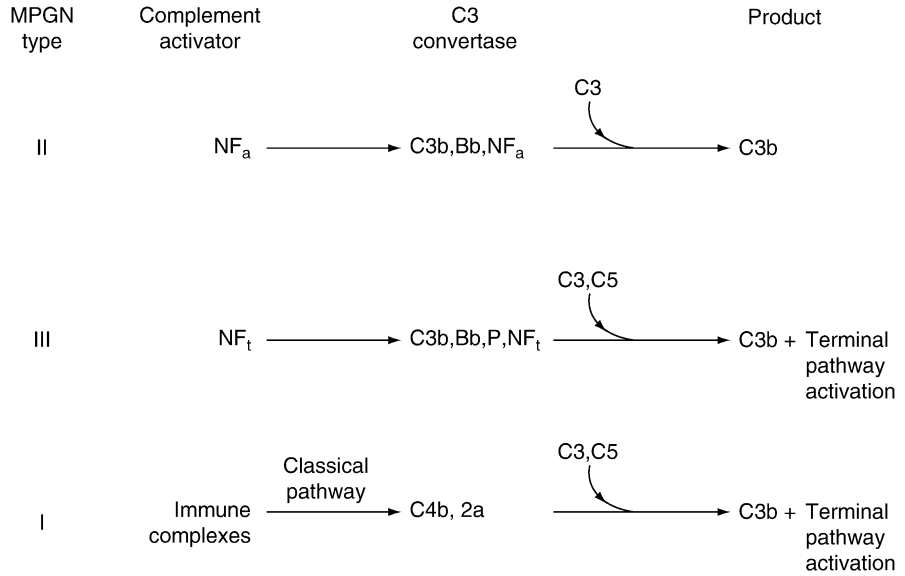


Table 32-2

Relative frequency of MPGN by type

Series	Patient #	Type I (%)	Type II (%)	Type III (%)
Iitaka (29)	41	78	5	17
ISKDC (31)	73	58	19	23
Braun (31)	78	36	23	41
Schwertz (32)	50	52	34	14
Antonovych (34)	100	55	35	10
Little (33)	70	43	33	24
Totals	412	213 (52%)	109 (26%)	90 (22%)

post-streptococcal glomerulonephritis difficult. A biopsy is often not performed until it is apparent that the C3 concentration has failed to return to a normal level as is typical for resolving acute post-streptococcal glomerulonephritis.

Associated clinical features (*Table 32-3*): Estimated glomerular filtration rate (GFR) is commonly lower at presentation in patients with MPGN type I when compared to those with type III (31). There is also a higher frequency of hypertension at presentation in patients with MPGN type I compared to type III. Hypertensive encephalopathy is rare at presentation, but has been reported during follow-up in both treated and untreated patients (30). Antecedent constitutional complaints, including

Table 32-3
Clinical presentation of MPGN types I and III

	Type I (%)	Type III (%)
Clinical Presentation		
Edema	19	32
Gross hematuria	28	24
Asymptomatic microhematuria/proteinuria	22	65
Additional Common Features at Presentation		
Symptoms of systemic disease	26	0
Hypertension	60	21
Low serum C3	68	86
Acute infection prior to presentation	33	26

fatigue, lassitude, and weight loss, characterize the onset in about 25% of patients with MPGN type I, but are not observed in those with MPGN type III (13). An occasional patient, especially with MPGN type I, will have a normochromic, normocytic anemia out of proportion to the degree of renal insufficiency.

Pathology

Glomeruli in MPGN type I by light microscopy (*Fig. 32-3*) usually have a uniform increase in cellularity due to an influx of leukocytes and mesangial proliferation. The mesangial proliferation causes glomerular enlargement and marked reduction in the number of open capillary lumens. With silver stain, capillary walls may show a double contour called *tram-tracking*. This is the result of new basement membrane formation at the site of mesangial interposition. With progression, the mesangial proliferation may become replaced by centro-lobular hyalinization, giving the glomeruli a progressively more lobular appearance. In MPGN type III the degree of mesangial proliferation is typically less in comparison to type I, and often focal in distribution (*Fig. 32-3*). Glomerular size is usually normal. Double contoured capillary walls are rare, due to less mesangial interposition. These differences, however, cannot reliably distinguish MPGN type I from type III by light microscopy.

Immunofluorescent microscopy (44) usually can distinguish type I from III (*Table 32-4*) in that C4 is usually present in type I, and staining for C3, C4, and IgG co-localize on the periphery of the glomerular lobules, the so called "fringe" pattern (*Fig. 32-4*). In MPGN type III, C3 deposition is typically in a mesangial and capillary loop pattern, C4 is rarely present and IgG is present in small amounts in about 50% of biopsies (13).

Ultrastructural studies are essential in order to distinguish MPGN type I from type III (*Table 32-5*). In MGPN type I uranyl-lead stained micrographs typically demonstrate a completely intact GBM without breaks or laminations (*Fig. 32-5*). There is prominent mesangial proliferation with extension of the mesangium at least partially around the glomerular capillaries (interposition) which, combined with the subendothelial deposits, causes narrowing of the capillary lumen.

In MPGN type III, uranyl-lead stains typically demonstrate a complex thickening and irregularity of the GBM. However, the type III lesion, characterized by a discontinuous fragmented, laminated appearance of the GBM (*Table 32-5*), is best seen with methenamine silver stains (*Fig. 32-5*). The distribution of deposits in this lesion has been shown to vary according to the C3 level at the time of the biopsy (*Table 32-5*).

Pathogenesis

Idiopathic MPGN type I is presumed to be secondary to circulating immune complexes composed of [IgG (antibody)-unknown antigen-C3] which deposit in the subendothelial glomerular space, giving rise to a response that includes marked mesangial proliferation. Evidence for this hypothesis is based on the observation that indistinguishable renal pathology is present in MPGN type I secondary to infectious, oncogenic and antigenic causes (*Table 32-1*). In addition, the pattern of complement activation is via the classical complement pathway in at least 40% of patients, favoring the presence of circulating immune complexes.

This hypothesis has been further strengthened by the clear association of MPGN type I and hepatitis C virus (HCV) infection (45). Recent studies have suggested that it is not the chronic antigen exposure, but rather the development of persistent cryoglobulinemia that is the critical pathogenic factor (46). In a large study of Italian patients with HCV and cryoglobulinemia, MPGN type I lesions were found in more than 80% of patients (47). Conversely in a study of French patients with chronic HCV infection renal disease was found only in those with detectable levels of cryoglobulins (48). However, there are no studies that convincingly identify circulating immune complexes in pediatric patients with MPGN type

Figure 32-3

Light microscopic appearance of MPGN type I (a) and type III (b). (a) The glomerulus is enlarged, very cellular, and has a lobulated appearance. The mesangium is markedly expanded. The capillary walls are thickened in appearance and frequently the capillary lumens are obscured by the mesangial expansion. In addition to the increase in mesangial cells, scattered neutrophils are also identified in many of the segments. (b) The glomerulus in type III is less cellular in appearance and there is less expansion of the mesangium. Most of the capillary loops are patent; however, thickened capillary walls are evident (Courtesy of David Witte, M.D.). (See color plate 13)

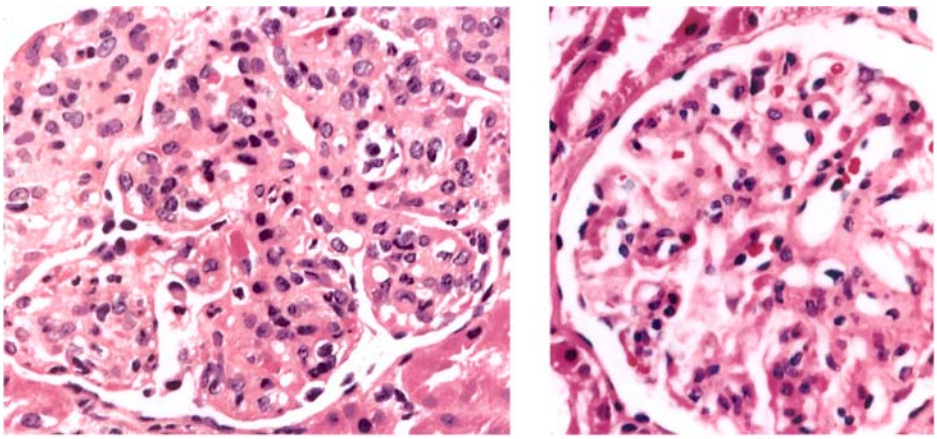


Table 32-4

Comparison of immunofluorescent microscopy in MPGN types I and III

MPGN	C4	C3	C5	IgG
Type I	75%	100%	100%	100%
Type III	0	100%	100%	50%

Adapted from reference (44)

I, and chronic HCV infection was not found retrospectively in a large cohort of pediatric patients with idiopathic type I (49).

Little is known regarding the pathogenesis of MPGN type III. Normal serum C4 levels and the absence of C4 in glomeruli suggest that immune complex formation does not play a major pathogenic role. West and associates (50, 51) have reported a strong association between hypocomplementemia and the presence of paramesangial and subendothelial deposits. Subendothelial deposits in particular were never found in normocomplementemic patients at biopsy (*Table 32-5*). This suggests that NF_t may play a significant role in the development and perpetuation of the ultrastructural changes that define MPGN type III. This hypothesis must be reconciled with other reports which have failed to demonstrate any relationship between the presence of NF, the duration or severity of hypocomplementemia, and either renal survival or disease progression in MPGN type III (31, 52).

Natural History

The natural history of MPGN was described in detail by Cameron et al. (53) in a report comparing the long-term outcome of 69 children and adults with MPGN type I (type III presumably was included in this group) to 35 with DDD (*Fig. 32-6*). The outcome was poor irrespective of type, with 50% losing renal function by 10 years and 90% by 20 years. Similar results have been observed by Habib et al. (5). Cameron et al. (53) noted that MPGN type I progressed more slowly in children than in adults during the first 10 years of follow-up. There are no reports that compare the courses of MPGN types I and III in the absence of effective treatment. Outcome has been shown to be worse in those presenting with nephrotic syndrome, renal insufficiency, and/or with crescents in their initial biopsy.

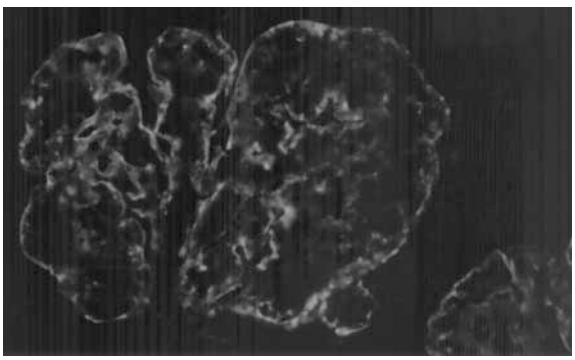
Treatment

The effectiveness of treatment regimens for MPGN has to be judged by the outcome of typically uncontrolled,

retrospective observational studies involving small numbers of patients. In several series, the effects of the treatment regimens were confounded by the inclusion of patients treated with other agents either prior to enrollment or during the study period. Despite these shortcomings, some consensus has emerged (54).

Figure 32-4

Immunofluorescence appearance of MPGN type I. The prominent peripheral distribution of IgG deposits in the capillary walls results in a "fringe" pattern (Courtesy of David Witte, M. D.). (See color plate 14)



Steroids. The treatment regimens that appear to improve outcome in patients with MPGN types I and III have used steroids. The Cincinnati group (31, 55–57) has used "high dose" (2 mg/Kg to a maximum of 80 mg) alternate day prednisone for a minimum of 2 years. Dose reduction thereafter is based on improvement of clinical parameters (urinalysis, serum albumin, serum C3 level) and glomerular morphology (degree of mesangial proliferation, number of open capillary lumens). Long-term, prednisone dose is slowly reduced if there is no evidence for disease reactivation (increase in proteinuria, and/or hematuria and/or decrease in serum C3 level). Since many, if not most patients will continue with at least some degree of proteinuria as a result of chronic glomerular damage, loss of microhematuria appears to be the best clinical indicator of disease remission (56). Most patients have continued on alternate day steroids for at least 5 years and many for much longer periods.

Reports of outcome with this regimen have been encouraging, but continue to be difficult to interpret because of small patient numbers, retrospective analysis of data, lack of a control group and use in some patients of adjunctive therapies. In the most recent report (31) using the same alternate day prednisone regimen, renal survival was 80% at 10 years in those with type I, and 70% in those

Table 32-5

Differences in glomerular ultrastructure in type I and type III MPGN as viewed in uranyl-lead and methenamine-silver stained preparations

	Type I	Type III
Uranyl-lead staining		
Basement membrane	*Intact*	Thickened areas with patchy dense deposits
Methenamine-silver staining		
Basement membrane	*Intact*	*Type III lesion*[a] present with both ↓ [C3] and nl [C3]
Uranyl-lead and methenamine-silver staining		
Subendothelial deposits	*Always present with ↓ [C3]* *Present in 50% with nl [C3]*	*Present in 50% with ↓ [C3]* *Never present with nl [C3]*
Subepithelial paramesangial deposits	*Very rare. Never with nl [C3]. In ~12% with ↓ [C3]*	*Present with ↓*[C3] *and in those with nl [C3] if level has been low in past year*
Subepithelial loop deposits	Frequent, accompany subendothelial deposits	May be present. Not clearly related to [C3]

↓ [C3], serum C3 level low at time of biopsy; nl [C3], serum C3 level normal at the time of biopsy

Note: Correlations of deposits with serum C3 levels are taken from references (4) and (50). Italics indicate key features differentiating types I and III

[a]Type III lesion is a complex lesion of the GBM that appears to originate from several generations of subepithelial and subendothelial deposits forming in conjunction with multiple interruptions of the lamina densa, such that the deposits are partially confluent. Lesions develop a complex laminated appearance because each generation of deposit is covered by new lamina densa-like material (From (31) with permission)

Figure 32-5

Ultrastructural features of MPGN type I and type III. (a) An electron photomicrograph of a glomerulus with MPGN type I. Note the prominent mesangial proliferation and deposits in the mesangium. The capillary walls are thickened due to extensive interposition (*arrow*) in addition to prominent subendothelial deposits (*asterisk*). In this silver-stained preparation, the lamina densa can be clearly identified and is generally intact. (b) The glomerulus with the type III lesion also shows by silver stain extensive mesangial proliferation and deposits. The capillary walls show extensive disruptions of the lamina densa with deposits that are subepithelial, subendothelial, and, at times, completely obscure the lamina densa (Courtesy of David Witte, M. D.).

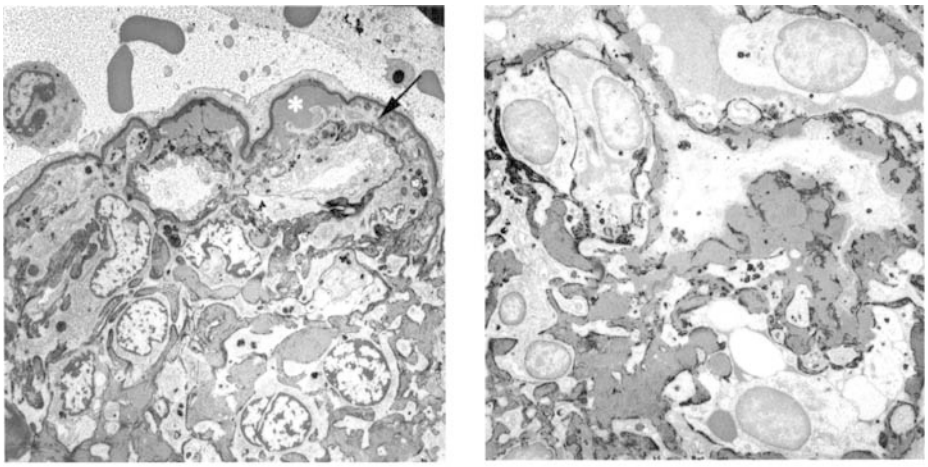
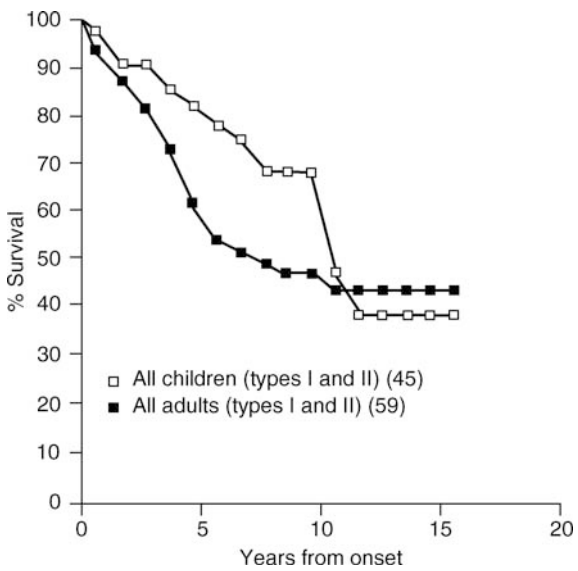


Figure 32-6

Comparison of renal survival between adults and children (From (53) with permission).



with type III. In addition to a lower renal survival, those with type III had at 10 years a significantly greater frequency of proteinuria and hematuria and a lower glomerular filtration rate.

Based on early reports of improved outcome with alternate day prednisone, the International Study of Kidney Disease in Children (ISKDC) sponsored a randomized, double-blinded, placebo-controlled 5-year trial of alternate day prednisone, 40 mg/m^2 versus placebo in 80 children with MPGN and heavy proteinuria (30). The study included 42 patients with MPGN type I and 17 with type III. Treatment failure was defined as an increase in serum creatinine of either $\geq$30% over baseline or $>$0.4 mg/dl. Outcome analysis evaluating patients with MPGN types I and III as a group showed that at last follow-up (mean 5.25 years) 33% of patients treated with prednisone and 58% in the placebo group were treatment failures ($p = 0.07$). The results favored treatment with prednisone.

Similar results were obtained by Mota-Hernandez et al. (58) who treated eight MPGN type I patients with alternate day prednisone and ten with placebo. After an

average follow-up of 6 years, treated patients had stable or improved renal function whereas 4 of 10 placebo-treated patients had progressed to end-stage renal disease. Recently a larger study of some 47 patients with MPGN type I by the Mexico City group reported similar findings (59).

Additional uncontrolled reports have favored the use of alternate day prednisone (29, 60–62). The observation that in the absence of treatment, the disease in those not nephrotic at presentation progresses more slowly (5, 53) has led to the recommendation that steroids be withheld in these patients (63) or used in a low dose (61, 62). However, others have shown significantly better outcome in patients treated with prednisone early in the course of the disease regardless of severity (29, 56).

Long term follow-up data on patients with MPGN type III has been published by a number of centers (31–33, 64). The report by Iitaka et al. (64) is by far the most promising with 100% renal survival after 16 years of follow-up. However, it is uncertain if the universally good outcome was due to early identification and aggressive therapy, or to an unknown factor which moderates the course of the disease in the Japanese population. The outcomes for patients with type III reported by Little et al. (33), Braun et al. (31) and Schwertz et al. (32) is less encouraging with renal survivals of 65% at 5 years, 70% at 10 years and 50% at 15 years, respectively.

Other therapies. Other controlled prospective treatment trials have shown no long-term benefit with the anti-platelet agent, dipyridamole (65) or with cyclophosphamide, warfarin and dipyridamole (66). Uncontrolled reports have suggested no or limited benefit from treatment with other cytotoxic agents, antimalarials, or anticoagulants. Cyclosporine was reported to improve the outcome of 8 patients with MPGN resistant to steroids and of 17 of 18 adult patients with idiopathic MPGN resistant to treatment with either steroids or anti-platelet agents (67). Two patients resistant to steroids were reported to improve after addition of tacrolimus (68). There have been several recent reports suggesting that mycophenolate may be of some benefit in the treatment of MPGN type I (69–71). While encouraging, these results should be viewed with caution as the follow-up times were brief, the numbers of patients small, and histologic and serologic characterization of the patient population was limited. There have been a number of larger treatment trials in adult populations with MPGN type I secondary to HCV (72). These studies, using either anti-viral agents combined with plasmapheresis or immunodepletion with anti-CD20 antibody, have shown promise in halting progression of disease. However, given the low frequency of secondary forms of MPGN type I in children, the rational for the use of these approaches in pediatrics is uncertain.

Disease Recurrence in Renal Transplant Recipients

MPGN type I frequently recurs after kidney transplantation (73). Lien and Scott (74) collated 61 reports of recurrence of MPGN (not separated into type I or III) in 218 renal transplants indicating the rate of recurrence to be about 30%. There is limited data on recurrence in type III; however the report by Little et al. suggests that the recurrence rates of type I and type III are similar, 36 and 34% respectively (33). Of those with recurrent type I, graft half-life was significantly shortened with graft loss from recurrence in more than half of patients (73). It is possible that the chance for recurrence may be decreased by the use of cyclosporine following transplantation (75). Once a recurrence manifests, however, the approach to treatment is unclear. Case reports have suggested that graft survival may be improved by the addition of cyclophosphamide to immunosuppressive drugs which include a calcineurin inhibitor (74, 76).

Dense Deposit Disease

Epidemiology and Genetics

Dense deposit disease (DDD) (MPGN type II) is the least frequent of the three MPGN types (*Table 32-2*). It constitutes 19–34% of the cases in whites and 5% in Japanese. DDD affects males and females equally and is primarily a disease of children and young adults with a median age at onset of approximately ten years (53, 54, 77, 78). In most patients there is no evidence for a genetic basis for the disease. Two families in which only one of a pair of identical twins has the disease have been reported (79). A small subset of patients with DDD has been identified to have mutations in the key complement regulator, Factor H, which results in un-inhibited C3 activation (80) Uninhibited C3 activation and glomerular pathology similar to DDD has been reproduced in mice and piglets with Factor H deficiency (reviewed in (11)).

A recently performed genotype-phenotype study suggested that there was co-segregation of specific allele

variants of the genes encoding for Factor H (*CFH*) and Factor H related protein 5 (*CFHR-5*) and DDD (81). Furthermore, the common Factor H variant H402Y, which has recently been identified as a susceptibility factor for age-related macular degeneration, is also found at higher prevalence in patients with DDD (70%) compared to healthy controls (29%) (82).

Presentation and Clinical Manifestation

Symptoms and signs at presentation can not distinguish DDD from MPGN types I or III. An upper airway infection or immunization may precede the clinical onset in about half of patients, suggesting that biological stress factors may initiate renal injury. Microscopic hematuria is always present and proteinuria is common. Nephrotic syndrome at onset is seen in 50% (32) and has been associated with a poor prognosis (83, 84). In 20% of patients onset is associated with macroscopic hematuria and 30% have hypertension (32). Occasionally, patients present with renal failure and have a rapidly progressive course (85, 86).

The clinical course frequently is characterized by persistent or evolving nephrotic syndrome and increasingly severe hypertension (83, 85, 87). Conversely, some patients may have periods after the onset of disease when their nephritis is "silent" (85); their urinary abnormalities completely disappear and only hypocomplementemia remains. Signs of overt nephritis may recur, most commonly with intercurrent infections, and these findings can persist.

In addition to renal injury, there is evidence of complement dependent injury in other organ systems such as the eye (82, 88), spleen (89, 90), and fat tissue (91) indicating the systemic nature of this disorder. Dense deposits similar to those found in the GBM can develop in the basement membranes of the retina which resemble those seen in adults with age-related macular degeneration (82, 88). Furthermore, dense deposits occur within the basement membrane of the sinusoids of the spleen (89, 90). It is of interest that these spleen and eye membranes, like the GBM, are unique in that they are bathed in plasma.

Partial lipodystrophy (PLD), characterized by the loss of fat tissue especially in the face and upper body, is associated with NFa, low C3, and subsequent development of DDD. A pathogenetic link between NFa and PLD is based on the fact that adipocytes produce key components of the alternative complement pathway, including Factor D, C3 and Factor B. In vitro, NFa containing sera results in adipocyte lysis associated with increased fluid-phase terminal complement complex formation (91). PLD may be present for years before clinical evidence for nephritis develops (92). Gerth et al. reported a female with PLD at age 11 who developed glomerulonephritis (DDD) at age 21, and eventually macular degeneration at age 54 (93). Recently a familial form of PLD also associated with DDD was reported in a patient with Dunnigan-Kobberling syndrome (94–96).

Pathology

In most cases, by light microscopy the glomeruli are uniformly hypercellular with increased mesangial matrix and the number of open capillary lumens is commensurately reduced. In contrast to MPGN type I, the proliferation is rarely great enough to increase glomerular size. The diagnosis of DDD can occasionally be made by light microscopy of silver stained preparations; the basement membrane is thickened and at sites of the intramembranous deposits, argyrophilia is lost. Deposits are located along the GBM, the Bowman's capsule, and the tubular basement membrane.

By immunofluorescence, IgG, IgA, IgM, and C4 are rarely found in glomerular deposits indicating that the classical pathway of complement is not activated. C3 deposits are abundant. Kim et al. (97) found C3 to be present along the margin but not within the central portion of the dense deposits in the GBM, giving a double linear appearance (railroad tracks). They also observed C3 deposits within the mesangium outlining circular structures that represent shed fragments of the basement membrane (mesangial rings). The railroad tracks are visualized with labeled anti-C3c and -C3d, indicating that they are composed of C3b, iC3b, and C3dg, whereas the paramesangial deposits stain only with anti-C3c, indicating that they are formed from C3c deposited from the circulation (98).

The diagnostic intramembranous deposits are best seen by electron microscopy of uranyl lead stained specimens (*Fig. 32-7*). They usually occupy only the lamina densa but occasionally may be present only in the lamina interna (99). The deposits may be discontinuous. Similar deposits may also be found in Bowman's capsule and in the basement membranes of isolated groups of tubules. If searched for, subepithelial deposits can be found on the parts of the basement membrane that overlay the mesangium (paramesangial) if the patient was biopsied when hypocomplementemic (100). Occasionally, hump-like subepithelial deposits are present on the capillary loops.

Pathogenesis

The pathophysiology of DDD *in humans* is still poorly understood. However, excessive activation of C3 by un-regulated C3 convertase, C3bBb, appears crucial for the pathogenesis (11, 12, 101). DDD is most often associated with NFa, an IgG autoantibody which binds to a neoantigen on the C3 convertase, C3bBb, making it resistant to inactivation by Factor H, resulting in continuous C3 activation. NFa is present in 55% of adult and 80% of child onset DDD (52). Whereas atypical HUS has commonly been observed in humans with deficient or dysfunctional Factor H, this association with DDD has only been rarely reported (11, 80, 102). Licht et al. described two siblings with DDD and mutation of the factor H gene resulting in functionally defective factor H protein. Both siblings were also positive for C3NeF (80). Meri et al. reported a patient with factor H autoantibody and hypocomplementemic DDD (103). Patients with Marder's disease have MPGN and a C3 mutation that renders the C3b molecule unable to bind factor H, resulting in permanent complement activation (104). Observations in a strain of Factor H deficient piglets that developed glomerular lesions similar to DDD support the hypothesis that unregulated C3 activation is important to the pathogenesis. In addition, Factor H deficient mice have also been shown to develop glomerular lesions similar DDD (105). In this model glomerular lesions were prevented when C3 activation was blocked by crossing Factor H deficient mice with Factor B deficient animals. Further observations in mice have shown that Factor I is required for GBM C3 deposition, confirming that C3b metabolites target the GBM (23). Last, in mouse kidney transplant studies, C3 deposited on the GBM was shown to be derived from plasma (11). Taken together, these observations support the hypothesis that in the pathogenesis of DDD C3 metabolites, formed from Factor I induced proteolytic breakdown of C3b to iC3b, C3dg, and C3c, are required, and deposit in the GBM. Currently however, it is not known if the deposition of C3 breakdown fragments on the GBM is responsible for formation of the dense deposits or if the dense deposits precede complement deposition. Finally, it is not understood why some individuals with un-regulated alternative pathway activation develop DDD while others develop atypical HUS or other glomerular lesions.

Natural Course

Children with DDD have a poor prognosis and half progress to end stage renal disease (ESRD) within 10 years with the degree of proteinuria and hypertension being negative predictors (32, 106). Interestingly, Little et al. recently reported that when comparing different MPGN types, the severity of the glomerular injury on the initial renal biopsy and presentation with nephrotic syndrome, rather than the MPGN type determined renal survival (33).

Treatment

Treatment options for patients with DDD are scarce and are primarily supportive in nature. Anti-platelet, anti-coagulant, and immunosuppressive drugs, usually in combination with corticosteroids, have been used but there is no clear evidence that such regimens are effective. Although not validated by a controlled trial (30), high dose alternate day prednisone is currently the most widely used treatment (107). However, response to treatment with this regimen is neither consistent nor unpredictable. In a series of 14 patients who have received this regimen, the disease in five is in remission; serial renal biopsies showed improvement in these patients to the extent that the dense deposit in the GBM was partially or completely lost (108). Experience with other immune suppressants including calcineurin inhibitors (109) and mycophenolate is either limited or not available.

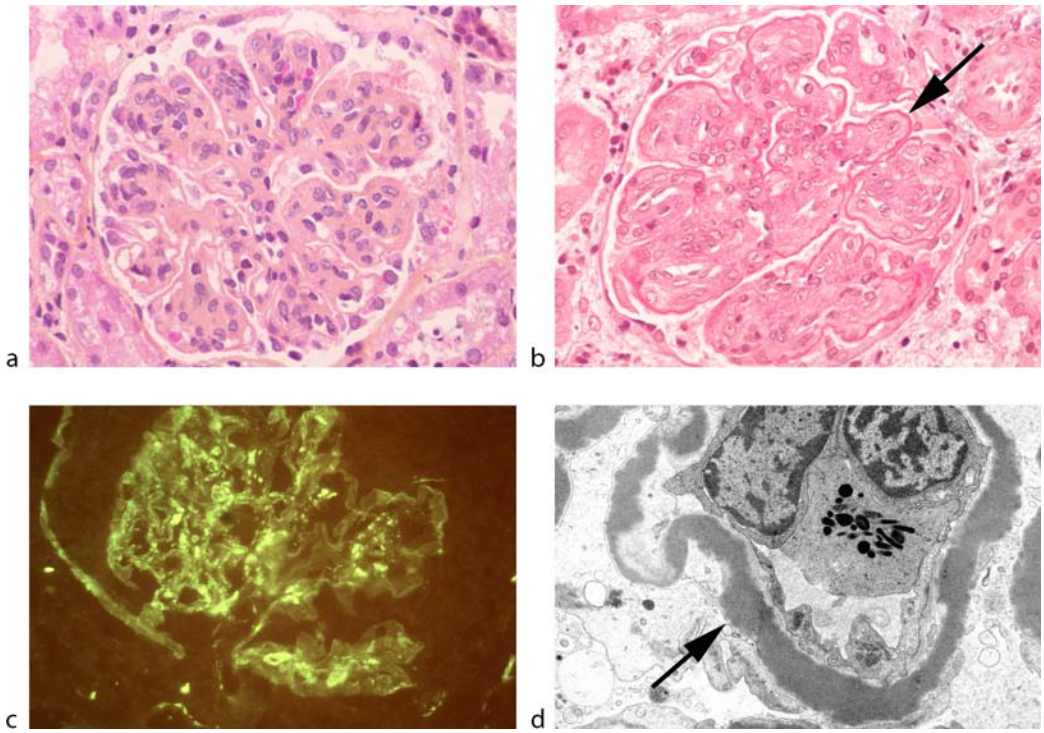
For patients with a genetic defect in the regulation of the alternative pathway, replacement of the abnormal protein by infusion of plasma, or the purified or recombinant protein has been successful. There are isolated case reports of the successful treatment of DDD with plasmapheresis (110–112) which reduces circulating NF. Novel therapies such as anti-CD20 antibody, humanized monoclonal anti-C5 IgG antibody, sulodexide (a combination of low molecular weight heparin and dermatan sulfate) with profibrinoloytic and antithrombotic activity, have been proposed (78). However their utility in the treatment of DDD is currently untested.

Disease Recurrence in Renal Transplant Recipients

The histological recurrence rate of DDD with occurrence of dense deposits within the GBM after renal transplantation is high, ranging from 50–100% (113, 114). Recurrence has been reported as early as 12 days posttransplantation (114). Histological recurrence can precede clinical recurrence of glomerulonephritis. It is uncertain, however, if the presence of dense deposits in the absence of clinical signs of active nephritis has an impact on overall graft survival. The development of

Figure 32-7

Pathology of dense deposit disease: (a) By light microscopy, glomeruli show accentuation of lobules with hypercellularity of mesangial regions and thickened capillary loops (hematoxylin-phloxine-safranin stain, original 600) (b) The capillary loop basement membranes often show a ribbon-like staining pattern (*arrow*) (Masson trichrome, original 600) (c) By immunofluorescence microscopy, glomeruli show mesangial and capillary loop deposition of C3, but immunoglobulins are usually negative (original × 600). By electron microscopy, capillary loop basement membranes show a characteristic electron dense transformation (*arrow*) that can vary from intramembranous to transmembranous and involve different loops to different degrees (original × 10,000) (Courtesy of Paul Thorner, M. D.). (See color plate 15)



dense deposits prior to C3 deposition within the GBM was described in 3 patients (114). A number of investigators have correlated disease recurrence with persistent or recurrent hypocomplementemia (115–117), whereas others could find no correlation with either low C3 levels or the presence of NF (118, 119). Published reports of graft loss due to recurrent DDD varies widely from 0–100% (83, 116, 117, 120–122). The North American Pediatric Renal Transplant Cooperative Study (NAPRTCS) transplantation database analyzed the long-term consequences of disease recurrence: five-year graft survival of DDD patients was worse than of the other patients in the database (50.0% vs. 74.3%). Five-year graft survival of living related donor grafts was better than with deceased donor grafts (65.9% vs. 34.1%). The primary cause of graft failure in 14.7% of patients was disease recurrence. While no correlation was found for pre- or posttransplant C3 levels and disease recurrence or graft failure, there was a strong association between the degree of proteinuria and disease recurrence (123). Little et al. (33) recently reported that the only factors predictive of recurrence risk were younger age at initial diagnosis and presence of cellular crescents on the initial native kidney biopsy.

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33 Membranous Nephropathy in the Pediatric Population

J. Ashley Jefferson · William G. Couser

Introduction

Membranous nephropathy is a disease that typically presents with nephrotic syndrome, and although more common in the adult population, has been described in children, and even in the newborn. The disease is defined by the presence of subepithelial immune deposits (between the glomerular basement membrane (GBM) and the podocyte) with subsequent expansion of the GBM resulting in a membrane-like (membranous) thickening of the glomerular capillary wall. The subepithelial immune complexes which are separated from the circulation by the GBM, do not cause inflammation or recruitment of circulating leukocytes (nephritis), but rather lead to complement dependent podocyte injury and nephrotic syndrome.

Epidemiology

Membranous nephropathy is the most common cause of nephrotic syndrome in older adults (1), but is an uncommon condition in children, with less than 200 cases described (see *Table 33-1*). In adults, the disease is mostly idiopathic, although about 20% of cases are associated with clinical conditions such as cancer, infections, autoimmune disease and drugs. In childhood, MN is more often due to secondary causes such as systemic lupus erythematosis, hepatitis B, drugs and toxins. By contrast, idiopathic MN is a relatively rare cause of nephrotic syndrome in children. Single center studies of pediatric biopsy series report that around 5% (range 1–7%) of renal biopsies for nephrotic syndrome were due to MN (2–4, 8, 9) with an estimated incidence of less than 1 per million childhood population (10). It should be remembered however, that the majority of children with nephrotic syndrome have minimal change disease and do not undergo renal biopsy until they have failed a course of steroid therapy, and thus, the biopsy data greatly overestimates the incidence of MN as a cause of nephrotic syndrome. The strong male predominance in adult patients is not found in children, and the disease can occur in all ethnic groups. Age at presentation can vary from neonatal to young adulthood, but the mean age is typically 8–10 years.

Etiology and Pathogenesis

Mechanisms of Immune Complex Formation in the Subepithelial Space

Membranous nephropathy is characterized by the development of immune complexes in the subepithelial space on the outer surface of the glomerular basement membrane. The immune deposits consist of immunoglobulin (IgG, predominantly IgG4), complement components (C3, C5b-9), and presumably antigen, which in most cases has not been identified. The subepithelial space is separated from the circulation by a layer of endothelial cells and the GBM, and this provides an effective barrier to the passage of pre-formed circulating immune complexes. The presence of immune complexes in the subepithelial space therefore suggests either *in situ* formation involving podocyte antigens or planted exogenous antigens, or the reassembly of circulating immune complexes that have deposited in subendothelial areas, dissociated and crossed the GBM, to reform *in situ* beneath the podocytes (reviewed in (11)). However, circulating immune complexes are rarely detected in idiopathic MN (12) and, in an *ex vivo* model, the isolated perfused kidney, subepithelial immune complexes of Heymann nephritis (see below) were produced with nephritogenic anti-podocyte antibodies in the absence of circulating antigen (13). The most likely scenario in idiopathic MN, is that autologous antibodies react to an endogenous antigen on the podocyte foot process in a mechanism similar to the Heymann nephritis model (14). In this model a glomerular lesion indistinguishable from human membranous nephropathy can be produced by immunization of rats with extracts of proximal tubular brush border (active Heymann nephritis) or injection of heterologous anti-serum or IgG to a crude tubular preparation called

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Table 33-1

Series of idiopathic membranous nephropathy in pediatric patients (modified from (2))

References	N	Follow-up period (yr)	Mean age at presentation (yr)	Age range	M:F	Biopsy incidence (%)	Nephrotic at presentation (%)	CKD (%)
(3)	50	1–10	NA	8 mo–14 yr	38:12	3.7	62	10
(4)	14	5.5	9	2–15 yr	6:8	6.7	79	29
(5)	22	4.7	12	11 mo–19.9 yr	11:11	5.7	77	37
(6)	14	6.4	10.5	3.5–14	9:5	1.3	79	21
(7)	12	5.9	7.7	2.9–15.8 yr	8:4	3	25	0
(8)	38	7.5 (SMGN); 12.4 (GMGN)	7.6	1.5–16 yr	25:13	2.4	24	0
(9)	19	28.5	9.5	1.7–14.9	9:10	1	89.5	15
(2)	13	3.5	9.6	4–17 yr	6:7	2.8	38	23

Abbreviations: GMGN, global membranous glomerulonephritis; HTN, hypertension; NA, not available; SMGN, segmental membranous glomerulonephritis

Fx1A (passive Heymann nephritis). This results in the development of subepithelial deposits of IgG, C3 and C5b-9 associated with heavy proteinuria. The immune deposits form *in situ* due to the binding of circulating IgG to antigenic epitopes expressed on the foot processes of the glomerular epithelial cell. Immune complexes may also form *in situ* by the reaction of antibody to an exogenous antigen which has become trapped in the subepithelial space (planted antigen). This mechanism may explain the association of membranous nephropathy with some infections and certain drugs (*Table 33-2*).

Potential Podocyte Antigens

In Heymann nephritis the antibodies are targeted against the Heymann nephritis antigenic complex (HNAC) consisting of two proteins, megalin and receptor associated protein (RAP) (15). Megalin is a 600 kDa molecular weight glycoprotein belonging to the LDL receptor family, which acts as a multi-functional receptor facilitating endocytosis. It is expressed in both the brush border of proximal tubular cells (16) and in the clathrin-coated pits on the sole of podocyte foot processes (17). Megalin forms a heterodimeric complex with RAP, and several epitopes on this complex seem to be involved in the formation of the immune deposits (18). Notably, in animal models, active immunization with megalin alone or passive immunization with anti-megalin antibody result in the accumulation of immune deposits in the subepithelial space, but no C3 or C5b-9 deposition is seen and no proteinuria occurs (19). We now recognize that other antibodies in the anti-Fx1A fraction inhibit complement regulatory proteins on the podocyte leading to local activation of the alternate complement pathway. Despite the similarities between Heymann nephritis and human disease, megalin, although expressed in human tubular cells, is not expressed by any human glomerular cells, and therefore, cannot be the pathogenic antigen responsible for human MN. However, recent studies by Salant et al. have identified antibodies to phospholipase A2 receptor (PLA2R) in a majority of patients with active MN and found correlations between this antibody and response to therapy or relapse of the disease (133). PLA2R is a transmembrane podocyte glycoprotein with a similar distribution to megalin and co-localizes with IgG in the subepithelial deposits in human MN.

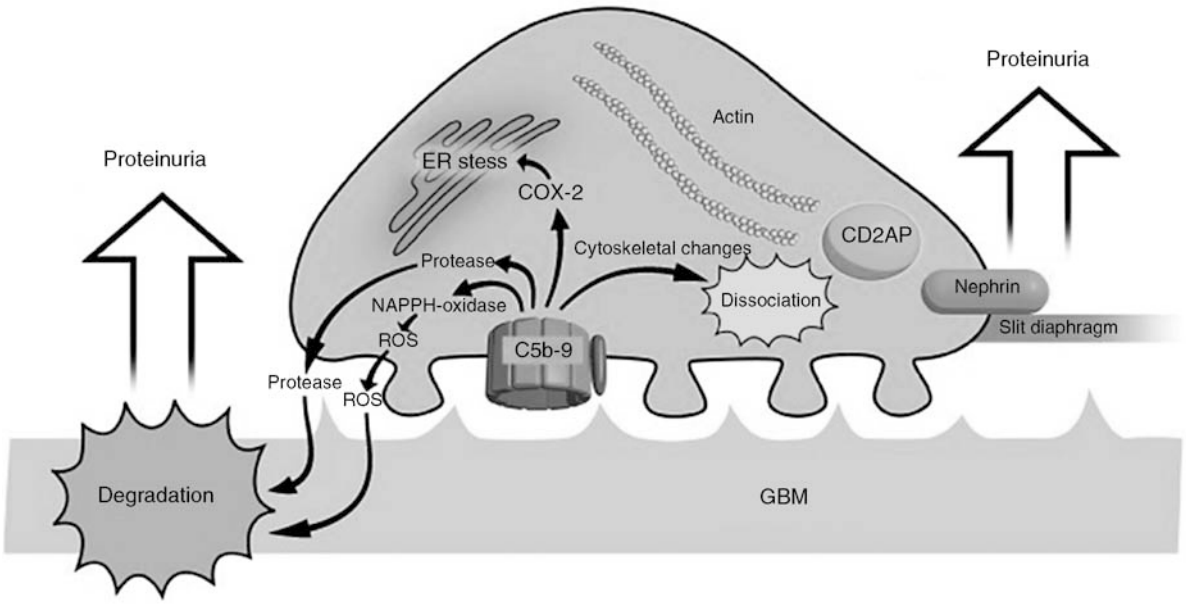
Two other animal models of MN have been produced by antibodies targeting dipeptidyl peptidase IV (DPPIV) in rats (20) and neutral endopeptidase (NEP) in rabbits (21). Importantly, both of these antigens are expressed on the human podocyte (as well as human glomerular endothelial cells). Subsequently, NEP was identified as the target antigen in several cases of neonatal MN (see below) (22).

Possible Antigens in Secondary Membranous Nephropathy

A range of antigens have been detected in the subepithelial immune deposits in the secondary forms of MN. These include tumor antigens (carcinoembryonic antigen, prostate specific antigen, others), thyroglobulin, infection antigens (hepatitis B, hepatitis C, helicobacter pylori, syphilis) and DNA associated antigens (dsDNA, histones, nucleosomes) (23). Although it has been postulated that

Figure 33-1

Pathogenesis of membranous nephropathy. Immune complex formation in the subepithelial space is associated with activation of complement, predominantly via the alternate pathway leading to the insertion of C5b-9 into the podocyte cell membrane. This results in podocyte cytoskeletal injury and alterations in slit diaphragm proteins leading to proteinuria. The release of reactive oxygen species (ROS) and proteases leads to remodeling of the glomerular basement membrane (GBM).



these antigens are pathogenic, they may merely reflect non-specific trapping of proteins due to the increased permeability of the glomerular endothelium and GBM.

Mediation of Injury: The Role of Complement and the Alternate Pathway

In MN, C3 and C5b-9, the membrane attack complex (MAC), can be detected in supepithelial immune deposits (24). The C5b-9 inserts into the podocyte membrane and causes cell activation with release of reactive oxygen species, proteases and eicosanoids which damage the GBM. C5b-9 is subsequently internalized by the cell, transported in multivesicular bodies to the luminal surface and exocytosed into the urinary space (25). Based on studies in rodent models, the urinary excretion of C5b-9 correlates with the period of active immune complex deposition (26, 27) Notably, generalized complement depletion with cobra venom factor (28) or specific C6 depletion with anti-C6 antibody (29) abolishes the proteinuria in passive Heymann nephritis (PHN). Similarly, C6 deficient rats are protected from Heymann nephritis (30) or the development of proteinuria is significantly delayed (31).

It had long been considered that the immune deposits in MN activate complement via the classical pathway with the production of C5b-9. Indeed, deposition of components of the MAC are detected in the subepithelial deposits in both Heymann nephritis (32) and human membranous nephropathy (24) However, in most cases of idiopathic MN, the predominant immunoglobulin deposited is IgG4 (which has little C1q fixing ability) (33). Similarly, there is minimal, if any, detectable deposition of C1q and C4 suggesting that the classical pathway is not the predominant means of complement activation in this disease (34).

Evidence in experimental MN suggests that inhibition of complement regulatory proteins allows complement activation to occur and C5b-9 to be assembled via the alternate pathway. Thus, proteinuria in the PHN model of MN cannot be induced by IgG antibody unless blocking antibody to Crry, the rodent analog of the human podocyte complement regulatory protein CR1, is also present. Of course, complement regulatory activity may also be inhibited by other mechanisms, and a complete

absence of the entire CR1 protein has been demonstrated in some forms of glomerulonephritis including MN (35).

Podocyte Response to Injury & Mechanisms of Proteinuria

Of the three cell types in the glomerulus, MN is a complement dependent disease of the podocyte. Following C5b-9 insertion into the lipid bilayer of the podocyte cell membrane, instead of cell lysis, a series of signaling events result in cell activation and changes in podocyte structure and function.

Extracellular Matrix and Glomerular Basement Membrane

The GBM is an acellular structure synthesized by the podocyte and the glomerular endothelial cell, and it is the GBM thickening that gives rise to the term "membranous" nephropathy. The extension of the GBM between the subepithelial immune deposits also leads to the prototypical "spike" formation seen with silver methenamine staining. The GBM matrix that accumulates in MN consists of normal GBM components that are produced by the podocyte, in particular, type IV collage and laminins (36, 37). Notably, this matrix accumulation occurs in the setting of an increase in matrix degrading proteases, suggesting a marked increase in matrix production or an increase in protease inhibitors (for example, tissue inhibitors of matrix metalloproteinases (TIMPs)). A marked upregulation in the production of laminins and type IV collagen *in vitro* has been confirmed in podocytes under C5b-9 attack (38). Transforming growth factor beta (TGF-β) plays a key role in the accumulation of matrix and a marked increase in the TGF-β2 isoform and its receptor has also been shown in podocytes in experimental MN (39).

Production of Oxidants, Proteases and Eicosanoids

The insertion of C5b-9 into the podocyte membrane leads to the production of reactive oxygen species (ROS). An increase in hydrogen peroxide (via NADPH oxidoreductase) (40) and superoxide ion (via xanthine oxidase) (41) have been described in the PHN model. ROS may directly injure the GBM, potentiate the effects of proteases. or cause further injury by lipid peroxidation of the podocyte cell membrane (reviewed in (42)). In the PHN model, the administration of scavengers of ROS (deferioxamine (43), DMTU (44)), inhibitors of lipid peroxidation (45) or inhibitors of xanthine oxidase (41) have all been shown to markedly decrease urine protein excretion.

Proteases have also been proposed to mediate some of the GBM injury in MN. In PHN, increased gelatinase activity (46) and enhanced expression of matrix metalloproteinase-9 (MMP-9) prior to the onset of proteinuria have been documented (47). There is also an increase in the amount of proinflammatory eicosanoids with increased inducible cyclooxygenase (COX2) (48), leukotriene B4 (49), prostaglandin E2 and thromboxane B2 (50, 51).

Reduction in Podocyte Number (Podocytopenia)

The podocyte has a limited ability to proliferate, and therefore, any process leading to podocyte loss (apoptosis, detachment) will result in a reduction in podocyte number. This podocytopenia has been associated with the development of glomerulosclerosis and is associated with progressive renal dysfunction in a range of glomerular diseases (52). The paucity of podocytes on the outer surface of the capillary loop may result in loss of support for the capillary loop and further podocyte injury from mechanical stress. In addition, the resulting uncovered areas of GBM promote both protein traffic across the glomerular filtration barrier and the development of glomerulosclerosis (53).

a. *Cell detachment.* Podocytes adhere to the outer side of the GBM due to interactions between GBM matrix proteins and the podocyte foot processes (notably via α3β1 integrins and α-dystroglycan). The detachment of podocytes from the GBM with a resultant decrease in podocyte cell number has been described in MN and may result in focal areas of increased protein permeability, (54, 55) in addition to promoting the development of glomerulosclerosis (53). The cells detach due to a combination of cytoskeletal changes in the podocyte and alterations in GBM structure which interfere with cell matrix interaction (56, 57) Once detached into the urinary space, the podocytes may subsequently be excreted and detected in the urine. Notably many of these cells are viable and can be cultured *in vitro* (58).
b. *Podocyte apoptosis.* Podocyte apoptosis has been difficult to demonstrate in MN, but this may be because

apoptotic cells may quickly detach into the urinary space (59). An imbalance between pro-apoptotic (e.g., ROS, TGFβ) and pro-survival factors (e.g., vascular endothelial growth factor) may mediate podocyte apoptosis (reviewed in (60)).

c. *Failure of podocyte proliferation.* A key characteristic of the podocyte is its limited ability to proliferate, and as podocyte loss is not readily replaced, this may lead to podocytopenia. In MN, this anti-proliferative phenotype may be augmented by upregulation of cyclin kinases inhibitors (p21 and p27) which inhibit progression through the cell cycle (59). Podocytes become arrested at the G2/M phase preventing cytokinesis (cell division) and bi-nucleated podocytes may be detected (61). Attempts at cell division may also result in weakening of attachment to the GBM, and loss of podocytes into the urinary space. C5b-9 has also been shown to induce DNA damage in podocytes further impairing the proliferative response (62).

Alterations in Podocyte Slit Diaphragm

The slit diaphragm between adjacent podocyte foot processes is now recognized as one of the major impediments to protein permeability across the glomerular capillary wall. Nephrin is a major component of the slit diaphragm, bridging the filtration slit, and is linked to the actin cytoskeleton by CD2AP and podocin. Studies have shown a reduction and redistribution of nephrin, and a dissociation from both podocin (63) and the actin cytoskeleton (64) in the PHN model of MN. Notably, this disruption in the slit diaphragm can be detected at the onset of proteinuria, prior to the development of foot process effacement (65).

Mechanisms of Progressive Renal Dysfunction

The podocyte and GBM abnormalities above can largely explain the marked proteinuria that typically develops in MN. The loss of renal function, however, is only partly explained by podocyte injury leading to glomerulosclerosis, but also reflects mechanisms leading to tubulointerstitial fibrosis. The rate of decline in renal function in MN correlates with the degree of proteinuria, and although this may reflect greater glomerular injury, there is increasing evidence that proteinuria itself may be injurious to tubular epithelial cells (reviewed in (66)). Proteinuria may be accompanied by intra-tubular complement activation leading to C5b-9 mediated injury to tubular epithelial cells and interstitial damage. Although most complement proteins are too big to be filtered at the glomerulus, tubular epithelial cells are able to synthesize complement components and may become activators of the alternate pathway (67). Proximal tubular cells only weakly express the complement regulatory protein CD59 on their apical membrane (68). Notably, rats that are deficient in C6 are protected from tubulointerstitial injury in several models of glomerular disease including the remnant kidney (69).

Pathology

Light Microscopy

At early stages, the glomeruli and interstitium look essentially normal, and MN may be impossible to differentiate from minimal change disease by light microscopy alone. As the disease progresses, the pathognomonic thickening of capillary loops becomes evident due to the accumulation of sub-epithelial immune complexes and the deposition of new basement membrane material by the podocyte (*Fig. 33-2b*). Staining with silver methenamine may reveal spikes representing basement membrane material projecting between the immune deposits (*Fig. 33-2c* and *d*). Glomerular cellularity is typically normal. The presence of mesangial hypercellularity or leukocyte infiltration (70) suggests a secondary form of MN. In some patients with heavy proteinuria and progressive disease, a reduction in podocyte number, partly due to detachment of podocytes into the urinary space, may lead to areas of denuded GBM, glomerular hypertrophy, attachment to Bowman's capsule, and subsequent capillary collapse, with an appearance similar to idiopathic focal segmental glomerulosclerosis (FSGS) (53, 71).

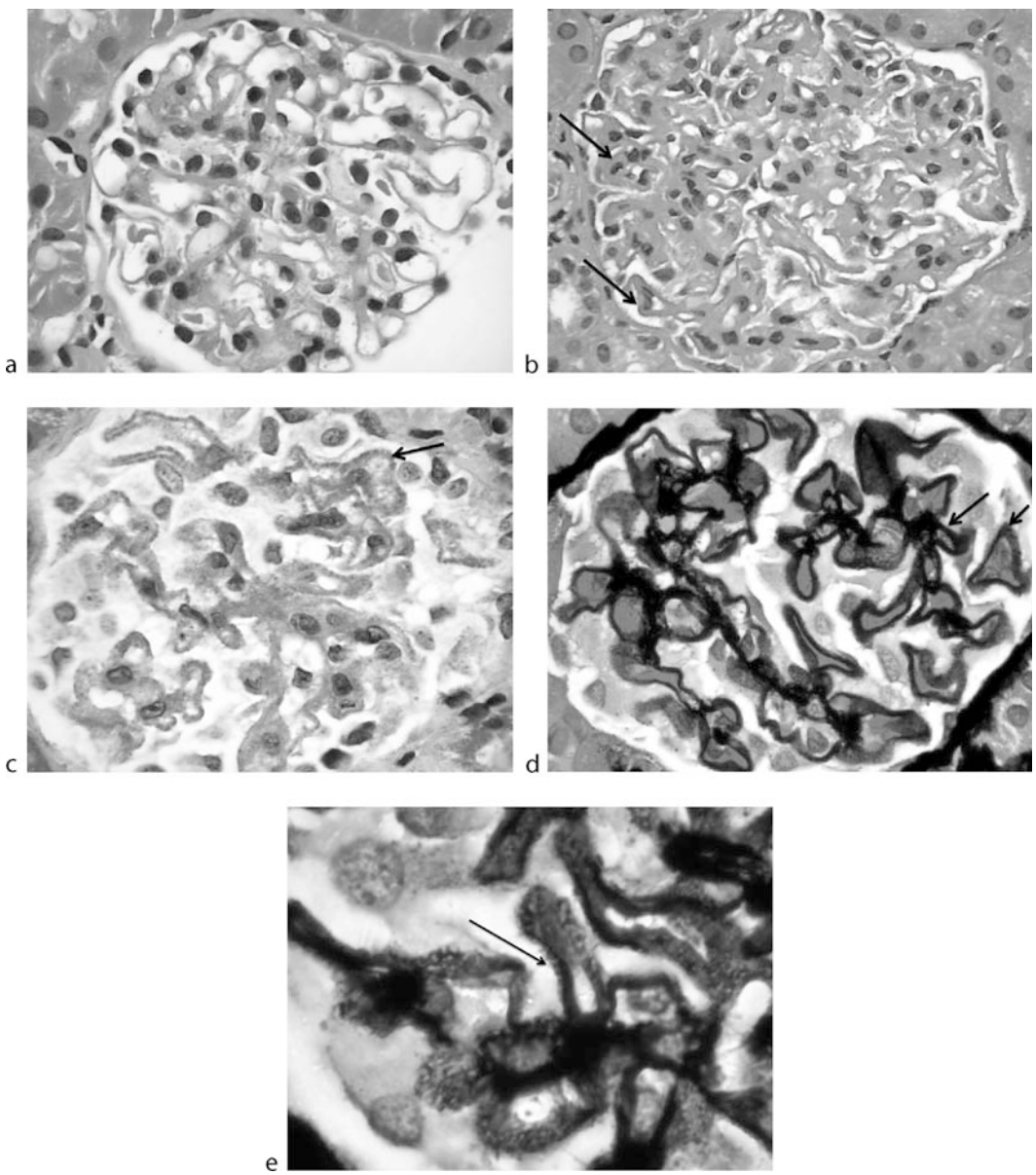
Interstitial changes are not specific to membranous nephropathy, and as in other nephrotic diseases, correlate better with renal function and prognosis than the glomerular abnormalities. Interstitial infiltrates consist of T cells, macrophages and B cells, and are typically associated with areas of tubular degeneration and interstitial fibrosis (mostly Type I collagen).

Immunofluorescence

Immunofluorescence reveals finely granular deposits of IgG (predominantly IgG4) in a subepithelial distribution on the outer surface of the capillary basement membrane (*Fig. 33-3*). Staining for IgA or IgM is negative, but if

Figure 33-2

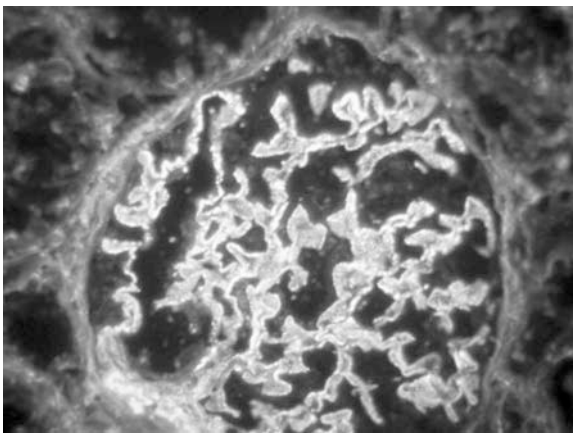
Light microscopy in a 12-year-old girl with membranous nephropathy and nephrotic syndrome. (a) Normal control. A normocellular glomerulus from an age matched control has thin, delicate glomerular capillary walls. Note that early membranous nephropathy may appear normal by light microscopy (hematoxalyn and eosin; x 400). (b) Membranous nephropathy. The normocellular glomerulus has relatively preserved architecture with only slight mesangial expansion. There is diffuse thickening of the glomerular capillary wall (arrows), that imparts a ''stiff'' appearance (hematoxalyn and eosin; x 400). (c) Numerous small subepithelial deposits (arrow), which appear as red dots studding the basement membrane, are highlighted by histochemical stains that differentiate the basement membrane from other proteins (masson trichrome; x 500). (d) The glomerular basement membranes are thick and mottled (arrows) as a result of small peripheral projections (methenamine silver-hematoxalyn; x 400). (e) ''Spikes'' project from the basement membrane (arrow), separating and surrounding the deposits of nonargyrophilic material that contribute to the membrane thickness (methenamine silver-hematoxalyn; x 1,000). (Generously provided by Dr. Laura Finn, Renal Pathologist, Children's Hospital, University of Washington, Seattle, WA.) (See color plate 16)



present suggests a secondary form of MN such as lupus. Complement C3 is present in about 50% of adult patients and usually reflects staining for C3c, a breakdown product of C3b that is rapidly cleared. Consequently, the presence C3 staining may suggest active, ongoing immune deposit formation and complement activation (72). Notably, C1q and C4 are typically absent in keeping activation of complement through the alternate pathway.

Figure 33-3

Immunofluorescence in membranous nephropathy. Immune complex deposits are detected as intense fine granular staining along the outer surface of the capillary loops (fluorescein conjugated anti-IgG; x 400). (See color plate 17)



Electron Microscopy

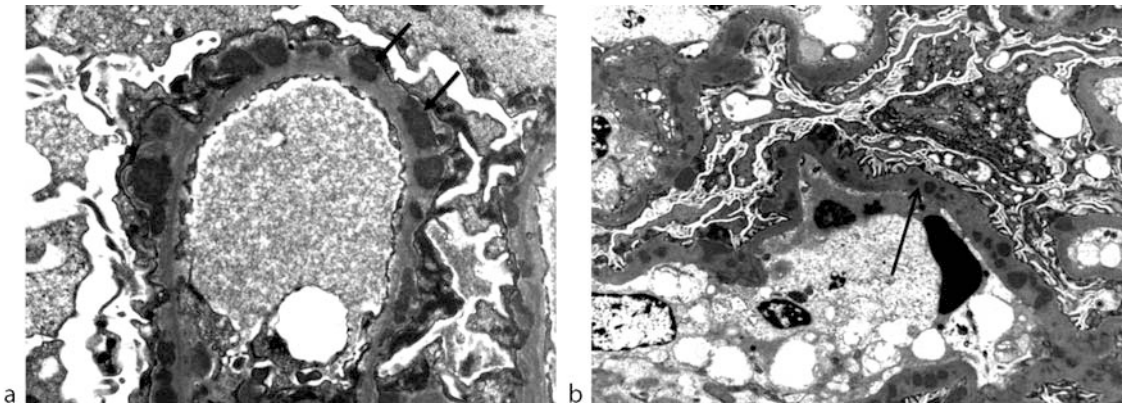
The characteristic feature of MN, subepithelial immune deposits, are seen on electron microscopy. These are initially small without a prominent basement membrane response, however with time, projections of basement membrane protrude around and enclose the immune deposits (*Fig. 33-4a* and *b*). Effacement of podocyte foot processes is found overlying the areas of electron dense deposits. The immune deposits typically involve the entire glomerular tuft (global), but in a subset of children, a segmental pattern has been described (8). Interestingly, an increase in C1q staining has been described in this group in the absence of any features of lupus. It has been argued that this may reflect a different disease entity with a more benign prognosis, but it may simply reflect an early, or resolving, stage of the disease. Electron dense deposits found in mesangial or subendothelial areas are suggestive of a secondary form of MN such as lupus or malignancy.

Genetics and Familial Membranous Nephropathy

A number of studies have shown associations of MN with various HLA antigens, particularly HLA DR3, HLA B8 and HLA B18 (73). There have also been reports of families with two affected family members (typically brothers), (74, 75) but these are relatively rare. The largest

Figure 33-4

Electron microscopy in membranous nephropathy. (a) Subepithelial electron-dense deposits (arrows) are separated by irregular projections of basement membrane material. The glomerular epithelial cells (podocytes) have diffuse foot-process effacement (Uranyl acetate-lead citrate; x 11,000). (b) Deposits become incorporated into the GBM (arrow) when new layers of GBM are laid down over the electron-dense deposits (Uranyl acetate-lead citrate; x 4,200).



family reported describes four affected male family members and suggests that there may be an X-linked susceptibility gene (76). Notably, in this family, the ages of onset were variable (1, 3, 10 and 67 years). The three children all initially responded to immunosuppression, however, with time a relapsing course of nephrotic syndrome occurred, with the development of stage four chronic kidney disease in one and ESRD in another by age 23 years.

Clinical Features of Idiopathic MN

Membranous nephropathy in childhood affects males and females fairly equally and typically presents with either asymptomatic proteinuria on screening, or with features of nephrotic syndrome (*Table 33-1*). Proteinuria is typically within the nephrotic range (90%). Microhematuria is common (80%) and gross hematuria occurs in approximately 40% (9). Hypertension at onset is uncommon (21%, range 6–50%), and may herald a worse prognosis (2–9), whereas renal function is typically normal at presentation. Children are at risk for complications of nephrotic syndrome including infection, however, renal vein thrombosis is a rare complication in children (77).

Natural History and Prognosis

The course of membranous nephropathy in adults is variable, but about 30–40% develop progressive disease (78–80). Prognostic risk factors for progression include a greater degree and duration of proteinuria, impaired renal function at presentation, age older than 50 years, non-Asian race and certain renal biopsy features (glomerulosclerosis, focal segmental glomerulosclerosis, Stage III/IV disease, tubulointerstitial fibrosis) (81–83). It has been argued that the pathological features on renal biopsy do not give further prognostic risk stratification independent of the clinical variables (83). The urinary excretion of biomarkers such as beta-2 microglobulin and/or IgG may be more accurate prognostic indicators than total urinary protein excretion, although these assays are not widely available (84).

The prognosis in children is much better than in adults, however, overall about 25% (10–28%) progress to chronic kidney disease (*Table 33-1*) (2–9). The most important prognostic indicator in children is the presence of nephrotic syndrome. Other adverse risk factors that have been reported include the renal biopsy findings (degree of glomerular sclerosis and interstitial fibrosis), age (older than 10 years), hypertension at onset and the presence of renal vein thrombosis. Children who present with asymptomatic proteinuria have a much more favorable outcome and progressive disease is rare (7, 9, 85). Proteinuria in this group often resolves around 12–18 months, although relapse has been described. Remission may occur earlier in those treated with steroids. By contrast, in children with nephrotic syndrome, the overall remission rate is only around 50% (26–74%), with progressive renal dysfunction developing in around one third (2–9). Of note, children with nephrotic syndrome typically have higher levels of proteinuria and greater glomerulosclerosis and tubulointerstitial fibrosis on renal biopsy.

Treatment of Idiopathic Membranous Nephropathy

The management of idiopathic MN in children relies on the exclusion of secondary causes of the disease. All children with idiopathic MN should be considered for therapy with the non-immunosuppressive strategies described below, and immunosuppression is usually reserved for those with adverse risk factors for disease progression.

Exclusion of Secondary Causes (see *Table 33-2*)

Idiopathic MN is uncommon in children and a thorough search for secondary causes, including medications, should be considered. Investigations may include liver function tests, hepatitis B and C serologies, serum complement levels, anti-nuclear antibody and anti-double stranded DNA (dsDNA), and fluorescent treponemal antibody or VDRL. Depressed complement levels may be found with lupus, hepatitis B or syphilis. Certain features on the renal biopsy may be suggestive of a secondary form of MN including the presence of mesangial cell proliferation, cellular infiltration, IgA or C1q deposition by immunofluorescence, or the presence of electron dense deposits in subendothelial or mesangial sites, or tubuloreticular structures in endothelial cells by electron microscopy. The treatment of secondary forms of the disease is directed at the underlying etiology.

Table 33-2

Conditions associated with membranous nephropathy in children

	Common	Uncommon
Autoimmune diseases	Systemic lupus erythematosis	Rheumatoid arthritis, mixed connective tissue disease, Sjogren's syndrome, Graves disease, Hashimoto's thyroiditis, dermatomyositis, primary biliary cirrhosis, Bullous pemphigoid, dermatitis herpetiformis, ankylosing spondylitis, Guillain-Barre syndrome, myasthenia gravis
Infections	Hepatitis B congenital syphilis	Tuberculosis, Streptococcus, hepatitis C, quartan malaria, schistosomiasis, filariasis, hydatid disease, leprosy
Drugs and toxins	Non-steroidal anti-inflammatory drugs (NSAID), penicillamine	Gold, captopril, probenecid, tiopronin, lithium, mercury, formaldehyde, hydrocarbons
Miscellaneous	–	Tumors, diabetes mellitus, sarcoidosis, sickle cell anemia, Kimura disease, sclerosing cholangitis, systemic mastocytosis, Gardner-Diamond syndrome

Non-Immunosuppressive Therapy

Angiotensin converting enzyme (ACE) inhibitor or angiotensin receptor blocker (ARB) therapy are renoprotective in adult patients with both diabetic (86, 87) and non-diabetic (88) glomerular disease. Although the use of ACE inhibitors or ARBs can be expected to reduce urine protein expression by about one third in adults, patients with MN may be resistant to RAS blockade (89), and this may not result in a clinically significant reduction in the rate of disease progression in MN (90, 91). Children with MN are frequently treated with renin angiotensin system (RAS) blockade, however, there is still no consensus on specific indications. Blood pressure should be controlled. Lipid lowering therapy with HMG-CoA reductase inhibitors (statins) is routinely used in adults, but the benefit of lipid lowering drugs in children remains controversial, particularly in pre-pubertal children. The incidence of renal vein thrombosis is lower in children and prophylactic anticoagulation is not recommended, although low dose aspirin therapy may be used. The risk of infection in children with nephrotic syndrome is significant, and if not already immunized, pneumococcal vaccination and varicella zoster immune globulin should be given to those receiving immunosuppression.

Immunosuppressive Therapy

Since MN is a rare condition in children, there are no randomized clinical trials to provide an evidence based approach to the treatment of this condition in childhood. However, the approach to immunosuppressive therapy can be extrapolated from controlled studies in adults (92–99), and from a series of uncontrolled studies in children (2–7, 9, 85, 100).

In adults, immunosuppressive therapy is typically reserved for patients with adverse prognostic factors as described above. It is generally accepted that steroid therapy alone is ineffective in adults (92, 94, 95), although this is typically the first line treatment in children who often do not undergo renal biopsy until they fail a course of steroids. Adults at a high risk for progression are typically treated with either a combination of steroid and an alkylating agent (most commonly cyclophosphamide) or a combination of steroid and cyclosporin. These combination therapies are typically reserved for children who have failed empiric steroid alone therapy. A rational approach to immunosuppression in children with MN may be based on the presence of nephrotic syndrome at presentation.

Children with asymptomatic proteinuria. In general, these children have a much better prognosis, and in the absence of evidence of disease progression, are typically treated with non-immunosuppressive therapy and do not receive immunosuppression.

Children with nephrotic syndrome. The majority of children with nephrotic syndrome will have received empiric steroid therapy (e.g., prednisone 60 mg/m^2 or 1 mg/kg) for presumed minimal change disease before MN is diagnosed on renal biopsy. This group has a worse prognosis and most children who respond (steroid sensitive) will do so within 2–3 months, although it is difficult to exclude spontaneous remissions in this group. In those who fail to respond to steroid therapy (steroid resistant), the addition of a second line immunosuppressive agent such as cyclophosphamide (2 mg/kg orally) or cyclosporin (3–5 mg/kg/d in two divided doses) may be considered.

There is only limited data on the use of cyclosporin in children with MN (9). If cyclosporin is going to be effective, children will typically enter remission within 6 months, although there is a risk of relapse when this medication is discontinued. Cyclosporin should be used with care if the renal function is already impaired.

We should also recognize when we are considering immunosuppressive therapy for MN, that although the patient may have nephrotic syndrome, the active immune process leading to antibody deposition may already have resolved. The resolution phase of MN, with reabsorption of the subepithelial deposits may take many months to years before the proteinuria resolves. This is best demonstrated by the example of drug induced MN, in which proteinuria may continue for a year or two after discontinuation of the offending agent. In idiopathic MN, ongoing immune activation may be suggested by the presence of glomerular C3c deposition (72) or the detection of urinary C5b-9 (26, 101). Many patients with MN may exhibit persistent injury to the glomerular filtration barrier and proteinuria in the absence of an active autoimmune process, and therefore would not be expected to respond well to immunosuppression. Unfortunately, at this time, we do not have the tools to accurately identify these patients.

Neonatal Membranous Nephropathy

Infants who are born with nephrotic syndrome typically have congenital abnormalities of the slit diaphragm or perinatal infections. A few cases of neonatal MN have been described secondary to feto-maternal alloimmunization with antibodies to neutral endopeptidase (NEP) (22, 102). MN associated with congenital syphilis should be considered in the differential diagnosis.

Clinical Presentation

Infants with antibodies to NEP may be born with oliguric acute renal failure and respiratory distress on the first day of life. Antenatal ultrasound shows oligohydramnios and enlarged fetal kidneys. As the renal failure improves they develop massive proteinuria and nephrotic syndrome. Hypertension may be present. The disease rapidly improves over the first few weeks of life as the maternal antibody disappears, however, some children have residual proteinuria and chronic kidney disease, possibly as a result of podocyte injury, nephron loss and focal segmental glomerulosclerosis.

Renal pathology shows the typical subepithelial immune deposits and GBM abnormalities, but additional renal arterial lesions and glomerular capillary tuft collapse may be present suggesting glomerular ischemia which may also lead to chronic kidney disease. It has been suggested that maternal antibodies inhibit the enzyme activity of NEP with failure to degrade various vasoactive peptides (22).

Pathogenesis

The presentation with antenatal MN suggested the possibility of feto-maternal alloimmunization, where the mother becomes sensitized to an antigen on the fetal podocyte, and produces anti-podocyte antibodies which cross the placenta. In the index case described, an IgG fraction from the mother produced immune deposits and proteinuria when injected into rabbits. Subsequently, antibodies to neutral endopeptidase, a membrane bound peptidase expressed on the podocyte surface, were identified in the maternal serum, and transiently in the infants serum in keeping with the maternal origin (22). Further investigations confirmed that each of the mothers in these cases had truncating mutations in the *MME* gene encoding NEP, and presumably developed anti-NEP antibodies due to prior exposure to this antigen (previous miscarriage or paternal sperm) (102). Notably, the NEP deficiency in the mothers was not associated with any obvious phenotype. The neonatal MN was more severe in mothers with high titer antibody, and in those with more IgG1, as opposed to IgG4 deposition. It is unclear if antibody mediated enzyme inhibition plays any role in this condition.

The possibility of MN secondary to alloimmunization to podocyte antigens should also be considered in *de novo* MN in the allograft following renal transplantation, and in native kidneys following stem cell transplantation.

Membranous Nephropathy Associated with Hepatitis B

Hepatitis B (HBV) infection is associated with a variety of renal diseases including membranous nephropathy, polyarteritis nodosa, membrano-proliferative glomerulonephritis and a serum sickness-like syndrome. MN is the most common renal manifestation and develops in the chronic carrier state (HBeAg + ve; HBsAg + ve).

In adults, HBV accounts for less than 1% of MN in the US, but in children idiopathic MN is uncommon and HBV accounts for about 20% of MN in children in the US (103).

In patients with HBV associated with MN (HBV-MN), the pathogenesis is considered to be due to the formation of immune complexes of HBeAg and HBeAb in the subepithelial space. Immunostaining reveals predominantly HBeAg deposition along glomerular capillary walls, but HBsAg and HBcAg may also be detected (104). Immunoelectron microscopy has co-localized HBeAg and C5b-9 to the subepithelial deposits (105) and the deposits presumably form *in situ*. The possibility that the disease results from autoimmune mechanisms involving podocyte antigens, and that the glomerular deposition of HBV antigens is secondary, has not been excluded as many patients with chronic liver disease also exhibit a variety of autoantibodies.

Clinical Features

HBVMN in children (typically boys aged 2–12 years) usually presents with proteinuria and edema, with hematuria in 40–60%. Renal function is usually normal. Children may have a mild transaminitis, but no evidence of chronic liver disease.

Hepatitis serology reveals the presence of HBsAg and anti-HBc antibody in most patients. The majority of patients also have circulating HBeAg (60–80%). Circulating HBeAg correlates with clinical activity and loss of antigen and conversion to HBeAb status correlates with recovery of nephrotic syndrome. This is followed by seroconversion to HbsAb (106). Complement studies commonly reveal a low C3 and C4 (15–64%) and circulating immune complexes (80%) (103), both of which are typically not found in idiopathic MN.

Renal biopsy reveals features similar to idiopathic MN with subepithelial deposits, although minor subendothelial and mesangial deposits are frequently present as well. Electron microscopy may reveal viral-like particles in subepithelial and subendothelial spaces and within glomerular cells (reviewed in (103)).

It should be recognized that proteinuria in patients with HBV may not be exclusively the result of HBV infection. A high incidence of proteinuria in non-HBV infected family members has been reported suggesting that other environmental or infectious agents may be contributing (107). Co-infection with hepatitis B and C in children with MN has been reported with no increase in severity of the disease (108).

Treatment

Most children with HBV associated MN undergo spontaneous resolution with a cumulative remission rate of 64% at 4 years (106, 109). Initial observation of these children is appropriate with general supportive measures. Anti-viral therapy can be considered in those who remain HBeAg positive and have persistent disease (for example greater than 1 year) or in those who are likely to progress (older children with focal sclerosis on biopsy). Interferon α therapy was shown to be effective in an open randomized study of children with hepatitis B associated MN (age <14 years) (110). Treated patients were free of proteinuria by 3 months with HBeAg seroconversion by 5–6 months. The interferon α therapy was surprisingly well tolerated in this study. Case reports of treatment with lamivudine are also described (111, 112). The treatment of HBV-associated MN with steroids may cause more harm than good by enhancing viral replication and impairing seroconversion, but may still be considered (113).

Syphilis & Other Infections

MN in neonates and infants may be secondary to congenital syphilis. In this setting it may be associated with other clinical features of treponemal infection such as rash, periostitis, nasal discharge and hepatosplenomegaly. Hypertension may be present.

The etiology is suggested by the presence of hypocomplementemia and may be confirmed by the demonstration of fluorescent treponemal IgM antibodies. Treponemal antigens have been localized to the subepithelial space, but it is unclear if this is merely non-specific trapping. Early treatment with penicillin leads to the resolution of the proteinuria.

MN has also been reported with a number of other infections although some of these associations remain debatable (*Table 33-1*). Although Hepatitis C (HCV) typically causes a membrano-proliferative glomerulonephritis (MPGN) related to mixed cryoglobulinemia, there are several reports of MN associated with HCV in adults (114–116). Glomerular disease and nephrotic syndrome occur much more commonly in tropical countries than in industrialized countries (reviewed in (117)). Most tropical glomerulonephritides are due to chronic parasitic infection. MN has been reported with quartan malaria and hepatosplenic schistosomiasis, although a steroid resistant membrano-proliferative lesion is much more typical with these infections. In general, these chronic

immune complex glomerulonephritides respond poorly to therapy for the underlying infection.

Lupus Membranous Nephropathy

MN accounts for 15–20% of lupus nephritis in children (118, 119) and several small series have been described demonstrating a variable prognosis. (118,120) The clinical presentation is typically with nephrotic syndrome with hematuria (50%), and hypertension and renal impairment are variable. Serum complement levels are often normal. Lupus membranous is commonly associated with, or evolves to, proliferative lupus nephritis lesions on biopsy which are associated with a worse prognosis. Notably, MN may be the initial presentation of lupus, with subsequent development of extra-renal manifestations at a later stage, particularly in women (9). Pathological features that may suggest the presence of lupus as the underlying etiology include mesangial hypercellularity, the presence of IgA, C1q or C4 by immunofluorescence, and on electron microscopy, the presence of subendothelial or mesangial immune deposits, or tubuloreticular structures in endothelial cells. This topic is covered in detail in Chapter 47.

Membranous Nephropathy in the Renal Allograft

MN may occur in the renal allograft as a result of recurrence of the original idiopathic disease or as a *de novo* condition. In adults, MN is the commonest de novo glomerulonephritis, occurring in about 2% of allografts (121) and typically presents with nephrotic syndrome late after transplantation (36–48 months). By contrast, recurrent disease occurs in 10–30% of those with prior MN, may be more common in living related donor kidneys, and tends to occur earlier in the post transplant course (12–24 months) (122, 123).

In view of the rarity of this condition in children, the recurrence rate following transplantation is unknown. De novo MN has been described in children and is estimated to be in the 1–2% range (124), although the incidence may be as high as 9% in patients who undergo protocol surveillance biopsies (125). De novo MN in the graft is generally associated with rejection (121). Given the recent demonstration of alloantibodies to a podocyte antigen (NEP) in neonatal MN, it remains to be determined if the development of MN represents a more generalized loss of tolerance to the graft with the development of anti-podocyte antibodies in addition to other anti-donor antibodies. De novo MN has also been associated with ureteral obstruction (124, 126). The clinical course is variable, but in general, graft loss may be more likely from the associated rejection process.

The treatment of de novo MN in children usually consists of intensified immunosuppression, for example, switching the calcineurin inhibitor to cyclophosphamide for a short period of time. In general, the use of intensified immunosuppression in the children with MN in the renal allograft needs to be balanced with the cumulative exposure to immunosuppression and the lifetime risks of lymphoma.

Membranous Nephropathy Associated with Other Conditions

MN is commonly associated with malignancy in older adults, but only a few cases have been described in children. In some cases, the disease has resolved following excision of the tumor (127). MN is also associated with drugs and toxins (*Table 33-2*), and cases are described with penicillamine treatment in children (128, 129). MN has also been described with a range of other autoimmune conditions other than systemic lupus erythematosis (*Table 33-2*).

A rare form of MN in early childhood has been described associated with an anti-tubular basement membrane (TBM) nephritis with linear or granular IgG and C3 deposition along the TBM (130). Antibodies directed against the tubulointerstitial nephritis antigen (TIN-Ag), a glycoprotein component of tubular basement membrane matrix found predominantly in proximal tubules (131), are detected in most cases. The disease typically affects young males and may be associated with a proximal tubulopathy, and with extrarenal manifestations including pulmonary hemorrhage and diarrhea (intestinal villous atrophy or autoimmune enteropathy). A familial form of this disease has been described (132).

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34 Crescentic Glomerulonephritis

Arvind Bagga

Crescentic glomerulonephritis (GN) is a rare condition in children, clinically characterized by features of rapidly progressive GN (RPGN) with rapid loss of renal function. Renal histology shows extracapillary proliferation in the Bowman's space resulting in formation of crescents in most glomeruli. These crescents are formed when breaks in glomerular capillaries allow leakage of cells and plasma proteins into Bowman's space, as might occur in almost any glomerulonephritis, and sometimes in diseases that do not primarily affect the glomeruli.

Improvements in our understanding have enabled categorization of crescentic GN into three broad entities (immune-complex GN, pauci-immune GN, antiglomerular basement membrane antibodies mediated GN) based on glomerular findings on immunohistology. A complete diagnosis of crescentic GN requires the integration of clinical data, renal biopsy findings and serological tests, which has significant implications for therapy and outcome. In all three groups, the severity of renal failure at presentation, the proportion of glomeruli containing crescents and the extent of tubulointerstitial scarring and fibrosis are considered important prognostic signs. RPGN is a medical emergency, which if untreated rapidly progresses to irreversible loss of renal function.

Definition

Crescentic GN is characterized by the presence of large epithelial crescents in the Bowman's space involving 50% or more glomeruli. However, criteria for histological appearance and the number of affected glomeruli required for diagnosis vary from 20 to 75% in various pediatric studies. The presence of crescents is a histologic marker of severe glomerular injury, which may occur in a number of conditions, including postinfectious GN, systemic and renal vasculitis, IgA nephropathy, systemic lupus erythematosus (SLE) and membranoproliferative GN (1, 2). The severity of clinical features correlates with the proportion of glomeruli that show crescents. While patients with circumferential crescents involving more than 80% of glomeruli present with severe renal failure, those with crescents in less than 50% of glomeruli, particularly if these are noncircumferential, often have an indolent course.

The clinical correlate of crescentic GN is RPGN, characterized by an acute nephritic syndrome with rapid loss of renal function over days to weeks (1). The terms RPGN and crescentic GN are often used interchangeably. *Table 34-1* lists the common conditions that present with crescentic GN.

Pathogenesis of Crescent Formation

The chief participants in formation of crescents are coagulation proteins, macrophages, T cells, fibroblasts and parietal and visceral epithelial cells (1, 3). Perturbations of humoral immunity as well as the Th1 cellular immune response contribute to the pathogenesis (1, 2).

The initiating event is the occurrence of a physical gap in the glomerular capillary wall and glomerular basement membrane (GBM), mediated by macrophages and T lymphocytes. Breaks in the integrity of the capillary wall lead to passage of plasma proteins and inflammatory mediators into the Bowman's space with fibrin formation, influx of macrophages and T cells, and release of proinflammatory cytokines, e.g., interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α). Breaks in the Bowman's capsule allow cells and mediators from the interstitium to enter Bowman's space and for contents of the latter to enter the interstitium, resulting in periglomerular inflammation.

Apart from macrophages, the other cellular components of the crescents are proliferating parietal and visceral epithelial cells (4). The development of a crescent results from the participation of coagulation factors and proliferating cells, chiefly macrophages, parietal glomerular epithelial cells and interstitial fibroblasts. The presence of coagulation factors in the Bowman's space results in formation of a fibrin clot and recruitment of circulating macrophages. Activated neutrophils and mononuclear cells release procoagulant tissue factor, IL-1 and TNF-α, serine proteinases (elastase, PR3) and matrix metalloproteinases. The proteases cause lysis of the GBM proteins and facilitate the entry of other mediators in the Bowman's space. Release of IL-1 and TNF-α also result in

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Table 34-1

Causes of crescentic glomerulonephritis (GN)

Immune complex GN
Post infectious GN. Poststreptococcal nephritis, infective endocarditis, shunt nephritis, visceral abscesses, *Staphylococcus aureus* sepsis, other infections: human immunodeficiency virus, hepatitis B and C, syphilis, legionella, mycoplasma, tuberculosis, leprosy
Systemic disease. Systemic lupus erythematosus, Henoch-Schonlein purpura, cryoglobulinemia, mixed connective tissue disorder, juvenile rheumatoid arthritis, Behcet's syndrome, relapsing polychondritis, mixed connective tissue disease, dermatomyositis
Primary GN. IgA nephropathy, membranoproliferative GN, membranous nephropathy, C1q nephropathy
Pauci-immune crescentic GN
Microscopic polyangiitis, Wegener's granulomatosis, renal limited vasculitis, Churg Strauss syndrome
Idiopathic crescentic GN
Medications: penicillamine, hydralazine, hydrocarbons, propylthiouracil
Anti glomerular basement membrane (GBM) GN
Anti-GBM nephritis, Goodpasture's syndrome, postrenal transplantation in Alport syndrome
Postrenal transplantation
Recurrence of IgA nephropathy, Henoch Schonlein purpura, membranoproliferative GN, systemic lupus erythematosus

upregulated expression of adhesion molecules, leading to macrophage recruitment and proliferation.

The stage of inflammation is followed by the development of fibrocellular and fibrous crescents. The expression of fibroblast growth factors and transforming growth factor (TGF-β) is important for fibroblast proliferation and production of type I collagen, responsible for the transition from cellular to fibrocellular and fibrous crescents. The transition from cellular to fibrous crescents, which occurs over days, is clinically important since the latter is not likely to resolve following immunosuppressive therapy. The plasminogen-plasmin system enables fibrinolysis and resolution of crescents.

Role of ANCA

Antineutrophil cytoplasmic autoantibodies (ANCA) are a heterogeneous group of circulating antibodies directed against antigens in the primary granules of neutrophils and the monocytes' lysosomes. The demonstration of ANCA in the blood in a proportion of patients with crescentic GN raises the issue of their pathogenicity. Evidence for pathogenicity includes the correlation between ANCA titers and increased surface expression of PR-3 on neutrophils and disease activity, and the induction of oxygen radical release, cell degranulation and inhibition of microbicidal function following ANCA binding to neutrophils and monocytes (5, 6). Experimental data suggest that ANCA binding to endothelial cells induces injury and that increased circulating endothelial cell concentration correlates with disease activity (7). Further evidence for their pathogenicity is derived from mice that are deficient in T and B lymphocytes. When these mice were induced to generate ANCA with myeloperoxidase specificity (8), they showed histological evidence of glomerulonephritis and vasculitis. On the other hand, there is evidence against the pathogenicity of ANCA, including the fact that (i) high titers of ANCA can be found in patients without active vasculitis (9) and (ii) the absence of disease in newborns of pregnant women with ANCA-associated vasculitis. It is likely that these autoantibodies are necessary but not sufficient for the development of crescentic GN and vasculitis.

Classification and Causes

Based on renal histology and patterns on immunofluorescence staining, crescentic GN may be classified into three types, reflecting different mechanisms of glomerular injury.

1. Immune-complex GN with deposits of immune complexes along capillary wall and mesangium.
2. Pauci-immune GN with minimal or no immune deposits, with or without systemic vasculitis.
3. Anti-GBM GN with linear deposits of anti-GBM antibodies.

Immune Complex Crescentic GN

These patients form a heterogeneous group in which multiple stimuli lead to proliferative GN with crescents. Immunohistology shows granular deposits of immunoglobulin and complement along capillary walls and in the mesangium. The causes include infections, systemic diseases and preexisting primary GN.

Poststreptococcal GN can rarely be complicated by the occurrence of crescentic GN. While most patients recover completely, the presence of nephrotic range proteinuria, sustained hypertension and crescents is associated with an unsatisfactory outcome (10, 11). Other infectious illnesses associated with crescentic GN include infective endocarditis, infected atrioventricular shunts and visceral abscesses. Crescentic GN associated with other infectious agents including methicillin resistant *Staphylococcus aureus,* hepatitis B and C virus, leprosy and syphilis are reported anecdotally.

RPGN with glomerular crescents might be seen in patients with class IV lupus nephritis, less commonly in type III, and rarely in patients with isolated membranous nephropathy. The outcome of disease in patients with class IV lupus nephritis and crescents is not satisfactory and the natural history of crescents is unclear. The presence of crescents is not uncommon in Henoch Schonlein purpura in both adults and children, but most series are biased towards patients with more severe disease in whom renal biopsies have been performed (10). Extensive crescent formation in patients with Henoch Schonlein purpura is associated with a poor prognosis. Patients with IgA nephropathy, membranoproliferative GN and rarely membranous nephropathy may present with rapid deterioration of renal function and crescentic GN (10, 12); the long-term outcome in such cases is unsatisfactory.

Pauci-Immune Crescentic GN

Microscopic polyangiitis, Wegener's granulomatosis and renal limited vasculitis are characterized by small vessel vasculitis that, if involving glomerular capillaries, results in necrotizing crescentic GN with few or no immune deposits on immunofluorescence microscopy (2, 13). Most patients have circulating ANCA.

The majority of patients with pauci-immune crescentic GN has or will develop clinical features of vasculitis. This variety is considered a part of the microscopic polyangiitis/Wegener's granulomatosis spectrum, since the histological features are similar and some patients who present with renal-limited vasculitis might later show systemic vasculitis (2). Some cases of ANCA positive disease might be induced by drugs, including penicillamine, propylthiouracil, minocycline and penicillamine.

Approximately 10–20% patients with pauci-immune crescentic GN do not have circulating ANCA (14). Such patients are, however, considered part of the spectrum of vasculitis, since they show similar clinical and histological features (15). Some reports suggest that patients with ANCA negative, pauci-immune crescentic GN show more proteinuria, higher prevalence of nephrotic syndrome and less extrarenal involvement than those who are ANCA positive (16). There is also evidence that the mechanism for negative-ANCA, pauci-immune RPGN might involve a primary defect within the glomerular podocytes (17). The deletion of the Von Hippel-Lindau gene (Vhlh) from glomerular cells of mice induces a necrotizing crescentic glomerulonephritis. Loss of Vhlh leads to stabilization of hypoxia-inducible factor alpha subunits (HIFs) and de novo expression of the HIF target gene Cxcr4 in glomeruli. The course of the disease is markedly improved in mice treated with a blocking antibody to Cxcr4, whereas overexpression of Cxcr4 alone in podocytes of transgenic mice is sufficient to cause glomerular disease. These data indicate an alternative mechanism for the pathogenesis of RPGN and glomerular disease in an animal model and suggest novel molecular pathways for intervention in this disease.

Anti-GBM Crescentic GN

This condition is uncommon in childhood, accounting for less than 5–10% of cases in children (10, 18–21). The nephritogenic autoantibody is directed against a 28 kDa monomer located on the α3 chain of type IV collagen (Goodpasture antigen). Pulmonary involvement (Goodpasture syndrome) is uncommon.

Approximately 5% of patients with Alport syndrome who receive a renal allograft show anti-GBM autoantibodies and anti-GBM nephritis within the first year of the transplant (22). Unlike de novo anti-GBM nephritis, pulmonary hemorrhage is not observed in these patients since the patient's lung tissue does not contain the putative antigen. The risk of post transplantation nephritis is low in subjects with normal hearing, late progression to end stage renal disease, or females with X-linked Alport syndrome.

Epidemiology

Crescentic GN comprises approximately 5% of unselected renal biopsies in children (21). While there are no

population based studies in children, a recent report suggests an annual incidence of 3.3 per million adult population (23). The 2006 NAPRTCS database shows that idiopathic crescentic GN accounts for 1.8% of all transplanted patients (24). This figure is an underestimate since other conditions, including membranoproliferative GN (2.7%), SLE (1.6%), systemic immune disorders (0.4%), Wegener's granulomatosis (0.5%), chronic glomerulonephritis (3.4%) and IgA nephropathy and Henoch Schonlein purpura (2.5%), often show crescentic GN.

Table 34-2 outlines the underlying etiologies in five series of patients with crescentic GN reported from India (10, 21), United States (18), United Kingdom (19) and France (20). Immune complex GN is the most common pattern of crescentic GN in children accounting for 75–80% cases in most reports. Pauci-immune crescentic GN, while common in adults, is infrequent in children, accounting for 15–20% cases. The decline in the incidence of postinfectious GN has resulted in a change in the etiological profile of crescentic GN, and a recent survey of 73 patients between 1 and 20 year old showed similar frequencies of immune complex (45%) and pauci-immune crescentic GN (42%) (1).

Clinical Features

Patients with crescentic GN typically present with the clinical syndrome of RPGN. The presenting symptoms are those of an acute nephritic syndrome. The spectrum of presenting features is variable, and includes macroscopic hematuria (in 60–90% patients), oliguria (60–100%), hypertension (60–80%) and edema (60–90%) (10, 13, 18). The illness may be complicated by the occurrence of hypertensive emergencies, pulmonary edema and cardiac failure. Occasionally, RPGN has an insidious onset with the initial symptoms being fatigue or edema. Nephrotic syndrome is rare and seen in patients with less severe renal insufficiency.

Systemic complaints, involving the upper respiratory tract (cough, sinusitis), skin (vasculitic rash over lower limbs), musculoskeletal (joint pain, swelling) and/or the nervous system (seizures, altered sensorium) are common in patients with pauci-immune crescentic GN, with or without ANCA positivity. Sixty to seventy five percent patients with Wegener's granulomatosis have crescentic GN, and 80% show pulmonary features. Relapses of systemic and renal symptoms occur in one-third of patients with vasculitis (2, 13).

Patients with anti-GBM antibody disease may present with hemoptysis and, less often, pulmonary hemorrhage. Similar complications may be found in Wegener's granulomatosis, SLE, Henoch Schonlein purpura and severe GN with pulmonary edema.

The severity of clinical, laboratory and histological features at presentation vary with the underlying cause, the most severe being anti-GBM disease, followed by pauci-immune GN and finally immune complex crescentic GN (1, 25).

Table 34-2

Causes of crescentic glomerulonephritis (GN) in children (%)

	SPNSG (18) (*N* = 50)	Srivastava, et al. (10) (*N* = 43)	Niaudet, Levy (20) (*N* = 41)	Jardim, et al. (19) (*N* = 30)	Dewan (21), et al. (*N* = 22)
Immune complex disease					
Unspecified, others	26	–	4.8	–	18.2
Systemic lupus erythematosus	18	2.3	2.4	3.3	9.1
Postinfectious GN	12	25.5	12.1	6.6	31.8
Henoch-Schonlein purpura, IgA nephropathy	14	6.9	34.1	30	13.6
Membranoproliferative GN	4	–	21.9	23.3	13.6
Vasculitis	6	–	7.3	16.6	4.5
Idiopathic crescentic GN	14	60.4	7.3	13.3	
Anti-glomerular basement disease	6	2.3	7.3	6.6	9.1
Others	–	2.3	2.4	–	

SPNSG Southwest Pediatric Nephrology Study Group

Investigations

Gross or microscopic hematuria with red cell casts is present in all patients. Most patients have a variable degree of nonselective proteinuria (2+ to 4+), with leukocyte, granular and tubular epithelial cell casts. Renal insufficiency is present at diagnosis in almost all cases. The degree of renal failure is usually more than that estimated by the serum creatinine. Anemia is mild; peripheral smear shows normocytic normochromic red cells.

Serology

Serological investigations assist in the evaluation of the cause and the monitoring of disease activity (*Table 34-3*, *Fig. 34-1*). Low levels of total hemolytic complement (CH50) and complement 3 (C3) are seen in postinfectious GN, SLE and membranoproliferative GN, and inversely correlate with disease activity. Positive antistreptolysin O titers and anti-deoxyribonuclease B suggests streptococcal infection in the past 3 months. Patients with SLE show antinuclear (ANA) and anti-double stranded DNA autoantibodies.

Elevated levels of ANCA suggest underlying vasculitis, and are present in patients with pauci-immune crescentic GN. Most ANCA have specificity for myeloperoxidase (MPO) or proteinase-3 (PR3). ANCA should be screened by indirect immunofluorescence and positive tests confirmed by both PR3-ELISA and MPO-ELISA. In patients with pauci-immune crescentic GN, negative results from indirect immunofluorescence should be tested by ELISA, because 5% of serum samples are positive only by the latter. Wegener's granulomatosis is associated with PR3 ANCA, which produces a cytoplasmic staining pattern on indirect immunofluorescence (c-ANCA) in 85% cases. Renal limited vasculitis and drug induced pauci-immune GN are associated with MPO ANCA that shows perinuclear staining on immunofluorescence (p-ANCA). Patients with microscopic polyangiitis have almost equal distribution of MPO ANCA/p-ANCA and PR3 ANCA/c-ANCA. Approximately 10% of patients with Wegener's granulomatosis or microscopic polyangiitis have negative assays for ANCA.

ANCA (usually p-ANCA) are also found in 20–30% patients with anti-GBM GN, and occasionally in idiopathic immune complex RPGN, inflammatory bowel disease, chronic liver disease, rheumatoid arthritis and SLE (26). Several medications have been associated with the induction of MPO ANCA, including propylthiouracil, hydralazine, minocycline and penicillamine.

Apart from diagnosis, ANCA titers have also been used for monitoring activity of systemic vasculitis. The risk of relapse in patients who show persistently negative ANCA titers is low. On the other hand, persistent or reappearing ANCA positivity in patients in remission may be associated with disease relapse in ANCA-associated vasculitides. The presence of a positive ANCA titer at the time of switch from cyclophosphamide to azathioprine increases chances of recurrent disease, particularly in those who are PR3-positive. Based on review of multiple studies, it is recommended that isolated rise in ANCA titers not be used for modifying treatment in patients with systemic vasculitis (27). Patients with ANCA-associated crescentic GN in remission, with persistent or reappearing ANCA positivity or rise in its titer, should be closely followed up and diagnostic efforts intensified to detect and treat relapses.

High titers of anti-GBM IgG antibodies, demonstrated by immunofluorescence or ELISA, are seen in anti-GBM nephritis or Goodpasture's syndrome and correlate with disease activity. About 5% of ANCA positive samples are

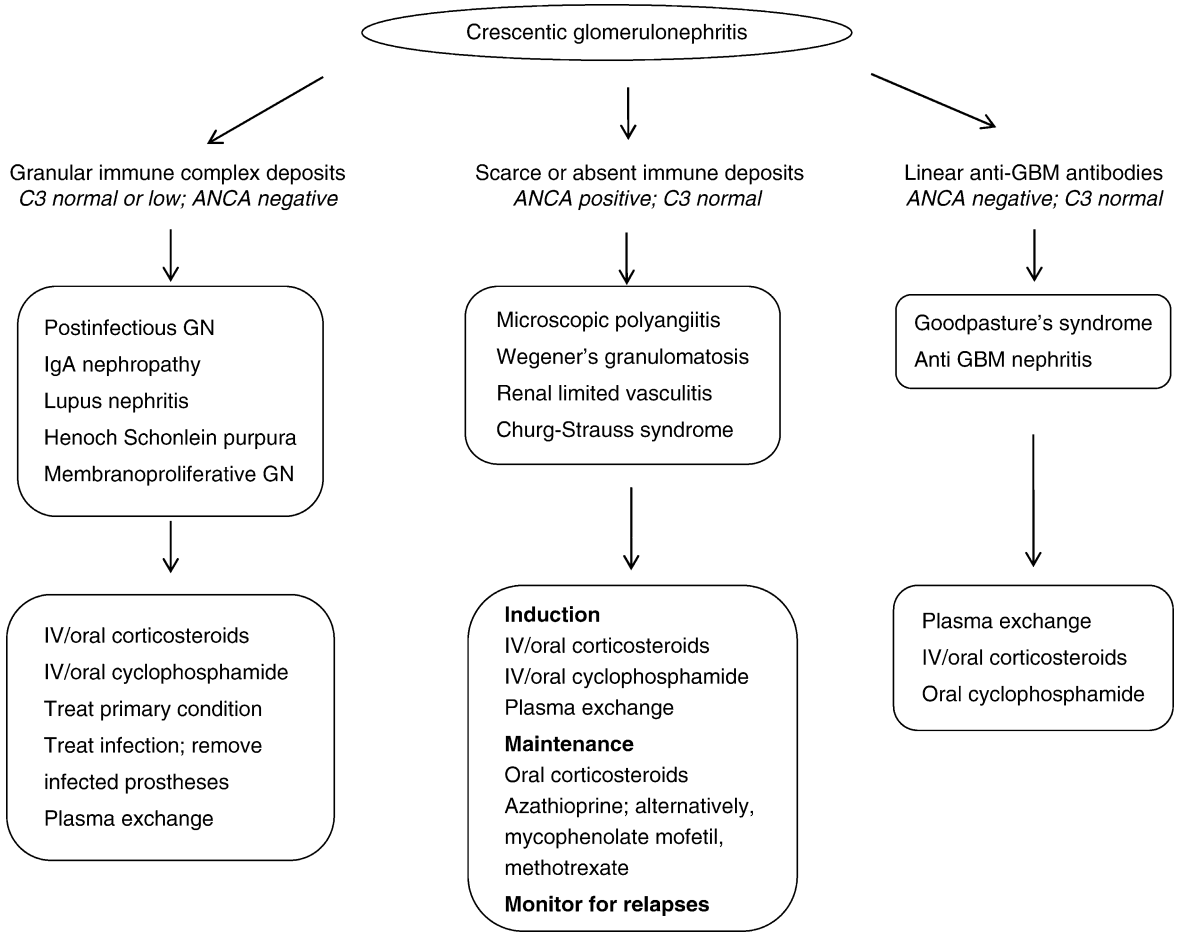
Table 34-3

Diagnostic evaluation of patients with crescentic and/or rapidly progressive GN

Complete blood counts; peripheral smear for type of anemia; reticulocyte count
Blood levels of urea, creatinine, electrolytes, calcium, phosphate
Urinalysis: proteinuria; microscopy for erythrocytes and leukocytes, casts
Complement (C3, C4, CH50)
Antistreptolysin O, antinuclear antibody, anti-double stranded DNA antibodies
Antinuclear cytoplasmic antibodies (anti-proteinase 3, anti-myeloperoxidase)
Anti-GBM (IgG) antibodies
Blood levels of cryoglobulin, hepatitis serology
Radiographs, CT scan for chest, sinuses (patients with Goodpasture's syndrome, Wegener's granulomatosis)
Renal biopsy (light microscopy, immunofluorescence, electron microscopy)

Figure 34-1

Diagnostic categories and principles of therapy of crescentic glomerulonephritis. The diagnostic categories are distinguished based on renal immunofluorescence findings and serology. Therapy is tailored to the specific diagnosis.



also anti-GBM positive and approximately 20–30% of anti-GBM positive samples are ANCA positive. Serology for ANCA is therefore recommended in all patients with either anti-GBM antibodies in blood or linear IgG deposition along the GBM. The initial clinical outcome for these patients is similar to that of anti-GBM disease, though relapses may occur as in systemic vasculitis (1).

Histopathology

Light Microscopy

A glomerular crescent is an accumulation of two or more layers of cells that partially or completely fill the Bowman's space. The crescent size varies from circumferential to segmental depending on the plane of the tissue section and the underlying disease. Crescents in anti-GBM nephritis or ANCA associated disease are usually circumferential, while they are often segmental in immune complex GN. Once the glomerular capillary loop is compressed by the crescent, tubules that derive their blood flow from that efferent arteriole show ischemic changes.

Crescents may be completely cellular or show variable scarring and fibrosis. Cellular crescents are characterized by proliferation of macrophages, epithelial cells and neutrophils (*Fig. 34-2a, b*). Fibrocellular crescents show admixture of collagen fibers and membrane proteins amongst the cells (*Fig. 34-3a, b*). In fibrous crescents, the cells are completely replaced by collagen (*Fig. 34-4a*). Interstitial changes range from acute inflammatory infiltrate (*Fig. 34-4b*) to chronic interstitial scarring and tubular atrophy.

Renal biopsies from patients with vasculitis often show crescents in various stages of progression indicating

Figure 34-2

(a) Large, cellular crescent with compression of glomerular tuft (PAS ×200); (b) cellular crescent compressing tuft (Silver methenamine ×200). (See color plate 18)

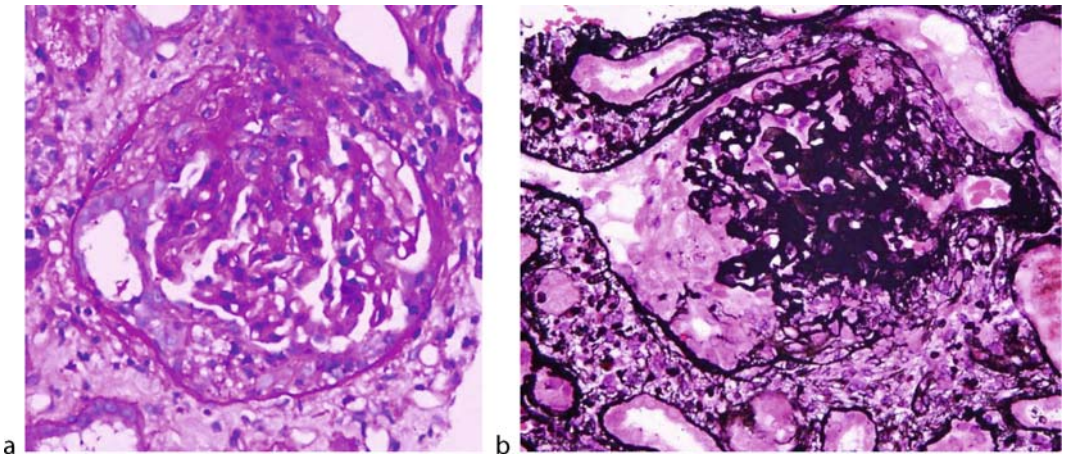
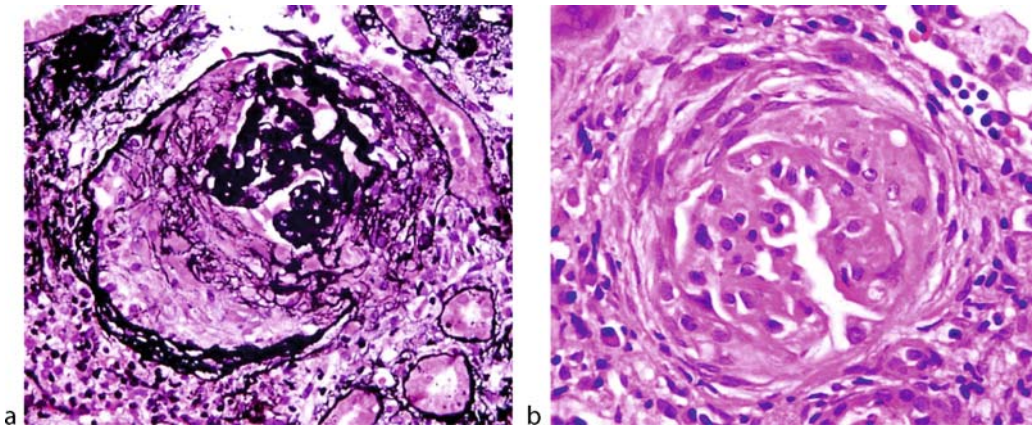


Figure 34-3

(a) Circumferential, fibrocellular crescent (Silver methenamine ×200). (b) Fibrocellular crescent with sclerosed glomerular tuft and fibrin deposition (Hematoxylin & eosin ×200). (See color plate 19)



episodic inflammation. Early lesions have segmental fibrinoid necrosis with or without an adjacent small crescent (*Fig. 34-5*). Severe acute lesions show focal or diffuse necrosis in association with circumferential crescents. Features of small vessel vasculitis, affecting interlobular arteries and rarely angiitis involving the vasa recta might be seen.

Immunofluorescence Microscopy

These investigations assist in determining the cause of crescentic GN, based on presence, location and nature of immune deposits. Crescents stain strongly for fibrin on immunofluorescence. Mesangial deposits of IgA are found in IgA nephropathy and Henoch Schonlein purpura; granular, subepithelial deposits of IgG and C3 in postinfectious GN; mesangial, subendothelial and intramembranous deposits of IgG and C3 in MPGN; and "full house" capillary wall and mesangial deposits of granular IgG, IgA, IgM, C3, C4 and C1q in SLE (*Fig. 34-6*). Glomeruli of patients with vasculitis, both with and without ANCA positivity, have few or no immune deposits. Anti-GBM disease is characterized by linear staining of the GBM with IgG (rarely IgM and IgA) and C3.

Figure 34-4

(a) Predominantly fibrous crescent in a partially sclerosed glomerulus (Hematoxylin & eosin ×200). (b) Fibrous crescent and glomerulosclerosis. Note the periglomerular mononuclear infiltrate (Hematoxylin & eosin ×200). (See color plate 20)

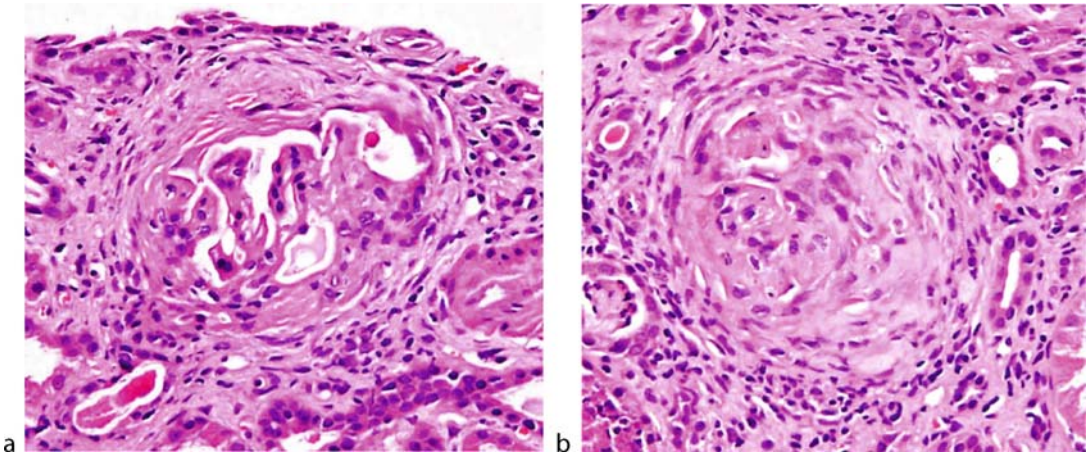
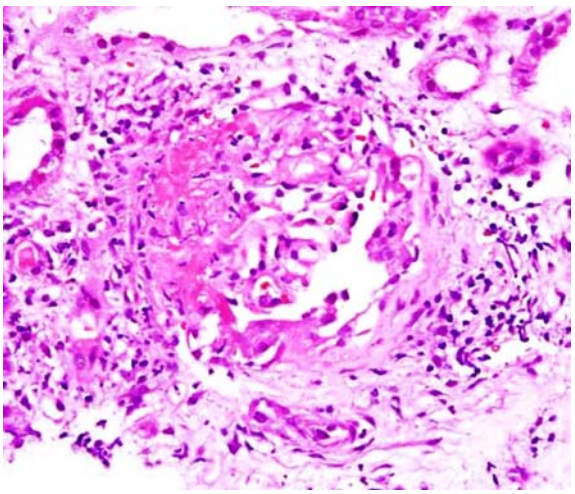


Figure 34-5

Glomerulus showing fibrocellular crescent with fibrinoid necrosis of part of the glomerular tuft. Note the disruption of the Bowman's capsule (Hematoxylin & eosin ×200). (See color plate 21)



Electron Microscopy

The findings on electron microscopy confirm those on immunofluorescence staining. Immune deposits are scant in ANCA-associated diseases and anti-GBM disease, but are common in immune complex GN, including SLE (where they are prominently present), postinfectious glomerulonephritis (chiefly subepithelial), and IgA and C_1q nephropathy disease (mesangial). Electron microscopy shows the ruptures of the Bowman's capsule and GBMs, wrinkling of the GBM and areas of mesangiolysis.

Diagnosis

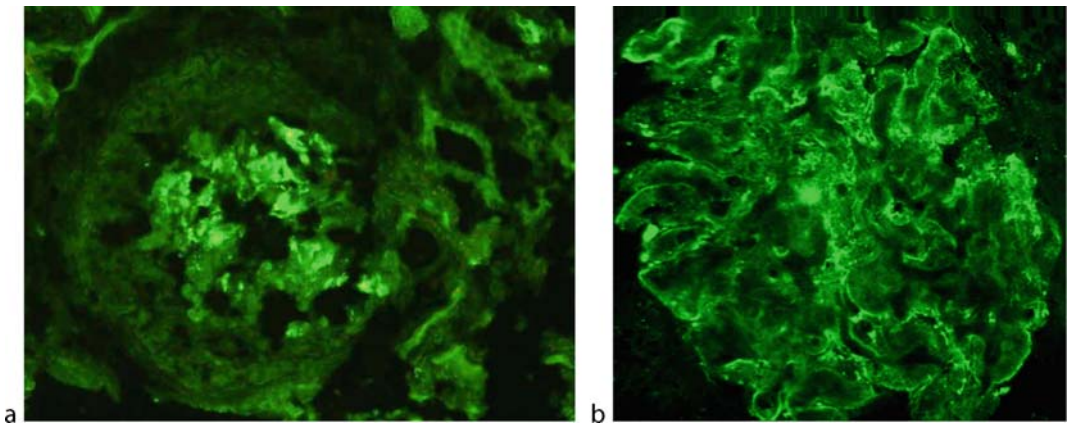
It is necessary to make an accurate and rapid diagnosis in RPGN as treatment strategies vary and delay in instituting treatment results in irreversible disease. All patients with RPGN should undergo a kidney biopsy promptly. While the majority shows the presence of crescentic GN, the detection of thrombotic microangiopathy (affecting interlobular arteries and arterioles) or diffuse proliferative GN is not unusual.

The diagnosis of the etiology of crescentic GN depends on integration of clinical data and findings on serology and renal histology (*Fig. 34-1*). In this way, anti-GBM disease or ANCA-associated RPGN can be distinguished from other causes of crescentic GN.

Patients with Wegener's granulomatosis have anemia, thrombocytosis and elevated acute-phase reactants (high erythrocyte sedimentation rate and C-reactive protein). The majority of these patients are positive for c-ANCA and for antibodies to proteinase 3. In the past, the diagnosis of Wegener's granulomatosis required biopsy of tissue from the lung, kidney, skin, nerve, sinus or nose. Currently the diagnosis is often made by the presence of typical clinical features, consistent

Figure 34-6

Immunofluorescence staining. (a) IgM deposits in a 7-year-old boy with rapidly progressive glomerulonephritis (GN) secondary to immune complex crescentic GN. (b) 12-year-old girl with class IV lupus nephritis; crescent formation shows granular deposition of IgG on the capillary wall ×1,200. (See color plate 22)



imaging studies (CT scans) and positive serology. When tissue is available, the characteristic pathologic features include the triad of granulomas, neutrophilic vasculitis and necrosis.

Microscopic polyangiitis is characterized by glomerulonephritis in 90% patients, pulmonary capillaritis and gastrointestinal involvement in 40–50% each; and skin and musculoskeletal features in 60% each. Microscopic polyangiitis is the most common cause of acute pulmonary-renal syndrome, although the differential diagnosis includes Wegener's granulomatosis, Churg-Strauss granulomatosis, lupus erythematosus and Goodpasture's disease.

Timely and appropriate therapy is indicated in view of the widely recognized unsatisfactory outcome in untreated patients.

Treatment

Evidence based data is limited and specific treatment guidelines for children are based on data from case series and prospective studies in adults (25). Besides specific therapy, supportive management includes maintenance of fluid and electrolyte balance, providing adequate nutrition, and control of infections and hypertension.

The specific treatment of RPGN comprises two phases: *induction* of remission and its *maintenance* (*Table 34-4*). The first phase aims at control of inflammation and the associated immune response. Once remission is induced, the maintenance phase attempts to prevent further renal damage and relapses.

Induction

Therapy with high dose corticosteroids is used initially. Treatment includes IV pulses of methylprednisolone (15–20 mg/kg, maximum 1 g/day) for 3–6 days, followed by high-dose oral prednisone (1.5–2 mg/kg daily) for 4 weeks, with tapering to 0.5 mg/kg daily by 3 months and alternate day prednisone for 6–12 months.

Cyclophosphamide is an important part of induction regimen for many authors, depending on the underlying disease, though there is debate on benefits of oral versus IV treatment. A meta-analysis of nonrandomized studies in ANCA positive pauci-immune GN showed that IV cyclophosphamide was significantly more likely to induce remission (odds ratio 0.29; 95% CI 0.12–0.73) and had a lower risk of infection and leukopenia. Pulse cyclophosphamide dosing was however associated with a greater risk of relapses, exposing patients to further immunosuppression (28). Oral and IV administration of cyclophosphamide were compared in the CYCLOPS trial of the European Vasculitis Study Group (EUVAS). Analysis of data from this trial shows that IV pulse cyclophosphamide is equally effective as daily oral treatment for induction of remission, but with significantly reduced dose and thereby lower toxicity (29). Cyclophosphamide is administered at an oral dose of 2 mg/kg/day, or IV starting at 500 mg/m^2 and increased every 3–4 weeks to a maximum dose of 750 mg/m^2. The dose should be adjusted to maintain a nadir leukocyte count, 2 weeks' post treatment, of 3,000–4,000/cu mm.

The patients are transferred to maintenance therapy at 3 months when receiving oral cyclophosphamide and

Table 34-4

Treatment of crescentic glomerulonephritis

Induction phase (3–6 months)	Maintenance phase (2–5 year)
Methylprednisolone 15–20 mg/kg (maximum 1 g) IV daily for 3–6 doses	Azathioprine 1.5–2 mg/kg/day for 12–18 months
Prednisone 1.5–2 mg/kg/day PO for 4 weeks; taper to 0.5 mg/kg daily by 3 months; 0.5–1 mg/kg on alternate day for 3 months	Prednisone 0.5–1 mg/kg on alternate days; later taper
[a]Cyclophosphamide 500–750 mg/m^2 IV every 3–4 weeks for 6 pulses	Consider mycophenolate mofetil (1,000–1,200 mg/m^2/day), if disease activity is not controlled
[b]Plasmapheresis (double volume) on alternate days for 2-weeks	
Agents for refractory disease	
Intravenous immunoglobulin, TNF-α antibody (infliximab), anti CD20 (rituximab)	

[a]The dose of cyclophosphamide is increased to 750 mg/m^2 if no leukopenia. Dose reduction is necessary in patients showing impaired renal function. Alternatively, the medication is given orally at a dose of 2 mg/kg daily for 12 weeks

[b]Plasmapheresis should begin early, especially if patient is dialysis dependent at presentation or if biopsy shows severe histological changes (>50% crescents). Plasma exchange is particularly useful in anti-GBM nephritis and ANCA-associated vasculitis. It might be considered in patients with immune complex GN with unsatisfactory renal recovery after steroid pulses

at 3–6 months when receiving the IV medication, once successful disease remission is achieved.

Plasmapheresis

Plasmapheresis or plasma exchange has been used for the treatment of crescentic GN with variable success. The mechanism of action is not clear, but is believed to involve removal of pathogenic autoantibodies, coagulation factors and cytokines. Plasma exchange has been shown, in randomized controlled trials in adults, to have therapeutic benefit in patients with anti-GBM disease with clearance of anti-GBM antibodies, lower serum creatinine and improved patient and renal survival (30). The benefits were limited in adults who were anuric with severe azotemia, dialysis dependent or having more than 85% crescents on renal biopsy.

The experience in other categories of crescentic GN is discussed below. Retrospective data in children with RPGN show benefits of plasma exchange if commenced within 1 month of onset of the disease (31). Prospective studies in pauci-immune crescentic GN suggest that discontinuation of dialysis and renal recovery was 91% and 38% when patients received plasma exchange with immunosuppression and immunosuppression alone respectively (32). Anecdotal reports confirm the effectiveness of plasmapheresis in patients with RPGN due to SLE, Henoch Schonlein purpura and severe proliferative GN, and in life-threatening pulmonary hemorrhage.

The role of intensive plasma exchange versus IV methylprednisolone, in addition to oral steroids and cyclophosphamide, was examined by the EUVAS MEPEX trial on 137 patients with renal vasculitis (33). This study investigated whether plasma exchanges were more effective than IV methylprednisolone in patients with ANCA-associated vasculitis and a serum creatinine >500 μmol/L (5.8 mg/dL). Patients were randomized to receive seven plasma exchanges ($n = 70$) or 3,000 mg of IV methylprednisolone ($n = 67$). Both groups received oral cyclophosphamide and oral prednisolone. At 3 months, 49% of those receiving IV methylprednisolone compared with 69% of the plasma exchange group were alive and independent of dialysis (95% CI for the difference 18–35%; $P = 0.02$). At 12 months, patients receiving plasma exchanges had a 24% lower risk for progression to ESRD (95% CI 6.1–41%). While this study supports the use of plasma exchange in the treatment of ANCA-associated vasculitis that presents with RPGN, it is important to note that there was no significant difference between the two groups in terms of rates of mortality at 3- and 12-months.

The role of combined induction with IV methylprednisolone, plasmapheresis and cyclophosphamide requires to be examined. It is emphasized that plasma exchanges have not been shown to be more effective than standard therapy in patients with less-severe renal dysfunction, or to benefit patients with advanced disease. In patients with extensive scarring and little or no activity on biopsy, intensive immunosuppression and/or plasma exchange should be avoided.

Maintenance

The requirement for maintenance therapy in crescentic GN depends on the underlying disease. Most patients with ANCA-associated disease need long-term maintenance immunosuppression, due to the risk of relapses. Extended treatment with cyclophosphamide has been used in adults, but carries significant risks and is currently not preferred for children. While azathioprine is not recommended during the induction phase, it is useful during the maintenance phase.

The timing of the switch from cyclophosphamide to azathioprine was clarified by the CYCAZAREM trial, which compared switching from cyclophosphamide to maintenance azathioprine at 3 versus 12 months (34). The conclusion of the trial was that, for patients in remission, the withdrawal of cyclophosphamide after 3 months and its substitution by azathioprine did not increase the rate of relapse; renal function and patient survival were also similar in the two groups.

The duration of maintenance treatment is debatable, with most patients of pauci-immune crescentic GN treated for 2 or more years (35). The length of maintenance therapy is extended in those with Wegner's granulomatosis or persistent PR3-ANCA positivity.

Supportive Treatment

Intensive immunosuppression is associated with an increased risk of infections. Prophylactic antimicrobials especially against *Pneumocystis carinii* and *Candida* may be required during induction. In areas of high prevalence, patients should be screened for tuberculosis. Patients on long-term therapy with corticosteroids should receive dietary calcium supplements. They should be counseled about the possibility of infertility following cyclophosphamide treatment. MESNA should be administered to patients receiving IV cyclophosphamide to protect against urothelial toxicity (35).

Specific Therapies

Immune Complex Crescentic GN

There are limited evidence-based recommendations on treatment for these patients. Therapy for immune complex GN largely depends on the underlying disease. The treatment of lupus nephritis, Henoch Schonlein purpura and IgA nephropathy presenting with RPGN is discussed in their respective chapters.

Postinfectious RPGN

Poststreptococcal GN presenting with extensive crescents is rare and the benefits of intensive immunosuppressive therapy are unclear, since most patients recover spontaneously. Nevertheless, immunosuppressive therapy with corticosteroids and alkylating agents has been used in patients with renal failure and extensive glomerular crescents (18, 36). Despite the lack of evidence-based data, patients with poststreptococcal RPGN and crescents involving 50% or more glomeruli should be treated with 3–6 IV pulses of methylprednisolone, followed by tapering doses of oral steroids. Some authors recommend the addition of cyclophosphamide to the therapeutic regimen.

Eradication of the infection and removal of infected prostheses are necessary for resolution of immune complex GN associated with active infections.

Pauci-Immune Crescentic GN

Induction therapy comprises of treatment with IV methylprednisolone (administered daily for 3–6 days) followed by oral prednisone and cyclophosphamide (given either orally for 3 months or by the IV route every 3–4 weeks for 6 months) (2, 25). Intensive PE for 2 weeks is advised for children who are dialysis dependent, those with pulmonary hemorrhage or not responding satisfactorily to induction treatment (33, 35).

Two recent studies compared the efficacy of treatment with mycophenolate mofetil, as an alternative to cyclophosphamide, for induction of remission (37, 38). These preliminary studies suggest that initial treatment with mycophenolate mofetil is promising in ameliorating disease activity and improving renal function in patients with crescentic GN.

Therapy is continued during the maintenance phase, with tapering doses of oral prednisone and azathioprine, for at least 24 months following successful disease remission (35). A longer duration of therapy, extended up to 5 year, is required in patients with Wegner's granulomatosis showing relapses, elevated ANCA titers and those with PR3-ANCA (2).

Treatment with mycophenolate mofetil, during the maintenance phase, has been examined in patients with systemic vasculitis. In an open-label investigation of the use of this agent for maintenance therapy of 11 patients with

Wegener's granulomatosis or microscopic polyangiitis and crescentic GN, treatment was associated with sustained remission in 91% (39). A National Institutes of Health (USA) trial on 14 patients examined the role of mycophenolate mofetil as maintenance therapy after induction with cyclophosphamide. While the agent was well tolerated, the relapse rate was high (43%) (40). Currently, the EUVAS IMPROVE trial is prospectively recruiting patients to compare the efficacy of mycophenolate mofetil to azathioprine for remission maintenance (41).

Approximately one-third patients with pauci-immune crescentic GN have one or more relapses. Minor relapse is treated with an increase in prednisone dosage. Major relapse is treated with reinstitution of induction therapy with cyclophosphamide with an increase in prednisone; IV methylprednisolone or plasma exchange may also be considered (35). Less intensive treatment with mycophenolate mofetil is proposed for relapses that are diagnosed early.

Anti-GBM Crescentic GN

Prompt institution of plasma exchange is necessary in subjects with anti-GBM nephritis. Double volume exchange is done daily, and subsequently on alternate days until anti-GBM antibodies are no longer detectable (usually 2–3 weeks) (1, 25). The patients are also treated with IV methylprednisolone (described above) followed by high-dose oral prednisone, with subsequent tapering over several months. Co-administration of cyclophosphamide (2 mg/kg daily for 3 months) is effective in suppressing further antibody production. Pulmonary hemorrhage responds to therapy with three-doses of methylprednisolone (20 mg/kg on alternate days); plasmapheresis is beneficial in these patients.

As anti-GBM disease does not usually have a relapsing course, prolonged maintenance therapy is not required and treatment with steroids is tapered over the next 6–9 months. Patients treated early in the course of their illness do satisfactorily. In patients who develop end stage renal disease, transplantation should be deferred until anti-GBM antibodies are undetectable for 12 months, at which point disease recurrence is unlikely.

A proportion of patients with anti-GBM nephritis also show positive ANCA, most often p-ANCA. While the precise significance of the dual positivity is unclear, the initial clinical outcome for these patients is similar to that for classical anti-GBM disease. In view of a higher risk of relapses, these patients require a longer course of maintenance immunosuppresive therapy (as for pauci-immune crescentic GN) (42).

Newer Agents

A number of studies have examined the efficacy of intravenous immunoglobulin in subjects with ANCA positive systemic vasculitis and RPGN, with benefit lasting for up to 3-months (43). The EUVAS NORAM study compared the effectiveness of orally administered methotrexate and cyclophosphamide in adult patients with early systemic vasculitis and mild renal involvement. Induction of remission were similar in the two groups at 6 months (90 vs. 94% respectively), but relapses were significantly more frequent after treatment withdrawal in the methotrexate treated patients (44). Methotrexate, however, accumulates in renal impairment and is therefore not recommended for patients with moderate or severe renal dysfunction.

Rituximab, a monoclonal antibody directed against CD20 antigen on B cells, has been used successfully in therapy resistant lupus nephritis and refractory Wegener's granulomatosis. Eleven patients with refractory small vessel vasculitis, not controlled by maximally tolerated doses of cyclophosphamide and corticosteroids or a contraindication to treatment with cyclophosphamide received treatment with 4 weekly injections of rituximab. Disease remission and reduction of ANCA titers were induced in all patients within 6 months; remission persisted if B cells remained absent and acute-phase reactants (C-reactive protein, sedimentation rate) were reduced (45).

The efficacy of other agents, including cyclosporin, leflunomide, mizoribine, deoxyspergualin, anti-thymocyte globulin, anti-CD52 antibodies and infliximab is being examined (46, 47). Although potentially useful for treatment of individual patients, there is insufficient evidence to recommend general use of these agents.

Outcome

The outcome of these patients has improved in the last decades, as almost 60–70% patients recover renal function. The course is largely determined by the severity of renal failure at presentation and the promptness of intervention, renal histology and the underlying disease (1, 2, 25). Patients with poststreptococcal crescentic GN have a better prognosis, most of them showing spontaneous improvement after supportive management. The outcome in patients with pauci-immune crescentic GN, membranoproliferative glomerulonephritis and idiopathic RPGN is less favorable than Henoch Schonlein purpura or systemic lupus erythematosus.

The potential for recovery is related to the relative proportion of cellular or fibrous crescents, and the extent

of tubular atrophy, interstitial fibrosis and glomerulosclerosis. In patients with severe crescentic GN, the presence of normal glomeruli are a positive predictor of dialysis independence and recovery of renal function, suggesting that the unaffected part of the kidney is vital in determining renal outcome (48).

Post Transplant Recurrence

The use of potent immunosuppressive medications following transplantation and antigenic characteristics of the graft prevent posttransplant recurrence in most patients. Better graft survival has however increased the likelihood of disease recurrence in the allografts. A positive ANCA titer at the time of transplantation does not increase the risk of posttransplant recurrence. Conditions associated with risk of histological recurrence include membranoproliferative GN type II, IgA nephropathy, Henoch Schonlein purpura and systemic lupus erythematosus.

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Tubular Disease

35 Nephronophthisis and Medullary Cystic Kidney Disease

Friedhelm Hildebrandt

Natural History of Nephronophthisis and Medullary Cystic Kidney Disease

Nephronophthisis (NPHP) is an autosomal recessive cystic kidney disease that constitutes the most frequent genetic cause for end-stage kidney disease (ESKD) in the first 3 decades of life (1–4). Three clinical forms of NPHP have been distinguished by age of onset of ESKD: infantile (5, 6), juvenile (7), and adolescent NPHP (8), which manifest with ESKD at median ages of 1 year, 13 years, and 15 years, respectively. Initial symptoms are relatively mild with the exception of infantile NPHP type 2. They consist of polyuria, polydipsia with regular fluid intake at nighttime, secondary enuresis, and anemia (9). A slightly raised serum creatinine is noted at an average age of 9 years, before ESKD invariably develops within a few years (› *Fig. 35-1*). Renal ultrasound reveals increased echogenicity (› *Fig. 35-2*). Beyond the age of 9 years cysts appear at the corticomedullary junction within kidneys of normal or slightly reduced size (› *Fig. 35-2*) (10). Renal histology reveals a characteristic triad of tubular basement membrane disruption, tubulointerstitial nephropathy, and cysts (› *Fig. 35-3*) (11, 12). In nephronophthisis cysts arise from the corticomedullary junction of the kidneys (› *Fig. 35-2*). Because kidney size is normal or slightly reduced (except in infantile NPHP type 2, where there is moderate renal enlargement), cysts seem to develop *e vacuo* through loss of normal tissue. This is in contrast to polycystic kidney disease, where cysts are distributed evenly and lead to gross enlargement of the kidneys (13).

NPHP is inherited in an autosomal recessive mode. This includes NPHP variants with extrarenal manifestations (1, 13). In more than 10% of cases NPHP can be associated with extrarenal involvement, primarily including retinal degeneration (Senior-Loken syndrome) (14, 15), cerebellar vermis aplasia (Joubert syndrome) (16, 17), liver fibrosis (18), and cone-shaped epiphyses (19). The extrarenal manifestations will be discussed below in light of the cilia/centrosome theory of NPHP. NPHP has previously been grouped together with the clinical entity of medullary cystic kidney disease (MCKD) (7, 11), due to similarities of clinical and pathologic features (20). Both, NPHP and MCKD, feature corticomedullary cysts in kidneys of normal or slightly reduced size. However, MCKD is clearly distinct from NPHP regarding multiple aspects: (1) MCKD follows autosomal dominant inheritance, (2) ESKD occurs in the fourth decade or later, and, (3) in MCKD there is no extrarenal involvement other than hyperuricemia and gout.

Nephronophthisis and dominant MCKD seem to be distributed evenly among males and females. Nephronophthisis has been reported from virtually all regions of the world (21)with an incidence of 9 patients/8.3 million (22) in the United States or 1 in 50,000 live births in Canada (23). The condition constitutes the most frequent genetic cause for ESKD in the first two decades of life, and is a major cause of end-stage renal disease in children, accounting for 10–25% of these patients (21, 24, 25). In the North American pediatric ESKD population pooled data indicate a prevalence of less than 5%. MCKD appears to be somewhat more rare.

NPHP was first described by Smith and Graham in 1945 (2) and by Fanconi et al. (3), who introduced the term "familial juvenile nephronophthisis". Since then over 300 cases have been published in the literature (11). In NPHP the earliest presenting symptoms are polyuria, polydipsia, decreased urinary concentrating ability, and secondary enuresis. They occur in over 80% of cases (21) and start at around 6 years of age. Anemia and growth retardation develop later in the course of the disease (9). Regular fluid intake at nighttime is a characteristic feature of the patients' history, and starts around age 6 years. Due to the mild nature of symptoms and the lack of edema, hypertension, and urinary tract infections there is often a delay in the diagnosis of NPHP. This may cause a risk of sudden death from fluid and electrolyte imbalances. Disease recurrence has never been reported in kidneys transplanted to NPHP patients (26). By positional cloning nine different recessive genes (*NPHP1-9*) have been identified

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to cause NPHP types 1–9 (see below). This has made definite molecular genetic diagnostics possible (www.renalgenes.org). Homozygous deletions in the *NPHP1* gene account for approximately 25% of all cases of NPHP, whereas the other genes contribute less than 3% each. As expected in a recessive disease, penetrance of the renal phenotype seems to be 100%.

In NPHP, chronic renal failure develops within the first 3 decades of life (8, 27, 28). Infantile NPHP, which is characterized by mutations in *NPHP2/inversin*, leads to ESKD between birth and 3 years of age (28, 29). In a study conducted in 46 children with juvenile NPHP type 1 caused by mutations of the *NPHP1* gene, a serum creatinine of 6 mg/dl was reached at a median age of 13 years of age, (range 4–20 years) (*Fig. 35-1*) (7, 27). Similarly, the median age of ESKD in patients with mutations in the *NPHP5* gene was 13 years (30). The median time lapse between a serum creatinine of 2 and 4 mg/dl was 32 months, and between 4 and 6 mg/dl 10 months (*Fig. 35-1*) (31). In patients with adolescent NPHP due to mutations in the *NPHP3* gene ESKD developed by 19 years of age (8). If renal failure has not developed by the age of 25 years, the diagnosis of recessive NPHP should be questioned and autosomal dominant MCKD considered as a differential diagnosis. In MCKD, which follows autosomal dominant inheritance, ESKD occurs later in life. MCKD types 1 and 2 show a median onset of ESKD at 62 years (32) and 32 years (33), respectively. MCKD type 2 can be positively diagnosed by detection of mutations in the *UMOD* gene encoding uromodulin/Tamm-Horsfall protein (34).

Figure 35-1

Progression chart representing the average course of deterioration of renal function in 19 patients of 8 families with NPHP type 1 as proven by homozygous deletion of the *NPHP1* gene. Median (solid line) and quartile (dashed lines) curves were calculated from 308 serial SCR values (Reproduced with permission from (27)).

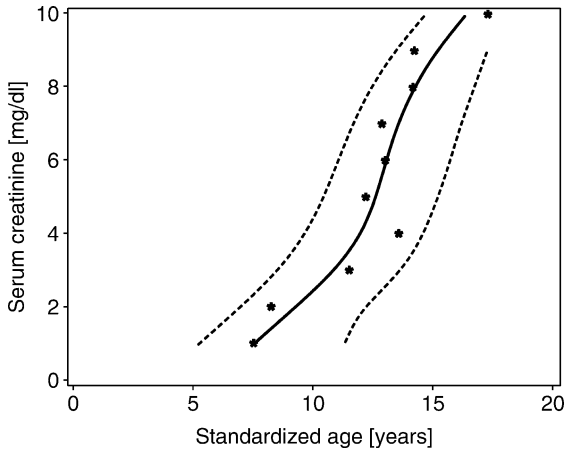


Figure 35-2

Renal ultrasound in nephronophthisis. Note kidney of normal size (12 cm between markings), loss of cortico-medullary differentiation, presence of cysts at the cortico-medullary border of the kidney, and increased echogenicity, which renders the ultrasound pattern similar to the pattern of liver (Courtesy of Dr. U. Vester, Essen).

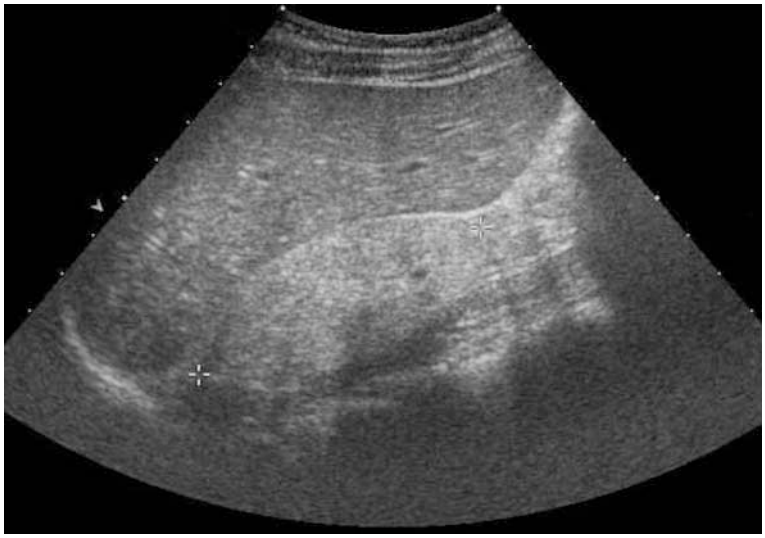
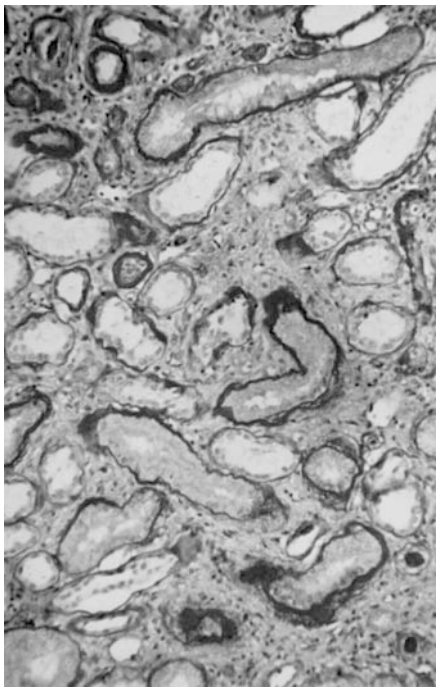


Figure 35-3

Renal histology of nephronophthisis. Note the characteristic triad of tubular basement membrane disruption, tubular cell atrophy with cysts, and interstitial infiltration with fibrosis. Hematoxylin/eosin stain (Courtesy of Prof. R Waldherr, Heidelberg).



Pathology and Histopathology

Renal histopathology is very similar in NPHP and MCKD, and has been described comprehensively in 27 patients with NPHP by Waldherr and associates (11, 12) (*Fig. 35-3*). Kidney size is normal or moderately reduced. There is always bilateral renal involvement. Macroscopically, the kidney surface has a finely granular appearance, most likely due to the protrusion of dilated cortical collecting ducts. There are between 5 to approximately 50 cysts of 1–15 mm in diameter, located preferentially at the corticomedullary border. The cysts arise primarily from the distal convoluted and medullary collecting tubules as shown by microdissection (35), but may also appear in the papilla. Cysts are observed only in about 70% of autopsy cases, and seem to arise late in the course of the disease (36). Therefore, the presence of cysts is not a prerequisite for diagnosis. No cysts are present in organs other than the kidney. The histologic changes of NPHP are characteristic but not specific for the disease and seem to develop only postnatally. Typically, there is pronounced thickening and multilayering of the tubular basement membrane (TBM), which represents the most characteristic histologic feature of NPHP and MCKD (*Fig. 35-3*). By light microscopy there appears to be a sequence of events, TBM disruption is followed by lymphocytic and histocytic peritubular infiltration. Subsequently, atrophic or dilated and tortuous tubules develop predominantly at the corticomedullary junction. In advanced stages the picture merges into a diffuse sclerosing tubulo-interstitial nephropathy. TBM changes and cyst formation are most prominent in distal tubules, where cysts are lined with a single layer of cuboidal or flattened epithelium (37–40). Glomeruli demonstrate periglomerular fibrosis with splitting and thickening of Bowman's capsule. Glomerular obsolescence is only present in nephrons that have been destroyed by tubular alterations. Leakage of Tamm-Horsfall protein from damaged collecting tubules into the interstitium has been demonstrated in patients with MCKD (41). On transmission electron microscopy there is thickening, splitting, attenuation, and granular disintegration of the tubular basement membrane without clear stages of transition (12). A marked increase of microfilaments is seen at the base of the tubular epithelial cells.

Molecular Genetics and Pathophysiology

A positional cloning approach was used to gain insight into the pathogenesis of NPHP and MCKD (*Table 35-1*). This has revealed recessive mutations in nine different novel genes as causing NPHP. These are *NPHP1* (42, 43), *NPHP2/inversin* (6), *NPHP3* (44), *NPHP4* (45, 46), *NPHP5* (30), *NPHP6/CEP290* (47, 48), *NPHP7/GLIS2* (49), *NPHP8/RPGRIP1L* (50–52), and *NPHP9/NEK8* (53), defining NPHP types 1 through 9, respectively (*Table 35-1*). These are monogenic recessive genes, implying that mutations in each single gene is sufficient in itself to cause NPHP in a patient, indicating that their gene products are necessary for normal kidney function. Gene identification thereby generated new insights into disease mechanisms of NPHP, and revealed that they are related to signaling mechanisms of primary cilia, centrosomes, and planar cell polarity (1, 6, 54, 55) (see below and *Fig. 35-4*). Gene identification has made definite molecular genetic diagnostics possible (www.renalgenes.org) for approximately 30% of cases. Homozygous deletions in the *NPHP1* gene account for approximately 21% of all NPHP cases, whereas the other genes (*Table 35-1*) contribute less than 3% each. Thus the causative genes are still unknown in about 70% of cases, indicating that

Table 35-1

Disease variants, gene loci, extrarenal manifestations, and mouse models of nephronophthisis (NPHP) and medullary cystic kidney disease (MCKD)

Disease	Gene	Onset of ESRD (Median in Years)	Chromosome	Gene (Product)	Extrarenal Association	Mouse Model
Nephronophthisis						
Type 1 (juvenile)	*NPHP1*	13	*2q12.3*	NPHP1/nephrocystin-1	SLSN, OMA, JBTS, MKS	–
Type 2 (infantile)	*NPHP2/ INVERSIN*	1–3	*9q22-q31*	NPHP2/Inversin	SLSN, ventricular septal defect, *situs inversus*	*inv/inv*[76]
Type 3 (adolescent)	*NPHP3*	19	*3q22*	NPHP3	SLSN, LF	*pcy*[82], *Nphp-/-*[83]
Type 4	*NPHP4*	20	*1p36*	NPHP4/nephroretinin	SLSN	–
Type 5	*NPHP5/ IQCB1*	13	*3q13.33*	NPHP5/IQ motif containing B1	SLSN (all patients)	–
Type 6	*NPHP6/ CEP290*	<13	*12q21.32*	NPHP6/centrosome protein Cep290	SLSN, JBTS, MKS	*rd16*[87]
Type 7	*NPHP7/ GLIS2*	~17	*16p13.3*	NPHP7/GLIS familiy zinc finger 2	–	*Glis*[2]*/-*[5]
Type 8	*NPHP8/ RPGRIP1L*	<13	*16q12.2*	NPHP8/RPGRIP1-like	JBTS, MKS	*Ftm*[51]
Type 9	*NPHP9/ NEK8*	~13	*17q11.2*	NPHP9/NIMA-related kinase 8	–	*jck*[93]
Medullary Cystic Kidney Disease						
MCKD type 1	*?*	62	*1q21*		Hyperuricemia, gout	
MCKD type 2	*UMOD*	32	*16p12*	Uromodulin/Tamm-Horsfall protein	Hyperuricemia, gout	*Umod-/-*[148]

AD autosomal dominant, *AR* autosomal recessive, *ESKD* end-stage kidney disease, *JBTS* Joubert syndrome, *LF* liver fibrosis, *MKS* Meckel syndrome, *OMA* oculomotor apraxia type Cogan, *SLSN* Senior-Loken syndrome, *UMOD* uromodulin/Tamm-Horsfall protein – = no data

further genes are involved in the pathogenesis of NPHP. Recently, evidence has been generated that more than one recessive gene may be mutated in individual patients with NPHP (56) as has been proposed for the related disorder Bardet-Biedl syndrome (BBS) (57, 58). In the following, the pathogenesis of NPHP will be discussed in the context of the discovery of each of the genes *NPHP1* – *NPHP9* (*Table 35-1*). The structure and function of primary cilia and basal bodies is delineated in *Fig. 35-2*.

NPHP1

In juvenile nephronophthisis (NPHP type 1) a gene locus has been mapped to chromosome 2q12.3 (59). This locus was further refined (60–64) and the gene (*NPHPP1*) responsible for NPHP1 was identified by positional cloning (42, 43). About 85% of patients with NPHP type 1 carry large homozygous deletions of the *NPHP1* gene (65, 66). Spontaneously occurring deletions of the *NPHP1* locus (67) as well as specific loss-of-function point mutations of *NPHP1* have been characterized (27, 68). In a subset of patients with large deletions in *NPHP1* there is an association with oculomotor apraxia type Cogan (66) (*Table 35-1*). Another subset shows an association with retinitis pigmentosa (68). Mutations in *NPHP1* were identified as causing juvenile nephronophthisis type 1 (42, 43). *NPHP1* encodes nephrocystin-1, a protein that interacts with components of cell-cell and cell-matrix signaling, including p130Cas (69), focal adhesion kinase 2 (70), tensin, filamin A and B (71, 72). It is located at adherens junctions and focal adhesions of

Figure 35-4

Cilia structure and intraflagellar transport. The cilium is a hair-like structure that extends from the cell surface into the extracellular space. Virtually all vertebrate cell types can produce cilia. Cilia consist of a microtubule-based axoneme covered by a specialized plasma membrane. The axoneme has nine peripheral microtubule doublets. There may be two central microtubules (9 + 2 vs. 9 + 0 axoneme). 9 + 2 cilia usually have dynein arms that link the microtubule doublets and are motile, while most 9 + 0 cilia lack dynein arms and are non-motile (''primary cilia'') with a few exceptions. The ciliary axoneme is anchored in the basal body, a microtubule-organizing center derived from the mother centriole. The transition zone at the junction of the basal body acts as a filter for the molecules that can pass into or out of the cilium. Nephrocystin-1 is localized at the transition zone of epithelial cells (73). During ciliogenesis, cilia elongate from the basal body by the addition of new axonemal subunits to the distal tip, the plus end of the microtubules. Axonemal and membrane components are transported in raft macromolecular particles (complex A and B) by so-called intraflagellar transport (IFT) along the axonemal doublet microtubules (159). Anterograde transport towards the tip is driven by heterotrimeric kinesin 2, which contains motor subunits Kif3a and Kif3b and a non-motor subunit. Mutations of Kif3a cause renal cysts and cerebellar vermis aplasia in mice (160). Retrograde transport back to the cell body occurs via the motor protein cytoplasmic dynein 1B (161) (modified from Bisgrove and Yost, 2006) (162).

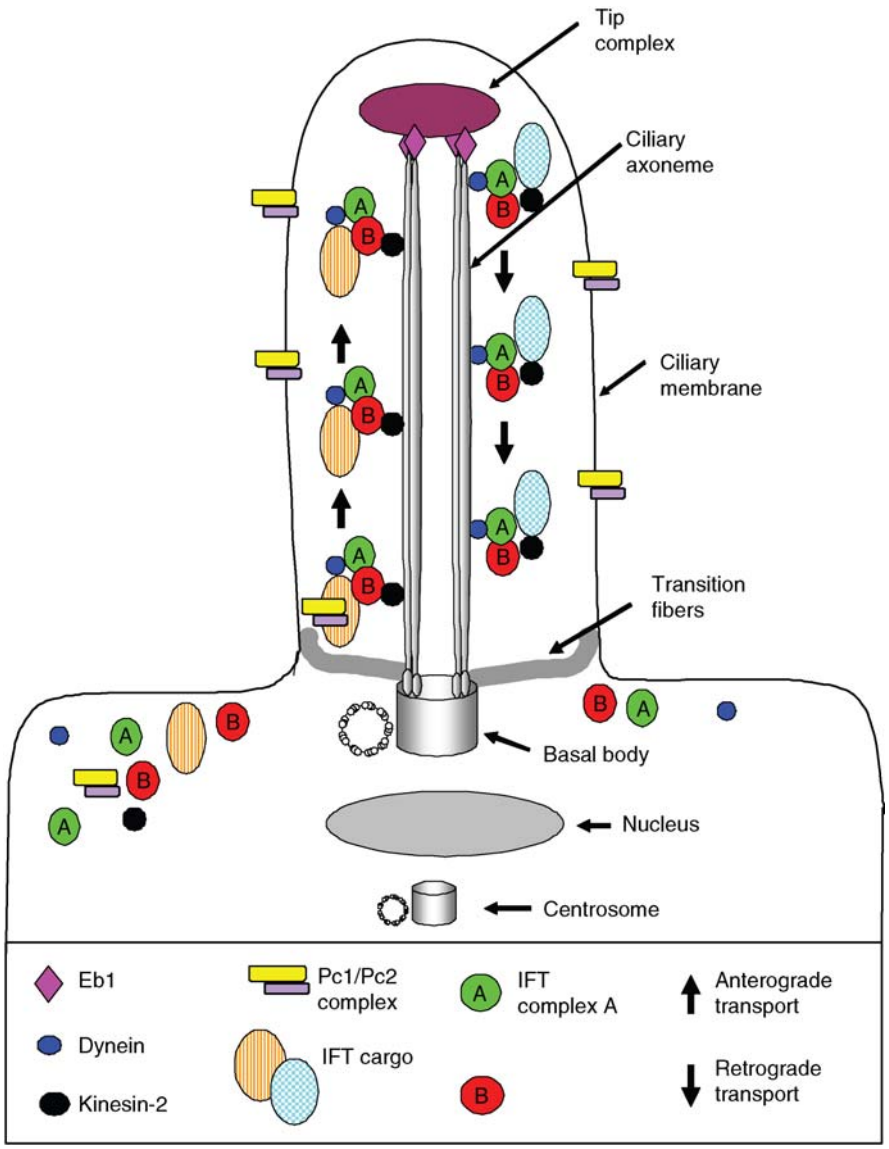
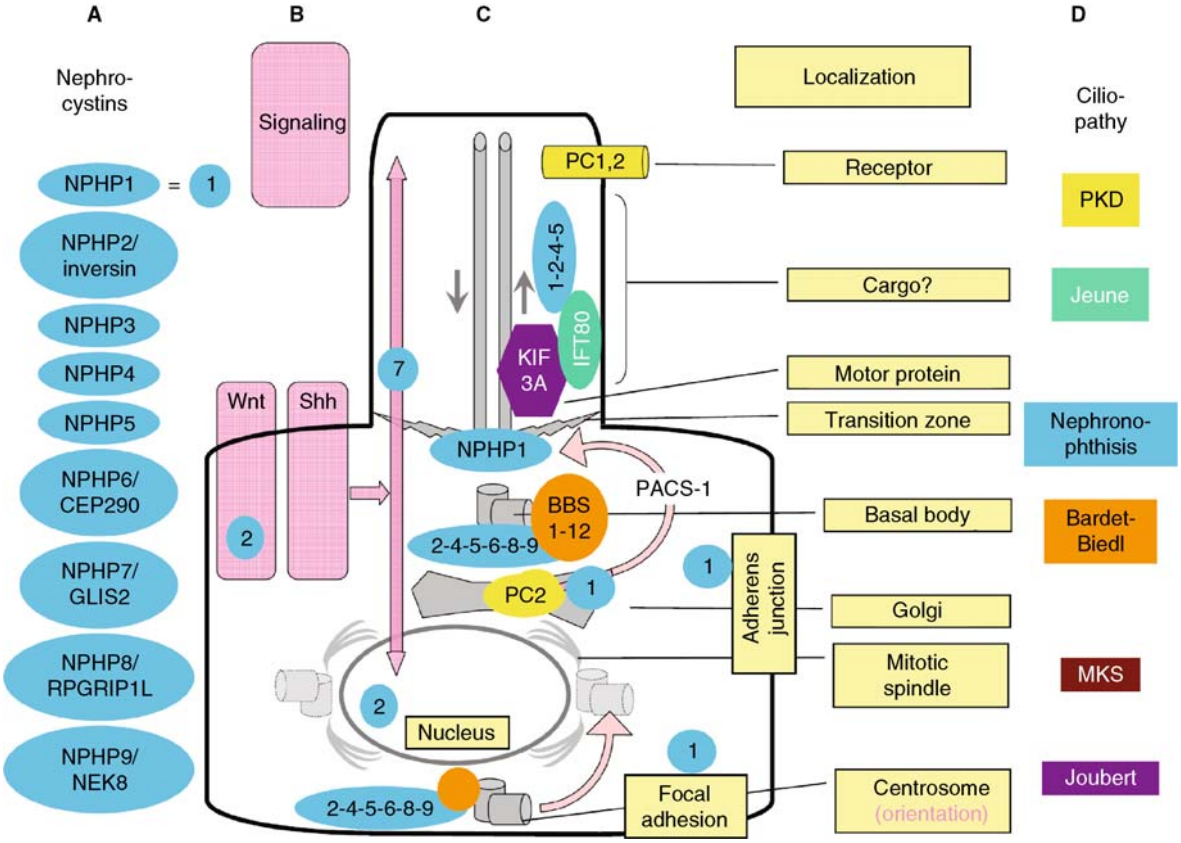


Figure 35-5

Subcellular localization of nephrocystins to primary cilia, basal bodies, the mitotic spindle, focal adhesions and adherens junctions, and functional interaction with other proteins mutated in renal "ciliopathies". "Cystoproteins" are proteins of genes mutated in cystic kidney diseases of humans, mice, or zebrafish. Depending on cell cycle stage, cystoproteins are localized at different subcellular organelles (shown in grey) (47, 163) including primary cilia, basal bodies, endoplasmic reticulum, the mitotic spindle, centrosomes, adherens junctions or focal adhesions. Arrows in the primary cilium indicate the direction of anterograde transport along the microtubule system mediated by kinesin-2 and retrograde transport by cytoplasmic dynein 1b. (A) Most nephrocystins (blue) are located at cilia, the basal body, and centrosome in a cell cycle dependent manner. NPHP1 is also at the transition zone, focal adhesions and adherens junctions. (B) Sensory cilia (*Fig. 35-4*) perceive and process cell external signals, and "cystoproteins" are involved in signaling mechanisms downstream of cilial signal recognition. Downstream of cilia (pink), Wnt signaling (*Fig. 35-6*) and hedgehog signaling play a role in planar cell polarity, which is mediated (C) partially through orientation of centrosomes and the mitotic spindle poles. (D) Cilia-dependent mechanisms of planar cell polarity seem to be the central to the pathogenesis of the ciliopathies, the most prominent of which are listed on the right. Wnt, the Wnt signaling pathway; Shh, the sonic hedgehog signaling pathway.



renal epithelial cells (71, 72), which are involved in cell-cell and cell-basement membrane contacts, respectively (*Fig. 35-5*). Nephrocystin-1 also interacts with the product of other nephronophthisis genes such as nephrocystin-2/inversin (6), nephrocystin-3 (44) and nephrocystin-4 (45, 46). More recently, it was shown that nephrocystin-1 is targeted to the transition zone of motile and primary cilia by the protein PACS-1 (phosphofurin acidic cluster sorting protein-1) (73, 74) (*Fig. 35-5*). This is initiated by casein kinase 2-mediated phosphorylation of three critical serine residues within a cluster of acidic amino acids in nephrocystin, leading to PACS-1 binding, and to colocalization of nephrocystin with PACS-1 at the base of cilia (74).

NPHP2/Inversin

Infantile nephronophthisis (NPHP type 2) was recognized as a distinct disease entity, in which end-stage renal failure occurs within the first 3 years of live (5, 75) (*Table 35-1*). Macroscopically, NPHP type 2 differs from other forms of NPHP by the presence of enlarged kidneys and cortical microcysts, and by the absence of medullary cysts. Histologically, there is no disruption of tubular basement membranes. Mutations of *NPHP2/inversin* (*INVS*) were identified as the cause of infantile NPHP (type 2) with and without situs inversus (6) by positional cloning (28) and using candidate gene data (76, 77). The renal cystic changes of infantile nephronophthisis combine clinical features of NPHP and of PKD (5). The gene products nephrocystin-1 and NPHP2/inversin interact with β-tubulin, which constitutes the microtubule axoneme of primary cilia (*Fig. 35-4*), and they are localized at primary cilia of renal tubular cells (*Fig. 35-5*) (6). These findings supported a unifying theory of renal cystogenesis (1, 54, 78, 79), which states that proteins ("cystoproteins") which are mutated in renal cystic disease in humans, mice or zebrafish, are expressed in primary cilia, basal bodies, or centrosomes (6, 54). Basal bodies are the foundations from which cilia are assembled. Once mitosis and cell division are completed, basal bodies derive from the *mother* centriole of the centriole pair that had previously organized the mitotic spindle in cell division. When cilia are formed from the basal body, the *daughter* centriole is placed on the side of the nucleus opposite to the basal body, thus specifying cell polarity (*Fig. 35-5*). It is becoming apparent that primary cilia are highly conserved structures that sense extracellular cues in a broad spectrum of epithelial tissues. There is a wide range of cues that can be received by specific ciliary receptors, including photosensation, mechanosensation, osmosensation, and olfactory sensation. In general, it seems that the pathogenesis of ciliopathies is based on an inability of epithelial cells to sense or process extracellular cues (80). Inversin was shown to localize to different subcellular locations, in a cell cycle dependent manner. Specifically, it is found at the mitotic spindle in mitosis, at the midbody in cytokinesis, and in cilia, at the basal body and centrosome in interphase (*Fig. 35-5*). All of these subcellular organelles are involved in regulation of planar cell polarity or the cell cycle (see below). In this context, a major breakthrough was made for the understanding of the pathogenesis of renal cystic diseases when Simons et al. demonstrated a role of inversin/NPHP2 in signaling mechanisms of planar cell polarity necessary to maintain normal tubular development and morphology (81) as outlined in *Fig. 35-6* (55, 81). As a consequence of this model, when inversin is defective (as in NPHP type 2) the canonical Wnt pathway will prevail (*Fig. 35-6*), which will interfere with proper apical-basolateral polarity of the renal epithelium (55) (*Fig. 35-7*).

NPHP3

In a large Venezuelan kindred we identified by positional cloning mutations in *NPHP3* as responsible for adolescent nephronophthisis (*Table 35-1*) (8, 44). The *pcy* mouse model demonstrated that mutations in the mouse ortholog *Nphp3* cause the renal cystic mouse mutant *pcy* (44), which was demonstrated to be responsive to treatment with a vasopressin receptor antagonist (82). Recently, it was shown that complete loss of *Nphp3* function results in *situs inversus*, congenital heart defects, and embryonic lethality in mice (83) and that truncating mutations of *NPHP3* in humans can cause a broad clinical pattern that resembles Meckel syndrome. This included *situs inversus*, polydactyly, central nervous system malformations, structural heart defects, preauricular fistulas, and congenital anomalies of the kidney and urinary tract (83).

NPHP4

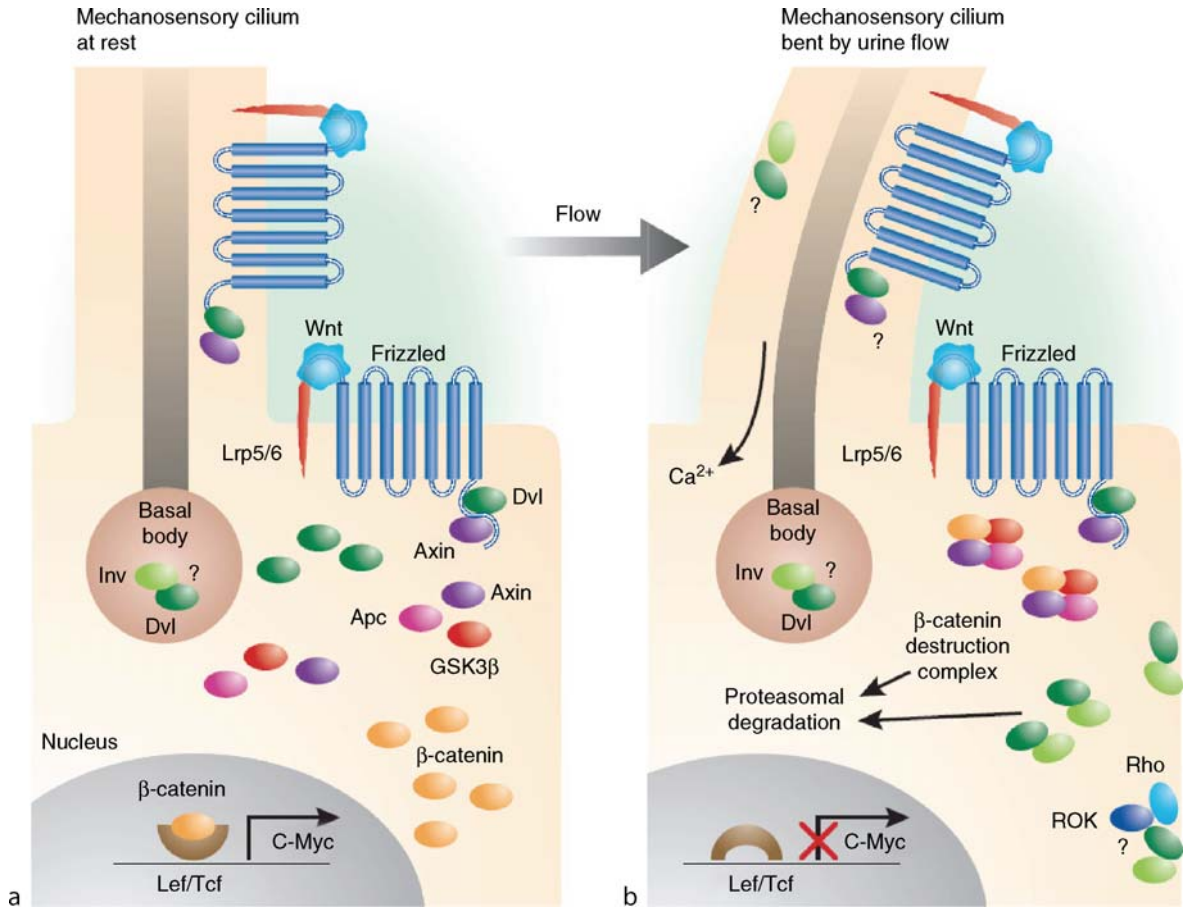
Mutations in the novel gene *NPHP4* were identified by homozygosity mapping and total genome search for linkage (45, 46, 84) (*Table 35-1*). Nephrocystin-4, like inversin, localizes to primary cilia, basal bodies, centrosomes, and the cortical actin cytoskeleton (85) (*Fig. 35-5*).

NPHP5

Recessive mutation in the novel gene *NPHP5* were identified as mutated in nephronophthisis type 5 (30). All mutations detected were truncations of the encoded protein nephrocystin-5, and all patients had an association with early-onset retinal degeneration. Thus, *NPHP5* represents the gene mutated in the typical early-onset form of Senior-Loken syndrome (SLSN) (*Table 35-1*). Nephrocystin-5 contains an IQ domain, which directly interacts with calmodulin (30), and is in a complex with the retinitis pigmentosa GTPase regulator (RPGR), which when defective causes X-linked retinitis pigmentosa. Both, nephrocystin-5 and RPGR are localized in connecting cilia of photoreceptors and in primary cilia of renal epithelial cells (30) (*Fig. 35-5*). The fact that connecting

Figure 35-6

Inversin/NPHP2 mediates a switch from the canonical the non-canonical Wnt signaling pathway, which plays a role in planar cell polarity maintenance (81). (a) This cartoon of a renal tubular epithelial cell shows how Wnt signaling occurs primarily through β-catenin–dependent pathways in the absence of urine flow. Ligand binding by the frizzled receptor results in inactivation of the β-catenin destruction complex through the presence of disheveled (Dvl), increased β-catenin levels, and upregulation of effector gene expression of the canonical Wnt signaling pathway. (b) Stimulation of the primary cilium, e.g. by urine flow, results in increased expression of inversin (Inv), which then reduces levels of cytoplasmic Dvl by increasing its proteasomal degradation. This allows reassembly and activation of the β-catenin destruction complex, thereby switching from the canonical to the non-canonical Wnt signaling pathway. The model is consistent with the finding that overexpression of β-catenin (equivalent to canonical Wnt signaling) leads to renal cysts in a mouse model (164) (from (55)).



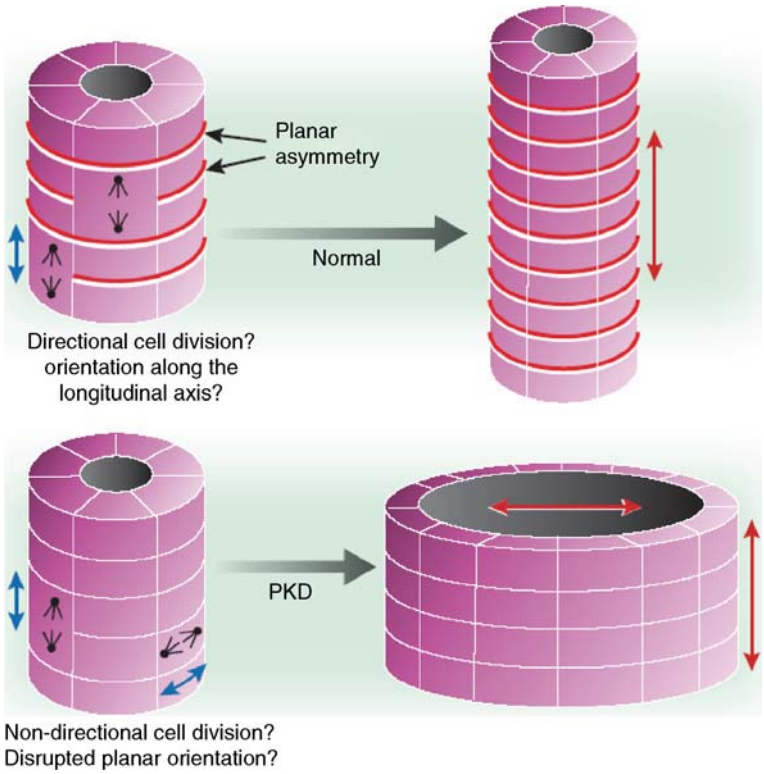
cilia of photoreceptors are the structural equivalents of primary cilia of renal epithelial cells rendered an explanation for retinal involvement in the retinal-renal syndrome Senior-Loken syndrome (*Fig. 35-8*).

NPHP6/CEP290

Recessive truncating mutations in the novel gene *NPHP6/CEP290* were identified as the cause of NPHP type 6 and Joubert syndrome type 5 by positional cloning (47). Its gene product nephrocystin-6/CEP290 is part of the centrosomal proteome (86) (*Table 35-1*). Similar to NPHP2/inversin and NPHP4, NPHP6/CEP290 is expressed in centrosomes and the mitotic spindle in a cell-cycle dependent manner. Abrogation of *NPHP6* function in zebrafish caused planar cell polarity defects and recapitulated the human phenotype of NPHP type 6, including renal cysts, retinal degeneration, and cerebellar defects (47). Nephrocystin-6 modulates the activity of ATF4/CREB2, a transcription factor that may be implicated in cAMP-dependent renal cyst formation (82).

Figure 35-7

Defects of cystoproteins lead to disruption of planar cell polarity, and thereby to renal cysts through to malorientation of the centrosome or mitotic spindle complex. Correct orientation of the mitotic spindle and centrosomes with respect to the longitudinal axis of the tubule is critical for proper planar cell polarity (i.e., the orientation of an epithelial cell layer in 3-dimensional space). Non-canonical Wnt signaling (see *Fig. 35-6*) is involved in regulation of planar cell polarity during renal tubular morphogenesis, when in rodents 2 weeks post partum the tubules still elongate. The structure that would result from disruption of this longitudinal orientation is a dilated tubule or cyst (from (55)).



Interestingly, a 300-amino acid in-frame deletion of *Nphp6/Cep290* caused retinal degeneration only, without renal or cerebellar involvement in the *rds16* mouse model (87) (*Table 35-1*). This is in accordance with the recent finding that a hypomorphic mutation of *NPHP6/CEP290* represents the most frequent cause of Leber's congenital amaurosis (88). Mutations in *NPHP6/CEP290* have been confirmed as causing JBTS with and without renal involvement (48). Furthermore, truncating mutations in *NPHP6* were shown to cause Meckel syndrome (89).

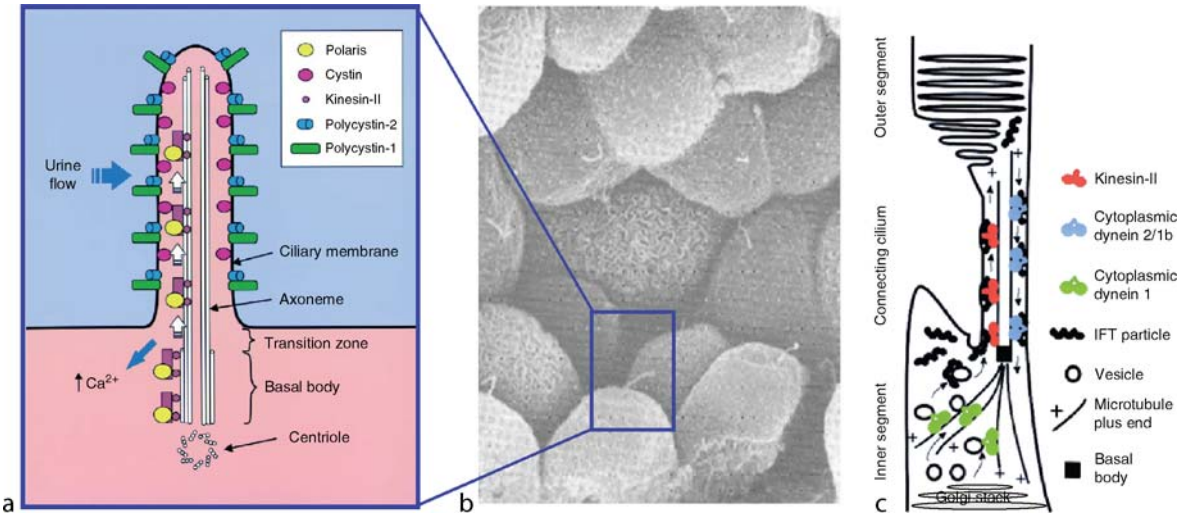
Correct orientation of the mitotic spindle and centrosomes with respect to the longitudinal axis of the renal tubule is critical for proper apical-basolateral polarity (*Fig. 35-7*). Non-canonical Wnt signaling (*Fig. 35-6*) is involved in these processes in renal tubular morphogenesis, when in rodents postnatally renal tubules still elongate. The structure resulting from disruption of the longitudinal growth would be a dilated tubule or cyst (*Fig. 35-7*). Recently, evidence was generated for a role of planar cell polarity in renal cystic diseases (90) by measuring orientation of the mitotic spindle through 3-D imaging of renal tubules. Comparison of the distribution of the mitotic angles in wild-type animals and rodent cystic kidney disease models revealed that mitotic angles of two rodent models of cystic kidneys, the HNF1β-deficient mouse model and the *pck* rat model where clearly different from wild-type littermates (90).

NPHP7/GLIS2

Recently, mutations in the *NPHP7/GLIS2* gene, encoding the transcription factor Gli-similar protein 2 were discovered as the cause of NPHP type 7 (*Fig. 35-4*) (49).

Figure 35-8

Primary (non-motile) cilia of renal epithelial cells and connecting cilia of retinal photoreceptors are analogous structures. In the primary cilium (a) of renal epithelial cells (b) "cargo" proteins are trafficked along the microtubule tracks from the region of the Golgi stack to the tip of the cilia via the motorprotein kinesin II and back down via cytoplasmic dynein 1b. (c) In an analogous fashion approximately 10^9 molecules of the visual pigment rhodopsin are transferred up and down the connecting cilia per human retina per day (modified from Somlo & Igarashi (78) and Pazour (165)).



In analogy, *Glis2* mutant mice showed severe renal atrophy and fibrosis resembling human nephronophthisis (49) (*Table 35-1*). Differential gene expression studies on *Glis2* mutant kidneys demonstrated that genes promoting epithelial-to-mesenchymal transition and fibrosis are upregulated in the absence of *Glis2* (49). There was also prominent apoptosis present in distal tubular segments of the kidney, which might provide an explanation why in PKD kidneys are enlarged with hyperproliferation prevailing, whereas in NPHP kidney size is reduced. As GLIS2 is related to the GLI transcription factor these findings implicated the hedgehog signaling pathway in the pathogenesis of cystic kidney diseases (91). It is a signaling pathway that controls, cell determination and tissue patterning during embryogenesis.

NPHP8/RPGRIP1L

Missense and truncating mutations in the *NPHP8/RPGRIP1L* gene were shown to cause Joubert syndrome and Meckel syndrome (*Table 35-1*) (51). RPGRIP1L colocalized at the basal body and centrosomes with the protein products of both *NPHP6* and *NPHP4* (51) (*Fig. 35-4*). Whereas the presence of two truncating mutations caused Meckel syndrome, missense mutations were seen in patients with Joubert syndrome (50, 51). These findings confirmed that there is a continuum for the multiorgan phenotypic abnormalities found in Meckel syndrome, Joubert syndrome, and nephronophthsisis on the basis of distinct mutations of identical genes (multiple allelism).

NPHP9/NEK8

Three different highly conserved amino acid changes were identified in the gene *NEK8* (never in mitosis kinase 8) as causing NPHP type 9 (*Table 35-1*) (53). One of the mutations identified is positioned in the same RCC1 domain, in which the missense mutation causing the renal cystic mouse model *jck* is positioned (92, 93). The notion that mutations in *NEK8* cause nephronophthisis (type 9) was supported by the finding that, upon expression in medullary collecting duct cells, all three mutant forms of *NEK8* showed defects in ciliary and centrosomal localization to varying degrees (53). As NEK8 plays a major role in cell cycle regulation, these data establish a direct link between a protein defective in renal cystic disease and the role of centrosomes for cell cycle regulation (*Fig. 35-4*). In this context it is interesting that two mouse models of polycystic kidney disease (*jck* and *cpk*) can be efficiently treated with the cyclin-dependent kinase inhibitor roscovitine (94).

Animal Models

Several spontaneously occurring mouse models of mutated genes that exhibit an NPHP-like phenotype or a phenotype of SLSN-like retinal degeneration were shown to represent orthologs of human NPHP genes. Examples are shown in *Table 35-1* for the genes *NPHP2/INVS*, *NPHP3*, *NPHP6/CEP290*, *NPHP7/GLIS2*, *NPHP8/RPGRIP1L*, and *NPHP9/NEK8*. Nephrocystin-4 is conserved in *C. elegans* and expressed in ciliated head and tail neurons of the nematode (95). Upon knockdown it exhibits a male mating phenotype, similar to the phenotype found upon knockdown of the polycystin-1 and polycystin-2 orthologs (96). Localization of nphp-1 and nphp-4 to some of these ciliated neurons also overlaps with localization of the cystoprotein orthologs polycystin-1 (*lov-1*), polycystin-2 (*pkd-2*), and with many orthologs of Bardet-Biedl syndrome (BBS) proteins (95, 97) similar to what has been described for *lov-1* and *pkd-2* mutants (96). These data have been recently refined for specific neuronal cell type (98, 99) and the necessity of *nphp-1* and *nphp-4* for morphologic integrity of ciliated neurons in *C. elegans* was demonstrated (100, 101). In addition, a role for *nphp-4* in life span of the worm has been demonstrated (102). Evolutionary conservation of nephrocystins and other cystoproteins goes even further: Some cystoproteins have been conserved over more than 1.5 billion years of evolution from the unicellular organism *Chlamydomonas Reinhardtii* to vertebrates. *Ch. Reinhardtii* uses two motor cilia (flagella) for locomotion. Strikingly, nephrocystin-4 and at least six proteins mutated in BBS are conserved in *Ch. Reinhardtii* where they are part of its basal body proteome (97, 103). Defects of cystoprotein orthologs in *Ch. Reinhardtii* have deficient intraflagellar transport and flagellar propulsion (104). This further supports the notion that cystoproteins play a role in functional modules that are conserved throughout evolution.

Extrarenal Clinical Manifestations of NPHP Occur on the Basis of Cilial Defects

A prominent feature of NPHP is involvement of multiple organs (pleiotropy) outside the kidney. Infantile NPHP type 2 (6) can be associated with *situs inversus* (29), retinitis pigmentosa (105), or cardiac ventricular septal defect (6). Defects in other organs are usually of degenerative or developmental nature. Specifically, NPHP may beassociated with tapetoretinal degeneration (Senior-Loken syndrome (14, 15), cerebellar vermis aplasia (Joubert syndrome (16, 17), ocular motor apraxia type Cogan (106), mental retardation (47), liver fibrosis (18), or cone-shaped epiphyses of the phalanges (Mainzer-Saldino syndrome (19)). In some instances there appears to be a genotype/phenotype correlation regarding pleiotropy. For instance, there is involvement of the retina in all known cases with mutations of *NPHP5* or *NPHP6*. In other instances, such as *NPHP1* mutations, the molecular basis of eye involvement is unknown.

Retinal Involvement (Senior-Loken Syndrome)

The renal-retinal involvement in Senior-Loken syndrome can be explained by the fact that the primary cilium of renal epithelial cells is a structural equivalent of the connecting cilium of photoreceptor cells in the retina (107) (*Fig. 35-8*). We have shown that nephrocystin-5 and nephrocystin-6 are expressed in the connecting cilia of photoreceptors (30, 87).

Cerebellar Vermis Aplasia (Joubert Syndrome)

In Joubert syndrome (JBTS) NPHP is associated with coloboma of the eye, with aplasia/hypoplasia of the cerebellar vermis causing ataxia, and with the inconstant symptoms of psychomotor retardation, and episodic neonatal tachy/dyspnea (16, 17, 108–110). The radiographic feature of JBTS on axial magnetic resonance brain imaging is the so-called "molar tooth sign" of the midbrain-hindbrain junction (110, 111). Ocular motor apraxia type Cogan, defined as the transient inability of horizontal eye movements in the first few years of life, may also be associated with JBTS. This symptom has been described in patients with mutations in the *NPHP1* (66, 106) ("JBTS4") and *NPHP4* (46) genes. Three different recessive genes, *NPHP1* (17, 110, 111), *AHI* (112, 113) (JBTS type 3), and *NPHP6* (48, 114), have been found mutated in JBTS. Three further loci for JBTS have been identified: *JBTS1* on chromosome 9q34.3 (115), *JBTS2/CORS2* on chromosome 11p12-q13.3 (116). In addition, mutations of *NPHP8/RPGRIP1L* can cause JBTS if at least one mutation is non-truncating (50, 51).

Liver Fibrosis

NPHP can be associated with periductal liver fibrosis (18, 117–119), as has been described for a patient with *NPHP3*

mutation, e.g. in NPHP type 3 (44). Children develop hepatomegaly and moderate portal fibrosis with mild bile duct proliferation. This pattern differs from congenital hepatic fibrosis, where biliary dysgenesis is prominent, and from hepatic involvement in ARPKD, Arima syndrome (cerebro-oculo-hepato-renal syndrome) (120–122), and Meckel syndrome, which exhibits bile duct proliferation. Bile duct involvement in these cystic kidney diseases may be explained by the ciliary theory, as the epithelial cells lining bile ducts (cholangiocytes) possess primary cilia.

Brain Malformations (Meckel Syndrome)

Within the spectrum of NPHP-associated ciliopathies Meckel syndrome (MKS) is the most severe. It leads to perinatal mortality with renal cystic dysplasia, occipital encephalocele, polydactyly, and biliary digenesis. Two recessive genes have been identified, *MKS1* (123) and *MKS3* (124), and another gene locus, *MKS2* (125), has been mapped. Recently, a Meckel-like phenotype has been described for truncating mutations of *NPHP3* (83), *NPHP6/CEP290* (89), and *NPHP8/RPGRIP1L* (50, 51). MKS represents the ciliopathy of the group that encompasses defects in most organs, and organ involvement is of developmental rather than degenerative nature. For instance, organ defects reveal cystic dysplasia rather than NPHP in the kidneys, microphthalmia of the eyes, bile duct dysgenesis in the liver, occipital encephalocele in the brain, bones involvement by postaxial polydactyly (126). The notion that MKS is at the most pronounced end of the clinical spectrum, is supported by the finding that the presence of two truncating mutations in *NPHP8/RPGRIP1L* causes MKS, whereas one "mild" mutation (missense rather than truncating) may cause the less severe phenotype of JBTS (51). In addition, the presence of 2 truncating mutations in *NPHP6/CEP290* may cause an MKS-like phenotype (MKS4) (89).

Cardiac Defects and *Situs Inversus*

In a patient with mutation of *NPHP2* a ventricular septal defect as a congenital cardiac malformation has been described (29). Thus, the role of inversin for left-right axis specification known from mouse models was confirmed in humans (76, 77). As this was associated with *situs inversus* cardiac ventricular septal defect may be viewed as a "heterotaxy" (left-right orientation) phenotype caused by the same mechanism (127). We confirmed the phenotypic combination of cystic kidney disease, *situs inversus,* and cardiac septal defect on the basis of *inversin* mutations is observed in humans, mice, and zebrafish (6).

Skeletal Defects

NPHP can be associated with skeletal defects, including Jeune syndrome (asphyxiating thoracic dysplasia) (128–131), Ellis van Creveld syndrome (132), RHYNS syndrome (retinitis pigmentosa, hypopituitarism, NPHP, skeletal dysplasia) (133), Meckel-Gruber syndrome (123, 124), and Sensenbrenner syndrome (cranioectodermal dysplasia) (134, 135). This strongly suggests a role of primary cilia function in skeletal development. The association of NPHP with cone-shaped epiphyses of the phalanges (type 28 and 28A) is known as *Mainzer-Saldino syndrome*, and occurred in patients who also had retinal degeneration and cerebellar ataxia (19). Interestingly, mutations in the ortholog of the intraflagellar transport protein IFT80 of *Ch. Reinhardtii* was found to be the cause of Jeune syndrome (136) (*Fig. 35-4*), which emphasized the strong evolutionary conservation of "ciliopathy genes".

Medullary Cystic Kidney Disease

Goldman and associates were the first to report a large kindred with dominant inheritance exhibiting an adult-onset medullary cystic kidney disease (MCKD) (137), followed by publication of two large pedigrees from the United States by Gardner et al. (138, 139). Whereas histopathology is very similar in MCKD and NPHP, MCKD differs from NPHP by its dominant mode of inheritance, and its onset of ESKD in the third decade of life, with an average at age 28.5 years (138). In MCKD penetrance appears to be very high by the age of 45 years. Another feature distinguishing NPHP from MCKD is the lack of extrarenal involvement in dominant disease, with the exception of hyperuricemia and gout (*Table 35-1*). A gene locus for MCKD type 1 has been mapped to chromosome 1q (32), but the responsible gene has not yet been identified (140–145). The locus for MCKD type 2 resides on chromosome 16 (146, 147). Recently, mutations in the *UMOD* gene encoding uromodulin/Tamm-Horsfall protein have been identified as responsible for MCKD type 2 (34, 148) and a group of "uromodulin associated kidney diseases" including familial juvenile hyperuricemic nephropathy (FJHN) and glomerulocystic kidney disease (GCKD) (149–152). Thus, MCKD type 2 can be positively diagnosed by mutation analysis of the UMOD gene.

Diagnosis

Laboratory Studies

Patients with NPHP are usually diagnosed when an increased serum creatinine value is detected fortuitously. Patient history generally reveals prolonged nocturia since school age. Specific gravity of a morning urine specimen will be low. Renal ultrasound will then corroborate the diagnosis (*Fig. 35-2*), which can be subsequently confirmed by molecular genetic diagnostics (www.renalgenes.org). A diagnostic algorithm for nephronophthisis has been suggested (153). Hematuria, proteinuria, and bacteriuria are uncommon in NPHP. In rare cases, where proteinuria is present, it is usually mild and of the tubular type. Laboratory studies are needed to assess the severity of renal failure and generally demonstrate elevation of serum creatinine, blood urea nitrogen and phosphorus, together with metabolic acidosis, hypocalcemia, and anemia. In SLS retinitis pigmentosa is diagnosed by its specific findings on ophthalmoscopy including increased retinal pigment, attenuation of retinal vessels, and pallor of the optic disc. If retinitis pigmentosa is present, electroretinography and electro-oculography can be employed to evaluate severity. Retinal degeneration is characterized by a constant and complete extinction of the electroretinogram, preceeding the development of visual and fundoscopic signs of retinitis pigmentosa. Ophthalmoscopy should be performed in any patient to evaluate for signs of retinal degeneration. Liver function test and hepatic ultrasonography are important to facilitate detection of patients with hepatic fibrosis.

Imaging

The most useful imaging technique in NPHP or MCKD is renal ultrasonography. Kidneys are of normal or moderately reduced size, show increased echogenicity, loss of cortico-medullary differentiation and, in later stages, cyst formation at the cortico-medullary border of the kidneys (10) (*Fig. 35-2*). Garel and associates have described medullary cysts in 13 of 15 children studied at the time of renal failure (mean age 9.7 years) (154). Roentgenography contributes little to the diagnosis of the disease. Medullary cysts can sometimes also be demonstrated on magnetic resonance imaging or computed tomography (155, 156). Histology is characteristic but not pathognomonic in NPHP or MCKD, because cysts may be absent and tubulointerstitial disease can be relatively unspecific. Renal biopsy can be circumvented as an initial procedure due to the availability of molecular genetic diagnostics in NPHP (www.renalgenes.org). If molecular genetic diagnostics do not detect a molecular defect, the diagnosis can be based on the combined results of typical history with polyuria, polydipsia and anemia, and the classical appearance of the kidney on ultrasound, and renal histology. A thorough pedigree analysis should be documented for each of three successive generations, to rule out autosomal dominant MCKD.

Molecular Genetic Diagnosis

Recently, molecular genetic diagnosis has become available mostly for NPHP type 1, but is theoretically possible also for the very rare forms of NPHP type 2 through 9 (see above). A diagnostic algorithm should be followed to avoid unnecessary renal biopsy (153). Molecular genetic analysis is the only diagnostic procedure, by which the diagnosis of NPHP can be made with certainty. However, due to the presence of additional loci for NPHP, the lack of detection of mutations in the *NPHP1* gene does not exclude the diagnosis of NPHP. If renal disease with features of the NPHP or MCKD complex occurs in a person older than 25 years, its presence should be thoroughly sought in preceding generations. This may frequently result in detection of a pattern of autosomal dominant inheritance. In this case mutation analysis in the *UMOD* gene is warranted.

Genetic Counseling

Molecular genetic testing should be performed only following consent within the guidelines of the National and International Societies for Human Genetics (http://www.ashg.org/press/healthprofessional.shtml). The acceptance of molecular genetic diagnostics may be strongly enhanced in the US due to the recent passing of the Genetic Information Nondiscrimination Act (GINA) by congress (http://www.ashg.org/press/ginaupdate.shtml). Before genetic counseling, a thorough pedigree analysis to distinguish recessive (early-onset) from dominant (late-onset) disease is mandatory, and extrarenal organ involvement should be sought. Since non-symptomatic potential carriers of recessive defects should not be examined by molecular genetic diagnostics, unaffected siblings below 25 years of age should be re-evaluated yearly for maximal urinary concentrating ability and renal ultrasound. If symptoms evolve and serum creatinine increases, molecular genetic diagnostics following informed consent is

warranted, to allow early prevention of complications from electrolyte disturbances, dehydration, anemia, and growth retardation. If a transplant recipient's renal histology suggests NPHP and a living related donor is considered, molecular genetic diagnostics may help to exclude or detect renal disease within the family. Because there is genetic locus heterogeneity in diseases of the NPHP and MCKD, prenatal diagnosis can only be performed by direct genetic testing. This requires a setting, in which a specific mutation or deletion of the *NPHPP1–9* genes have already been characterized in an affected sibling.

Differential Diagnosis

On histopathology NPHP and MCKD have to be differentiated from other forms of interstitial nephropathies like chronic pyelonephritis or drug injury. In oligomeganephronic dysplasia kidney size is reduced and histology is distinct from NPHP. The paucity of urinary abnormalities, the frequent lack of hypertension, normal kidney size, and the localization of renal cysts (if present) readily differentiate variants of the NPHP and MCKD from recessive or dominant polycystic kidney disease. Finally, medullary sponge kidney (157) can easily be distinguished from NPHP or MCKD, since it does usually not lead to chronic renal failure and shows calcifications and calculi on renal ultrasound.

Prognosis and Therapy

There is no causative therapy for NPHP or MCKD. Therapy is symptomatic and is directed towards the treatment of hypertension, if present, as well as the correction of disturbances of electrolyte, acid-base and water balance. Hypokalemia may contribute to the polyuria, so that oral potassium supplementation may alleviate this symptom. Metabolic acidosis should be corrected, and osteodystrophy and secondary hyperparathyroidism treated with adequate calcium supplementation, phosphorus restriction, phosphate binders, and vitamin D therapy. Anemia will be treated with iron supplementation and erythropoetin, and growth retardation may require administration of growth hormone. Adequate nutrition should be maintained with the help of a dietician. Psychological counseling of the patients is an integral part of therapy, because of the poor self-image associated with growth retardation and to alleviate pressures resulting from the need to comply with complicated medications and dietary prescriptions. All patients will require renal replacement therapy by dialysis and renal transplantation during childhood, adolescence or, in dominant disease, in early adult life.

Gattone et al. have recently shown that the renal cystic phenotype of *pcy* mice, which is the equivalent of human NPHP type 3 (*Table 35-1*) can be strongly mitigated or even reversed by treatment with the vasopressin V2 receptor antagonist OPC31260 (82). Similar results were obtained using a *pkd2* mouse model (158). This effect is thought to be mediated by a reduction in intracellular cAMP levels (47). An important future challenge will be the development of therapies that capitalizes on what has been learnt about the pathobiology of NPHP, MCKD and other cystic diseases of the kidney.

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36 Polycystic Kidney Disease

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Introduction

Polycystic kidney disease (PKD) is a heritable disorder with diffuse cystic involvement of both kidneys without dysplasia (1). All forms of PKD can have clinical manifestations in infants and children. The major clinical entities of autosomal-recessive polycystic kidney disease (ARPKD) and autosomal-dominant polycystic kidney disease (ADPKD) have considerable overlap in clinical presentation and radiographic features. Glomerulocystic kidney disease (GCKD) can be a feature of several inherited, sporadic, and syndromal conditions, as well as an expression of ADPKD.

Differential Diagnosis of Polycystic Kidneys in Childhood

The clinical presentation of polycystic kidneys and/or enlarged echogenic kidneys can be associated with a number of kidney disorders. Not all of these represent the classic genetic disorders of polycystic kidney *disease* (i.e., ARPKD and ADPKD). Additional diagnoses to consider include bilateral cystic dysplasia, which is often associated with congenital syndromes, and multicystic dysplastic kidney (MCDK). Both of these diseases generally occur sporadically and are reviewed in detail in Chapter 5. The inherited disorder of juvenile nephronophthisis (JN) is associated with cystic kidneys, but these are usually small or normal in size. JN is reviewed in Chapter 35. Other rarer causes of polycystic and/or enlarged echogenic kidneys are outlined in *Table 36-1*.

In most clinical settings, the major challenge in the differential diagnosis of polycystic kidneys in the pediatric patient is clearly delineating ARPKD from ADPKD. In fact, ADPKD presenting in the neonatal period may be indistinguishable clinically from ARPKD (2, 3). In such instances, a staged evaluation including careful history, physical examination, imaging, and histologic examination is recommended. As shown in *Table 36-2*, certain clinical features can help differentiate between ARPKD and ADPKD, although no single finding is diagnostic. A complete family history is often the most important element in difficult cases. Parents should have standard, or if available, high resolution renal ultrasonography. If the parents of a child with undiagnosed PKD are under 30, the grandparents should also be evaluated because 4–5% of patients with ADPKD may not have visible renal cysts before age 30. The absence of any cystic disease in family members makes the diagnosis of ARPKD more likely. It does not, however, exclude the diagnosis of ADPKD, since approximately 8–10% of all ADPKD cases are the result of new gene mutations (4). Radiographic studies, particular MRI imaging if the kidneys and liver, may clearly distinguish ARPKD and ADPKD in some cases (*Table 36-2*). However, in clinical practice, up to 20% of all cases will show certain features of both diseases on radiographic studies of the kidneys, making definitive diagnosis difficult unless extra-renal features of either disease are present (see below). Tissue diagnosis (biopsy of kidney and liver) is generally deferred given the availability of molecular diagnostics. Molecular genetic testing for ARPKD and ADPKD is available and is increasingly utilized given mutation detection rates of 85–90% in high quality laboratories (5). Such testing is indicated for the subset of patients in whom the clinical and/or tissue diagnosis is equivocal, and/or additional information is needed for genetic counseling. In the United States, passage of recent legislation (2008) preventing discrimination by employers or insurers against any individual with a genetic disorder (Genetic Information Non-Discrimination Act or GINA) will remove a major obstacle to diagnostic testing of asymptomatic, at risk PKD patients. Early diagnosis of asymptomatic individuals with ADPKD and ARPKD affords the current opportunity for maximal anticipatory care (i.e., BP control), and the future opportunity to benefit from new therapies (i.e., early treatment with therapies that will limit cyst development and enlargement) (6).

Pathophysiology of Cyst Formation in PKD

In the last decade, major advances have been made in understanding the molecular genetics of PKD. Through

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Table 36-1
Differential Diagnosis of Polycystic and/or Echogenic Kidneys in the Pediatric Patient

Polycystic Kidney Diseases (PKD)
Autosomal-recessive polycystic kidney disease (ARPKD)
Autosomal-dominant polycystic kidney disease (ADPRD)
Glomerulocystic kidney disease (GCKD)
Inherited Disorders Associated with Polycystic Kidneys
Tuberous sclerosis complex
Meckel–Gruber syndrome
Jeune syndrome and other chondrodysplasia syndromes
Ivemark syndrome
Bardet–Biedl syndrome
Oro-facial-digital syndrome Type I
Zellweger cerebrohepatorenal syndrome
Beckwith–Wiedemann syndrome
Trisomy 9 and 13
Juvenile nephronophthisis (JN)/medullary cystic disease (MCD) complex
Von Hippel-Lindau Syndrome
Hajdu-Cheney Syndrome
Sporadic Disorders Associated with Cystic Kidneys
Isolated cystic dysplasia
Multicystic dysplastic kidney (MCDK)
Unilateral/localized cystic kidney disease
Caliceal diverticula
Miscellaneous Causes of Cystic and/or Enlarged Echogenic Kidneys
Nephroblastomatosis
Bilateral Wilms' tumor
Leukemia or lymphoma
Pyelonephritis
Glomerulonephritis
Radiocontrast nephropathy
Bilateral renal vein thrombosis
Transient nephromegaly
Congenital nephrotic syndrome
Glycogen storage disease
Acquired cystic kidney disease

Table 36-2
Differential Clinical Features of Childhood PKD

Major clinical features of *both* ARPKD and ADPKD
Enlarged kidneys
Hypertension
Concentrating defect
Sterile pyuria
Clinical features suggesting ARPKD rather than ADPKD
Neonatal presentation
Progression to end-stage renal disease as a child
Hepatosplenomegaly
Portal hypertension and esophageal varices
Bacterial cholangitis
Negative family history
Clinical features suggesting ADPKD rather than ARPKD
Positive family history
Extrarenal cysts
Cerebral aneurysms
Asymptomatic presentation
Unilateral renal presentation
Hematuria
Urinary tract infection

Adapted from Avner ED. Polycystic kidney disease. In Pediatric Nephrology. Drukker A, Grushkin A (eds.). In Pediatric and Adolescent Medicine. Branski D (series ed.). Basel, AG Karger, 1993

a combination of positional cloning, direct sequencing and utilization of the rapidly expanding genome databases, the major causative genes for both ADPKD (*PKD1* and *PKD2*) as well as ARPKD (*PKHD1*) have been identified (7–10) (*Table 36-3*). Details specific to the molecular genetics of ARPKD and ADPKD are addressed in the respective sections that follow. Numerous studies have demonstrated that the protein products of the ADPKD and ARPKD genes are membrane-bound proteins which interact and generally exist in multimeric protein complexes at various sites in cells. The primary sites of "cystoprotein complex" localization have been reported to be apical cell membranes (particularly on or adjacent to the primary cilium), adherins junctions, desmosomes, and focal adhesions (11–15). Multimeric cystoprotein complexes thus interact with a number of distinct signal transduction pathways which appear to be critical in normal tubular growth and differentiation. Mutations in PKD genes result in abnormal cystoprotein structure and function, with subsequent aberrant integration of complex signaling events resulting in the unique phenotype of the cystic epithelial cell. Although the precise mechanisms by which specific PKD gene mutations result in cyst formation have not yet been fully elucidated, considerable progress has been made in understanding the pathophysiology of cyst formation. Key pathogenic

Table 36-3
Human Polycystic Kidney Disease Genes and Proteins

Disease	Gene	Mode of inheritance	Chromosome location	Protein	Function/Role
ADPKD	*PKD1*	AD	16p13.3-p13.12	Polycystin 1	?Receptor
ADPKD	*PKD2*	AD	4q21-q23	Polycystin 2	Cation channel
ARPKD	*PKHD1*	AR	6p21	Fibrocystin (polyductin)	?Receptor
GCKD (hypoplastic variant)	*HNF-1β*	AD	17cen-q21.3	Hepatocyte nuclear factor – 1beta	Transcription factor

AD, Autosomal Dominant, *AR*, Autosomal Recessive

features of the cystic phenotype have been identified (6, 15, 16). Such features are critical as targets for the development of future therapies, and include:

- Abnormalities of expression and function of the epidermal growth factor (EGFR) – axis
- Decreased intracellular calcium with aberrant intracellular cAMP signaling
- Abnormal structure and/or function of the primary cilia
- Alterations in cell-cell, and cell-matrix interactions

Each of these pathogenic processes likely contributes to some extent to one or more of the fundamental features of renal cyst formation and progressive enlargement, namely: (1) tubular cell hyperplasia; (2) tubular fluid secretion; and (3) abnormalities in tubular extracellular matrix, structure, and/or function (*Fig. 36-1*) (16–20). As the following sections will illustrate, key insights into the pathogenesis of cyst formation in PKD have been provided by rodent models of ARPKD and ADPKD (*Table 36-4*). This Table does not include the increasing number of reported genetically-manipulated "knockout" or conditionally targeted genetic models, many of which have not been fully characterized as of this writing. The interested reader is referred to the following readings which review the most significant of these models produced to date (21–24). Several of these models have been used to test the efficacy of novel therapies.

Renal Tubular Cell Hyperplasia

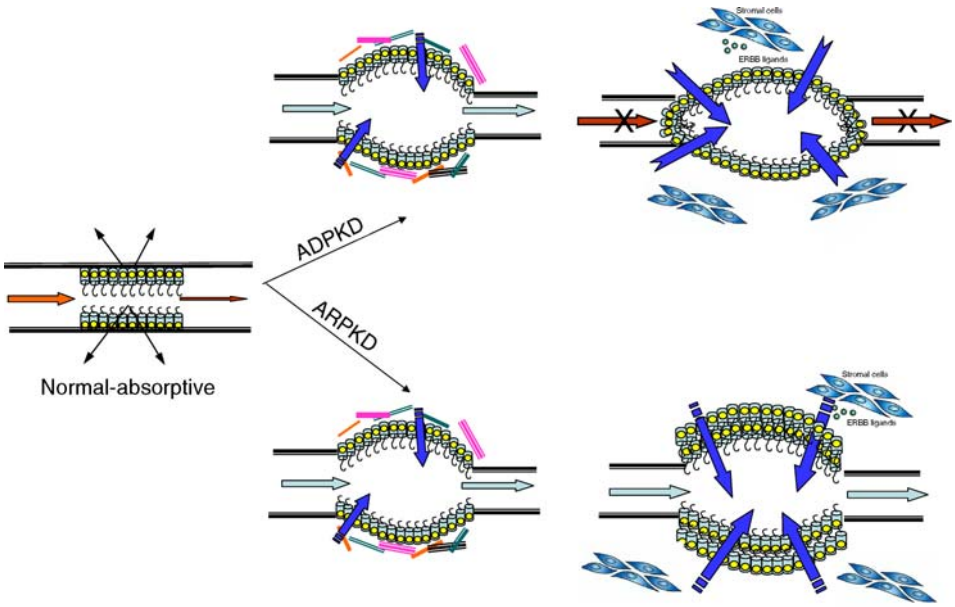
Renal tubular hyperplasia is a central morphologic feature of all described human renal cystic diseases (25, 26). On the basis of mathematic modeling of cyst growth, it has been shown that tubular cell hyperplasia, with expansion of tubular wall segments to accommodate an increased cellular mass, is an essential factor in cyst formation and enlargement (27). Multiple studies in vivo and *in vitro* have demonstrated abnormal renal tubule epithelial proliferation. Increased renal tubular epithelial cell proliferation is a feature of both cystic and non-cystic tubular epithelium from ADPKD and ARPKD kidneys (28). Cyst-derived epithelial cells from ADPKD and ARPKD demonstrate increased cell growth potential compared with controls (29, 30).

The EGFR-Axis

Dysregulation of growth factors/receptors have a primary role in tubular cell hyperplasia. A growing body of evidence implicates one or more members of the ErbB receptor family, including the epidermal growth factor receptor (EGFR), as well as the related receptors, ErbB2 and ErbB4. In both human ADPKD and ARPKD and in every rodent models of PKD published to date, cystic kidneys display characteristic alterations in EGFR expression. Both quantitative abnormalities, including increased mRNA and protein, and qualitative differences, in particular, the appearance of "mislocalized" EGFR expressed on the apical surface of tubular epithelium, are seen (31–33). Apical EGFR is functional and capable of transmitting mitogenic signals *in vitro* (34). Inhibition of EGFR function *in vitro* by treatment with either an inhibitor of tyrosine kinase function or a blocking antibody inhibits formation of proximal tubule cysts and significantly decreased explant growth and distal nephron differentiation in metanephric organ culture models (35, 36). Additional support for a central role for EGFR in the pathogenesis of cyst formation is provided by *in vivo* data. Inhibition or reduction of EGFR function, either by treatment with a novel tyrosine kinase inhibitor (37), or genetic manipulation (38), leads to a marked reduction in cyst formation and progressive enlargement in animal models.

Figure 36-1

Pathophysiology of renal cyst formation. Studies in a variety of experimental models, in addition to human ADPKD and ARPKD tissue, implicate three major factors in renal cyst formation and progressive enlargement. Normal renal tubular absorptive epithelium can become cystic if (a) hyperplasia, localized to a distinct nephron segment, requires accommodation of increased renal mass; (b) secretion, as opposed to absorption, leads to the accumulation of intratubular fluid; and (c) extracellular matrix (ECM) abnormalities alter the epithelial microenvironment to further stimulate proliferation and secretion. The figure depicts the difference between ADPKD epithelia, where proliferation leads to isolated cysts which are not connected to intratubular flow and can grow only through transtubular fluid movement; and ARPKD epithelia, where proliferation leads to thickened tubular ectasia and where fluid secreted across tubular walls remains part of urinary flow. These processes are not mutually exclusive, may reflect characteristics of undifferentiated epithelium, and operate in concert during tubular cysr formation and progressive enlargement.



The central role of EGFR-family members in the pathogenesis of ARPKD has been recently confirmed by the demonstration that ErbB2, rather than EGFR (or ErbB1) is predominantly overexpressed and apically mislocated in the PCK rat, an orthologous ARPKD rodent model. This model, which does not respond to ErbB1 inhibition (39) responds dramatically in-vivo to therapies which decrease active phosphorylated ErbB2 (40, 41) This response includes a dramatic reduction in renal cyst formation and progressive enlargement, an improvement in biliary tract ectasia and periportal fibrosis, and dramatic improvements in renal function and renal concentrating ability. Given the importance of G-protein coupled receptors in cystogenesis previously noted, it is significant that activation of such receptors leads to significant transactivation of the EGFR family (42).

ErbB2 (HER-2) overexpression is seen in some cysts of ADPKD kidneys but not seen in late-stage ARPKD kidneys (43). Late-gestation/early post-natal human ARPKD kidneys samples, however, show increased ErbB2 expression compared with normal human fetal and postnatal kidneys (44). Wilson et al. (45) demonstrated that apical localized EGFR complexes in normal fetal and ADPKD epithelia are heterodimers of EGFR (ErbB1) and ErbB2, while basal membrane localized EGFR in normal adult renal epithelia are comprised of EGFR (ErbB1) homodimers. They further showed that inhibition of ErbB2 corrected the migratory phenotype seen in ADPKD cells. Overexpression and mislocalization of another ErbB family member, ErbB4, has been demonstrated in cystic collecting tubule epithelia of two ARPKD rodent models (46).

Overexpression of several EGF related growth factors/ErbB ligands, is also a prominent feature of both ARPKD and ADPKD cystic epithelia. Renal cyst fluid contains EGF or EGF-like peptides in mitogenic concentrations, despite apparent reductions in EGF tissue expression

Table 36-4
Rodent Models of PKD[a]

Mouse model	Mode of inheritance	Chromosome location	Gene	Protein product	Function/Role/Comments
bpk	AR	10	*Bicc1*	bicaudal C	RNA-binding protein
cpk	AR	12	*Cys1*	cystin	Cilia-associated protein
inv	AR	4	*Inv*	inversin	Role in left-right axis development
jck	AR	11	*Nek8*	nek8	Function unknown
kat	AR	8	*Nek1*	nek1	Function unknown
jcpk	AR	10	*Bicc1*	bicaudal C	Allelic with *bpk*
orpk	AR	14	*Ift88 (TgN737)*	IFT88 (polaris)	Role in left-right axis development; Cilia-associated protein
pcy	AR	9	*Nphp3*	Nephrocystin3	Model of JN
Rat model	**Mode of inheritance**	**Chromosome location**	**Gene**	**Protein product**	**Function/Role/Comments**
Han-SPRD	AD	5	*PKDR (Cy)*	SamCystin	Function unknown
LPK	AR	?	?	?	Function unknown
PCK	AR	9	*PKHD1*	fibrocystin (polyductin)	Orthologous model of human ARPKD, with some clinical features of ADPKD
WPK	AR	5	*MKS3*	Meckelin	Function unknown

AD Autosomal Dominant, *AR* Autosomal Recessive
[a]"Knockout" models for PKD are not included in this listing

(29, 47–50). Treatment with EGF transiently improves renal function in murine models (51), but has no effect on histopathologic abnormalities and continued EGF treatment worsens disease and shortens survival (52). TGF-α and EGF are cystogenic in both murine embryonic organ cultures (53) and normal human kidney cells grown in a unique collagen gel system in vitro (54). ADPKD kidneys and cells derived from ADPKD have increased mRNA or protein levels of TGF-α (31, 55), and transgenic mice that overexpress TGF-α develop cystic kidneys (56). Loss of TGF-α, however, does not modify cystic kidney disease in an ARPKD mouse model, suggesting that there is significant redundancy in EGFR ligands which can promote cyst formation or growth in ARPKD (57).

Additional data suggest that inhibiting EGFR-ligand function may also partially ameliorate cystic disease. Treatment with an inhibitor of TACE, a metalloproteinase implicated in the processing of several EGF-related growth factors, decreased cystic kidney disease in a murine model, less effectively than EGFR inhibition (58). Combining inhibition of EGFR ligand release with EGFR inhibition maximized therapeutic effectiveness while minimizing toxicity (59). Additional EGFR ligands, including amphiregulin and heparin-binding EGF, are abnormally expressed in PKD and may prove to also have a role in proliferation of cystic epithelium (50).

c-Src is an important intermediate in several key cystogenic signaling pathways. Src is a critical intermediate which integrates proliferation from both G-coupled protein receptors and the EGFR-axis (6, 16). Increased Src activity (pY418) was found to be associated with a more severe renal cystic disease in two ARPKD rodent models. Furthermore, treatment of these models with an inhibitor of Src ameliorated both the renal and hepatic disease through inhibition of G-protein coupled receptor and EGFR-axis triggered phosphorylation cascades (41).

cAMP and Intracellular Calcium Ion

Another major contributor to cellular proliferation in both ADPKD and ARPKD is intracellular cyclic AMP (cAMP). Unlike normal renal epithelia, ADPKD and ARPKD cystic epithelia respond to increased intracellular cyclic AMP (cAMP) with an increase, rather than decrease, in proliferation due to phosphorylation of B-Raf (60–62).

Normal and polycystic kidney epithelia from an ARPKD rodent model also demonstrate differences in regulation of cAMP-dependent protein kinase (PKA), which is associated with increased proliferation *in vitro* (63).

Intracellular calcium concentrations have been identified as a critical component of the pro-mitogenic cAMP response of cystic epithelia. Several studies have demonstrated that calcium restriction induces a switch to this cAMP dependent growth phenotype, whereas addition of calcium to PKD cells in culture restores the normal anti-mitogenic response to cAMP (64, 65). In addition, cells that do not express *PKHD1*, the mutated gene in ARPKD, have decreased intracellular calcium and increased epidermal growth factor (EGF)-induced proliferation, suggesting that loss of one or more PKD proteins may lead to abnormal proliferation by modulation of intracellular calcium (66). In addition, Leuenroth et al. (67) recently showed that triptolide (a Chinese herb) induces calcium release in a polycystin-2 dependent manner. Further, triptolide treatment of a mouse model of ADPKD resulted in attenuated cyst formation and decreased tubular cellular proliferation.

The cAMP pro-mitogenic response in PKD cells is associated with phosphorylated B-Raf activation of the mitogen-activated protein kinase (MAPK) pathway, which can mediate a variety of cellular processes, most notably cell proliferation. There is considerable "cross talk" between the cAMP and MAPK signaling pathways in both normal and disease states (68). Increased phosphorylation of several MAPK pathway members is a prominent feature of rodent and human ADPKD and ARPKD kidneys and tubular epithelial cells (23, 69, 70). There are conflicting animal data, however, as to whether inhibition of ERK 1/2 impacts progression of cystic kidney disease (23, 71).

Apoptosis

Dysregulation of apoptosis, or the balance between apoptosis and proliferation, may also contribute to the progression of ARPKD and ADPKD (72–74). Increased rates of apoptosis and increased caspase 3 and 4 activity have been demonstrated in kidneys from ARPKD rodent models (75, 76). A marked increase in caspase 3 and 7 activity has also been reported in an ADPKD rodent model (77, 78). Furthermore, caspase inhibition reduced tubular apoptosis and proliferation and slowed cystic kidney disease progression in that same model (79).

Mice deficient in the anti-apoptotic molecule, bcl-2, develop severe multicystic hypoplasia characterized by proximal and distal tubular cysts and hyperproliferation of epithelium and interstitium (80). Alternatively, increased bcl-2 expression has been demonstrated in animal models of both ARPKD and ADPKD (73, 76, 77). These finding suggest that the balance of pro- and anti-apoptotic mediators, rather than the absolute expression levels, may be a critical factor in the development of cystic kidney disease (77).

Proto-oncogenes

Abnormal expression of proto-oncogenes, in particular, c-Myc may also contribute to abnormalities in proliferation and apoptosis, leading to cyst development. In both murine ARPKD and human ADPKD kidneys, c-Myc is overexpressed in cystic tissue (73, 81, 82) and is associated with a marked increase in both tubular cellular proliferation and apoptosis (83–85). In addition, c-Myc antisense oligonucleotides have been used to ameliorated cystic kidney disease in a murine ARPKD model (85).

mTOR

The target of rapamycin (mTOR) pathway is currently a growing area of interest in the pathophysiology PKD because it integrates signals from growth factors (including EGFR), G-protein coupled receptors (which generate cAMP), cellular energy levels, nutrient status and stress conditions to stimulate protein synthesis and cell growth through activation through phosphorylation of S6K1 and eIF4E (86, 87). The *TSC1* and *TSC2* genes, when mutated, cause tuberous sclerosis, a disease in which renal cystic lesions may accompany the more classical angiomyolipomas. *TSC1/2* mutations upregulate mTOR signaling. The fact that the *TSC2* and *PKD1* genes lie adjacent to each other on human chromosome 16p13.3, as well as the fact that the cytoplasmic tail of polycystin-1 interacts with mTOR led to an evaluation of mTOR activity in polycystic kidney disease. In a variety of animal models, as well as in human ADPKD and ARPKD cyst-lining epithelia, expression of phospho-mTOR and p70S6K is increased (88). The mTOR pathway is regulated by polycystin-1, and its inhibition reverses renal cystogenesis in polycystic kidney disease. These findings, combined with therapeutic efficacy of rapamycin (an mTOR inhibitor) in ameliorating cystic disease in the orpk, bpk and Han:SPRD rodent models has led to the development of pilot clinical trials for rapamycin in patients with ADPKD (87).

Arachidonic Acid Metabolites

20-hydroxyeicosatetratraenoic acid (20-HETE), is formed by the ω-hydroxylation of arachidonic acid by cytochrome P450 (CYP) 4A and 4F enzymes (89). Recent evidence has implicated 20 HETE as a mediator of cellular proliferation in normal and malignant renal cells (90–94). Further, norepinephrine, angiotensin II, and EGFR activity (all upregulated in PKD) stimulate the synthesis and release of 20-HETE and increase proliferation in both vascular smooth muscle cells and renal tubular epithelial cells (90–92, 95). With this rationale, recent studies have demonstrated a significant role of 20-HETE in mediating collecting tubular epithelial proliferation in the murine BPK and rodent orthologous PCK models of ARPKD (96); Further, in these studies, when 20-HETE synthesis was selectively decreased, or 20-HETE activity was specifically inhibited using genetic or pharmacological inhibition, both in vitro and in vivo, cystic epithelial proliferation, cyst formation, and progressive cystic enlargement were markedly inhibited (96). Notably, the decrease in proliferation and amelioration of cystic disease was associated with a dramatic decrease in EGFR-activity and downstream signaling. Clinical development of 20-HETE inhibitors as potential therapeutic agents in PKD is being actively pursued.

Fluid Secretion

In addition to epithelial hyperplasia, tubular fluid secretion is an important feature of renal cyst formation and progressive enlargement (25, 26, 29). Given the anatomical differences discussed below, it is likely that tubular fluid secretion is quantitatively and qualitatively different in the pathophysiology of cyst formation in ADPKD and ARPKD. On a theoretical basis, tubular fluid secretion in addition to hyperplasia, fulfills the requirements for cyst growth predicted by mathematic modeling (27). Cellular proliferation without tubular secretion would produce solid tumor nests of epithelial cells rather than cysts. In ADPKD more than 70% of cysts have no afferent or efferent tubular connections, and thus must fill by transepithelial secretion of solute and fluid (97, 98). In contrast, microdissection studies confirm that enlarged, ecstatic collecting tubules in ARPKD are in continuity with the urinary space (99). One would not expect transtubular secretion to be as critical in the pathogenesis of changes in ARPKD as in ADPKD, unless there is downstream obstruction (which is not a characteristic finding in ARPKD). Studies in a variety of model systems have evaluated possible mechanisms involved in PKD tubular fluid secretion. These include alterations in ciliary structure and function, intracellular calcium transport, cyclic-AMP activity, epithelial sodium channel function, and sodium potassium ATPase localization and activity.

Cilia

Studies in ARPKD and ADPKD animal models, and human kidneys and cells, (as well as studies in other renal cystic diseases, such as nephronophthisis), have demonstrated that many of the disease-associated proteins appear to be present as multimeric complexes on, or in close proximity to, the primary cilia that are present on the apical membranes of renal tubular epithelial cells (100). In tubular epithelial cells, cilia project into the lumen and are thought to have a mechanosensory role (101, 102). Abnormalities in ciliary structure and/or function have been demonstrated in many PKD animal models, as well as in epithelium isolated from human ADPKD and ARPKD kidneys (103–107).

Pkd1 −/− cells have normal-appearing cilia, but lack the flow-induced Ca^{++} response noted in normal cells (101, 108). This finding suggests that polycystin complexes in or near the cilia act as flow-sensors, and that Ca^{++} influx occurs via a functional polycystin channel (predominantly mediated by polycystin 2). The Ca^{++} influx consequently induces release of Ca^{++} from intracellular stores. As previously noted, the polycystin complex is also found at desmosomes, adherens junctions, and focal adhesions (11–15). Primary cilia can sense changes in shear stress and fluid flow at the apical cell surface, whereas focal adhesions can sense tensile strength of cell-matrix attachments, and cell-cell junctional complexes sense forces between cells. Therefore, polycystin complex localization to these sites suggests a complex integration of mechanosensation with multiple signal transduction pathways.

It should be noted that data localizing a large number of cystoproteins to apical cilia have recently been called into question. To co-localize proteins of interest to cilia, tagged-antibodies have often been mixed with an anti-acetylated alpha tubulin antibody prior to tissue staining. It now appears that this procedure gives many false positives through non-specific protein-protein interactions of the antibodies prior to tissue staining (personal communication, Joel Rosenbaum PhD; Yale University, August 2008).

At the time of this writing, a primary role for isolated ciliary abnormalities in cystogenesis remains controversial in ADPKD and ARPKD. One cannot ignore the findings

that ciliary structural and functional abnormalities appear to be present in cystic tissue. However, in addition to the methodological problems noted above in protein co-localization studies, recent data suggest that polycystin-fibrocystin complexes may be found in subapical endosomes adjacent to, but not superimposed upon, ciliary basal bodies (centrosomes) in electron micrographs of human ADPKD and ARPKD kidneys. These endosomes bud around cilial axonema and appear in cyst fluid and urine. (Chris Ward, PhD, Mayo Research Foundation, personal communication, August 2008). Current data suggest that ciliary abnormalities may have a more primary role in syndromic renal cysts and other organ abnormalities seen in so called "ciliopathies" such as Meckel-Gruber syndrome, Bardet-Biedl syndrome, Oral-facial-Digital syndrome and perhaps nephronophthisis (see chapter 6 on Syndromes in Section One of this Textbook and Chapter 36 on Nephronophthisis in this Textbook). However, as suggested above, in ADPKD and ARPKD, it appears that cilia are part of a complex pathophysiological process linking mechanosensation to integration of multiple cell-signaling pathways emanating from multiple sites within the cell.

cAMP and Transport

Several studies support a major role for cyclic adenosine monophosphate (cAMP)-mediated chloride secretion during *in vitro* cyst formation (60, 109–111). A putative lipid "secretagogue" isolated from cyst fluid of human ADPKD kidneys was found to stimulate intracellular cAMP and stimulate fluid secretion (112) and more recently was confirmed to be the cAMP agonist forskolin (113).

Pharmacologic interventions directed towards down-regulating cAMP levels in cystic epithelia are therapeutic in both ARPKD and ADPKD animal models. Inhibitors of the vasopressin V2 receptor, a G-protein coupled, adenylate cyclase activating receptor present in the collecting duct, which modulates levels of intracellular cAMP, ameliorates renal cyst disease in the PCK rat model of ARPKD and the $Pkd2^{(-/WS25)}$ model of ADPKD (114–116). In both models, improvement in cystic kidney disease was associated with decreased levels of cAMP and aquaporin 2. Interestingly, increased water intake, which functionally down-regulates V2R activity, also improved cystic kidney disease and decreased kidney cAMP levels (117). In a related study, orthologous ARPKD (PCK) rats were bred to a Brattelboro rat strain (which lacks a functional renal vasopressin axis), and resultant double mutants demonstrated significant amelioration of renal cystic disease (118). Although background genetic modifiers may have influenced the results, such genetic complementation studies further support the primary role of G-protein mediated increases in renal epithelial cAMP in cystogenesis. Accordingly, controlled Phase 3 clinical trials with a VPV2R inhibitor (Tolvaptan) in patients with ADPKD have been initiated in both the United States and Japan. In addition, studies in animals and European pilot studies in ADPKD patients suggest that octreotide, a somatostatin analogue that decreases cAMP activity in both renal and biliary epithelium, may be effective in ameliorating cystic kidney and liver disease in ADPKD (119–121). However, recent data suggest that increased cAMP activity may not be the final "effector" for mediating cyst growth. The therapeutic effect of Src inhibition on renal and biliary disease in bpk and PCK rodent models, was not associated with significant changes in intracellular cAMP levels. These findings suggest that Src activity in PKD is downstream and independent from cAMP activation (41).

It has been hypothesized that cAMP-stimulated chloride and fluid secretion occurs in PKD through activity of the CFTR (cystic fibrosis transmembrane receptor), the chloride channel mutated in cystic fibrosis (122). CFTR Cl- channels exist in apical membranes of epithelial cells and are major mediators of forskolin-stimulated chloride and fluid secretion by epithelial cells of human polycystic kidneys *in vitro* (122, 123). CFTR is required for cAMP-dependant *in vitro* renal cyst formation (124). *In vivo* support for a role of CFTR in the pathogenesis of PKD was provided by a report of an ADPKD kindred in which cystic fibrosis was also present. Patients with ADPKD and CF (which results in a loss of functioning CFTR) were found to have less severe disease than those with ADPKD who did not have CF (125). However, a subsequent report failed to confirm such a protective effect (126). An *in vitro* study of CFTR inhibitors and cyst growth in 3D collagen gels demonstrated that cyst growth inhibition correlated with cAMP-stimulated chloride current inhibition, but not cell proliferation, suggesting that an effect, if present, is related to inhibition of fluid secretion only (127). Studies of ARPKD rodent models bred with a CFTR null mouse failed to show improvement in kidney disease in cystic animals lacking CFTR compared to those that expressed CFTR (128). Thus, these data demonstrate that CFTR does not have a significant impact on ARPKD cyst formation and expansion. The most attractive alternative hypothesis to date, supported by an increasing body of electophysiological data in normal and cystic tissue implicates abnormal EGFR-mediated down-regulation of the amiloride-sensitive Na^+ channel in tubular secretion in ARPKD (129–131). EGFR-expression

leads to decreased ENac subunit production at both the mRNA and protein level with consequent electrophysiological alterations. A block in active Na^+ transport at the luminal membrane results in intratubular Na^+ accumulation which obligates anion and fluid transport through active channels and perhaps, the paracellular pathway. With the availability of new reagents and technologies to perform definitive transport studies in human kidney epithelia, studies are underway in many laboratories to delineate both the similarities and differences underlying abnormal tubular secretion in ADPKD and ARPKD.

Additional *in vivo* and *in vitro* studies demonstrate a potential role for quantitative and qualitative alterations in Na^+-K^+-ATPase activity in mediating tubular fluid secretion in cystogenesis (132–137). In proximal tubules, it has been postulated that increases in Na^+-K^+-ATPase activity modulate tubular secretion and cyst formation through activation of a secondary active transport process (e.g., tubular organic anion secretion), which osmotically obligates intratubular fluid accumulation cystogenesis (132–134). In collecting tubules, apical, as opposed to normal basolateral cell surface Na^+-K^+-ATPase expression may mediate basal to apical vectorial sodium transport and thus directly drive fluid secretion in affected nephron segments in ADPKD and ARPKD (135–137). Apical Na^+-K^+-ATPase expression in murine ARPKD may reflect an exaggeration of the normal developmental profile of collecting tubule sodium pump expression (137). This, in association with the relatively undifferentiated ultrastructural and genetic profile of cystic tubular epithelium (18), suggests that abnormalities in the differentiation program of cystic tubular cells are fundamental to the process of cystogenesis.

Alternatively, apical misclocation of the Na^+-K^+-ATPase in many PKD specimens from end stage kidney (or those shipped under less than optimal conditions may), may reflect the results of ischemic injury. As ADPKD kidneys enlarge and vessels splay around enlarging tubular cysts, there are parenchymal areas which are underperfused and chronically ischemic. These changes not only result in mislocalization of the Na^+-K^+-ATPase and many additional changes, but contribute to the characteristic chronic fibrosis in ADPKD kidneys which leads to end stage renal disease. Similar alterations of the Na^+-K^+-ATPase and other polarized proteins can also artificially result from 24–48 h of cold ichemia time in ADPKD kidneys studied post-nephrectomy. These variables may explain, in part, conflicting reports demonstrating normal, basolateral Na^+-K^+-ATPase expression in freshly isolated PKD kidneys and some PKD models (138).

Extracellular Matrix

The third major mediator of tubular cyst formation and progressive enlargement is abnormalities involving the extracellular matrix (18, 25, 139, 140). Diffuse ultrastructural and biochemical abnormalities of tubular basement membranes have been demonstrated in human and animal models of PKD. Specific defects in the biosynthesis and transport of sulfated proteoglycans have also been identified (141–143). Renal tubular cells from patients with ADPKD grown *in vitro* produce increase amounts of extracellular matrix when compared with normal tubular epithelia (144).

It does not appear that matrix abnormalities mediate simple changes in the compliance or viscoelastic properties of tubular basement membranes leading to distension under normal intratubular pressures (145). Rather, it would appear that altered matrix composition modulates cyst formation through altered tubular epithelial cell–matrix interactions. These interactions regulate various aspects of cell growth, cell surface protein expression, cytodifferentiation, and gene expression (18, 19). Conceivably, altered epithelial cell-matrix interaction could modulate or amplify the processes of hyperplasia and fluid secretion discussed above. β-4 integrin and its ligand, laminin α-5, a component of the basement membrane, are aberrantly expressed in polycystic kidney disease and may have a role in cell adhesion and migration abnormalities seen in ADPKD cyst-lining epithelial cells (146). A recent study reported the development of PKD in a mouse harboring a hypomorphic mutation in the laminin α-5 gene (147). These and other findings suggest that a primary defect in one ECM component is sufficient to cause aberrant cell proliferation and development of renal cysts (148).

Experimental evidence suggests that matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) may also play a role in progression of disease in PKD (149–151). Elevated serum levels of MMPs, including MMP-1, TIMP-1 and MMP-9 have been demonstrated in a cohort of ADPKD patients when compared to normal controls (152). Although it is difficult to determine whether abnormal MMP expression is a reflection of a primary abnormality or a secondary effect, data suggest that inhibition of MMPs may have an impact on the severity of disease in animal models of ADPKD and ARPKD (58, 153). Overexpression of other basement membrane, extracellular matrix and cell adhesion components have also been demonstrated in PKD. Tenascin, an ECM glycoprotein, is abnormally expressed in human ARPKD and ADPKD fetal kidneys and in a

murine model of ARPKD (154, 155). Irregular expression of alpha-integrin subunits has also been demonstrated in fetal PKD kidneys (156).

Abnormal processes within the interstitium leading to interstitial inflammation and fibrosis contribute to progression in all cystic kidney diseases. For instance, MCP-1, a chemoattractant and mediator of interstitial inflammation, is upregulated in ADPKD rats (157). In addition, oxidant stress is increased and protective effects of antioxidants decreased in the kidneys of animal models of both ARPKD and ADPKD models (158). Abnormalities in steroid and lipid metabolism have also been demonstrated in murine ARPKD (159–161).

Angiogenesis may also have a role in the pathogenesis of cyst expansion in ADPKD. When cysts enlarge, their nutrient requirements may outstrip their blood supply, in a manner analogous to tumor progression in cancer. ADPKD kidneys show increased vascularity around cysts and evidence of ongoing angiogenesis (162). Endothelin levels are increased in human and rodent ADPKD kidneys (163). ET-1 overexpressing mice also develop polycystic kidneys and interstitial fibrosis, although they do not develop hypertension. Yet, interestingly, blockade of either endothelin A or B increased the severity of polycystic kidney disease in two ADPKD animal models (164, 165). The authors speculated that acceleration of cystic kidney disease was due to altered balance between ETA and ETB. Whether angiogenesis has a role in cyst expansion in ARPKD remains to be determined.

Theories of renal cyst formation generated in experimental models are not mutually exclusive and are largely complementary. A mutant gene or environmental factors can directly lead to alterations in tubular epithelial proliferation. In addition, there is increasing recognition that modifying genes can significantly alter the cystic kidney disease caused by the mutated PKD gene (161, 166–171). Environmental factors can also modulate the expression of a mutant gene or directly lead to tubular cell death. Resultant alteration of tubular cell metabolism may subsequently lead directly to the abnormal sorting of transport proteins, growth factor receptors, or cell adhesion molecules, with resultant abnormal extracellular matrix production, or production of growth factors mediating tubular hyperplasia. Induced changes in transtubular transport energetics may lead to hyperplasia secondary to increased transmembrane sodium flux, whereas programmed cell death may lead to further hyperplasia secondary to tubular regeneration. Alterations in sodium or chloride-mediated transtubular transport could lead to net intratubular fluid accumulation. Subsequent increases in tubular wall tension may further increase stimulation of epithelial proliferation, leading to tubular hyperplasia. The presence of a particular pattern of tubular hyperplasia, along with necrotic debris from cell death, may lead to partial tubular obstruction and further increases in tubular wall tension. Finally, abnormal extracellular matrix production could alter the epithelial microenvironment, further increasing hyperplasia and transtubular transport, thereby contributing to cyst formation and progressive cyst enlargement. Such an overall hypothetical schema of renal cyst formation appropriately focuses future investigations on the molecular mechanisms by which tubular epithelial hyperplasia is controlled and tubular metabolism are altered in both experimental and human cystic diseases.

The Cystic Phenotype and Targeted Future Therapy

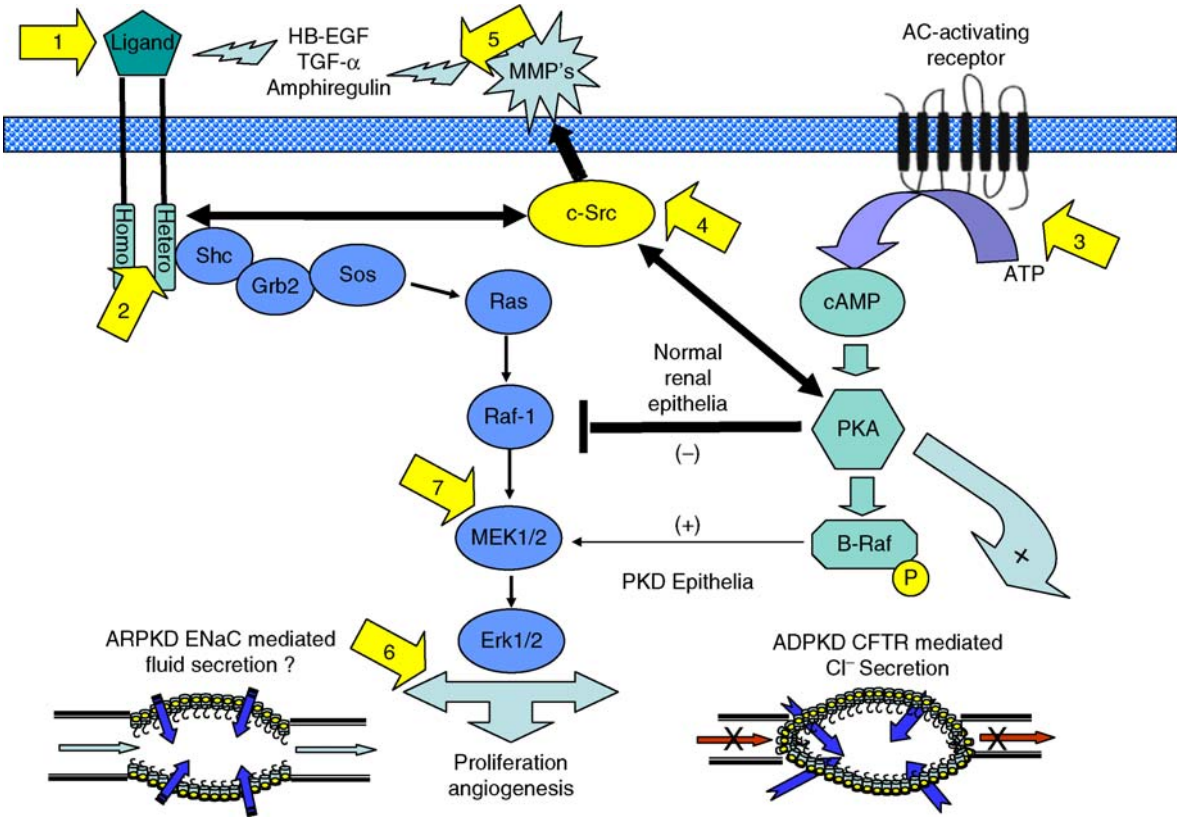
As discussed in the previous sections, the extensive studies delineating the molecular and cellular biology of ADPKD and ARPKD over the past decade have defined a unique "cystic phenotype", which provides a number of potential targets for future genetic and pharmacological therapy (*Fig. 36-2*). Relative to controls, the cystic ADPKD and ARPKD epithelial cell:

1. Demonstrates quantitative (increased amount) and qualitative (apical vs. basolateral) expression of various members of the EGFR-family of receptors and ligands. This initiates an autocrine-paracrine cycle of proliferation through activation of the Ras-Raf-MEK-ERK pathway and stimulates tubular fluid secretion by inhibiting amiloride sensitive sodium transport.
2. Demonstrates increased intracellular cAMP, which mediates proliferation through activation of PKA, phosphorylation and activation of the B-Raf/MEK-ERK pathway and stimulates tubular fluid secretion through activation of PKA and apical CFTR-mediated chloride transport.
3. Interacts with an abnormal microenvironment which includes poorly characterized abnormalities of ECM structure and function, increased cytokines, and angiogenesis that secondarily increases proliferation and tubular secretion.

Delineation of the "cystic phenotype" identifies key targets for future therapeutic intervention. As noted in the text and *Fig. 36-2*, the most promising therapies for future development and clinical trial target the abnormal

Figure 36-2

The Cystic Phenotype and Therapeutic Interventions. The figure depicts the two primary signaling pathways which mediate progressive cyst formation and enlargement in ADPKD and ARPKD: Abnormal expression of the EGFR-axis and adenylate cyclase activating receptor activity leading to increased cAMP (see text for details). Superimposed on the figure are key sites of therapeutic targeting: 1. Monoclonal antibodies against the EFGR-family (e.g., cetuximab; transtuzumab; nimotuzumab); 2. Small molecule inhibitors of EGFR-family tyrosine kinase activity (e.g., erlotinib, lapatinib, HKI-272); 3. Inhibitors of adenylate cyclase activating receptors (e.g., tolvaptan; somatostatin analogues); 4. Inhibitors of c-Src kinase activity which decrease EGFR-family ligand availability and inhibit tyrosine kinase activity, as well as decrease B-Raf activity (e.g., bosutinib, SKI-758, SU-6656); 5. Matrix metalloproteinase inhibitors which inhibit release of bioavailable EGFR-ligands (e.g., XL-784); 6. mTOR inhibitors (e.g., rapamycin); 7. Inhibitors of MEK kinase activity (e.g., UO126, PD98059). Additional therapies described in the text and recent reviews (21, 173, 285) but not depicted include: 20-HETE inhibitors (96); CDK inhibitors (458), Reactive Oxygen Species inhibitors and TNF-alpha inhibitors (459).



EGFR-axis and adenylate cyclase activation at multiple sites. Perhaps the most promising therapies will target key signaling intermediates which appear to integrate these separate pathways, such as Src kinase (*Fig. 36-2*) (6, 16, 21, 41, 172, 173) and/or utilize multiple agents in combination. At this writing, a number of agents are in advanced states of pre-clinical development or Phase 2–3 pilot clinical trials. The interested reader is referred to regularly updated listings of ongoing clinical trials for ADPKD and ARPKD at (www.pkdcure.org; and http://clinicaltrials.gov/.).

Autosomal Recessive Polycystic Kidney Disease (ARPKD)

ARPKD is an inherited disorder characterized by cystic dilations of renal collecting ducts and varying degrees of hepatic abnormalities consisting of biliary dysgenesis and periportal fibrosis (5). ARPKD has alternatively been referred to as "infantile" polycystic kidney disease. This term, however, is generally no longer used because of recognition that the disease can present any time from the prenatal period through adolescence, and rarely even

in adulthood. Furthermore, other forms of PKD, including ADPKD, can present in the neonatal period (2, 174).

Epidemiology and Genetics

Based on published reports, the incidence of ARPKD is 1:10,000–1:40,000 (175, 176). The frequency of the gene in the population is estimated to be approximately 1:70 (177). However, the exact incidence is unknown, since published reports vary in the populations studied (e.g., autopsied patients versus survivors), and affected children may die in the perinatal period without a definitive diagnosis. With improvements in neonatal management leading to improved survival rates, as well as formal reporting mechanisms (such as a newly developed ARPKD registry (178)), more accurate incidence rates may become established.

Consistent with autosomal recessive disease, heterozygotes (carriers) are unaffected. The recurrence risk for subsequent pregnancies is 25%, and unaffected siblings have a 66% risk of being a carrier for ARPKD (5). Males and females are affected equally and ARPKD affects all racial and ethnic groups.

ARPKD is caused by mutations in *PKHD1* (polycystic kidney and hepatic disease 1), a large, novel gene that localizes to chromosome 6p21 (179). To date, all kindreds with features typical of ARPKD have demonstrated linkage to this locus (177). Thus, there is no evidence for genetic heterogeneity in patients with the typical features of ARPKD. Of note, kindred with features of ARPKD as well as additional extrarenal abnormalities including skeletal and facial anomalies has been described and linkage to the 6q21 locus excluded (180). Intrafamilial variability in ARPKD disease phenotype was originally reported to be unusual (181) in contrast to the wide variability often seen in some ADPKD kindreds (see below). However recent data suggest that up to 20% of ARPKD multiplex pedigrees exhibit significant intrafamilial phenotypic variability (182). Among families with at least one neonatal survivor, the risk for perinatal demise of a subsequent affected child is 37%. These data are important for appropriate genetic counseling.

PKHD1 was cloned by two independent research groups in 2002 (9, 10). The gene spans a region of over 400-kb of genomic DNA and contains at least 66 and possibly over 86 exons. The mRNA for the gene is produced as multiple alternative transcripts. The primary transcript of approximately 14–16 kb in length encodes a novel protein termed fibrocystin (alternatively named polyductin). Several alternative transcripts have also been described, several of which lack the transmembrane domain, suggesting that (if translated) they may result in production of secreted forms of fibrocystin (10). Fibrocystin is a very large protein with a predicted molecular weight of 447 kD, similar in size to polycystin 1. The precise function of fibrocystin is unknown at present. However, protein modeling suggests that it is a membrane-bound protein with immunoglobulin-like properties including the presence of several TIG/IPT domains (immunoglobulin-like folds shared by plexins and transcription factors). These motifs suggest that fibrocystin may function as a receptor (9, 10). Recent studies have demonstrated that fibrocystin undergoes proteolytic cleavage and that the C-terminal fragment of fibrocystin translocates to the nucleus (183). In addition, further studies have shown that fibrocystin regulates the expression and function of polycystin-2 (184). The precise functional significances of these observations are as yet undefined but they again highlight the complexities of PKD protein and signal transduction biology.

Pathogenesis

With the cloning and identification of *PKHD1* as the causative gene in ARPKD, detailed observations about mutations and genotype-phenotype correlations have begun to emerge. Several published series of multiple ARPKD kindreds have demonstrated different *PKHD1* mutations throughout the gene, without a clear clustering at specific sites, and the majority of families have unique ("private") mutations (9, 185–187). In addition, most patients studied in an ethnically diverse population are compound heterozygotes (188), i.e., has a different mutation on each *PKHD1* allele. Mutations identified include both missense and truncating mutations.

Genotype-phenotype analyses have also been performed, although these studies are complicated by the large number of mutations and high rate of compound heterozygotes. The locus-specific database for *PKHD1* contains over 350 different mutations (www.humgen.rwth-aachen.de). Several studies have confirmed, however, that patients with more pathogenic mutations (those with two truncating mutations) displayed a very severe phenotype, associated with a high rate of perinatal/neonatal mortality (185, 188, 189). In contrast, amino acid substitutions (missense mutations) were found to be more commonly associated with a nonlethal presentation (190). The actual position of the mutation along the gene, however, did not appear to correlate overall with phenotype (188).

Despite recent advances in the understanding of the molecular genetics of ARPKD, the pathogenesis remains

poorly defined. Northern analyses and RT-PCR demonstrated that *PKHD1* is expressed in both fetal and adult kidney, and to a much lesser extent in liver, pancreas and lung. Expression in other organs was not seen (9, 10). With the development of antibodies to the fibrocystin protein, additional tissue and cellular localizations have been delineated. During development, fibrocystin is expressed in the branching ureteric bud/collecting ducts of the developing kidney and is also present in developing neural tube, gut, bronchi and vascular system (191, 192). In the postnatal kidney, fibrocystin is primarily expressed in the collecting duct, the site of cyst formation in ARPKD. It is also present in the bile ducts and pancreatic ducts and islets. This expression pattern persists into adulthood (191). On a cellular level, fibrocystin localizes to primary cilia of renal collecting tubule and loop of Henle epithelia as well as biliary and pancreatic ductal epithelia (191–193). On a subcellular level, fibrocystin colocalizes with polycystin 2 in the polycystin complex adjacent to the basal bodies of cilia on the apical cell surface, as well as at adherens junctions and focal adhesions (192, 194). Recent data suggest that fibrocystin can undergo a complex pattern of Notch-like processing in which a large extracellular domain is shed in a process mediated by ADAM family metalloproteinases. Concomitantly, an intracellular fragment is release by gamma secretase actions (195). A similar pattern of processing is also seen with polycystin 1, which appears to translocate to the nucleus where it presumably affects expression of multiple genes (196).

Studies of animals with *PKHD1* mutations (either spontaneous or genetically engineered) have provided important insights into the abnormalities that can develop when fibrocystin is not expressed normally. The PCK rat harbors a 157-bp deletion in exon 36 of the rat orthologue of the human ARPKD gene, and developed spontaneously in a colony of Sprague-Dawley rats (9, 197). Although it is a genetic model of ARPKD, this model has clinical features of both ARPKD and ADPKD kidney and liver disease (9, 197). Affected animals develop progressive cystic enlargement of the kidneys after the first week of life. Renal cysts develop predominantly in distal tubules and collecting ducts. The animals also develop features consistent with Caroli's disease/congenital hepatic fibrosis (198). Several *Pkhd1* "knockout" models have also been developed and harbor mutations at various points along the gene. Interestingly, the phenotypes are quite varied and quite dissimilar to human ARPKD (199, 200). Insights into the regulation of *PKHD1* expression, have been provided by the observation that mice with mutations in hepatocyte-nuclear factor 1(HNF-1β), the gene mutated in the human disease MODY5, develop renal cysts and show a decrease in *PKHD1* expression (201). Further data demonstrate that HNF-1β itself directly regulates the activity of the *PKHD1* promoter (202).

Recent studies have provided new information regarding the pathogenesis of congenital hepatic fibrosis in ARPKD. CHF is characterized primarily by fibrosis and bile duct proliferation although biliary cyst formation is not a prominent feature. Increased expression of pro-fibrotic molecules transforming growth factor-beta (TGF-β) and thrombospondin–1 have been demonstrated in human ARPKD livers (203). Livers from orthologous PCK rats show a pattern consistent with the ductal plate malformation of patients with CHF. Intrahepatic bile duct dilatation with cystic changes and marked portal fibrosis are particularly prominent (198, 204). Biliary epithelium in the PCK show abnormalities in proliferative activity, related to abnormal EGFR-axis expression as well as apoptosis (41, 198, 205). Cholangiocytes in this model have abnormal cilia, the length of which is related to the level of *PKDH1* expression (206).

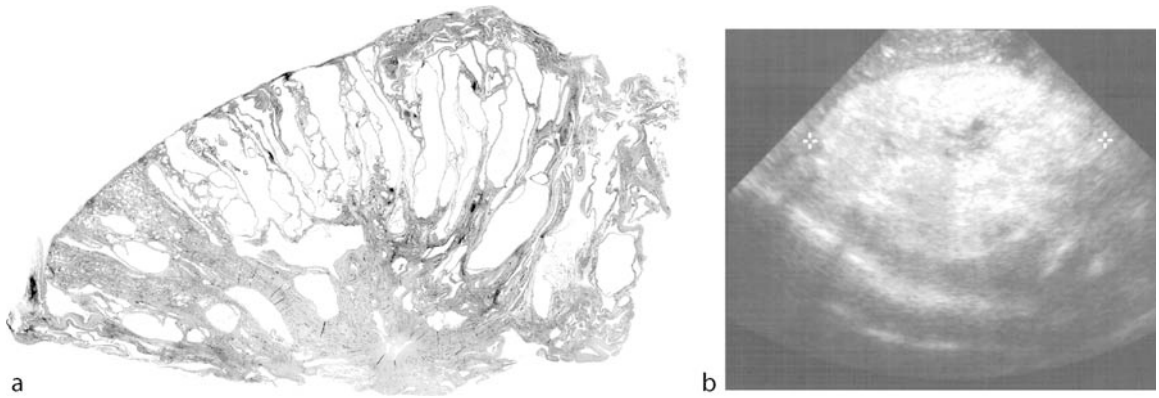
Liver disease is also evident in other ARPKD models, including the *bpk* and *cpk* mouse models. (170, 207–209). Biliary epithelial hyperplasia, like renal tubule hyperplasia appears to be mediated by a mitogenic cycle driven by abnormal EGFR-axis expression (205, 210).

Pathology

In infants and young children, the kidneys are reniform but grossly enlarged. Pinpoint opalescent dots are visible on the capsular surface and correspond to cystic cortical collecting ducts (211). Microscopically (see *Fig. 36-3a*), the cysts are usually less than 2 mm in size ("microcysts") and have been shown by microdissection, histochemical, and immunologic studies to be dilated collecting ducts lined by low columnar or cuboidal epithelium (212–215). The glomeruli and other tubular structures appear to be decreased in number because of marked collecting duct ectasia and interstitial edema. In fetal kidneys, proximal tubular cystic lesions have also been identified (216), but are largely absent by birth. The pelvicaliceal system and renal vessels appear normal. Unlike ADPKD, in which the cysts become discontinuous with the tubule, the cystic tubules in ARPKD are fusiform in shape and remain in contact with the urinary stream. Microdissection studies and scanning electron microscopy demonstrate that obstruction of urinary flow is not a component of ARPKD (211, 212). With increased patient survival, the development of larger renal cysts, interstitial fibrosis, and

Figure 36-3

Kidney histology and ultrasonography of autosomal recessive polycystic kidney disease (ARPKD). (a) Microscopic appearance of a kidney biopsy from a 7-month-old with ARPKD demonstrating multiple radially oriented collecting tubule cysts extending from the medulla to the peripheral cortex. A small amount of residual parenchyma contains glomeruli and noncystic tubules situated between the cysts. No glomerular cysts or signs of renal dysplasia are present. (Hematoxylin and eosin stain; original magnification x1.) (Specimen kindly provided by Dr. Steven Emancipator, Case Western Reserve University.). (b) Renal ultrasound (right kidney) of a newborn with ARPKD demonstrates the typical appearance of echogenic, enlarged kidneys (length = 6.5 cm; normal for age = 4.48 ± 0.62 cm) with poor corticomedullary differentiation.



hyperplasia produces a pattern more like ADPKD (see below) (217). Gang and Herrin (218) describe increasing fibrosis and inflammation in later specimens from patients who had typical collecting duct microcysts during infancy.

Some degree of biliary dysgenesis and hepatic fibrosis is always present in ARPKD. Although hepatic involvement is invariably present microscopically at birth, it is clinically evident in only 40–50% of neonates (219). The classic liver lesion shows a typical ductal plate abnormality consisting of portal fibrosis surrounding increased numbers of hyperplastic, ectatic biliary ducts with normal hepatocellular histology (217, 220, 221). With time, hepatomegaly and portal hypertension become evident in many patients. Intrahepatic biliary ectasia may result in macrocysts and dilation of extrahepatic bile ducts sometimes resulting in an enlarged gallbladder (222) or choledochal cysts (223). Although the combination of collecting tubule and biliary ectasia with periportal fibrosis is unique to ARPKD, portal fibrosis and bile duct proliferation may be associated with other types of renal disease, including ADPKD (224, 225).

Clinical and Radiographic Features

Historically, ARPKD was originally separated into four distinct clinical entities based on age at presentation and relative degrees of renal and hepatic involvement (226). Although such distinctions were useful as clinicopathological classifications, they are now recognized to be the result of different mutations within the same gene and are not used clinically (5, 227).

The majority of patients with ARPKD present in infancy (178, 219, 228, 229). A subset of patients with ARPKD may present as older infants with abdominal enlargement secondary to enlarged kidneys or hepatosplenomegaly without the full spectrum of clinical symptoms outlined below (178, 229). A smaller, though increasingly recognized subset of patients with ARPKD are diagnosed as older children or adults (178, 230, 231). These patients typically present with signs and symptoms related to congenital hepatic fibrosis, including hepatosplenomegaly and portal hypertension (177, 232). A recent series showed that almost one-third of individuals with mutations in *PKHD1* and hepatic involvement were 20 years or older at the time of initial presentation, suggesting that the clinical spectrum of the disease is broader than previously appreciated (231).

With the widespread use of prenatal ultrasound, most patients with ARPKD are now detected *in utero*. Prenatal ultrasound may demonstrate the findings of oligohydramnios, large renal masses, or absence of fetal bladder filling (233). At birth, patients usually have large, palpable flank masses that may be large enough to complicate delivery. Urine output is usually normal; however,

oliguric acute renal failure may occur (175). In such patients, increased urine output and a corresponding improvement in renal function may be seen following improvement in respiratory status (234). Most patients (70–80%) have some evidence of impaired renal function in the newborn period (3, 219). However, death from renal insufficiency is uncommon (226). Transient hyponatremia related to a urinary dilution defect is often present, but usually resolves over time (219, 228). The treatment consists of water restriction. Metabolic acidosis has also been reported (3, 228). As might be predicted from a pathological process that affects the collecting tubule, most patients have a urinary concentrating defect and symptoms of polyuria and polydipsia (3, 217, 228, 229).

Hypertension, which may be severe, is common in both infants and children and may well be a presenting feature (175, 217). It can be present in patients with normal renal function and eventually affects almost all children with the disease (3, 178). However, the pathophysiology of hypertension in ARPKD is poorly understood (172). In ADPKD patients the renin-angiotensin aldosterone system (RAAS) is upregulated and thought to occur as the result of expanding cysts causing local ischemia (235). The role of the RAAS in mediating hypertension in ARPKD is less clear. Systemic renin levels are not usually elevated in hypertensive ARPKD patients or in an ARPKD rat model (228, 229, 236). In addition, kidney size in ARPKD stabilizes over time and does not show the progressive macrocystic enlargement classically seen in ADPKD. Thus, it is unknown whether the same mechanism accounts for hypertension in both ADPKD and ARPKD. Local (intrarenal) RAAS activation is suggested by a recent histologic study that demonstrated increased expression of several renin-angiotensin axis components in two kidneys of individuals with ARPKD (237). Similarly, intrarenal RAAS activation has also been demonstrated in the orthologous PCK rat (238).

Pulmonary insufficiency, as manifest by respiratory distress, is a major cause of morbidity and mortality in neonates with ARPKD. Oligohydramnios results in pulmonary hypoplasia, which may be complicated by restriction of diaphragmatic movement due to massively enlarged kidneys. Additional causes of respiratory distress in these patients include pneumothorax and atelectasis, or a variety of common neonatal pulmonary disorders such as surfactant deficiency, bacterial pneumonia, meconium aspiration, or persistent fetal circulation. Severely affected infants may demonstrate all features of the "oligohydramnios sequence", including pulmonary hypoplasia, abnormal extremities and characteristic Potter's facies (239). Infants with true pulmonary hypoplasia often die soon after birth secondary to pulmonary insufficiency.

The typical appearance of ARPKD by ultrasonography is one of large echogenic kidneys with poor corticomedullary differentiation (*Fig. 36-3b*). Macrocysts, a feature of ADPKD, are usually not present at birth, but are not uncommon with progression of disease (240). In a study of sonographic features of adult patients with ARPKD, Nicolau et al. (241) noted the presence of multiple small cysts in normal-sized kidneys, increased cortical echogenicity and loss of corticomedullary differentiation as common features. Stein-Wexler and Jain (242) proposed that the ultrasonographic findings of "focal rosettes," corresponding to the macroscopic appearance of radially-oriented collecting tubule cysts, are specific for ARPKD. In addition, although kidneys may be markedly enlarged at birth, over time, the majority show stable to decreased renal size (243, 244). In a preliminary report from a prospective, NIH-supported study of the natural history of ARPKD, kidney volumes of enlarged kidneys increased at approximately 1/3 of the normal rate for age over 2–4 years (245).

Findings on magnetic resonance imaging include enlarged kidneys with hyperintense T2-weighted signals (246). Kern et al. showed that ARPKD kidneys have a characteristic hyperintense, linear radial pattern in the cortex and medulla by RARE-MR urography that may reflect the microcystic dilatation seen histologically (246, 247).

ARPKD kidneys have also been reported to have characteristic features by nuclear medicine studies. DMSA scanning demonstrated loss of the normal kidney outline and internal structure and patchy tracer uptake with focal defects throughout the kidneys, particularly at the poles. In the majority of cases, these DMSA changes did not correlate with the ultrasonographic findings, in which the kidneys appeared more uniformly affected (248).

Ultrasonographic findings in the liver include hepatomegaly, increased echogenicity and poor visualization of the peripheral portal veins. Reversal of normal venous flow by Doppler study, suggestive of portal hypertension, may also be seen. Hypertrophy of the left lateral segment of the liver is also occasionally seen (249) and a subset of patients will have overt evidence of biliary ductal dilatation (Caroli's disease) (249). In a preliminary report of the NIH-supported natural history study, over 75% of ARPKD patients demonstrated intra and extra-hepatic biliary dilatations, with dilated common bile ducts and enlarged gall bladders (245). Macroscopic liver cysts are uncommon (250), although choledochal cysts have been reported (223), and MRCP demonstrates diffuse intrahepatic bile duct dilatation with periportal fibrosis (249).

Diagnosis

With the advent of modern obstetrical ultrasonography, many patients with ARPKD are identified in the prenatal period. Enlarged echogenic kidneys, oligohydramnios, and the absence of urine in the bladder, are very suggestive of ARPKD (251). Older literature suggests that sonographic features of ARPKD may present in the second trimester but usually are not apparent until after 30 weeks' gestation (252). Both false-positive and false-negative results have been reported (253). However, with newer high-resolution obstetrical ultrasonography it is probable that diagnostic sensitivity and detection rates will improve. Wisser et al. (254) reported a case of a fetus with pathologically-confirmed ARPKD who demonstrated echogenic, normal sized kidneys at 15 + 4 weeks gestation.

As noted previously, other cystic kidney diseases in infancy, including ADPKD and cystic dysplasia, may have antenatal sonographic appearances that are difficult to distinguish from ARPKD (2). It has been proposed that fetal MRI may be a useful additional diagnostic study in fetuses with inconclusive ultrasonography in the third trimester of pregnancy (255). However, its accuracy in confirming the diagnosis earlier in pregnancy has not been assessed. Increased maternal alpha fetoprotein and amniotic fluid trehalase activity have been identified as potential markers for ARPKD, but neither has been confirmed as specific or sensitive for disease detection *in utero* (256, 257).

Definitive diagnostic criteria for ARPKD have not been established. Those proposed by Zerres et al, with modifications (5, 219), are used by many pediatric nephrologists, and include:

1. Ultrasonographic features typical of ARPKD, including enlarged, echogenic kidneys, with poor corticomedullary differentiation; and
2. One or more of the following:
   a) Absence of renal cysts in both parents, particularly if they are at least 30 years old,
   b) Clinical, laboratory or radiographic evidence of hepatic fibrosis,
   c) Hepatic pathology demonstrating characteristic ductal plate abnormality,
   d) Previous affected sibling with pathologically confirmed disease,
   e) Parental consanguinity suggestive of autosomal recessive inheritance

As noted above, renal ultrasonography may be less diagnostic in children who present later in childhood. Furthermore, in the subset of patients who present as older children and adolescents, hepatic abnormalities are often the prominent presenting feature.

Although renal biopsies will clearly differentiate the isolated fusiform cortical collecting tubular cysts of ARPKD (*Fig. 36-3*) from the heterogeneous cystic nephron involvement of ADPKD (*Fig. 36-4*) (214, 258), they are generally not indicated for patients who fulfill the classic criteria for ARPKD and/or those for whom genetic testing is definitive (see below) (5). In certain instances, liver biopsy may provide additional information and reveal the characteristic biliary dysgenesis of ARPKD. However, hepatic portal fibrosis and bile duct ectasia have been associated with other types of renal cystic disease, including ADPKD.

Genetic testing is also typically not required for patients with classic ARPKD diagnostic criteria. Genetic testing is useful, however, for families who already have an affected child, in identifying sibling carriers and in instances in which the diagnosis is less clear. Prenatal diagnosis may be made in a family with at least one known affected child through the techniques of linkage analysis or mutation analysis. Linkage analysis uses analysis of polymorphic markers that flank the location of a known disease gene to "track" the disease. This technique can also be used to identify whether the unaffected sibling is a carrier of the disease. In informative families, the accuracy of prenatal diagnosis using linkage analysis was >95% (259). An accurate genetic diagnosis by linkage analysis, however, is critically dependent on the diagnosis of ARPKD in the affected sibling (259).

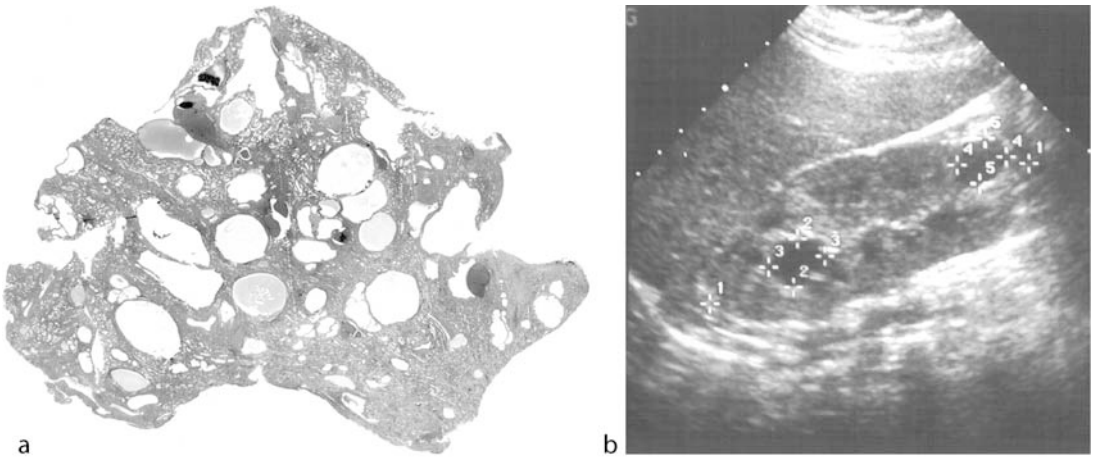
With the identification and cloning of *PKHD1*, molecular analysis is now available. Although initial studies reported a relatively low mutation detection rate (40–60%) by sequencing methods, newer studies using mutation screening by DHPLC demonstrate an overall mutation detection rate of 82–87% in individuals (including fetuses) with ARPKD (188, 260–263). In addition, pre-implantation genetic diagnosis (PGD) is now offered by a limited number of genetic laboratories (264). A complete list of laboratories offering clinical and research testing for ARPKD is available at www.geneclinics.org.

Treatment and Complications

Survival of neonates with ARPKD has improved in concert with overall medical advances in neonatal artificial ventilation and intensive care. It is currently impossible to predict which neonates with ARPKD who require immediate artificial ventilation have critical degrees of pulmonary hypoplasia incompatible with survival (3, 5). In some

Figure 36-4

Kidney histology and ultrasonography of autosomal dominant polycystic kidney disease (ADPKD). (a) Microscopic appearance of a kidney biopsy from an adult with ADPKD demonstrating multiple thin-walled cysts of varying sizes involving different nephron segments. Focal hemorrhage is noted within some cysts. (Hematoxylin and eosin stain; original magnification x1.) (Specimen kindly provided by Dr. Steven Emancipator, Case Western Reserve University.). (b) Renal ultrasound (right kidney) of a 13- year-old with ADPKD demonstrates several cysts (the largest measuring 1.5 cm × 1.6 cm). The left kidney also had several cysts not present on an ultrasound 2 years before this study. Kidneys are 11.4 cm (*right*) and 11.7 cm (*left*) (normal for age = 9.79 cm ± 1.5 cm).



instances, severe pulmonary distress may be secondary to potentially reversible fluid overload, neonatal lung disease, or restricted diaphragmatic motion secondary to massively enlarged kidneys. In selected cases, some authors have advocated continuous venovenous hemofiltration, unilateral or bilateral nephrectomy coupled with peritoneal dialysis to allow optimal ventilation and thereby assess the long-term pulmonary prognosis of the patient (265–268).

Infants and young children with ARPKD, including those without significant renal insufficiency must be followed closely. Because most children with ARPKD have urinary concentrating defects, significant dehydration is a particular risk during intercurrent illnesses, which may increase insensible water loss (fever), limit free water intake (nausea), or increase extrarenal water loss (vomiting, diarrhea). In patients with severe polyuria, thiazide diuretics may be of benefit to decrease distal nephron solute and water delivery. Supplemental bicarbonate therapy is required for those with metabolic acidosis.

Hypertension can be difficult to manage and may require multiple medications (175). Despite the fact that peripheral renin values are not usually elevated in hypertensive ARPKD patients, most patients respond well to angiotensin-converting enzyme inhibitors or angiotensin-II receptor blockers, which are considered by many to be the treatments of choice. It should be noted, however, that the safety of ACEi or ARBs in neonates has been called into question by recent studies in neonatal rats that demonstrated adverse effects on tubular maturation and exacerbation of injury associated with obstructive uropathy (269). In addition, ACEi can precipitate acute renal failure in PKD patients (270), as well as in infants in general (271), particularly with dehydration. If additional medications are required, second-line agents include calcium channel blockers, β-blockers (in those without chronic lung disease or signs of congestive heart failure), and diuretics. Recent studies on the pathophysiology of cyst formation (see above) raise the theoretical concern that Ca^{++} channel blockers may exacerbate low intracellular Ca^{++} in cystic renal epithelia and increase abnormal proliferation and disease progression.

Urinary abnormalities may be present or develop over the course of disease. Pyuria is a relatively common finding and can be seen in the absence of demonstrable bacteriuria or documented infection (217). Urinary tract infection has been reported as a common complication in at least one uncontrolled series (3), but it is unclear whether children with ARPKD truly have an increased incidence of upper or lower urinary tract infections (UTIs) when compared with appropriately age-matched controls. Thus, as in any child with an abnormal urinalysis, clinical features and

appropriately obtained urine cultures must guide antibiotic therapy. If a UTI is documented, a voiding cystourethrogram and renal ultrasound should be performed to determine the possible presence of vesicoureteral reflux and rule out obstruction or superimposed upper tract structural abnormalities (218). Microscopic or gross hematuria and proteinuria may also be seen (3, 175). In infants and children who develop chronic kidney disease, the consequences of progressive CKD (e.g. growth failure, anemia, and renal osteodystrophy) become apparent as renal function decreases.

Dialysis and/or transplantation are indicated when children with ARPKD reach symptomatic end-stage renal failure or if progressive uremia results in growth failure or developmental delay. Peritoneal dialysis is often the preferred method of dialysis and may be the only practical long-term option in the young child. Peritoneal dialysis in ARPKD is usually successful even in the face of large kidneys and hepatosplenomegaly. Kidney transplantation offers definitive renal replacement therapy in children with ARPKD. Successful kidney transplantation prolongs survival and often accelerates growth and development in young uremic children. Nephrectomies may be indicated prior to, or at the time of, transplantation to control hypertension and/or to permit room for transplant placement in patients with massively enlarged kidneys.

Difficulties in feeding, even in patients without renal insufficiency, are often noted. This is presumably due to the presence of enlarged kidneys and or liver, interfering with normal gastrointestinal function. Supplemental feeding via nasogastric or gastrostomy tubes is often required to optimize weight gain and growth. Although growth failure in ARPKD may also occur as the result of chronic kidney disease, a study by Lilova et al. (272) suggests that growth failure in this population is common, may be attributable to factors other than CKD alone, and responds well to growth hormone treatment. In contrast, the preliminary report from the NIH-supported Natural History Study of ARPKD suggests that completely normal growth curves may not be uncommon (245).

With improved patient survival and advances in renal replacement therapy, hepatic complications progressively dominate the clinical picture of many patients with ARPKD (178, 219, 221, 232, 273). These include hepatosplenomegaly, bleeding esophageal varices, portal vein thrombosis, and hypersplenism causing thrombocytopenia, anemia, and leucopenia. Data from two recent series showed that portal hypertension occurred in 37–44% of neonatal survivors and was age-related (178, 274). Although significant complications related to portal hypertension develop, liver synthetic function is usually intact.

One serious and potentially lethal complication in ARPKD patients with significant hepatic involvement is bacterial cholangitis, which has been reported as early as a few weeks of age (3). Fever or elevation of liver function tests at any time should lead to the suspicion of cholangitis and result in complete evaluation and appropriate antimicrobial therapy. However, patients may not present with the classic clinical findings of cholangitis and the diagnosis should be strongly considered in ARPKD patients with unexplained recurrent sepsis with gram negative organisms (275). Caroli's disease (dilated intrahepatic bile ducts) has been identified as a potential risk factor for bacterial cholangitis (276, 277). Cholangiocarcinoma has also been reported in patients with CHF/Caroli's (278).

In infants and children with hepatic involvement, close monitoring for complications of portal hypertension is mandated, particularly since typical "liver function" tests (such as serum albumin and transaminases) may be normal. Yearly ultrasonography to determine changes in liver or spleen size to identify portal hypertension by reversal of venous flow is non-invasive and may be of value. Endoscopy is necessary to evaluate suspected esophageal varices that can be treated by sclerotherapy or banding prior to life-threatening hemorrhage. Periodic monitoring should reveal the hematologic profile of hypersplenism. Sudden worsening of anemia should raise the possibility of occult gastrointestinal blood loss secondary to splenic sequestration or variceal bleeding. Porto-systemic shunting may be indicated in some cases (228, 279), but concerns have been raised about reported cases of ultimately fatal recurrent hepatic encephalopathy in children with porto-caval shunts who progressed to ESRD (280). It has been hypothesized that the loss of kidney function results in impaired clearance of toxins that are shunted from the liver. This finding has raised concerns about whether liver transplantation should be considered as an alternative therapy for ARPKD patients with portal hypertension being evaluated for possible shunts or those with recurrent episodes of cholangitis (281). The increased use and successful outcome of living-related partial liver transplants makes this a more realistic option. In fact, successful sequential liver and kidney living-related transplants have been reported (282).

In addition to the significant medical problems, the psychosocial stresses of ARPKD on the patient and family can be overwhelming. Social support measures and periods of respite care are often necessary. A team approach using the skills of pediatric nephrologists in concert with

other pediatric medical subspecialists, specialized nurses, dietitians, social workers, psychiatrists, and other support staff is required to provide optimal comprehensive care for children with ARPKD.

Prognosis

Prognosis is difficult to assess, although it is now clear that survival of all but the most severely affected neonates who demonstrate pulmonary hypoplasia is possible (1, 217). Published reports vary with respect to neonatal survival rates, but suggest that approximately 70–80% of patients survive the newborn period with aggressive neonatal intensive care (3, 228, 232). Actuarial survival rates calculated from birth for 55 patients with ARPKD referred to a pediatric tertiary care center revealed that 86% were alive at 3 months, 79% at 1 year, 51% at 10 years, and 46% at 15 years (228). Calculations based on patients who survived to 1 year of age showed that 82% were alive at 10 years and 79% at 15 years (228). Similar findings were reported in a more recent study of 164 neonatal survivors with confirmed *PKHD1* mutations. Patients in that cohort had a 1 year survival rate of 85% and a 10 year survival rate of 82% (274). In a cohort of 166 ARPKD patients born after 1990, 75% were alive at a median age of 5.4 years (178).

Patients who survive the neonatal period usually have a decreased glomerular filtration rate (GFR), but studies have demonstrated subsequent improvement in renal function consistent with some degree of continued renal maturation (175). However, a significant number of patients with ARPKD will progress to end-stage kidney disease. In a cohort of patients surviving the first month of life, Roy et al. (232) reported renal survival of 86% at 1 year and 67% at 15 years. A more recent study of patient with confirmed *PKHD1* mutations showed actuarial renal survival rates of 86% at 5 years, 71% at 10 years, and 42% at 20 years (274).

With the success of renal transplantation and improved survival of patients with ARPKD, morbidity and mortality of complications related to congenital hepatic fibrosis are more common and clinically relevant. Whether these complications result in significant mortality post-kidney transplant is a subject of some debate. Khan et al. reported the outcome of 14 patients with ARPKD after renal transplantation (283). With a mean follow-up of 14 years, the study showed 1 and 5 year patient survival rates of 93% and 86% respectively. Overall 36% of patients died and, in 4 of 5 of those patients, death was directly related to complications of hepatic disease. In those who survived, 63% had portal hypertension. Thus, complications of CHF developed in almost 80% of patients following renal transplantation for ARPKD. In contrast, in a retrospective study of patients included in the North American Pediatric Renal Transplantation Cooperative Study (NAPRTCS) registry, Davis et al. (284) reported similar patient and graft survival rates in kidney transplant patients with ARPKD compared to those without. It is interesting to note, however, that among those patients who died, sepsis was the cause in 64% of those with PKD versus 32% in those without PKD, suggesting that ARPKD patients may be at increased risk of infection compared to the general pediatric transplant population.

Autosomal Dominant Polycystic Kidney Disease (ADPKD)

ADPKD is a systemic inherited disease characterized by progressive renal cystic enlargement of all nephron segments coupled with variable extrarenal manifestations involving the gastrointestinal tract, cardiovascular system, reproductive organs and the brain (4, 173, 285). ADPKD has alternatively been called "adult" polycystic kidney disease. However, this term is a misnomer because ADPKD has been diagnosed in the fetus, newborns, older children and adolescents (175, 229, 286, 287).

Epidemiology and Genetics

ADPKD is the most common inherited human kidney disease and occurs at an incidence of approximately 1:400 to 1:1000. It affects all races and males and females are both affected; however, the kidney phenotype may be more severe in males (288). ADPKD is a rare cause of ESRD in the pediatric population, but accounts for approximately 5–10% of ESRD in adults. The two major disease-causing genes are *PKD1* and *PKD2*. In the general population, *PKD1* accounts for approximately 85% of ADPKD and *PKD2* the remaining 15%. A third ADPKD locus has been suggested by a few case reports (289–291), but has not been substantiated (4). Mutations in *PKD1* and *PKD2* produce similar phenotypes, however, the age of onset of cystic disease, hypertension, and renal insufficiency is delayed in the latter (21, 292–294).

PKD1 has been mapped to chromosome 16p13.3 (295). Like *PKHD1*, *PKD1* is a very large gene, spanning 53 kb of genomic DNA with 46 exons encoding a 14.5 kb transcript (7). A portion of the gene is duplicated in the proximal portion of chromosome 16. The gene encodes a large, novel 4304 amino acid protein product,

polycystin-1 (PC-1), a 460 kD protein with a large extracellular domain that contains motifs likely to function as protein and carbohydrate binding sites. The PKD repeats in the extracellular domain may mediate homodimerization of PC-1. The extracellular domain of PC-1 also contains a physiologically important G-protein-coupled receptor proteolytic site (GPS). Taken together these motifs suggest that PC-1 may function as a receptor and/or have roles in cell-cell interactions. *PKD2* has been linked to chromosome 4q13-q23 (8, 296) and expresses a 5.4 kb mRNA, which encodes a 968 amino acid polypeptide, polycystin-2 (PC-2) (8). Polycystin-2, also called TRPP2, is a calcium permeable, non-selective cation channel (297, 298) whose NH2 and COOH termini are both cytoplasmic.

Pathogenesis

ADPKD is characterized by considerable intrafamilial and interfamilial phenotypic variation (189, 299). Several studies have supported a role for genetic background/genetic modifiers as a cause of this variability (300). A number of candidate genes have been examined as potential modifiers of the disease phenotype. These include members of the renin-angiotensin system, including the angiotensin converting enzyme (ACE) gene, the endothelin system and the cystic fibrosis gene, *CFTR*. However, positive results suggesting an effect have not been reproducible (189).

One gene that has been found to influence disease severity is the tuberous sclerosis 2 (*TSC2*) gene. Several kindred's were identified that had co-existent *TSC2* and *PKD1* mutations with severe childhood-onset ADPKD. These kindreds were subsequently found to have large deletions in an area containing both *PKD1* and *TSC2*, resulting in a contiguous gene syndrome (301).

One explanation for phenotypic variability may be related to the so-called "second hit" theory of ADPKD which result in heterozygous mutations at the level of individual cysts. By analyzing two closely linked polymorphic markers within the PKD1 gene, Qian et al. revealed that the renal epithelia from single cysts are monoclonal, containing only the mutant haplotype (302). A subsequent study by Brasier et al. (303) confirmed these findings. These two studies suggest that patients harboring a germline mutation in the one allele of a PKD gene undergo a somatic "second hit", which results in the loss of the remaining normal allele and genetic heterozygosity in those affected cells. These studies provide a possible molecular explanation for both the focal nature of cysts (<5% of tubules are cystic in ADPKD) as well as the phenotypic variability within families harboring the same germline mutation. The extent to which this two-hit phenomenon contributes to the overall phenotypic variability remains a subject of some debate (304).

Because of the significant intrafamilial variability in ADPKD, including kindreds with more severe disease noted in successive generations, the genetic phenomenon of anticipation has been postulated to be an explanation for this heterogeneity. However, a recent study of ADPKD patients with *PKD1* mutations failed to find evidence for anticipation in this disease (305).

PKD1

Numerous different mutations throughout the *PKD1* gene have been identified in patients with ADPKD with no specific mutational "hot spots" identified (306). The majority of mutations are predicted to result in truncation of the PC-1 protein. Substantial phenotypic variability has made genotype-phenotype correlations unachievable, but a number of recent studies have made significant observations about the nature of *PKD1* mutations and disease phenotype. Rosetti et al. (307) found that, even taking into consideration the significant inter- and intrafamilial phenotypic heterogeneity, patients with mutations in the 5′ region of *PKD1* had significantly more severe kidney disease than those with mutations in the 3′ portion of the gene. Thus, the location, rather than the type of *PKD1* mutation, was found to be the factor that correlated with the onset of ESRD. In addition, 5′ *PKD1* mutations have also been reported to be predictive for the development of cerebral aneurysms (308).

PC-1, the protein product of *PKD1*, is expressed in multiple tissues, including kidney, liver, pancreas, intestine and cerebral blood vessels (all sites of pathologic changes in ADPKD), as well as in the lung, testis, and other tissues (309–312). Localization studies demonstrated robust PC-1 expression in human fetal renal tubular epithelia that diminished with age, but persisted at low levels into adulthood (309, 313), suggesting a role in renal development and tubular maintenance.

PC-1 possesses a large extracellular domain, multiple transmembrane spanning regions, and an intracellular carboxy-terminus (314, 315). The extracellular domain is dominated by immunoglobulin-like repeats (the PKD domain) as well as a leucine-rich repeat, a LDL-A domain, a REJ domain, and a calcium-dependent lectin domain. These structural components, taken as a whole, suggest that the extracellular portion of PC-1 may be capable of

binding an as yet undefined ligand (315). PC-1 is anchored to the cell membrane by 7–11 putative transmembrane domains. These numerous features suggest that PC-1 is a large, multifunctional molecule involved in carbohydrate motif recognition, ligand binding, and Ca^{2+} regulation. It may engage in cell–cell and/or cell–matrix interactions, which regulate signal transduction pathways mediated by cell surface protein–protein interactions or directly participate in regulating transcriptional programs.

PC-1 has been localized to the plasma membrane of renal epithelial cells in a basal distribution at areas of cell-cell contact (adherens junctions), cell-matrix contacts (focal adhesions), and in the primary cilia. PC-1 has been shown to bind to polycystin 2 (316, 317) and regulate the channel activity of polycystin-2 (a non-selective cation channel) (318). The polycystin complex at the cilia appears to play a role in mechanosensation as previously discussed and loss of function of one of the complex members can result in loss of flow induced-calcium response (101). Interestingly, Chauvet et al. (319) showed that mechanical stimuli can induce proteolytic cleavage and nuclear translocation of the polycystin-1 carboxy terminus tail suggesting that polycystin-1 may have a role in regulating gene expression.

PC-1 co-localizes with and forms multimeric complexes with a wide variety of other proteins at other sites along the plasma membrane as previously discussed. These include those involved with cell-matrix interactions (such as $\alpha_2$beta 4 integrins and focal adhesion complexes) as well as cell-cell interactions (including E-cadherin-β-catenin complexes) (11, 13, 315, 320). PC-1 has also been shown to interact with intermediate filaments at the desmosomes (321) and cells lacking polycystin 1 show mislocalization of desmosomal proteins (14).

Additional data demonstrate that polycystin-1 induces resistance to apoptosis via the phosphatidylinositol 3-kinase/Akt signaling pathway and promotes spontaneous tubulogenesis in MDCK cells (322, 323). The carboxy terminal of polycystin-1 triggers branching morphogenesis and migration of inner medullary collecting duct (IMCD) cells, and supports *in vitro* tubule formation (324).

Both under- and over-expression of polycystin-1 is associated with cyst formation and/or developmental abnormalities. In cystic epithelium of human ADPKD kidneys (309), polycystin 1 is overexpressed and data from animal studies suggest that *PKD1* overexpression is sufficient to induce cysts (325). "Knockout" mouse models, in which a variety of PKD1 mutations have been introduced that result in the loss of functional polycystin, have also provided important clues to its function, particularly during development. Animals lacking polycystin-1 die *in utero* or soon after birth and demonstrate abnormalities in multiple organs, including the heart, blood vessels kidneys and pancreas (326–328). To overcome this lethal phenotype, investigators have developed PKD1 mutant mice that either produce low levels of PC-1 (hypomorphs) or have a "conditional" mutation (floxed allele) that is controlled via breeding with a Cre-recombinase expressing mouse or by pharmacologically driven Cre-gene expression. These animals survive, but develop kidney, pancreatic and vascular disease of variability severity (22, 329). Interestingly, the timing of the loss of PC-1 post-natally has a significant impact on the disease phenotype. Neonatal mice that lose PC-1 develop massive cystic kidney enlargement within 4 weeks, whereas older mice who lose PC-1 expression develop only mild cystic kidney disease (330). These observations suggest that cyst formation requires not only loss of PC-1, but also concomitant cell proliferation, such as is seen during early post-natal kidney development.

PKD2

Multiple mutations in *PKD2* have been identified in affected families, and as with *PKD1*, most families have unique mutations (331, 332). These mutations truncate polycystin-2 (PC-2) and appear to be loss-of-function mutations. As with PKD1, considerable intrafamilial phenotypic variability is reported in families with PKD2 mutations (189, 332). Genotype-phenotype studies have suggested that, unlike *PKD1*, the location of *PKD2* mutations does not appear to influence the age of onset of ESRD.

Similar to PC-1, PC-2 is widely expressed. The highest levels of expression within the kidney are the thick ascending loop of Henle and the distal convoluted tubule, where PC-2 localizes to the basolateral plasma membrane of renal tubular epithelium (333). PC-2, like polycystin-1, is expressed in the vasculature, including porcine aorta and normal human elastic and intracranial arteries (334). On a subcellular level, PC-2 localizes to the plasma membrane as well as to the endoplasmic reticulum and Golgi apparatus (12, 333).

PC-2 contains six transmembrane regions and has intracellular domains at both its amino- and carboxy-termini. The transmembrane regions share significant homology with voltage-activated Ca^{2+}/Na^{+} channels, which suggested that polycystin-2 may be a channel protein. The carboxy-terminus contains an EF-hand domain

that binds Ca^{2+} in addition to several potential phosphorylation sites. Koulen et al. (297) confirmed by single channel studies that PC-2 (a member of the subfamily of the transient receptor potential (TRP) channel superfamily) functions as a calcium-activated intracellular ion release channel *in vivo* and hypothesized that polycystic kidney disease results from the loss of regulation of an intracellular calcium release signaling pathway.

As noted previously, PC-2 interacts with PC-1 to form a complex located at the cilia. Data suggest that PC-2 located on the endoplasmic reticulum also interacts with PC-1 present at the plasma membrane (173). Recent data also demonstrate that PC-2 has a role in regulating the cell cycle through direct interaction with Id2, a member of the helix-loop-helix (HLH) protein family known to regulate cell proliferation and differentiation. This interaction requires PC-1-dependent phosphorylation of PC-2 (335). PC-2 also interacts with the protein, kidney injury molecule-1 (KIM1), a chemosensor present on the cilia (336, 337). Finally, Li et al. (338) reported that intracellular portions of PC-2 associate with alpha-actinins, which are actin-binding and actin-bundling proteins. They hypothesized that the aberrant interactions between PC-2 and alpha-actinins could play a role in the cell proliferation, adhesion and migration abnormalities seen in PKD epithelia.

Animal models with reduced or absent *PKD2* expression have also provided important insights into the function of PC-2. Similar to *PKD1*, PKD2 knockout mice die *in utero* or soon after birth and demonstrate cardiac defects in septum formation as well as kidney and pancreatic cysts (339). Studies of two *PKD2* mutant models expressing variable levels of PC-2 demonstrate that increased cell proliferation is an early event associated with the loss of *PKD2* expression and precedes cyst formation (340). This *in vivo* finding was supported by *in vitro* studies of cell lines lacking PC-2. In those cell lines, the loss of *PKD2* was associated with increased proliferation rates, suggesting that PC-2 is a negative regulator of cell growth (341). In addition, loss of *PKD2* has been reported to induce changes in the localization of *PKD1*, suggesting it has a role in mediating *PKD1* subcellular localization (342).

Pathology

In ADPKD (see *Fig. 36-4a*), kidney cysts form in glomeruli and all tubular segments. Glomerular cysts may be seen as a component of ADPKD or as a separate disease entity. Unlike ARPKD, in which the cystic dilatations are fusiform in nature and remain in connection with the tubular lumen, in ADPKD the enlarging cysts eventually "pinch off" and become disconnected from the tubular lumen and urinary space.

Clinical and Radiographic Features

Patients with ADPKD are usually diagnosed and become symptomatic in adulthood (173). However, children affected with ADPKD may also become symptomatic or be diagnosed as an incidental finding. The clinical spectrum of pediatric ADPKD ranges from severe neonatal manifestations indistinguishable from ARPKD to renal cysts noted on ultrasound in asymptomatic adolescents (2, 175, 229, 286, 343, 344).

As with ARPKD, hypertension can present during the newborn or infant periods and is common in pediatric and young adult ADPKD patients, despite the presence of normal renal function (229, 286, 345, 346). The acceptance of ambulatory blood pressure monitoring (ABPM) as an important tool for blood pressure assessment has allowed for more in depth studies of blood pressure abnormalities in patients with ADPKD. A significant proportion of normotensive young adults with ADPKD have "prehypertension" by ambulatory blood pressure monitoring (347). Blunted "nocturnal dipping" on ambulatory blood pressure monitoring has also been reported to be associated with endothelial dysfunction in this population (348). It is also notable that in a study of adults with ADPKD, a history of hypertension in affected parents was associated with an earlier onset of hypertension in their affected offspring (349).

Hypertension in ADPKD has been hypothesized to be due to reduced renal blood flow due to cyst compression with subsequent activation of the renin?angiotensin system, and increased sodium retention (350, 351). Recent case–controlled studies that have specifically controlled for sodium intake have shown no differences in the systemic RAS activation in hypertensive ADPKD patients compared to patients with essential hypertension (352). It is still possible, however, that local (intrarenal) RAS activation could still play a role in the pathogenesis of both hypertension as well as progressive kidney damage associated with ADPKD (353, 354). In addition, increased ACE independent generation of angiotensin II (via mast cell production of chymase) has been reported (355).

Increased left ventricular mass been reported to occur in normotensive children and young adults with ADPKD (356) and is associated with impaired relaxation time during exercise testing (357). Doppler abnormalities

consistent with early diastolic dysfunction has been reported in some patients, although the data are conflicting (356, 358). In addition, Oflaz et al. (359) reported an increased rate of biventricular dysfunction in both hypertensive and normotensive ADPKD patients, suggesting early cardiac involvement prior to the development of overt hypertension. An intrinsic cardiac abnormality is not unexpected given the diffuse vascular localization of *PKD1* and *PKD2* and the severe vascular phenotypes associated with null mutations in these genes. On the other hand, in light of the data about prehypertension diagnosed by ABPM in apparently normotensive ADPKD patients, it is possible that patients who were reported to be "normotensive" may, in fact, have had subtle blood pressure abnormalities not recognized by standard casual blood pressure measurements.

The increased incidence of cardiac valvular abnormalities such as mitral valve prolapse, commonly seen in the adult ADPKD population (360, 361), has also been reported in children with ADPKD (362). There have also been several reports of endocardial fibroelastosis in children with ADPKD (363, 364). An increased risk of coronary aneurysms has been reported in adults, but no pediatric cases have been reported, to date (365). An increased risk of pericardial effusion has also been reported in adults with ADPKD (366). Although rarely detected before the age of 20, there are reports of clinically significant cerebral vessel aneurysms in pediatric ADPKD patients as well (367).

In addition to hypertension, other presenting symptoms can include abdominal pain, palpable abdominal masses, gross or microscopic hematuria, UTIs, abdominal or inguinal hernias. The occurrence of gross hematuria after seemingly minor trauma to the flank region should raise the possibility of ADPKD. Renal insufficiency is rare, but can occur in childhood (286, 344). A renal concentrating defect, which may be associated with clinical evidence of polyuria and polydipsia, may be present in up to 58% of children with ADPKD (3, 368) and its presence correlates with the presence of hypertension by ABPM as well as the number of renal cysts (369). These findings suggest that impaired renal concentrating ability may be another clinical indicator of cystic kidney disease severity in children with ADPKD. Renal infections are common in adult patients with ADPKD and can be a presenting feature in the affected infant and child (229). Pain can also result from urolithiasis, a common finding in adults with ADPKD, as well as cyst rupture.

The typical appearance of ADPKD in children by ultrasonography is one or more renal cyst. ADPKD renal involvement in children is commonly asymmetric and may be unilateral in a small minority (370). The Consortium for Radiologic Imaging Studies in Polycystic Kidney Disease (CRISP) is a longitudinal prospective study of adult ADPKD patients, which uses high-resolution magnetic resonance (MR) imaging (371). This series of studies has demonstrated that individual cysts, as well as total cyst and kidney volumes, are well-delineated by magnetic resonance imaging (MRI), which can also be used to monitor cyst and kidney growth and blood flow over time (372, 373). These insights have allowed the development of clinical trials with novel therapeutic agents by providing a non-invasive means of monitoring response to therapy over a relatively short (one to three) year period.

The extrarenal cysts seen commonly in adults with ADPKD (360, 374) are uncommon in pediatric patients. Although hepatic, pancreatic, or testicular cysts are rarely detected before puberty, they have been reported in affected children, even in the first year of life (375, 376). Although liver cysts (detected by ultrasonography) were thought to be uncommon in children, a recent MRI study suggested that liver cysts may be present in up to 55% of adolescents and young adults (377). The prevalence in that study was reported to be directly related to the kidney volume. Liver cysts in children, when present, are not generally associated with pain, infection, and hepatomegaly as noted in adult patients. Congenital hepatic fibrosis with severe portal hypertension in children and adults with ADPKD has been reported rarely (224, 225). The presence of pancreatic cysts has been found exclusively in *PKD1* patients and do not appear to contribute to morbidity or mortality (378).

Diagnosis

There are no specific clinical diagnostic criteria for children with suspected ADPKD. As noted previously, ADPKD can present in any age group, including fetuses and neonates. The diagnosis of ADPKD has been made *in utero* by ultrasound, and affected newborns can present with Potter's phenotype and die from pulmonary hypoplasia. Affected infants can be born with large hyperechoic kidneys with or without macrocysts and variable degrees of renal insufficiency. Prenatal diagnosis is suggested by antenatal ultrasound findings of moderately enlarged hyperechogenic kidneys with or without cysts, with increased corticomedullary differentiation (379). However, these findings may not be evident until the third trimester (380, 381).

In families with known ADPKD, asymptomatic children may be identified by ultrasonographic examination

or as an incidental finding during evaluation for an unrelated problem (*Fig. 36-4b*). In pediatric patients with a 50% risk of ADPKD, the finding of one cyst or enlarged echogenic kidneys without cysts may be considered diagnostic (4, 286).

Even in families not known to have ADPKD, the finding of one or more kidney cysts in a fetus or child should alert the clinician to the possibility of ADPKD, since approximately 8–10% of patients with ADPKD will have *de novo* (new) mutations (4). Although radiographic studies may report the presence of a "simple cyst" and note it as a normal finding, in fact, such cysts are extremely rare in childhood (382). If ADPKD is clinically suspected in a child, the parents (and/or grandparents if the parents are younger than 30) should be considered for radiographic evaluation (383). It is not uncommon that the diagnosis of ADPKD in a child can lead to the diagnosis of ADPKD in asymptomatic adults following parental radiographic studies. Additional rare causes of solitary or even grouped unilateral cysts in a patient without a family history should also be considered. These include caliceal diverticula or isolated renal cystic disease (384, 385). In such instances, additional diagnostic studies, including contrast enhanced CT or IVP can help to exclude these diagnoses (385).

Screening evaluations of asymptomatic children at risk for ADPKD is currently not recommended. Because cysts may not be evident until adulthood, the finding of a negative ultrasound may be falsely reassuring (386). Conversely, there may be significant psychosocial and financial implications of the diagnosis of ADPKD in an asymptomatic patient who may not develop clinical signs of disease for several decades (387, 388). Some adults with ADPKD choose not to be tested, and testing of asymptomatic children eliminates their ability to make that decision as an adult. Thus, it is currently recommended that for "adult onset" genetic diseases such as ADPKD, screening should not be done unless a there is anticipated benefit to the child (389). With the emergence of novel, potentially disease-modifying therapies for ADPKD, and the passage of legislation in the United States preventing discrimination against individuals with genetic disorders (Genetic Information Nondiscrimination Act, or GINA) recommendations regarding screening are being re-evaluated as previously noted.

Genetic testing (including prenatal testing) is available for ADPKD. Previously, genetic testing was via the technique of linkage analysis (229). However, because of the need for a relatively large number of family members willing to be tested, this technique may be appropriate for fewer than 50% of families (173). With improvement in mutation detection techniques, direct sequence analysis is now the more commonly used methodology. Current mutation detection rates are approximately 85% using this methodology (331, 390).

While prenatal genetic testing, including preimplantation genetic diagnosis (PGD) (391) is available, it is not widely used. In the majority of kindreds, fetuses harboring an ADPKD mutation will not show any obvious renal or other abnormalities and patients may be asymptomatic for 2–3 decades. Surveys of ADPKD families indicate that only 4% would consider pregnancy termination if the fetus were affected (392). An up-to-date listing of laboratory currently performing genetic testing of ADPKD patients for clinical or research purposes is available at www.geneclinics.org.

Treatment and Complications

Treatment of ADPKD is primarily focused on detecting and managing renal and extra-renal complications. Asymptomatic children at risk for ADPKD should be followed annually for the development of hypertension, hematuria (gross or microscopic), polyuria, proteinuria or palpable abdominal masses. Any of these findings is an indication for ultrasound examination and close clinical follow-up.

As with other forms of chronic kidney disease, identification and treatment of hypertension is essential in slowing progression to ESRD in ADPKD. In adults with ADPKD, more intensive blood pressure control (<120/80) has been reported to have a greater impact on LVH reduction than standard control (<140/90)(393). It has been suggested that ACE inhibitors and AII receptor antagonists (ARB), alone or in combination, may offer benefits in addition to anti-hypertensive effects (394–396); however, the data are not entirely conclusive (397). A longitudinal study of children with ADPKD treated with ACE inhibitors is currently underway. A larger-scale multicenter NIH trial (HALT/PKD) is underway to address the question of whether ACE inhibitor plus ARB is more beneficial than ACE alone in modifying disease progression. In addition, a smaller study of 49 hypertensive ADPKD patient: found that treatment with an ARB appeared to be more favorable than that of a calcium channel blocker (CCB) in terms of rates of decline in renal function and proteinuria. As previously noted, reports in rodent PKD models suggested that CCBs exacerbate cystic kidney disease because of depletion of intracellular calcium (398). Although the results of these animal studies are intriguing, avoidance of CCBs is not

currently recommended for ADPKD, or as previously noted, ARPKD (399). It is also notable that reversible acute renal failure may be precipitated by ACE inhibitors in ADPKD patients with diminished kidney function and massive cystic involvement (270). Although acute renal failure would be extremely unlikely in children (given the absence of massive cystic involvement and intact renal function during childhood), it is prudent to obtain follow-up serum chemistries after initiation of ACE and/or ARB therapy.

Urinary tract infection, in particular, cyst infection may occur in children and adults with ADPKD. It has been reported that the risk of pyuria and bacteriuria in ADPKD increases progressively from 2% in the second decade to 32% in the seventh decade. Most adult ADPKD patients have some degree of renal insufficiency when UTIs develop (213). Although no data are available regarding specific features of UTIs or renal cyst infections in pediatric ADPKD patients, it is reasonable to presume that their clinical course is similar to that described for adult ADPKD patients (400). Sterile pyuria is common, and appropriate cultures are needed to determine whether an infection is present. Most renal infections are caused by Gram-negative enteric organisms and can be complicated by cyst infection. Eradication of cyst infections is often difficult, despite *in vitro* sensitivity of responsible organisms; thus, the use of antibiotics that penetrate cyst walls is mandated (401). Antibiotics that generally penetrate cyst walls include ciprofloxacin (402) and sulfonamides. Penicillins and aminoglycosides (standard treatments for urinary tract infection) are generally ineffective in treating cyst infection (401, 403). Aggressive antibiotic treatment is critical because recurrent or ineffectively treated UTIs appear to be a definite risk factor in progression of renal disease (404). Occasionally, cyst drainage may be required to control infection, and MRI or PET scan may be a useful in identifying which cyst is infected (405, 406). In extreme cases, nephrectomy may be indicated (401). Prophylactic antibiotics should be considered before the introduction of any urinary tract instrumentation in children with ADPKD.

Episodes of flank pain are unusual in pediatric patients with few cysts. However, with progressive disease, particularly in adolescents, flank pain may become a more prominent feature. In the majority of instances, the painful episodes will resolve within a few days. Pain relief is accomplished with acetaminophen or brief courses of oral narcotics. Non-steroidal anti-inflammatory agents should be avoided. Long term narcotic use is discouraged, due to abuse potential. Non-pharmacologic interventions and referral to a chronic pain management center should be considered (407). In cases of severe pain, laparoscopic denervation and nephropexy has been reported to significantly relieve pain in adolescents (408, 409). Laparascopic cyst decortication is an addition therapeutic option, particularly in instances of recurrent pain and infection (410). Renal calculi, a common finding in adult patients with ADPKD (411), and a frequent cause of flank pain, are rare in childhood.

Hepatic cysts are relatively uncommon in the pediatric population, but have been recognized more frequently with the increased resolution of imaging studies. Patients with hepatic cysts may develop cyst infections, which typically present as right upper quadrant pain, fever, leukocytosis, and a rise in liver enzymes (412). Antibiotics alone may be ineffective, and the addition of surgical drainage is generally recommended (413). Intestinal diverticular disease (360) has not been reported in pediatric ADPKD patients, to date.

Cerebral aneurysms occur in approximately 10% of ADPKD patients. The risk of rupture of asymptomatic aneurysms in adults is related to the size, with the risk ranging from 0.05% per year for those less than 10 mm to 6% within one year for those greater than 25 mm (414). The risk of rupture for symptomatic aneurysms is about 4% per year (414). Although aneurysms are found in patients with negative family histories, intrafamilial clustering of aneurysms and aneurismal bleeding has been reported in ADPKD populations (415–417). Use of magnetic resonance angiography (MRA) may permit effective, noninvasive detection of significant aneurysms (418). However, routine screening of all ADPKD patients is not recommended, since many patients are asymptomatic and the incidence of rupture is low (419). Screening MRA may be recommended for patients with symptoms or a positive family history (415).

The incidence of clinically significant aneurysms and/or aneurismal rupture in children with ADPKD is thought to be very low, although data are limited. Ruptured aneurysms have been reported in children as young as 4 years old (420). Given the intrafamilial clustering of aneurysms, it is important to obtain a detailed family history. If such a history is present, and/or the patient complains of headache, further evaluation by MRA should be considered.

There are currently no disease-specific treatments available for ADPKD. Newer therapies, however, are being investigated in preclinical studies, and Phase II and III trials of adults with ADPKD. As previously noted (see *Fig. 36-2*) these include trials of inhibitors of the EGFR-axis, the vasopressin receptor antagonist, Tolvaptan, the mTOR inhibitor, Rapamycin, and the somatostatin analogue, octreotide (120, 421–423). A regularly

updated list of clinical trials in the US and around the world is available at www.clinicaltrials.gov, and www.pkdcure.org. A number of dietary interventions have been shown to slow progression of disease in animal models. These include dietary flaxseed, soy protein or protein restriction, sodium citrate, or caffeine restriction. To date, none has proven to significantly alter the clinical course of disease in humans (424).

Prognosis

The prognosis of ADPKD presenting in the fetus or neonate was once thought to be very poor. However, a number of recent studies of "very early onset" ADPKD suggest that it may be compatible with favorable long-term patient and renal survival (425, 426). Prognosis in the older child is also very favorable and progression to ESRD in childhood is rare in ADPKD (427). However, disease progression does occur in childhood, particularly in children with evidence of severe renal enlargement at a young age (427). Proteinuria has been identified as a potential early marker of severe cystic disease in children (428). The CRISP studies confirm that significant cyst growth, parenchymal damage and volume progression occurs in ADPKD well before changes in measured GFR are seen. Thus assessments of renal function, such as serum creatinine measurements are poor indicators of overall disease severity. Recent data, including that of the CRISP investigators, has shown that kidney volume and its rate of change are the most predictive factors for subsequent decline in renal function and clinical outcomes (288, 429) (430, 431). The CRISP study established that mean renal volume increases 5.3% per year in patients with ADPKD, providing a valuable non-invasive, short term parameter to monitor effectiveness of new therapies.

Approximately 50% of adult patients with ADPKD will progress to ESRD. On average, patients with PKD1 typically progress at an earlier age, with a mean age at ESRD of 53.0 years, whereas those with PKD2 progress to ESRD at a mean age at ESRD of 69.1 years (293). In light of the significant inter- and intrafamilial phenotypic heterogeneity, it is difficult to predict at what age a given patient with ADPKD will develop renal failure.

Glomerulocystic Kidney Disease

The term *glomerulocystic disease* (GCKD), coined by Taxy and Filmer in 1976, is used to describe the morphologic appearance of glomerular cysts, which occur in a variety of conditions (432). GCKD was first described clinically by Ross in 1941(433). Glomerulocystic kidney disease can be categorized into three major groups: *(a)* nonsyndromal inherited and sporadic forms of GCKD; *(b)* GCKD as the major component of congenital malformation syndromes; and *(c)* glomerular cysts as a minor component of abnormal or dysplastic kidney disease, some of which are syndromic.

Epidemiology and Genetics

Primary GCKD with isolated renal involvement can be an autosomal dominant disease, a familial hypoplastic disease, as well as a sporadic occurrence. Reports exist of infants with GCKD who have family members affected with ADPKD, which raises the question of whether these two entities are different expressions of the same genetic defect. Sporadic GCKD and GCKD occurring in the context of familial ADPKD are clinically, sonographically, and histopathologically indistinguishable. However, several recent studies of kindreds with autosomal dominant inheritance of GCKD excluded mutations in one or more of the PKD genes, including *PKD1*, *PKD2* and *HNF-1beta* (434, 435). Thus, with emerging molecular diagnostic techniques, the genetic basis for this rare and heterogenous disease may be more fully defined.

An apparently distinct entity is hypoplastic glomerulocystic kidney disease, a dominantly inherited disease reported in only a few families (436, 437). These kidneys, apart from being glomerulocystic, are small, and imaging studies show abnormal pyelocaliceal anatomy. Mutations in the hepatocyte nuclear factor-1beta (HNF-1β) gene were been identified in 4 kindreds with this hypoplastic GCKD variant (438).

GCKD can be associated with congenital syndromes such as orofaciodigital syndrome, type I (439); brachymesomelia–renal syndrome (440); trisomy 13 (441); Majewski-type short rib–polydactyly syndrome (441); and Jeune syndrome (442) and can be seen as a component of the renal abnormalities in nephronophthisis (443). Although tubular sclerosis generally includes tubular cysts, glomerular cysts can be present (443). Glomerular cysts also occur as a minor component in several other syndromes including Zellweger cerebrohepatorenal syndrome (441, 442) in which the cysts are typically present but rarely serious enough to affect renal function.

Other syndromes that may be associated with glomerular cysts as a component of renal dysplasia include Meckel syndrome, glutaric aciduria type II and renal–hepatic–pancreatic dysplasia (443). The glomerular cysts

are minor in comparison with the dysplastic components of the renal disease, although they may be present in sufficient numbers to create confusion with other glomerulocystic conditions.

Pathogenesis and Pathology

The pathogenesis of GCKD remains unknown. Clinically, GCKD can be difficult to distinguish from other cystic kidney diseases. The diagnosis can only be established by histologic examination of renal tissue. Sporadic GCKD in young infants is histopathologically indistinguishable from ADPKD-related GCKD. The kidneys in both the familial and sporadic forms are variably enlarged, with the degree of renal enlargement related to the degree of cyst formation (443). The cysts in both groups may be diffuse but can also be clustered, which may be responsible for asymmetric and asynchronous clinical presentations. Diffuse involvement is associated with interstitial edema, whereas patchy involvement is associated with better preservation of overall renal structure and function.

Characteristically, the cysts are dilated Bowman's spaces, comprising a sphere lined with cuboidal or columnar cells and containing abortive or primitive-appearing glomeruli (432), which occur as small scattered cysts separated by normal parenchyma. The cysts are located in the cortex, with preservation of the medulla. This lack of tubular involvement differentiates GCKD from other cystic diseases in which cysts generally arise from tubular dilation. In rare cases, they are more diffuse, surrounded by atrophic and fibrotic parenchyma. They may be found in association with tubular cysts and dysplasia (443).

The kidneys in sporadic GCKD and the GCKD form of ADPKD often contain abnormally differentiated pyramids, a type of medullary dysplasia. Both forms of GCKD are associated with biliary dysgenesis in approximately 10% of cases (443).

Clinical and Radiographic Features

Most GCKD patients described in the literature have some degree of renal failure and many have hypertension at presentation. The typical presentation is that of an infant with abdominal masses, renal insufficiency, and enlarged cystic kidneys on sonography. GCKD may manifest in adulthood with hypertension, flank pain, and hematuria. Variable degrees of renal dysfunction are seen. Later detection may be consistent with a milder course (286, 444). Clinically, hepatic cysts have also been described (443).

Patients with the familial hypoplastic glomerulocystic kidney disease variant have small kidneys with abnormal collecting systems and abnormal or absent papillae (436, 437). Family studies show a pattern compatible with autosomal-dominant inheritance. Most patients appear to have chronic kidney disease with some degree of renal impairment early in life but subsequently have stable courses without progression to ESRD.

Several reports of GCKD describe patients with no clear familial or syndromic association (441, 445, 446). Histologically and clinically, these patients resemble familial cases with large, hyperechoic kidneys. It remains unclear whether these sporadic cases are a distinct entity or are associated with unrecognized syndromal or familial cases. Reports on an infant with GCKD and multiple cardiac rhabdomyomas and an infant with severe GCKD who later developed skin findings consistent with tuberous sclerosis strongly suggest an association of GCKD with tuberous sclerosis (447, 448). This, together with the new information regarding the molecular basis of ADPKD and tuberous sclerosis and the reported familial association of GCKD and ADPKD, raises the possibility that autosomal dominant GCKD, ADPKD, and tuberous sclerosis are genetically linked in some kindreds. Single case studies have also reported GCKD in association with Henoch-Schoenlein purpura (449), hepatoblastoma (450), and as a sequelae of hemolytic-uremic syndrome (451, 452).

Ultrasonography demonstrates bilateral renal enlargement without distortion of the renal contour, increased echogenicity of the cortex and medulla, loss of corticomedullary junction differentiation, and small cortical cysts (445, 453). Radiographically, a feature that can help distinguish GCKD from ARPKD is abnormal medullary pyramids in the latter. In the future, CT and nuclear MRI may be of some value in differentiating between these two diseases (454). Reduced intensity of cortex on T1-weighted images and abnormalities of corticomedullary differentiation may help confirm the diagnosis.

In summary, GCKD represents a heterogeneous collection of heritable and nonheritable clinical entities. This clinical course and prognosis is quite variable and often dependent on the presence of associated disorders.

Polycystic Kidney Disease Associated with Congenital Syndromes

Many diseases can present with enlarged kidneys or cysts in the infant and young child and can initially be confused

with PKD (*Table 36-1*). Most syndromic and other inherited disorders can usually be differentiated from ARPKD and ADPKD by associated clinical features, with the exception of GCKD, occasionally tuberous sclerosis and von Hippel-Lindau disease (1, 5, 455). GCKD can be a feature of several inherited, sporadic, or syndromic conditions as discussed above. In addition, GCKD may be an early histopathologic expression of the ADPKD gene in young patients. Tuberous sclerosis is an autosomal-dominant neurocutaneous disorder, in which hyperplastic cystic lesions may affect any portion of the nephron (447). Genetic linkage of the chromosome 16 loci for tuberous sclerosis and ADPKD1 has been demonstrated (456). The tuberous sclerosis 2 (TSC2) gene has been identified and encodes a novel protein, tuberin. Uncommonly, patients show polycystic renal involvement without clinical neurocutaneous involvement or positive family history. Several kindreds have been identified with tuberous sclerosis and severe childhood-onset ADPKD; they have large deletions in the area containing *PKD1* and adjacent tuberous sclerosis 2 (TSC2) gene (301). Analysis of the deletions indicates that they inactivate *PKD1*, in contrast to mutations reported in ADPKD patients in which abnormal transcripts have been detected. Von Hippel-Lindau disease is a dominantly inherited cancer syndrome characterized by renal cell carcinoma, pheochromocytoma and hemangioblastomas of the eye, spine and cerebellum. Cystic kidneys and pancreas may be seen and, rarely, patients may present with "typical" features of ADPKD (457). To differentiate GCKD, tuberous sclerosis and von Hippel-Lindau from ARPKD and ADPKD, detailed family history, physical examination and close clinical follow-up are necessary.

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37 Aminoaciduria and Glycosuria

Israel Zelikovic

Introduction

Only negligible amounts of amino acids and glucose are normally present in the final urine, reflecting very efficient reabsorption mechanisms for these organic solutes in the proximal tubule. Renal tubular transport defects or specific metabolic abnormalities result in excretion of significant quantities of amino acids or glucose in the urine. Although hereditary defects in renal tubular transport of most of these substances are uncommon, they are of major biologic importance. First, some of these membrane transport disorders (e.g., cystinuria, lysinuric protein intolerance, Hartnup disease) are associated with significant morbidity. Second, the study of these disorders has provided much insight into the physiology of renal tubular reclamation of amino acids and glucose and into the specific metabolic pathways that control their reabsorption and has been crucial in understanding the genetics of tubular transport systems.

This chapter summarizes the general characteristics of renal tubular transport of amino acids and glucose, outlines the three main classifications used for amino acid transport systems (based on chemical properties, substrate specificity/ion dependence and sequence homology), reviews recent studies on the molecular biology of the transporters, describes the ontogeny of these transport processes, and discusses the specific hereditary membrane transport disorders that result in abnormal aminoaciduria and glycosuria. Special emphasis is given to classic cystinuria, lysinuric protein intolerance and Hartnup disease, including molecular genetic aspects of these diseases. Not discussed in this chapter are overflow aminoaciduria and glycosuria, which occur when the filtered load of these solutes exceeds the transport capacity of the renal tubule. This tubular overload is characteristic of various inborn errors of amino acid metabolism and diabetes mellitus, which result in elevated plasma levels of amino acids and glucose, respectively. Fanconi syndrome, a proximal tubular disorder characterized by generalized aminoaciduria, and urinary hyperexcretion of glucose, bicarbonate, phosphate, and other solutes, is discussed in Chapter _____.

Aminoaciduria

General Characteristics of Tubular Amino Acid Transport

Circulating free amino acids are derived from dietary protein that is hydrolyzed and absorbed in the intestine, from intracellular catabolism of peptides, and from de novo synthesis within cells. More than 99% of the load of free amino acid filtered by the kidneys of humans and other mammals is reabsorbed in the renal tubule and returned to plasma (1–3). Amino acid reabsorption occurs predominantly in the pars convoluta of the proximal tubule and, to a small extent, in the pars recta (1, 2). Amino acids are reabsorbed primarily from tubular lumen by an active uphill transport across the luminal membrane (1). In studies using renal brush-border membrane vesicles (BBMV) from various animals to explore amino acid transport across this membrane, the rate of accumulation by vesicles and the magnitude of the overshoot, which indicates active concentrative transport, was greatly augmented by an external Na^+ gradient across the vesicle membrane (1). Hence, it is widely accepted that uptake of most amino acids at the brush-border surface occurs by Na^+-amino acid cotransport driven by the electrochemical Na^+ gradient from tubular lumen to cell (1, 2) (*Fig. 37-1*). The energy maintaining the Na^+ gradient is established by the Na^+-K^+-ATPase, which is located at the basolateral membrane and translocates Na^+ out and K^+ into the cell. An additional driving force for amino acid transport across the luminal membrane is the H^+ gradient (luminal > intracellular) which has been shown to drive uptake of proline (4) and glycine (5) by BBMV. Finally, cationic acids and cystine are reabsorbed across the luminal membrane in exchange for recycled neutral amino acids (3, 6) (see Molecular Structure of Amino Acid Transporters *Fig. 37-1*).

Active amino acid transport across the brush-border membrane is followed by efflux, mainly via carrier-mediated, Na^+-independent, facilitated diffusion or exchange from the cell into the peritubular space across the basolateral membrane (3, 7) (*Fig. 37-1*). Thus, under

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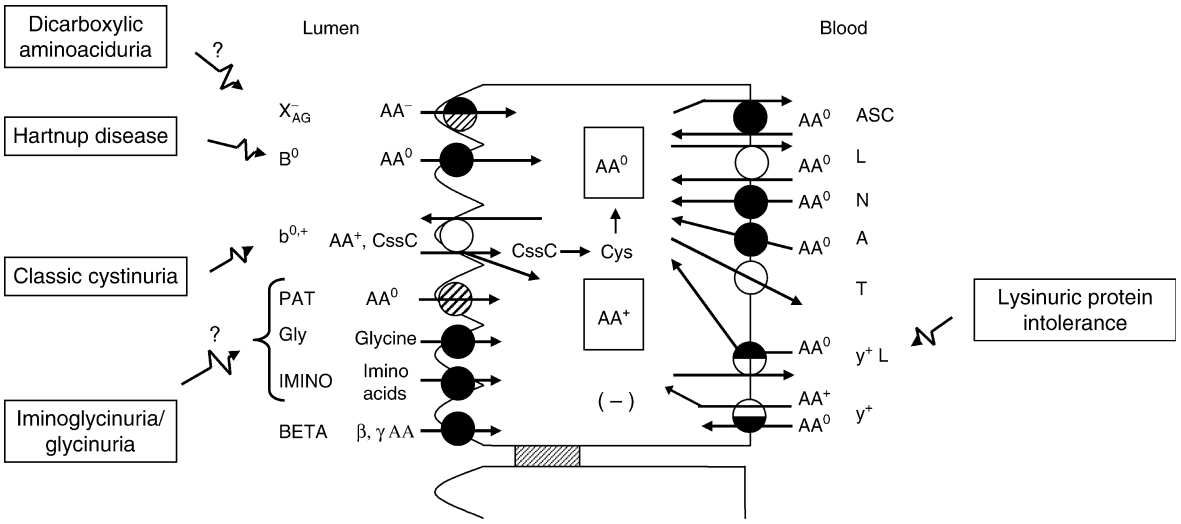
Figure 37-1

Summary of amino acid transport mechanisms in the proximal tubule. *Filled circles* indicate Na^+-dependent, active, carrier mediated cotransport or antiport. *Dashed circles* indicate H^+-dependent, active, carrier – mediated cotransport. *Empty circles* indicate Na^+-independent antiport or uniport (facilitated diffusion). *Half-filled*, *half dashed* or *half-empty circles* indicate various combinations of the above. Depicted are hereditary amonoacidurias known or postulated to be caused by defects in these transport mechanisms; AA^0 neutral amino acids, AA^+ basic amino acids, AA^- acidic amino acids, *CssC* cystine, *Cys* cysteine. See text for details.



normal conditions, net transepithelial movement of amino acids occurs from the tubular lumen to the peritubular space. However, net transepithelial flux of amino acids is composed of amino acid transport in both directions, namely lumen to interstitium and interstitium to lumen (3, 7, 8). Indeed, the basolateral membrane harbors active Na^+-dependent and Na^+-independent transport and exchange systems mediating amino acid uptake in the tubular cell (*Fig. 37-1*), and diffusional backflux of amino acids from cell into the tubular lumen is a well-documented phenomenon (3, 7). Interstitium to lumen oriented backflux through paracellular pathways also occurs. The sum of these vectorial fluxes determines the direction and the rate of transepithelial amino acid transport. This notion may be of major importance in understanding renal tubular amino acid transport, particularly in disease states and during maturation (8, 9).

Na^+-amino acid symport across the luminal membrane is a carrier-mediated saturable process obeying Michaelis-Menten kinetics (1, 2). The effectiveness of the active reabsorption process for a specific amino acid depends on the ratio V_{max}:K_m (1). A low V_{max} (decreased transport capacity) or a high K_m (diminished transporter-substrate affinity) for a given amino acid results in decreased reabsorption rate of this amino acid. Changes in efficiency or capacity of amino acid transport also play an important role in both neonatal aminoaciduria and hereditary aminoacidurias.

In analyzing the data obtained from microperfusion and micropuncture experiments and studies using BBMV for various amino acids (1), two or more Na^+-linked transport systems with different kinetic characteristics have been described. The demonstration of multiple transport systems for the same amino acid becomes meaningful if the reduced concentrations of filtered amino acid presented to the proximal straight tubule are considered. Thus, in the case of glycine, for example, two Na^+-dependent, active transport systems have been demonstrated along the luminal membrane of the isolated perfused proximal tubule (10): a low-affinity, high-capacity system in the convoluted segment and a high-affinity, low-capacity system in the straight segment. The latter system, which also operates in parallel to lower apical membrane backflux permeability in the proximal straight tubule (7), absorbs less glycine against a greater concentration gradient and probably permits the reduction of the luminal glycine concentration to lower levels than could be achieved in the proximal convoluted tubule (7, 10). This axial heterogeneity of Na^+-linked amino acid uptake systems with respect to kinetic characteristics has been demonstrated for several amino acids in BBMV derived from pars convoluta and pars recta of the

proximal tubule (1). The recognition of several transport systems for the same amino acid as well as their axial heterogeneity is of major importance in understanding the pathophysiology of hereditary aminoaciduria.

One or more Na^+ ions are transported for each amino acid molecule translocated, and with most amino acids this process is electrogenic-positive favored by a negative cell interior (1, 11). Na^+-amino acid stoichiometry determines the electrogenicity and efficiency of the transport system (7). Additional ions besides Na^+ are involved in the translocation of the amino acid carrier complex across the brush-border membrane (1). Taurine (12), glycine (13), and proline (14) transport, for example, operates by means of 2 or 3 Na^+:1 Cl^-:1 amino acid carrier complex.

Specificity of Transport

It has been well established that several distinct chemical group-specific Na^+-dependent transport systems for amino acids exist in the tubular luminal membrane (1–3). Evidence for these systems has been derived from a variety of microperfusion experiments and vesicle studies and, in humans, from the existence of inborn errors of renal tubular transport that can be explained only by defects in specific transport pathways (1, 3, 15). These include systems for dibasic (cationic) amino acids, cystine, acidic (anionic) amino acids, neutral α-amino acids, imino acids, glycine, as well as β- and γ-amino acids.

Ample evidence exists that in addition to separate systems for L-cystine and the dibasic amino acids (L-lysine, L-arginine, and L-ornithine), these amino acids share a common transport pathway (16–18), as also suggested by the urinary hyperexcretion of all four amino acids in classic cystinuria (see Classic Cystinuria). Transport of L-cystine but not L-cysteine can also proceed via Na^+-dependent transport pathways for neutral amino acids (1, 3). L-Cysteine is probably reabsorbed by an additional, separate, and specific transport system (2, 3).

The broad-specificity transport pathway for neutral α-amino acids, which is a low-affinity, high-capacity system, is located in the proximal convoluted tubule (15). However, there are several alternative specific renal transport systems for neutral amino acids, including high-affinity systems located in the proximal straight tubule. The presence of such pathways is also suggested by the finding of isolated transport defects for neutral amino acids (see Neutral Aminoaciduria). The imino acids proline and hydroxyproline are reabsorbed by at least three systems: a low-affinity/high-capacity system in the proximal convoluted tubule shared with glycine, an imino acid-specific, high-affinity/low-capacity system in the proximal straight tubule (10, 19) as well as a separate high-affinity/low-capacity system for glycine in the late proximal tubule (10).

The investigation of amino acid transport pathways in the plasma membrane of mammalian cells has delineated several transport systems which are classified according to their substrate specificity (3, 6, 20–24) (*Table 37-1*; *Fig. 37-1*). Most of these transport systems also have been identified in the kidney (3, 6, 20, 21, 23, 25). These systems include the Na^+-dependent, concentrative B^0 system (for most neutral amino acids), A and ASC systems (for small neutral amino acids), and N system (for glutamine, asparagine and histidine), the Na^+- and H^+-dependent X^-_{AG} system (for acidic amino acids), the Na^+- and Cl^--dependent IMINO system (for proline and hydroxyproline), Gly system (for glycine and sarcosine), and β system (for taurine, β-alanine and γ-amino butyric acid), the H^+ gradient-dependent PAT system (for imino acids), as well as the Na^+-independent, nonconcentrative L system (for bulky neural, branched chain amino acids and cysteine), T system (for aromatic amino acids), $b^{0,+}$ system (for dibasic and neutral amino acids including cystine), y^+ system (for the dibasic amino acids lysine, arginine, and ornithine), and y^+L system (for dibasic and neutral amino acids excluding cystine). Systems B^0, X^-_{AG}, IMINO, Gly, β, PAT, and $b^{0,+}$ operate in the luminal membrane, whereas systems A, ASC, N, L, T, y^+, and y^+L operate in the basolateral membrane (3, 22, 23, 25) (*Fig. 37-1*). The basolateral membrane-bound systems ASC, L, y^+ and y^{+L} as well as the luminal membrane-bound $b^{0,+}$ system function as antiport (exchange) systems (3, 23, 24) (*Fig. 37-1*). Recent progress in molecular cloning of amino acid transporters has helped to characterize, classify, and define the nature and the role of most of these tubular amino acid transport mechanisms at the cellular/molecular level (see Molecular Structure of Amino Acid Transporters).

Adaptation and Regulation of Amino Acid Transport

Although the exact mechanisms regulating renal tubular amino acid reclamation have not been established, several factors are known to modulate transmembrane amino acid transport (1, 26–28). These include ionic and voltage conditions (discussed earlier), as well as availability of amino acid substrate, osmotic changes, and protein phosphorylation.

Table 37-1

Amino acid transport systems operating in the renal tubule

Amino acid transport system	Cloned cDNA	Gene	Amino acids transported	Mechanism of action	Localization in proximal tubule	Cellular localization
I. Neutral						
B^0	B^0AT1, 2	SLC1A19, 15	Most neutral	Na^+-AA cotransport	EPT	BBM
ASC	ASCT 1, 2	SLC1A4, 5	Short chain neutral	Na^+-dependent AA antiport	EPT and LPT	BLM
L	4F2hc/LAT-1	SLC3A2/ SLC7A5	Large, branched chain neutral	AA antiport	EPT	BLM
	4F2hc/LAT-2	SLC3A2/ SLC7A8				
A	SNAT2, 4	SLC38A2, 4	Short chain neutral	Na^+-AA cotransport	EPT and LPT	BLM
N	SNAT 3, 5	SLC38A3, 5	Glutamine, asparagine, histidine	1 Na^+/AA cotransport 1 H^+ antiport	LPT	BLM
T	TAT1	SLC16A10	Aromatic	AA uniport	EPT	BLM
PAT	PAT1, 2	SLC36A1, 2	Proline, glycine, alanine	H^+- AA cotransport	EPT	BBM
Gly	XT2	SLC6A18	Glycine, sarcosine	2–3 Na^+/1Cl^-/1AA cotransport	LPT	BBM
Imino	IMINO	SLC6A20	Proline, hydroxyproline	2–3 Na^+/1Cl^-/1AA cotransport	EPT and LPT	BBM
Beta	TAUT	SLC6A6	Taurine, β-alanine	2–3 Na^+/1Cl^-/1AA cotransport	LPT	BBM
	BGT-1	SLC6A12	betaine, GABA,			
II. Cationic						
$b^{0,+}$	rBAT/$b^{0,+}$ AT	SLC3A1/ SLC7A9	Cationic and neutral (including cystine)	AA antiport	EPT and LPT	BBM
y^+L	4F2hc/y^+LAT-1	SLC3A2/ SLC7A7	Cationic and neutral (excluding cystine)	Na^+-dependent AA antiport	EPT	BLM
	4F2hc/y^+LAT-2	SLC3A2/ SLC7A6				
y^+	CAT 1	SLC7A1	Cationic and neutral (excluding cystine)	AA uniport (facilitative transport)	EPT and LPT	BLM
III. Anionic						
X^-_{AG}	EAAT 3, 2	SLC1A1, 2	Anionic	3 Na^+/1 H^+/AA cotransport 1 K^+-antiport	LPT	BBM

EPT – Early proximal tubule (S1, S2 segments); LPT – Late proximal tubule (S3 segment); BBM – Brush border membrane; BLM – Basolateral membrane

Reabsorption of amino acids in the proximal tubule increases during periods of reduced amino acid intake and decreases with dietary excess (1). This renal adaptive response to diet is expressed at the tubular luminal membrane surface. It has been suggested (26, 29) that both new synthesis of transporter protein and shuttling of preformed transporters are required for expression of the adaptive response. An expression study in *Xenopus*

oocytes (30) has demonstrated that the rat renal taurine transporter is regulated by dietary taurine at the level of both mRNA accumulation and protein synthesis.

Amino acids are known to serve as regulatory osmolytes in mammalian cells, including kidney cells (31–33). The main amino acids involved in this function are taurine, proline, and glutamic acid (31, 32, 34). Studies using MDCK cells, a cell line of distal tubular origin, demonstrate changes in taurine transport in response to changes in osmolarity of the medium (35). It has been shown that osmotic regulation of taurine transport depends on changes in taurine transporter gene expression (36).

Serine/threonine protein kinases play a central role in signal transduction by phosphorylating and thereby activating effector proteins (37, 38). It has been shown that the three main groups of serine/threonine protein kinases, namely cyclic adenosine monophosphate (cAMP)-dependent protein kinase (protein kinase A: PKA), Ca^{2+}- and phospholipid-dependent protein kinase (protein kinase C, or PKC), and multifunctional Ca^{2+}/calmodulin-dependent protein kinase II (CaMK II) alter amino acid transport across the tubular brush-border membrane (39–41). However, the exact role of protein kinase-induced phosphorylation in renal tubular amino acid transport remains to be established.

Molecular Structure of Amino Acid Transporters

Extensive efforts to isolate and identify the amino acid transport proteins in various tissues, including the kidney, have been largely unsuccessful. The isolation and molecular characterization of amino acid carrier proteins has been hindered by their very low abundance in the membrane, their poor stability in vitro, and the lack of specific tight-binding labels or inhibitors. Indirect approaches such as solubilization of proteins by organic solvents and incorporation into proteoliposomes, lectin-affinity chromatography, immunoprecipitation using monoclonal antibodies, and radiation inactivation analysis (42, 43) have yielded little structural data. Over the past two decades, however, by using molecular biology techniques, much progress has been made in elucidating the molecular structure of various membrane-bound transport proteins, including amino acid transporters. This new area in the study of brush-border membrane transporters was pioneered by Hediger et al. (44), who cloned the small intestinal Na^+-glucose cotransporter using the powerful method of expression cloning in *Xenopus* oocytes. This strategy involves isolation of mRNA from the tissue of interest, microinjection into *Xenopus* oocytes, and analysis of expressed transport activity by measuring uptake of radiolabeled substrate. After size fractionation of mRNA, cDNA synthesis, and cDNA library screening by functional expression in oocytes, a single clone encoding the transporter activity is isolated. A similar approach has been used to clone, functionally express, and sequence various amino acid transporters. This has led to the classification of amino acid transporters based on gene homology using the solute carrier family (SLC) nomenclature introduced be the Human Genome Organization (HUGO) (45).

The scquence homology and structural features of amino acid transporters have led to their categorization into seven different gene families (3, 6, 23, 24, 46–48): The SLC6 family (Na^+- and $C1^-$-dependent and "orphan" transporters), the SLC1 or EAAT/ASCT family (Na^+-dependent anionic/neutral amino acid transporters), the SLC36 or PAT family (proton-coupled imino acid transporters), the SLC7 or CAT family (cationic amino acid transporters), the SLC3/SLC7 or HAT family (cationic/neutral amino acid transporters), the SLC38 or SNAT family (Na^+-coupled neutral amino acid transporters), and the SLC16 or TAT family (aromatic amino acid transporters). Investigation of the primary structure of amino acid transporters has elucidated that as opposed to all other SLC families outlined here, which present multiple transmembrane domains (*Fig. 37-2*) and are therefore considered to function as monomeric carriers, members of the SLC3 family do not fit this model (*Figs. 37-3* and *37-4*) and are known to function as components or subunits of heteromeric carriers (see later).

The SLC6 Family (the Na^+- and CI^--Dependent and "Orphan" Transporters)

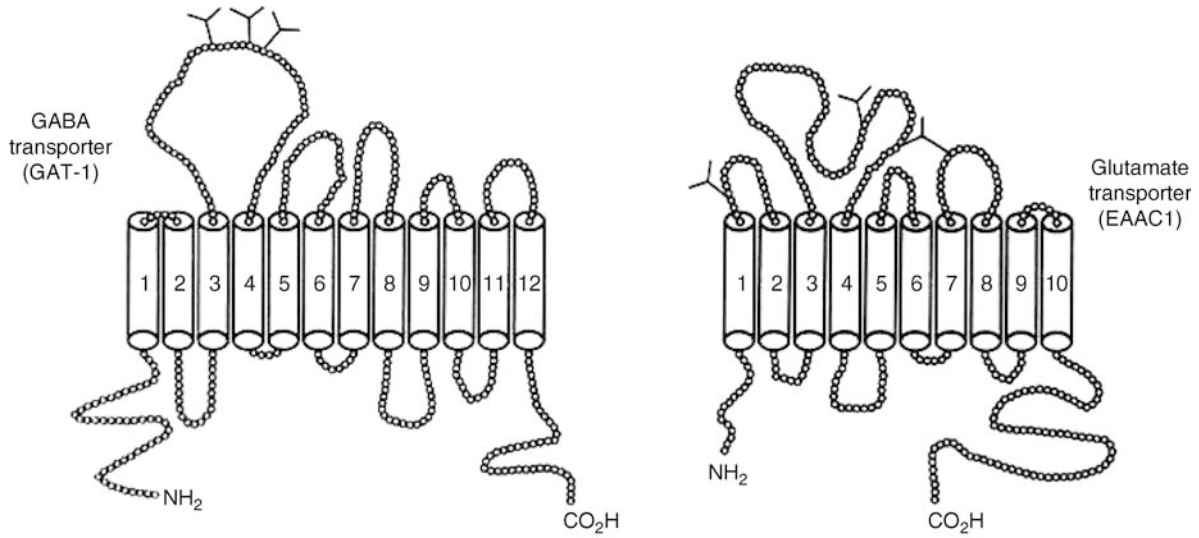
The SLC6 family is a diverse set of amino acid transporters comprising 20 members (51, 52). The family includes, among others, the Na^+- and CI^--dependent transporters for the neurotransmitters GABA (GAT; 53) glycine (GLYT; 54) and proline (PROT; 55), the Na^+- and CI^--dependent transporters for the osmolytes taurine (TAUT; 56, 57) and betaine (BGT; 58) operating in the brain and kidney, as well as a group of "orphan transporters," some of which play an important role in amino acid transport in the kidney (see later).

All these transporters show high homology in sequence and structure, and most of them have an absolute requirement for Na^+ and Cl^-.

The proposed model of the GABA transporter (GAT1; SLC6A1) is shown in *Fig. 37-2*. It is a 655-residue

Figure 37-2

The putative transmembrane orientations of the GABA transporter (GAT-1; SLC6A1), and the glutamate transporter (EAAC1; SLC1A1) are shown as representative of the SLC6 and SLC1 families, respectively, of amino acid transporters (modified with permission from (46)).



protein with a relative molecular mass of 73,925 Da. The predicted secondary structure shows 12 hydrophobic, membrane-spanning domains with a large extracellular hydrophilic loop between spans 3 and 4. The molecular structure contains putative *N*-glycosylation and phosphorylation sites. Recent studies investigating the crystal structure of the Na^+- and Cl^--dependent neurotransmitter transporters (59) and site-directed mutation analysis (60) have identified distinct domains which are similar between the family members and are important in Na^+, Cl^-, and substrate coupling.

The expression and function of various SLC6 family members in the kidney have been extensively investigated. Northern hybridization and polymerase chain reaction analysis have identified two GABA transporter isoforms, called GAT 2 (SLC6A13) and GAT 3 (SLC6A11) (61, 62), as well as glycine transporter isoforms called GLYT 1 (SLC6A9) and GLYT 2 (SLC6A6) (63–65) in the kidney. However, the physiological role of the GAT and GLYT transporters in renal amino acid handling has not been established.

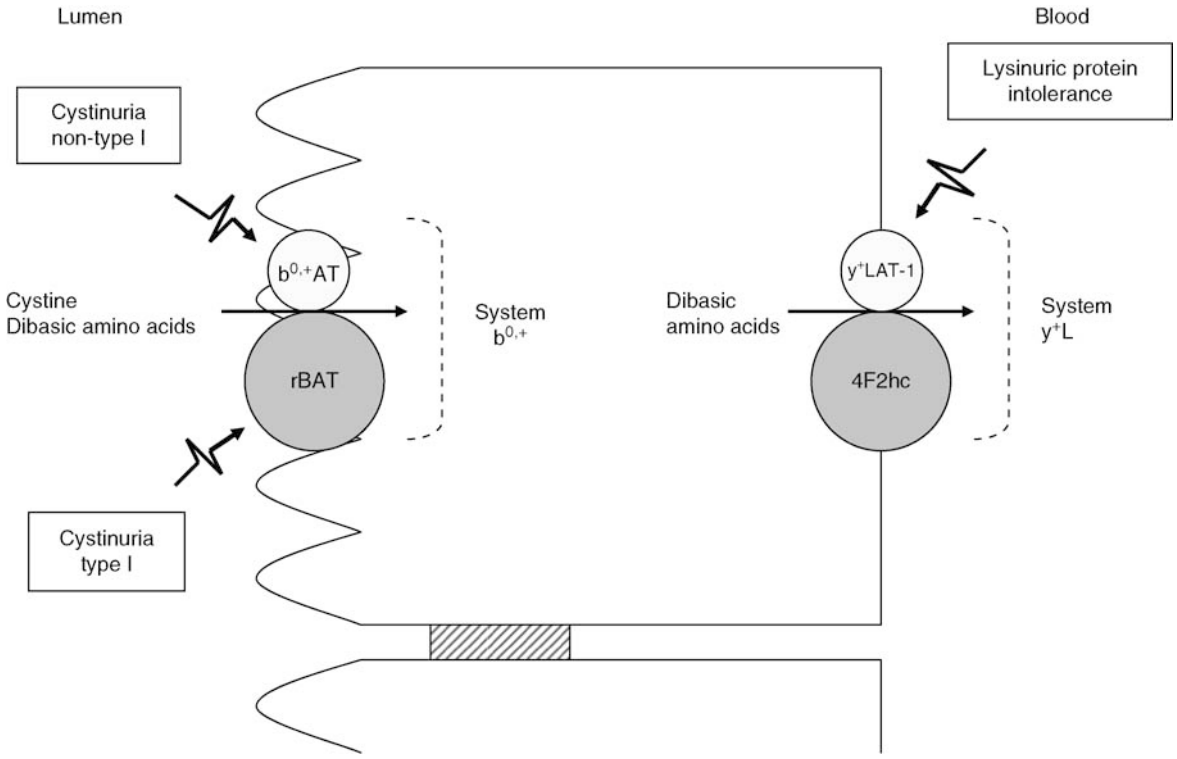
The mRNAs for the Na^+–Cl^- – taurine (TAUT; SLC6A6) and Na^+–Cl^- – betaine (BGT; SLC6A12) transporters are expressed in the kidney medulla (2) and TAUT mRNA is expressed also in the S3 segment of the proximal tubule (57). Both transporters are known to play a major role in cell volume regulation in the renal medulla (32, 35, 57). Upregulation of the activity of these transport systems by tonicity is mediated by a tonicity – responsive enhancer element (TonE) on the promoters of BGT1 (66) and TAUT (67) genes. TAUT and BGT1 (the latter transports also GABA) appear to be the molecular correlates of the amino acid transport system β (see Specificity of Transport).

The "orphan transporters" branch of the SLC6 family has been recently the subject of thorough investigation and several of its members have been cloned and shown to play an important role in amino acid transport in the kidney (3, 6, 48).

Following the mapping of Hartnup disorder to the tip of chromosome 5 (5p 15) (68), Bröer's group focused on a region in the mouse genome (chromosome 13), syntenic with this 5p15 locus in humans, and cloned a new member of the SLC6 "orphan transporters" group termed B^0AT1/SLC6A19 (69). The transporter has been further characterized by flux studies and electrophysiological techniques (70, 71). B^0AT1 has been shown to be the molecular correlate of the major apical neutral amino acid transport system B^0 (see Specificity of Transport). It is a Na^+-dependent but, unlike other SLC6 family members, Cl^--independent transporter (70). Immunohistochemical analysis demonstrated strong expression of B^0AT1 in the

Figure 37-3

Transport pathways for cystine and dibasic amino acids at the luminal and basolateral membranes of a proximal tubular cell. *Large circles* represent the heavy subunits and *small circles* the light subunits of the heteromeric amino acid transporters $b^{0,+}$ and y^+L. Depicted are hereditary aminoacidurias caused by defects in these transporters (modified with permission from (49)).



apical membrane of early segments (S1, S2) of the proximal (convoluted) tubule and intestinal microvilli (69, 72). SLC6A19 has been indeed identified as the mutated gene in Hartnup disorder (see Neutral Aminoaciduria).

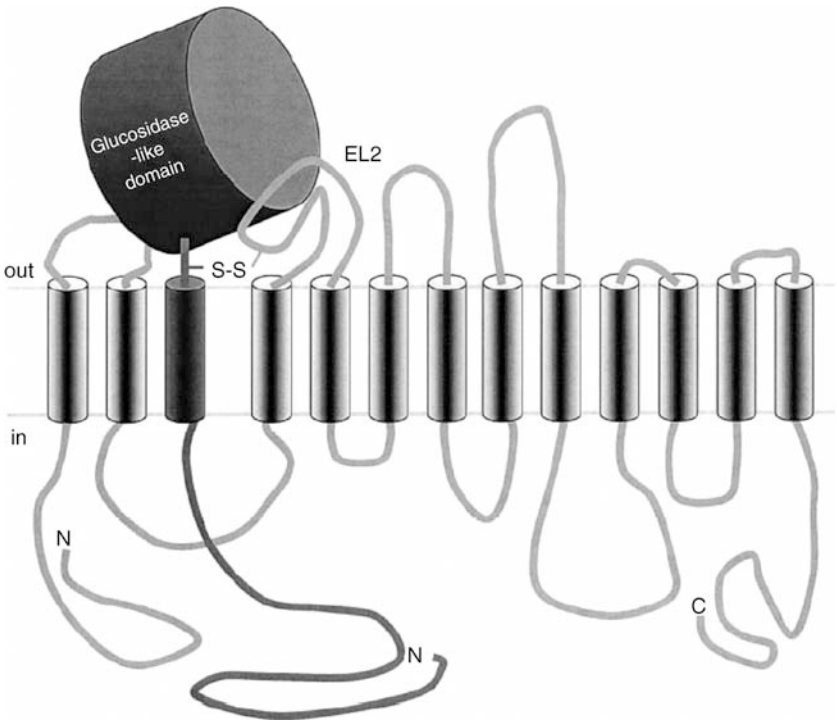
An additional orphan transporters, B^0AT2 (SLC6A15), which is functionally similar and sequence-related to B^0AT1, has been shown to transport branched – chain amino acids and proline and to be expressed in the brain and the kidney (73). Its exact function and localization, however, remain to be established.

Two additional members of the SLC6 "orphan transporter" branch have been recently cloned and characterized:

1. IMINO (SLC6A20, SIT1, XT3), the molecular correlate of system IMINO (see Specificity of Transport) is a high affinity (low Km), Na^+- and Cl^--dependent transporter for proline, hydroxyproline and other *N*-methylated amino acids (but not glycine) (74, 75), and is expressed in the brush border membrane of the early and late proximal tubule and the intestine (72, 74). SLC6A20 has been proposed as a candidate gene for iminoglycinuria (see Iminoaciduria and Glycinuria) (3, 48).
2. XT2 (SLC6A18, ROSIT) is a Na^+- and Cl^--coupled organic solute transporter (ROSIT) in the rat kidney (76) which is expressed in the luminal membrane of the late proximal tubule (S3 segment) (76, 77). XT2 knockout mice show hyperglycinuria as well as decreased glycine transport in BBMV derived from the kidney cortex of these mice (78), suggesting a role for this transporter in renal glycine transport. The potential involvement of SLC6A18 in hereditary iminoglycinuria or isolated glycinuria remains a subject for investigation (see Iminoaciduria and Glycinuria) (3, 48).

Figure 37-4

Schematic representation of the heteromeric amino acid transporters (HAT). The heavy subunit of HAT (HSHAT; *dark gray*) is linked by a disulfide bridge to the corresponding light subunit of HAT (LSHAT; *light gray*). The cysteine residues involved in this bond (S-S) are located extracellularly, just after the transmembrane (TM) domain of HSHAT and in the proposed extracellular loop 2 (EL2) of LSHAT. Loops and TM domains are not drawn to scale. See text for details (adapted with permission from (50)).



The SLC1 Family [Na^+-Coupled Anionic (EAAT)/Neutral (ASCT) Amino Acid Transporters]

This family of transporters is made up of five Na^+-, H^+-, and K^+-dependent, Cl^--independent anionic amino acid transporters and two Na^+-dependent neutral amino acid transporters (3, 23, 24, 79). The anionic amino acid transporters include the GLAST (or EAAT1; SLC1A3) (80), GLT-1 (or EAAT2; SLC1A2) (81), EAAC1 (or EAAT3; SLC1A1) (82), as well as EAAT4 (SLC1A6) (83), and EAAT5 (SLC1A7) (84), which serve as neurotransmitters and show marked similarity in sequence and structure. EAAC1, a neuronal and epithelial high-affinity glutamate transporter, first cloned from rabbit small intestine (82), was also identified in the kidney by Northern hybridization analysis (82). In situ hybridization and immunofluorescence studies revealed that EAAC1 is expressed predominantly in the apical membrane in the S2 and S3 segments of the proximal tubule (85). It mediates transport of glutamate and aspartate and is coupled to $3Na^+$ and $1H^+$ in exchange for $1K^+$ (79).

The proposed model of EAAC1 (*Fig. 37-2*), the prototype of the SLC1 family, shows a protein of 524 amino acids with a predicted molecular mass of 57,000 Da and 10 hydrophobic, membrane-spanning domains. The kinetics and specificity of this protein when expressed in *Xenopus* oocytes were similar to those of the X^-_{AG} transport system (see Specificity of Transport; *Fig. 37-1*). The gene for human EAAC1 (SLC1A1) has been localized to chromosome 9p24 (86). A defect in the EAAC1 gene is a likely, albeit unproven, cause of dicarboxylic aminoaciduria (see Dicarboxylic Aminoaciduria).

The neutral amino acid transporters include the ASCT1 (SLC1A4 or SATT) (87), and ASCT2 (SLC1A5 or AAAT) (88). ASCT1 and ASCT2, which were cloned from human brain (87) and mouse testis (88), respectively, have structural similarity to the anionic amino acid transporter gene group and appear to encode Na^+-dependent neutral amino acid transporters with specificity characteristics

of system ASC (see Specificity of Transport; *Fig. 37-1*). Northern blot analysis (87, 88) revealed ubiquitous expression of these genes in several tissues, including expression in the kidney, consistent with the general metabolic role ascribed to system ASC. While ASCT1 accepts only small neutral amino acids, ASCT2 also transports L-glutamine and L- asparagine at high affinity. In one study (89), ASCT2 has been reported to be expressed in the brush border membrane of the proximal tubule. However, it has been hypothesized that, in line with ASC system properties, ASCT2 is likely involved in transepithelial amino acid transport at the basolateral membrane level (6).

It is noteworthy that the transport systems EAAT1-5 (90), and ASCT1 (91) have a Cl^- channel mode of action in addition to their amino acid transport mode of activity. The Cl^- transport is not thermodynamically coupled to and is not necessary for amino acid translocation (79).

The SLC36 Family (Proton-Coupled Imino Acid Transporters; PAT)

The SLC36 family comprises several, recently identified proton-coupled amino acid transporters operating in the lysosomal and plasma membrane of cells (3, 92). The first member of the SLC36 family, SLC36A1, was identified independently as a lysosomal amino acid transporter (LYAAT1) in rat brain responsible for the export of lysosomal proteolysis products into the cytosol (93) and as a proton –amino acid transporter (PAT1) responsible for amino acid absorption in the gut (94, 95).

The proton–amino acid transport activity of PAT1 is electrogenic and operates with a stoichiometry of 1:1 (94). PAT1 transports proline, glycine, alanine as well as GABA at low affinity (high Km) and is expressed in several organs including brain (mainly lysosomes), liver, small intestine and kidney. The PAT1 protein was found apically in small intestinal Caco-2 cells (96). PAT1 appears to be the major shared imino acid/glycine carrier expressed in the brush border membrane of the small intestine and the kidney proximal tubule (6, 48) and corresponds to the H^+ gradient-driven proline (4) and glycine (5) transport demonstrated in renal BBMV. It is noteworthy that the H^+ gradient (luminal > intracellular) necessary for PAT1-mediated amino acid transport into the epithelial cell is likely generated by the activity of the Na^+/H^+ exchanger operating in the luminal membrane (6, 92).

Based on its substrate specificity, PAT1 has been proposed, like SLC6A18 and SLC6A20 (see earlier), as a candidate for the transporter mutated in iminoglycinuria (see Iminoaciduria and Glycinuria) (3, 48).

An additional member of the PAT family, PAT2 (SLC36A2), has significant sequence and functional similarity to PAT1 (94, 97). PAT2 is, however, expressed in the kidney, but not the intestine (92), and its physiological role remains to be determined.

The SLC7 (CAT) Family

The SLC7 family is divided into two subgroups: (1). Cationic amino acid transporters (CAT; SLC7A1–4) (98, 99) (discussed in this section), and (2). The glycoprotein-associated amino acid transporters (SLC7A5–11) also called the light subunits of the heteromeric amino acid transporters (LSHAT) (99, 100) are discussed in the next section. The associated glycoproteins (heavy chains) form the SLC3 family (101). The heavy (SLC3) and light (SLC7A5–11) subunits combined to the heteromeric amino acid transporters (HAT) constitute the distinct SLC3/SLC7 family (see later).

The CAT genes were the first mammalian amino acid transporters cloned (47, 98). Expression studies in *Xenopus* oocytes identified the ecotropic murine leukemia virus as the ubiquitous y^+ system (now called CAT1; SLC7A1), a Na^+-independent transport system that accepts dibasic amino acids and excludes cystine (102, 103). In addition, it catalyzes transport of neutral amino acids only in the presence of Na^+ (23) (*Fig. 37-1*). Northern hybridization analysis revealed the CAT1 gene in various mouse tissues including the kidney (102). Since then, four other homologous murine cDNAs have been found to express a similar y^+ system amino acid transport activity, namely CAT2A, CAT2B, CAT3 and CAT4 (SLC7A2–4), which are not expressed in the kidney (23, 47, 98, 104). The CAT transporters allow accumulation of cationic amino acids within the cell for general metabolic purposes. CAT-mediated arginine flux into cells plays a role in modulating nitric oxide synthesis in various cell types including kidney cells (98, 104, 105). The CAT1 cDNA predicts a 629 amino acid protein with 14 membrane-spanning domains and a molecular mass of 68,000 Da.

The SLC3/SLC7 Family (HAT)

As indicated earlier, the heteromeric amino acid transporters (HAT) are composed of a heavy subunit (SLC3; HSHAT) and a light subunit (SLC7; LSHAT) (98, 99).

1. *The SLC3 (rBAT/4F2hc; HSHAT) Family*: To gain insight into the transport defect in cystinuria, research has focused on the molecular structure of the family of carrier proteins responsible for transport of cystine and other dibasic amino acids. Several groups have demonstrated the expression of cystine (106) and dibasic and neutral amino acids (107–109) in *Xenopus* oocytes injected with mRNA of small intestine and kidney. In 1992 (110, 111), kidney cortex cDNAs from rabbit and rat (named rBAT (110) and D2 (111), respectively) have been cloned. Upon in vitro transcription to cRNA and injection into oocytes, they induce system $b^{0,+}$, the Na^+-independent transporter for neutral amino acids, dibasic amino acids, and cystine (see Specificity of Transport; *Figs. 37-1* and *37-3*). The predicted proteins for rBAT and D2 demonstrate significant homology with a family of the carbohydrate-metabolizing enzymes α-glucosidases. Similarly, cRNA from the human 4F2 heavy chain (4F2hc) surface antigen, a glycoprotein highly regulated at the onset of cell proliferation, stimulated system y^+L amino acid transport (see Specificity of Transport; *Figs. 37-1* and *37-3*) in *Xenopus* oocytes (112).

The rBAT cDNA contains an open reading frame of 2,049 nucleotides coding for a protein of 685 amino acids with a molecular mass of about 90,000 Da (47). The predicted proteins for rBAT and D2, as well as the 4F2 heavy chain antigen (all of which show high structural similarity), were found to contain only one putative membrane-spanning domain (23, 101). This structure, which is atypical of the known membrane transport proteins, raised the possibility that these proteins function as activators of transport systems y^+L and $b^{0,+}$ or as regulatory subunits of these transporters. The functional promoter of the rat rBAT gene has been identified (113), and the gene for the human rBAT, named D2H, was localized to chromosome 2p21 (113, 114). The human rBAT (D2H) gene has been termed SLC3A1 (see later).

In situ hybridization and immunolocalization studies have localized rBAT mRNA expression in the brush border membrane of the proximal straight tubule (S3 segment) and the small intestinal mucosa (115, 116). In contrast to rBAT, 4F2hc mRNA is almost ubiquitous with marked expression in kidney, where it localizes to the basolateral membrane of proximal tubular cells (23). The human 4F2hc gene has been termed SLC3A2. Studies from several laboratories have demonstrated that both rBAT and 4F2hc are type II membrane glycoproteins, which constitute heavy subunits of heteromeric amino acid transporters (HSHAT) (50, 117–119). These studies showed that rBAT (90 kDa) and 4F2hc (85 kDa) associated by disulfide bridges with a light subunit (40 kDa) forming a heterodimeric complex of 125 kDa (50, 101, 119) (*Fig. 37-4*).

2. *The SLC7 family (LSHAT)*:The light subunit of rBAT, termed $b^{0,+}$ AT, (SLC7A9), and the light subunit of 4F2hc, termed y^+LAT-1 (SLC7A7), which were identified in 1998–1999 (120–122), are members of the family of light subunits of the heteromeric amino acid transporters (LSHAT) (50, 99, 100, 119). To date, nine members of the LSHAT family have been identified (99, 100). Recent research on the characteristics of LSHATs (50, 99) has revealed that they are unglycosylated proteins, which contain 12 putative transmembrane domains (*Fig. 37-4*), they need coexpression with the corresponding heavy subunit to reach the plasma membrane, they confer the specific amino acid transport activity to the heteromeric complex and, finally, all the amino acid transport activities associated with the LSHATs behave as amino acid exchangers (50, 99, 119, 123–125). In the kidney, the apical membrane-bound system $b^{0,+}$ (induced by rBAT and $b^{0,+}$ AT; *Figs. 37-1* and *37-3*) acts as tertiary active exchange mechanism of tubular reabsorption of dibasic amino acids and cystine. This tertiary transport mechanism is linked to a high intracellular concentration of neutral amino acids (*Fig. 37-1*). System $b^{0,+}$-mediated efflux of neutral amino acids from renal epithelial cells is the driving force for cystine and cationic amino acid reabsorption from lumen to cell (118, 126, 127) (*Fig. 37-1*). Reabsorption of cystine and cationic amino acids is also favored by the intracellular negative membrane potential and by the reduction of cystine to cysteine (*Fig. 37-1*) (118, 127). The basolateral membrane-bound system y^+L (induced by 4F2hc and y^+LAT-1; *Figs. 37-1* and *37-3*) mediates an electroneutral exchange mechanism in which efflux of cationic amino acids (against the intracellular negative voltage) is enhanced by the influx of neutral amino acids in the presence of Na^+ (*Fig. 37-1*) (118, 127). The tissue distribution of rBAT/$b^{0,+}$ AT and 4F2hc/y^+LAT-1 and their role in renal uptake of cystine and dibasic amino acids have made them candidates for the defective genes in cystinuria and lysinuric protein intolerance, respectively (see Cationic Aminoaciduria).

The family of LSHATs includes, among others, the light subunits LAT-1(SLC7A5) and LAT-2 (SLC7A8) that combine with 4F2hc (SLC3A2) to form the L system (see Specificity of Transport; *Table 37-1*; *Fig. 37-1*). The 4F2hc/LAT2 transporter is an obligatory exchanger found in the basolateral membrane of the intestine and the kidney proximal tubule (128). The transporter has a broad substrate specificity including all neutral amino acids except proline (129). A study using the proximal

tubular cell line, OK, has demonstrated that 4F2hc/LAT2 mediates basolateral efflux of L-cysteine (130). This finding suggests that this transporter may play an important role in exchanging intracellular cysteine for extracellular neutral amino acids thereby participating in the transepithelial flux of cysteine in the proximal tubule (3, 6).

Two additional members of the LSHATs include asc1 (SLC7A10) (131) and xCT (SLC7A11) (132) both of which combine with the heavy subunit 4F2hc (SLC3A2) to form the basolateral membrane-bound amino acid transport system asc and x_c^-, respectively. 4F2hc/asc1 is expressed in the loop of Henle, distal tubule and collecting duct, but not in the proximal tubule (133), where it functions as a Na^+-independent exchanger of small neutral amino acids, thereby contributing to the nutritional supply and/or osmotic adaptation of kidney cells (3, 133). 4F2hc/xCT is a Na^+- independent exchanger for anionic amino acids that is expressed in plasma membrane of various cells including kidney cells (132, 134). Cystine/glutamate exchange mediated by this transporters plays an important role in controlling the intracellular level of glutathione which protects the cell against oxidative stress (134). These two biologically important transporters do not appear to be involved in reabsorbing the bulk of amino acids in the proximal tubule.

The SLC 38 (SNAT) Family

The recently cloned Na^+-coupled neutral amino acid transporters (SNAT; SLC38) are the molecular correlates of system A and system N activities (135) (see Specificity of Transport), as follows:

1. The system A subfamily includes SNAT1(SLC38A1), SNAT2 (SLC38A2) and SNAT4 (SLC38A4) subtypes of which only the latter two are expressed in the kidney (3, 135). Hydropathy analysis of SNAT1 sequence predicts a transporter with 11 transmembrane domains (135). System A transports small neutral amino acids (in particular alanine, serine and glutamine) in an electrogenic and pH-sensitive mode with a Na^+: amino acid stoichiometry of 1:1 (135). SNAT3 is expressed in the medulla and, to a lesser extent, in the cortex of the kidney (136) and is upregulated by amino acid deprivation (137). SNAT2 and SNAT4 most likely localize to the basolateral membrane to provide amino acids to renal cells (2) (*Fig. 37-1*).
2. The system N subfamily includes, among others, SNAT3 (SLC38A3) and SNAT5 (SLC38A5) both of which are expressed in the kidney (3, 135). Substrates for system N include glutamine, asparagine, and histidine (3). SNAT3 operates with coupling stoichiometry of 1 Na^+:1 glutamine in exchange for 1 H^+ (138). It is expressed in the basolateral membrane of the late (S3 segment) proximal tubule (139) (*Fig. 37-1*). Recent studies have demonstrated a marked induction in renal SNAT3 mRNA level (140, 141) and increased uptake of glutamine (NH_4 precursor) by renal cortical membrane vesicles (140) of acidotic rodents, providing evidence that this transporter plays an important role in the renal adaptive response to metabolic acidosis.

The SLC 16 (TAT) Family

The aromatic amino acid transporter TAT1 (SLC16A10), which was cloned in 2001 (142), is the molecular correlate of system T (see Specificity of Transport) (143). It is a member of the SLC16 family of monocarboxylate transporters (143). TAT 1, which is a protein of 534 amino acids with 12 transmembrane domains, is expressed in the basolateral membrane of the proximal convoluted tubule (144) and functions as an electroneutral, Na^+- and H^+-independent facilitative diffusion (uniport) system mediating efflux of aromatic amino acids across the basolateral membrane (144) (*Fig. 37-1*). A recent study has provided evidence that TAT1 can also control neutral amino acid efflux via the neighboring exchanger 4F2hc-LAT2 by recycling the aromatic influx substrates of the exchanger (145).

Future investigations into the molecular and biochemical characteristics of this expanding group of cloned amino acid transporters may yield important insight into the inherited human diseases that result from a defective transport of amino acids (see Hereditary Aminoacidurias).

Maturation of Tubular Amino Acid Transport

Urinary fractional excretion of almost all amino acids in humans (146) and animals (8) is higher in the newborn than later in life. A very high rate of urinary amino acid excretion is found in immature, very-low birth weight infants (147). Some amino acids, including glycine, alanine, proline, dibasic amino acids, and taurine (8, 147), have been shown to contribute more to neonatal aminoaciduria than other amino acids. Theoretically, a structural, quantitative, or regulatory change in any one

of the membrane-related events depicted in *Fig. 37-1* may underlie the diminished reabsorptive capacity of the renal tubule during early life (8, 9).

As indicated earlier, (see Specificity of Transport), proline and glycine reabsorption in the proximal tubule occurs predominantly by a shared low-affinity/high-capacity transport system in the proximal convoluted tubule (likely PAT2/SLC36A2) as well as by two specific, high-affinity/low-capacity systems located in the proximal straight tubule, one for proline (likely IMINO/SLC6A20) and one for glycine (likely XT2/SLC6A18) (1, 3, 10, 48). Lasley and Scriver studied infants affected with familial renal iminoglycinuria to explore the ontogeny of proline and glycine reabsorption in the renal tubule (148). In iminoglycinuria the shared transport system for proline and glycine (possibly PAT1), which normally seems to be the dominant transporter for these amino acids in the proximal tubule of the newborn, is affected by mutation (see Iminoaciduria and Glycinuria). Using the occurrence of ontogeny and transport defect together, Scriver's group provided evidence for the appearance of the two specific high-affinity transport systems in succession, the proline transporter (possibly IMINO) by 3 months of age and the glycine transporter (possibly XT2) by 6 months of age (148). Similarly, earlier studies on glycine and proline transport in rat renal cortical slices (8) showed that the specific high-affinity transporters were not present at birth yet appeared when amino acid reabsorption reached adult levels. In contrast, subsequent animal studies demonstrated the existence of both low-affinity/high-capacity and high-affinity/low-capacity systems for several amino acids (8) in neonatal and adult kidneys; no new systems were acquired with maturation.

Studies using cortical slices and isolated tubules from several species have provided evidence for an impaired basolateral membrane exit step of amino acids from immature tubular cells (8). Decreased Na^+-dependent taurine transport has been demonstrated in basolateral membrane vesicles from hypertaurinuric mice (149). It remains to be established whether a similar alteration in basolateral membrane amino acid transport during early life contributes to neonatal hyperaminoaciduria.

Several studies explored the maturation of the first step of amino acid reabsorption, namely transport across the brush-border membrane. A gradual age-related increase in Na^+-coupled uptake of taurine (150) and proline (151) by rat renal BBMV and of cystine by isolated dog renal cortical tubules (152) has been demonstrated. Whereas the maturation of proline transport involved an increase in affinity (decrease in K_m) of transport (151), the maturation of cystine transport was associated with an increase in capacity (increased V_{max}) of transport (152).

Alterations in phospholipid composition have been documented during rat tubular brush-border membrane maturation (8), suggesting that changes in membrane fluidity may account for the observed maturational changes in Na^+-linked tubular amino acid transport. In addition, an increased permeability to Na^+ (151) and an enhanced amiloride-sensitive Na^+–H^+ exchange activity (153) has been demonstrated in neonatal rat renal BBMV. This alteration in ionic permeability and the increased luminal membrane Na^+–H^+ antiport (coupled with a diminished Na^+–K^+-ATPase activity known to exist in the basolateral membrane of the neonatal proximal tubular epithelium (154)) may result in a rapid dissipation of the electrochemical Na^+ gradient necessary for Na^+-amino acid cotransport, thereby contributing to the aminoaciduria of early life (151, 153).

Protein kinases modulate renal tubular amino acid transport (see above). Recent studies (38, 155) demonstrate higher activity of PKC and CaMK II in the cytosol and the brush-border membrane derived from immature kidneys than in adult kidneys. Furthermore, the studies provide evidence for differential regulation of PKC (155) and CaMK II (38) isoenzymes during kidney development. Age-related changes in the activity and expression of protein kinases may underlie the developmental changes in tubular reclamation of amino acids and other solutes.

In summary, hyperaminoaciduria is a characteristic of the immature mammalian tubule. Although the mechanisms governing the developmental changes in tubular amino acid transport have not been fully established, evidence has accumulated that both luminal and antiluminal membrane-related events play a role in the maturation of amino acid transport. Most studies indicate that transport maturation does not represent the acquisition of new transport systems but rather a change in affinity or capacity of transporters. The role of protein kinase-induced phosphorylation in the maturation of renal tubular amino acid transport remains to be established. The exact cellular mechanisms and the biologic signals responsible for the observed developmental changes await future studies. Studies into the molecular structure of amino acid transporters will undoubtedly shed light on the mechanisms underlying the development of tubular amino acid reclamation.

Hereditary Aminoacidurias

Aminoacidurias are a group of disorders in which a single amino acid or a group of amino acids are excreted in excess amounts in the urine. The defective tubular reabsorption is assumed to result from a genetic defect in a

specific transport system that directs the reabsorption of these amino acids under normal conditions. Some of these disorders also involve a similar transport abnormality in the intestine. As opposed to inborn errors of amino acid metabolism, in which plasma levels of amino acids are elevated, resulting in overflow aminoaciduria, plasma levels of amino acids in hereditary aminoacidurias are largely normal.

The aminoacidurias are generally categorized into five major groups according to the group-specific transport pathway presumed to be affected (*Table 37-2*). The groups are further subdivided into several disorders, based on the profile of the affected amino acids within that group (*Table 37-2*; *Fig. 37-1*).

Cationic Aminoaciduria

Five distinct inborn errors of cationic amino acid transport have been identified: (1) classic cystinuria, (2) isolated cystinuria, (3) hyperdibasic aminoaciduria, (4) lysinuric protein intolerance, and (5) isolated lysinuria. These diseases differ in defined or putative transport systems affected, pathophysiology, organs involved, and clinical features. Classic cystinuria is the prototype for this group of hereditary aminoacidurias.

Classic Cystinuria: Cystinuria is a disorder of amino acid transport characterized by excessive urinary excretion of cystine and the dibasic amino acids lysine, arginine, and ornithine. The pathogenic mechanism of cystinuria is defective transepithelial transport of these amino acids in the proximal tubule and the small intestine (156, 157). The very low solubility of cystine in the urine results in cystine stone formation in homozygous patients. Lysine, arginine, and ornithine do not form urinary stones. Urinary cystine calculi may produce considerable morbidity including urinary obstruction, colic, infection, and in severe cases, loss of kidney function. Cystinuria accounts for 1–2% of all urolithiasis and 6–8% of urolithiasis in children (158, 159). The defective gastrointestinal transport of cystine and dibasic amino acids in cystinuria does not result in intestinal disease.

The disease was first recognized in 1810 by Wollaston (160) and later by Berzelius (161), who called the stones "cystic oxide" and "cystine," respectively, assuming that the stones they analyzed originated in the bladder. In 1908, Garrod (162) postulated that cystinuria was an inborn error of cystine metabolism. In the 1950s, Dent and Rose (163) first recognized the true nature of the disease, suggesting that cystine and the dibasic amino acids lysine, arginine, and ornithine that have structural similarity (two amino groups separated by 4–6 chemical bonds) share a carrier protein in the brush-border membrane of the renal tubule and the small intestine. They postulated that this transport mechanism was defective in cystinuria.

Transport Defect: Normally 1% of the filtered cystine and dibasic amino acids is excreted. In classic cystinuria, cystine clearance may be near or equal to the glomerular filtration rate (GFR), and in some patients even twice as high as the GFR, suggesting active cystine secretion (164). Lysine and ornithine clearance is 30–80% of the GFR, and arginine excretion is less abnormal.

Dent's postulate about a defective shared transport system for cystine and dibasic acids in cystinuria has been supported by the in vivo experiments of Kato (165) and Robson and Rose (166), demonstrating that in normal subjects and cystinuric patients, increasing the filtered load of one of these amino acids decreased reabsorption of the others. However, this hypothesis was challenged by studies showing that cystine uptake was not impaired in kidney slices from cystinuric patients (167) and that cystine and dibasic amino acids did not share a common transport system in kidney slices of normal and cystinuric patients (167). The reports of isolated cystinuria (168), isolated dibasic aminoaciduria (169), and isolated lysinuria (170) cast further doubts on Dent's hypothesis and suggested the existence of specific transport systems for these amino acids. Furthermore, the occurrence of several transport systems for cystine and dibasic amino acids was supported by the observations of Brodehl (146) and Scriver (171) that tubular reabsorption capacity matures at different rates for different amino acids.

Subsequent studies using isolated cortical tubules and BBMV (16, 17, 172) have clarified the picture of tubular amino acid transport. These studies, coupled with the recognition that kidney slices preferentially expose the basolateral membrane, provided evidence for three brush-border membrane-bound and two basolateral membrane-bound carrier systems for cationic amino acids in the kidney. Transport at the brush-border membrane occurs by a system shared by cystine and the dibasic amino acids, a system specific for cystine, and a system specific for dibasic amino acids. It is the shared high-affinity, low-K_m system located in the S3 segment of the proximal tubule and identified as system $b^{0,+}$ (see Specificity of Transport; *Figs. 37-1* and *37-3*), that is defective in classic cystinuria. The low-affinity, high-K_m, unshared cystine system, and the low-affinity, unshared dibasic amino acid system, both located in the S1-S2 segments of the proximal tubule (and both unidentified at the molecular level) may be abnormal in isolated cystinuria and dibasic aminoaciduria, respectively (see later). The antiluminal membrane harbors two specific

Table 37-2

Hereditary aminoacidurias

Disorder	Defective gene	Locus	Amino acid transport system defective	Individual amino acids affected	Localization of defect	Mode of inheritance	Prevalence	Clinical manifestations	Organs involved	OMIM No.[a]
I. Cationic aminoaciduria										
1) Classic cystinuria Type I	SLC3A1	2p16.3	rBAT (heavy subunit of $b^{0,+}$)	Cystine, lysine, arginine, ornithine	BBM	Autosomal recessive	1:7,000–1:15,000	Urolithiasis	Kidney, intestine	220100
Non-type I	SLC7A9	19q13.1	$b^{0,+}$ AT (light subunit of $b^{0,+}$)		BBM	Autosomal dominant (incomplete penetrance)				600918
2) Isolated cystinuria			Cystine	Cystine	BBM	Autosomal recessive	2 siblings reported	Benign	Kidney	238200
3) Hyperdibasic aminoaciduria Type I			Dibasic amino acids	Lysine, arginine, ornithine	BBM	Autosomal dominant	2 families reported	Mental retardation	Kidney, intestine	222690
4) Lysinuric protein intolerance (Hyperdibasic aminoaciduria Type II)	SLC7A7	14q11	y^+LAT-1 (light subunit of y^+L)	Lysine, arginine, ornithine	BLM	Autosomal recessive	1:60,000 (Finland)	Protein malnutrition, failure to thrive, hyperammonemia, seizures, coma	Kidney, intestine, lungs, bones	222700
5) Isolated lysinuria			Lysine (?)	Lysine	BBM (?)	Autosomal recessive	1 patient reported	failure to thrive, seizures, mental retardation	Kidney, intestine	

II. Neutral Aminoaciduria										
1) Hartnup Disease	SLC6A19	5p15.33	B^0(neutral, monoamino monocarboxylic α-amino acids)	Alanine, serine, threonine, valine, leucine, isoleucine, phenylalanine tyrosine, tryptophan, histidine, glutamine, asparagine	BBM	Autosomal recessive	1:20,000	Skin rash, cerebellar ataxia, psychiatric illnesses	Kidney, intestine	234500
2) Methioninuria			Methionine	Methionine	BBM	Autosomal recessive	2 cases reported	Edema, seizures, mental retardation	Kidney, intestine	250900
3) Histidinuria			Histidine	Histidine	BBM	Autosomal recessive	4 cases reports	Mental retardation	Kidney, intestine	235830
III. Iminoaciduria and Glycinuria										
1) Imino-glycinuria	SLC36A1 (PAT1) (?)	5q33.1	Imino acids, and glycine	Proline, hydroxyproline, glycine	BBM	Autosomal recessive	1:15,000	Benign	Kidney, intestine	242600
	SLCA20 (IMINO) (?)	3p21.3								
2) Isolated glycinuria	SLC6A18 (XT2) (?)	5p15.33	Glycine	Glycine	BBM	Autosomal dominant	2 families reported	Benign	Kidney	138500
IV. Dicarboxylic aminoaciduria	SLC1A1 (EAAT3) (?)	9q24	X^-_{AG} (acidic amino acids)	Glutamate, aspartate	BBM	Autosomal recessive	1:29,000 (French-Canadian)	Benign	Kidney, intestine	222730
V. β-amino-aciduria (mouse)			β-amino acids	Taurine	BLM (?)	Autosomal recessive		Benign	Kidney	

BBM brush border membrane; *BLM* basolateral membrane

[a]Online Mendelian Inheritance in Man (database at http://www.ncbi.nlm.nih.gov/omim)

systems, one for dibasic amino acids identified as system y^+L (see Specificity of Transport; *Figs. 37-1* and *37-3*), and one for cystine, but no shared system. These transport mechanisms mediate uptake and efflux of these amino acids across the basolateral membrane. The brush-border membrane of the intestinal cell has a single high-affinity, low-K_m, shared $b^{0,+}$ transport system for cystine and dibasic amino acids that is defective in classic cystinuria. Like kidney cells, intestinal cells have two basolateral membrane-bound, unshared specific transport systems, one (system y^+L) for dibasic amino acids and one for cystine (2, 3). The basolateral membrane-bound y^+L transporter for dibasic amino acids in the kidney and the intestine is defective in lysinuric protein intolerance (see Lysinuric Protein Intolerance). Amino acid transport in parenchymal cells and leukocytes from cystinuric patients is not impaired (2, 3) because the defect is not expressed in the plasma membrane of these cells.

Genetics: Classic cystinuria is inherited in an autosomal-recessive fashion. It is a common disorder with an overall prevalence of 1:7,000 to 1:15,000 and estimated gene frequency of 0.01 (156). A very high prevalence, 1:2,500, is observed in Israeli Jews of Libyan origin (173).

Although it is a recessive disease, phenotypic heterogeneity in homozygotes and heterozygotes is evident. The excretion patterns of cystine and dibasic amino acids in heterozygotes have delineated the traditional classification into three cystinuric subtypes (174). In type I, the most common phenotype, heterozygotes have normal urinary amino acid excretion. In type II, heterozygotes have high excretion of cystine and dibasic amino acids. Type III heterozygotes have excretion rates intermediate between the other two. The three subtypes were considered to be allelic, namely mild, moderate and severe mutations at a single cystinuria gene locus (15). However, Goodyer et al. (175) provided evidence that type I and type III cystinuria mutations might involve two distinct genetic loci. This was demonstrated by the finding that type I/III compounds excreted less cystine than type I/I probands, although type III/N heterozygotes excrete higher levels of cystine than their type I/N counterparts. These findings could be explained by genetic complementation between nonallelic cystinuria genes. Subsequent genetic studies have supported this hypothesis (see later – Molecular Genetics) and have resulted in a revision of the above classification of cystinuria to type I (OMIM # 220100) and non-type I (OMIM # 600918) (100, 157) (*Table 37-2*). While type I cystinuria is inherited as a fully recessive trait, type II and type III (collectively now termed non-type I) subtypes are inherited as autosomal domainant traits with incomplete penetrance (100).

Renal ontogeny has important implications for genetic counseling in cystinuria. This was demonstrated by Scriver's finding that heterozygous infants under 6 months of age who have immature tubular function can excrete cystine and dibasic amino acids at levels equivalent to those found in homozygous adults (171). Urinary excretion of these amino acids decreased steadily with age, to reach the variant parental value in heterozygous infants, but not in homozygotes. Hence, final classification of a cystinuric phenotype should not be done before the age of 6 months.

Molecular Genetics: The identification and characterization of the rBAT/D2H gene have led to the speculation that a defect in the human form of rBAT (SLC3A1) causes cystinuria. Pras et al. (176), using linkage analysis in 17 cystinuric families, demonstrated linkage between cystinuria and three genetic markers on chromosome 2p, providing strong evidence that SLC3A1 was indeed the gene causing the disease. Calonge et al. (177) identified six specific mutations in the SLC3A1 gene that segregated with a cystinuria phenotype thereby establishing the rBAT gene as the cystinuria gene (*Fig. 37-3*). Subsequent studies over the past decade have revealed numerous additional mutations in SLC3A1 (178–181). To date, over 100 different rBAT mutations have been reported in patients with type I cystinuria (100, 181). These mutations include nonsense, missense, splice site, frameshift mutations, as well as large deletions and chromosome rearrangements (100, 156, 181, 182). A recent exhaustive mutation analysis on 164 probands of the International Cystinuria Consortium (ICC) identified 90.5% of the affected alleles in type I cystinuria patients (181). Defective transport of cystine and dibasic amino acids has been demonstrated for many of these mutations when expressed in *Xenopus* oocytes (118). The most commonly occurring mutation is methionine 467 to threonine (M467T), which causes a defect in trafficking to the plasma membrane (49, 183). This trafficking defect, which has also been demonstrated for other rBAT mutations (182, 184), is consistent with the proposed role of rBAT as a chaperon of the corresponding light subunit in the heteromeric amino acid transporter. To date, no SLC3A1 mutations have been found in non-type I patients (180, 181, 185). Recently, a mouse model homozygous for the rBAT mutation D140G, which displays type I cyctinuria with urolithiasis, has been reported (186).

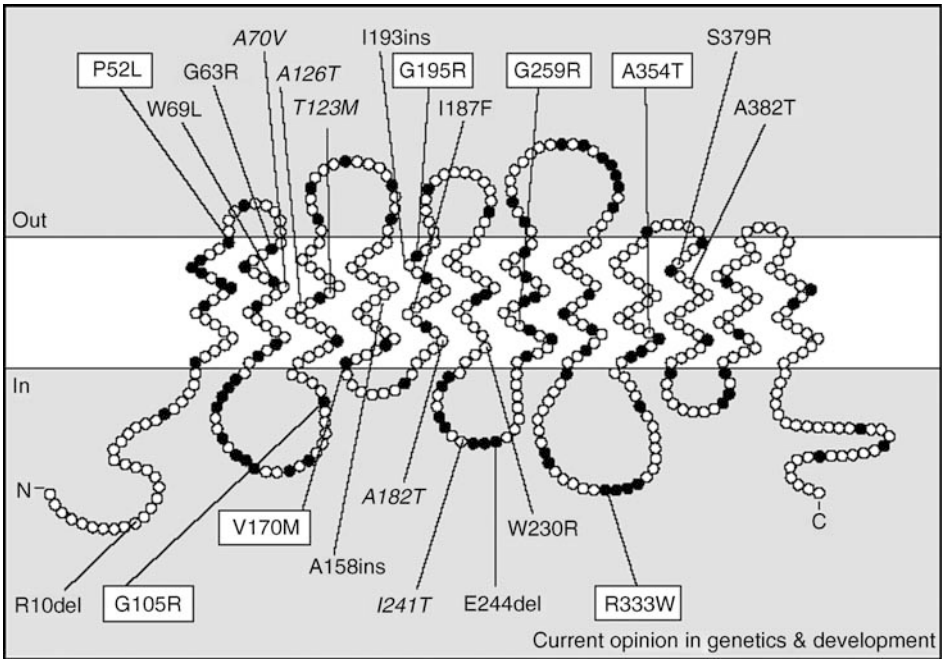
In 1997 Wartenfeld et al. (187) and Bisceglia et al. (188) demonstrated that cystinuria type III (and possibly

also cystinuria type II) is linked to a locus on chromosome 19q13.1. Subsequently, the ICC (189) identified the gene SLC7A9 which encodes the 487 amino acid protein $b^{0,+}$ AT that belongs to the family of light subunits of amino acid transporters (see Molecular Structure of Amino Acid Transporters; *Fig. 37-3*). The gene localized to the non-type I cystinuria 19q locus. Cotransfection of $b^{0,+}$ AT and rBAT in COS cells resulted in trafficking of rBAT to the plasma membrane and induced L-arginine uptake by cells. SLC7A9 mutations were found in Spanish, Italian, North American and Libyan–Jewish cystinuria patients (189, 190). Mutation G105R is the most frequent SLC7A9 mutation in the ICC cohort of patients (100). The mutation in Jews of Libyan origin is valine 170 to methionine (V170M), which leads to complete loss of $b^{0,+}$ AT amino acid uptake activity when cotransfected with rBAT in COS cells. To date, over 60 different $b^{0,+}$ AT mutations have been identified in non-type I cystinuria patients (400, 181) (*Fig. 37-5*). These mutations have explained 87.6% of the alleles in non-type I cystinuria patients of the ICC included in a recent study (181). The unexplained alleles in non-type I (and type I) patients might be due to mutations outside the open reading frame of the SLC7A9 (and SLC3A1) genes (intronic or promoter regions) or due to mutations in unidentified genes (50, 100, 182). Two patients with I/III phenotype had a dual mutation in both SLC3A1 and SLC7A9, suggesting the existence of digenic form of the disease (181, 189).

Interestingly, although most heterozygotes for SLC7A9 mutation show type II or III trait, a minority (about 15%) have type I phenotype (181, 191). Moreover, mutations in SLC7A9 have been shown to cause all three phenotypic subtypes (192). These data, indicating the lack of a direct relationship between the mutated cystinuria gene and the type of cystinuria, have prompted the ICC

Figure 37-5

Representation of the cystinuria-specific missense or single amino-acid point mutations identified in the $b^{0,+}$AT amino-acid transporter. Twenty-three missense or single amino-acid point mutations in *SLC7A9* ($b^{0,+}$AT) are depicted. Amino-acid residues conserved in all the human members of the LSHAT family are indicated in *black*. Mutations that are associated mainly with a severe urinary phenotype in heterozygotes (urine cystine levels similar to or below 200 μmol/g of creatinine and the sum of urine levels of cystine and the three dibasic amino acids similar to or below 1000 μmol/g of creatinine) are boxed. Mutations that are associated mainly with a mild urinary phenotype in heterozygotes (urinary levels of amino acids below the above indicated limits) are indicated in italics. The rest of mutations (with undefined or ambiguous phenotype) are indicated in smaller font. See text for details (adapted with permission from (182)).



to introduce an additional classification of cystinuria subtypes based on genotype rather than phenotype. This new classification includes: Type A – due to two mutations on SLC3A1 on chromosome 2; Type B – due to two mutations on SLC7A9 on chromosome 19; and type AB with one mutation on each SLC3A1 and SLC7A9 (compound heterozygote) (191). The ICC has reported that type A, type B, and type AB account for 38, 47 and 14% of cystinuria patients in their registry, respectively (100, 181).

Similar to the human disease, the SLC7a9 knockout mouse displays non-type I cystinuria with urolithiasis (193). This mouse model (and the cystinuria type I mouse model (186)) may prove to be very important tools in exploring the pathophysiology of cystinuria, the various factors influencing the formation of cystine stones and the efficacy of therapy (194).

Noteworthy is the recently described autosomal recessive disorder, hypotonia-cystinuria syndrome, characterized by generalized hypotonia at birth, failure to thrive, growth retardation and cystinuria type I (195). The syndrome is caused by microdeletions of SLC3A1 and a prolyl oligopeptidase-like gene (PREPL) which are adjacent on chromosome 2p21 (195).

Diagnosis and Clinical Features: The simplest diagnostic test is the microscopic examination of the urinary sediment of a freshly voided morning urine (156). The presence of typical flat hexagonal cystine crystals is diagnostic. Acidification of the urine precipitates cystine crystals and may improve the yield of the test. The best screening procedure is the cyanide-nitroprusside test (156). A positive reaction occurs with as little as 75–125 mg cystine per gram of creatinine, which is well below that of homozygotes, who excrete at least 250 mg/g creatinine. Some heterozygotes may also be detected by this procedure (160). The test is not specific and may detect acetone or homocystine as well. The definite test is a measurement of urine cystine and dibasic amino acid concentration by ion exchange chromatography. The upper limits of normal are 18, 130, 16, and 22 mg/g creatinine for cystine, lysine, arginine, and ornithine, respectively (159). In establishing the diagnosis of classic cystinuria, it is important to exclude other conditions associated with increased urinary cystine excretion, including isolated cystinuria, tubular immaturity in young infants, generalized aminoaciduria (Fanconi syndrome), and organic acidemias (156, 159).

Cystine stones are radiopaque because of the density of the sulfur molecule, and on a roentgenogram, they appear smooth. Occasionally, they form staghorn calculi. Cystine also may act as a nidus for calcium oxalate so that mixed stones may be found (196). Factors contributing to mixed stone formation in cystinuria include alkalinization of urine and urinary tract infections.

The disease usually presents with renal colic. Occasionally, infection, hypertension, or renal failure may be the first manifestation (156). Cystinuria occurs with equal frequency in males and females, but males are more severely affected because of a greater likelihood of urethral obstruction in the male. Clinical manifestations usually occur in the second and third decades of life, with a 62% probability of stones by age 25 years (197). Most patients have recurrent stone formation. Cystinuric patients who receive a kidney transplant have normal urinary cystine and dibasic amino acid excretion following transplantation (198, 199).

Treatment: Cystine crystalluria occurs when the cystine content of the urine exceeds 300 mg/L at pH 4.5–7. Cystine solubility increases sharply at a urine pH above 7 (159). The major therapeutic approaches to cystinuria are designed to increase the solubility of cystine, reduce excretion of cystine, and convert cystine to more soluble compounds (156). Therapies used in the management of cystinuria include the following:

1. Increased oral fluid intake to increase urine volume and cystine solubility. Because cystinuric patients excrete 0.5–1 g cystine/day, intake of 3–4 L could be required to keep the urinary cystine concentration below 300 mg/L. Patients should develop a 24-h schedule for drinking and voiding, with particular attention to night time hours, when urine may become supersaturated with cystine. Water should be taken at bedtime and whenever the patient awakens at night. Rigid adherence to fluid therapy is effective in approximately 70% of patients (156).
2. Oral alkali in addition to high fluid intake to further increase cystine solubility in the urine (159). A urine pH of 7.5–8 can be maintained by the provision of 1–2 mEq/kg/day of bicarbonate or citrate in divided doses. Because high sodium intake increases cystine excretion (see later), potassium citrate is preferred (159). Because urine alkalinization may result in formation of mixed calcium-containing stones, adherence to high fluid intake is crucial.
3. Dietary therapy to reduce cystine production and excretion. Studies examining the effect of dietary restriction of methionine (a metabolic precursor to cystine) on urine cystine excretion have yielded variable results (159). Also, diets low in methionine are very difficult to follow and may be harmful to growing children. Therefore, dietary methionine restriction is not recommended (159). Urinary excretion of cystine

and dibasic amino acids in cystinuric patients has been shown to correlate with urinary sodium excretion (200, 201). Hence, dietary sodium restriction has been recommended by some authors as a safe approach to the treatment of cystinuria (156). L-Glutamine administered orally or intravenously in conjunction with low salt intake reduces cystine excretion (200, 202), but this effect was not observed in patients receiving a normal salt diet (203). The mechanism of the anticystinuric effect of glutamine is unclear.

4. Pharmacologic therapy to increase cystine solubility and decrease cystine excretion. The sulfhydryl-binding compound D-penicillamine (β-dimethylcysteine) leads to the formation of the mixed disulfide penicillamine-cysteine following a disulfide exchange reaction. This mixed disulfide is far more water-soluble than cystine. Hence, penicillamine acts by reducing cystine excretion as well as by permitting the excretion of a more soluble compound. Penicillamine, given at a dosage of 1–2 g/24 h (30 mg/kg in children), is highly effective and reduces urinary cystine excretion to under 200 mg/g creatinine (204). Unfortunately, penicillamine produces serious side effects in 50% of patients (205). These reactions include rashes (including pemphigus), fever, arthralgia, nephrotoxicity (including nephrotic syndrome in up to 30% of patients, and rapidly progressive glomerulonephritis), pancytopenia, and loss of taste. Penicillamine also increases copper and zinc excretion in the urine. The loss of taste may be reversed by copper administration (159). Pyridoxine metabolism may be impaired, and pyridoxine supplementation should be provided for patients receiving D-penicillamine. Most of these side effects revert to normal upon discontinuation of the drug. Because of the serious side effects, D-penicillamine therapy should be reserved for patients unresponsive to conservative management, and stepwise dosing is recommended (156). D-Acetyl penicillamine, another anticystinuric sulfhydryl agent, has fewer side effects than D-penicillamine. Mercaptopropionyl glycine (MPG), another agent undergoing a disulfide exchange reaction, is as effective as D-penicillamine in the treatment of cystinuria (206). MPG has the same toxicity as D-penicillamine, but serious renal and hematologic reactions requiring cessation of therapy are much less common with MPG (206). Because of the lower incidence of side effects and because this compound can be used in patients who develop allergic reactions to D-penicillamine, MPG is the pharmacologic agent of choice in the therapy of cystinuria (206, 207).

Several studies have examined the effect of captopril, an angiotensin-converting enzyme inhibitor, on urinary cystine excretion in cystinuric patients (208–210). This nontoxic sulfhydryl compound builds highly soluble captopril-cysteine disulfides. Although a reduction in cystine excretion has been demonstrated in some studies (208, 209), others (210) have failed to show an effect. Further studies are needed to evaluate the efficacy of captopril therapy in cystinuria. It has been proposed that *meso*-1,3 dimercaptosuccinic acid (DMSA), an additional compound forming disulfide linkage with cysteine, might be a useful therapeutic agent in cystinuria (211). The efficacy of this agent in cystinuria remains to be established.

Ascorbic acid, which acts as a reducing agent to convert cystine to the more soluble cysteine, has been suggested as a therapeutic modality in patients with cystinuria (207). To date, the results of the use of this compound on a limited number of patients have been variable (159, 207). Also, concerns have been raised that ascorbic acid therapy in cystinuria is potentially lithogenic because of the hyperoxaluric and hypocitraturic effect of this agent.

Several urologic procedures have been used to treat cystine stones:

1. Chemolysis of stones by irrigation through a percutaneous nephrostomy. Successful dissolution of stones has been achieved using *N*-acetylcysteine, D-penicillamine, α-MPG, and a very alkaline agent, tromethamine (158, 159).
2. Extracorporal shock wave lithotripsy (ESWL). This therapeutic modality has been only partially successful because of the organic nature and the uniform crystal structure of cystine stones (158, 212). Percutaneous ultrasonic lithotripsy has been somewhat more effective (213).
3. Lithotomy. Surgical removal of stones is necessary only in rare patients with obstructing or infected stones unresponsive to a more conservative approach.

In summary, the mainstays of therapy in cystinuria include hydration, alkalinization of the urine, and dietary sodium restriction. Full compliance with this regimen results in significantly reduced urinary cystine excretion and good long-term prognosis in most patients. Pharmacologic treatment with sulfhydryl agents should be reserved for patients in whom conservative therapy fails. Urologic intervention may be indicated in selected patients.

Isolated Cystinuria: Brodehl et al. (168) report two siblings who showed high urinary excretion rates of

cystine but normal dibasic amino acid excretion. The children did not develop renal stones. This report, along with the detection of a similar abnormality in dogs (214), provides evidence of a separate cystine transporter not shared with dibasic amino acids in the tubular brush-border membrane, which appears to be defective in isolated cystinuria. The molecular nature of this proposed transporter is unknown.

Lysinuric Protein Intolerance (LPI) (Hyperdibasic Aminoaciduria Type II): Lysinuric protein intolerance is a rare autosomal-recessive disorder characterized by excessive urinary excretion of dibasic amino acids (especially lysine), normal cystine excretion, and poor intestinal absorption of dibasic amino acids (215–217). Plasma values of dibasic amino acids are low. The disease is relatively common in Finland, where the prevalence of the disease is 1:60,000 (217). About 130 patients, Finish as well as non-Finish, have been described (217, 218). Homozygous patients show massive dibasic aminoaciduria as well as hyperammonemia after a protein overload; heterozygotes have normal urinary amino acid excretion but impaired renal and intestinal transport of dibasic amino acids at increased loads. The clinical manifestations in homozygotes for LPI are those of protein malnutrition and postprandial hyperammonemia. They include failure to thrive, marked protein intolerance, anorexia, vomiting, diarrhea, hepatosplenomegaly, muscle hypotonia, interstitial lung disease, osteoporosis, seizures, and coma (217).

The pathogenic mechanism of LPI appears to be a defective transport of dibasic amino acids in the basolateral membrane of renal and intestinal epithelial cells, resulting in impaired efflux from cell to interstitium (219, 220). This has been confirmed by a measurement of fluxes in jejunal biopsy specimens from LPI patients (219) as well as by the observation that infusion of citrulline to these patients results in massive argininuria and ornithinuria (220). Citrulline is reabsorbed from the tubular lumen by a neutral amino acid transport mechanism and is converted to arginine and ornithine in the renal cell. Impaired exit at the antiluminal membrane results in backflux of accumulated arginine and ornithine at the brush-border membrane surface. It is the high-affinity, specific dibasic amino acid transporter y^+L that is affected in this disease (100, 221). Because basolateral membrane transporters of epithelial cells and plasma membrane transporters of parenchymal cells are homologous carriers, it is not surprising that granulocytes (222), and cultured skin fibroblasts (223) from patients with LPI show impaired transport of dibasic amino acids. Erythrocytes from LPI patients, which do not have the y^+L system, show normal cationic amino acid transport (224).

It is presumed that the poor intestinal absorption and excessive renal loss of dibasic amino acids deprive hepatic cells of ornithine and arginine, which are necessary for urea production (217). This results in protein intolerance, hyperammonemia, and low urea formation. This notion is supported by the observation that L-citrulline supplements improve protein tolerance in LPI patients (220). This amino acid, a metabolic precursor of ornithine and arginine, is absorbed in the intestine, enters the hepatic cell via neutral amino acid transport mechanisms, is metabolized in the liver to ornithine and arginine, and restores the pathway for ammonia disposal (217). Several manifestations of LPI patients including glomerular dysfunction (225), erythroblastophagia and alveolar proteinosis (226) suggest that the immune system is deranged in some patients.

In 1992, the rBAT homologous protein, the human cell surface glycoprotein 4F2 heavy chain (4F2hc, encoded by the gene CD98, now termed SLC3A2), was shown to induce y^+L cationic amino acid transport in *Xenopus* oocytes (112). Like rBAT, 4F2hc represents the heavy subunit of a disulfide linked heteromeric amino acid transporter (HAT) (100, 227) (see Molecular Structure of Amino Acid Transporters; *Figs. 37-3* and *37-4*). Molecular analysis excluded SLC3A2, which has been localized to chromosome 11, as a candidate gene for LPI (228). Linkage studies in Finnish and non-Finnish LPI families placed the gene of the disease to 14q11–13 (229, 230).

In 1998, Torents et al. (120) identified a human cDNA, SLC7A7, as encoding y^+LAT-1, a member of the family of light subunits that combine with 4F2hc to form heteromeric amino acid transporters (see Molecular Structure of Amino Acid Transporters; *Fig. 37-3*). The 4F2hc/y^+LAT-1 transporter has been shown to have the activity of amino acid transport system y^+L that is responsible for the efflux of basic amino acids at the basolateral plasma membrane of epithelial cells (49) (see Specificity of Transport; *Figs. 37-1* and *37-3*). The SLC7A7 gene localized to the LP1 locus (120). Subsequently, two groups (228, 231) have demonstrated that mutations in SLC7A7 cause LPI in Finnish, Italian and Spanish patients. To date, more than 40 SLC7A7 mutations, spread along the entire SLC7A7 gene, have been found in LPI patients from different ethnic groups (100, 218). Expression studies in *Xenopus* oocytes showed that various LPI mutations result in proteins that fail to co-induce amino acid transport activity when expressed with 4F2hc (232). Two of the mutants reached the oocyte plasma membrane when coexpressed with 4F2hc demonstrating that they are transport inactivating mutations (232). While

4F2hc (SLC3A2) is ubiquitously expressed, y^+LAT1 (SLC7A7) is primarily expressed in tissues affected in LPI including kidney, small intestine, lung and white blood cells (100, 120).

LPI is characterized by poor genotype/phenotype correlation. Even Finnish patients, who share the same founder mutation, show variation in the clinical picture (232). Similarly, both interfamilial and intrafamilial phenotypic variability were observed in Italian LPI patients heterozygotes for the same mutation (218). This suggests that, in addition to SLC7A7 mutations, hitherto unknown factors play a role in the pathogenesis of LPI (49, 182). A recently reported SLC7A7 knockout mouse model (233) had severe growth retardation leading to neonatal death in most SLCA7−/− mice. Surviving mice that received high protein diet displayed human LP1-like metabolic dysfunction.

Therapy of LPI consists of protein restriction to prevent hyperammonemia, as well as oral supplements of lysine (234), arginine, ornithine, and most important, citrulline (235). Administration of the latter amino acid, which corrects the hepatic deficiency in ornithine and arginine, results in clinical improvement and catch-up growth.

Hyperdibasic Aminoaciduria Type I:An autosomal-dominant cationic aminoaciduria has been described in two families containing several heterozygotes and a single homozygote (169, 236). This disease, called hyperdibasic aminoaciduria type I (HDBA I), is characterized by impaired renal reabsorption and intestinal absorption of the dibasic amino acids lysine, arginine, and ornithine, but not of cystine. Plasma values of dibasic amino acids are normal, and there is no protein intolerance or hyperammonemia. The reported homozygous patient had mental retardation (236). HDBA I heterozygotes have modest cationic aminoaciduria, whereas LPI heterozygotes have no hyperaminoaciduria. It has been speculated that the brush-border membrane-bound high-capacity transporter for dibasic amino acids, which excludes cystine, is defective in this disease. The molecular nature of this transporter is unknown.

Isolated Lysinuria: Omura et al. (170) report a single child with increased urinary excretion of lysine, impaired intestinal absorption of this amino acid, low plasma lysine levels, and normal renal and intestinal transport of ornithine, arginine, and cystine. The patient did not have hyperammonemia but had failure to thrive and mental retardation, probably secondary to the deficiency of the essential amino acid lysine. This case implies a defect in a selective transport system for lysine in the kidney and the intestine. Such a system has not been identified in physiologic or molecular studies.

Neutral Aminoaciduria

Three distinct disorders of neutral amino acid transport have been identified: Hartnup disorder, methioninuria, and histidinuria.

Hartnup Disease: Hartnup disease, which may have afflicted Julius Caesar and his family (237), was first recognized in two siblings in England in 1956 (238). This disease is characterized by intestinal malabsorption and massive aminoaciduria of the neutral monoamino monocarboxylic amino acids: alanine, serine, threonine, valine, leucine, isoleucine, phenylalanine, tyrosine, tryptophan, histidine, glutamine, and asparagine (239). Most patients also have increased excretion of indolic compounds that originate in the gut from bacterial degradation of tryptophan (240). Transport of other neutral amino acids including cystine, imino acids, glycine, and β-amino acids is unaffected. The disease is inherited as an autosomal-recessive trait and has an estimated incidence of 1:20,000 live births. Heterozygotes have normal urinary acid excretion under physiologic conditions. Clinical features in homozygotes may include photosensitive rash, cerebellar ataxia, and a variety of psychiatric manifestations-the features of pellagra (239). The pellagra- like manifestations are primarily caused by intestinal malabsorption and urinary loss of tryptophan, an amino acid that is required for niacin synthesis. The diagnosis should be suspected in any patient with pellagra who has no history of niacin or nicotinamide deficiency and should be made by chromatographic analysis of the urine (239).

A defect in the broad-specificity neutral, α-amino acid transport mechanism in the renal and intestinal brush-border membrane was presumed to be the pathogenic mechanism underlying this disorder (15, 241). Two lines of evidence have supported the hypothesis that the lesion is localized in the brush-border membrane (15). First, plasma amino acid response in patients with Hartnup disease was attenuated after oral feeding of free α-amino acids but not when appropriate dipeptides were given orally (242). The dipeptides are presumed to be reabsorbed by a dipeptide-specific brush-border membrane transporter and hydrolyzed in the enterocyte; the free amino acids exit the cell via a nondefective basolateral membrane-bound carrier. Second, tryptophan transport is normal in various parenchymal cells from patients with Hartnup disease including leukocytes, placenta, and cultured skin fibroblasts (239, 243). As indicated earlier, carriers in plasma membranes of parenchymal cells are homologous to basolateral membrane carriers in epithelial cells, which are supposed to be normal in Hartnup disease.

The transport characteristics and the epithelial distribution of the Na^+-dependent neutral amino acid transport B^o (see Specificity of Transport; *Fig. 37-1*), have led to the conclusion that this transporter is the defective one in Hartnup disorder. In 2001, the gene responsible for Hartnup disease was localized to chromosome 5p15 (68). Subsequently, Bröer's group (69) cloned from the syntenic region in the mouse the B^oAT1 (SLC6A19) gene (see Molecular Structure of Amino Acid Transporters). This SLC6 "orphan transporter" gene encodes a Na^+-dependent neutral amino acid transporter which is expressed in the brush border membrane of the early proximal tubule and the intestine and corresponds to the B^o transport system (3) (see Specificity of Transport).

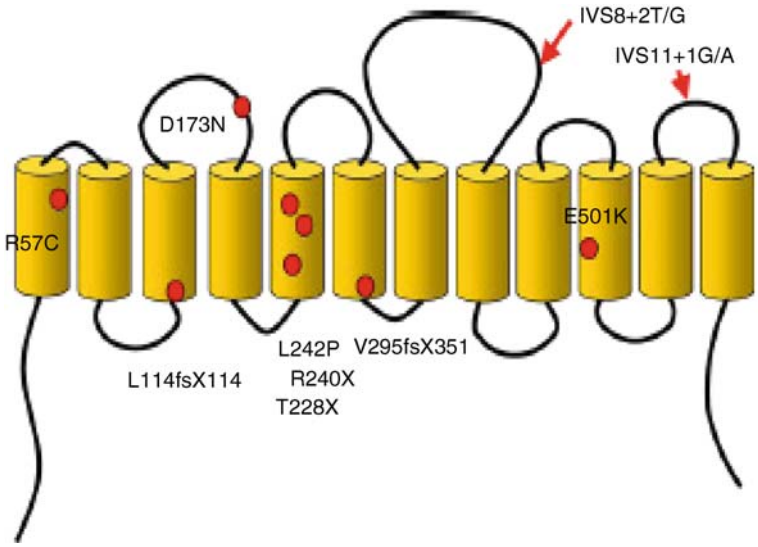
In 2004, two groups (244, 245) cloned the human SLCA19 gene from chromosome 5p15 and have found several mutations in this gene in British, Japanese and Australian patients with Hartnup disease, thereby identifying SLCA19 as the disease causing gene. To date, a total of 17 mutations have been identified that cause Hartnup disorder (246, 247) (*Fig. 37-6*). Interestingly, it has been demonstrated (247) that most of the probands with Hartnup disease analyzed so far display allelic heterogeneity in that they are compound heterozygotes for the SLC6A19 mutation.

Very recently, it has been demonstrated that collectrin (Tmem 27), a transmembrane glycoprotein with homology to angiotensin converting enzyme II, which is highly expressed in the collecting duct as well as the proximal tubule, is required for expression of the B^oAT1 protein and other SLC6 family members in the renal brush border membrane (248, 249). Collectrin knockout mice displayed decreased expression of B^oAT1 in the luminal membrane derived from the kidneys of the mice as well as massive generalized aminoaciduria (248, 249). Expression of collectrin in *Xenopus* oocytes and MDCK cells enhanced B^oAT1-mediated amino acid transport (248). These data identify collectrin as a regulator of amino acid reabsorption and may shed light on the molecular pathogenesis of deranged amino acid transport in various hereditary aminoacidurias including Hartnup disorder (250, 251).

The presence of alternative transport pathways for neutral amino acids in the renal tubule explains why the defect in the broad-specificity neutral amino acid transport in Hartnup disease results in only partial loss of some amino acids (e.g., phenylalanine) (15). These alternative systems, as well as the oligopeptide-preferring carriers in the kidney and intestine, are responsible for normal plasma amino acid values and lack of symptoms in most patients (252). As suggested by Scriver et al. (252), Hartnup disease is multifactorial in its pathogenesis, and only patients genetically predisposed to low plasma amino acid levels and impaired tryptophan metabolism develop symptoms. Given the phenotypic heterogeneity of patients with Hartnup disorder (246, 251), it is likely

Figure 37-6

SLC6A19 mutations associated with Hartnup disorder. A topological model of B^oAT1 (SLC6A19) is depicted, containing mutations associated with Hartnup disorder; *X* stop codon, *fs* frameshift mutation, *IVS* splice mutations (adapted with permission from (246)).



that other mutations (or, alternatively, polymorphisms) in other genes encoding for transport systems for neutral amino acids will be identified in the near future.

Patients with Hartnup disease respond well to oral therapy with nicotinamide 40–100 mg/day (253). Also, oral administration of tryptophan ethylester, a lipid-soluble form of tryptophan, has been shown to increase serum tryptophan and reverse clinical symptoms in patients with Hartnup disease (254).

Methioninuria: There are two case reports of patients with isolated increased urinary excretion of methionine and its metabolic breakdown products (255, 256). The patients had malodorous urine, edema, episodic hyperventilation, seizures, and mental retardation. The underlying defect appeared to be abnormal transport of methionine in the kidney and in the intestine. α-hydroxybutyric acid, a bacterial degradation product of unabsorbed intestinal methionine, appeared in the urine of these patients. This organic acid may have been responsible for the neurologic manifestations in these patients. Low-methionine diet resulted in significant clinical improvement. These cases of isolated methioninuria, as well as the observation that methionine, which shares the broad-spectrum amino acid transport system with other neutral amino acids, is not hyperexcreted in Hartnup disease, provide evidence for the existence of a specific transport pathway for methionine in renal and intestinal epithelium.

Histidinuria: Two siblings (257) and two other patients (258, 259) have been reported with isolated histidinuria. All showed significant mental retardation. Investigation revealed impaired transport of histidine in the kidney and intestine. Parents of the two affected siblings showed normal urinary histidine excretion under normal conditions, but hyperhistidinuria after an oral histidine load. The mode of inheritance of this disease is uncertain, but autosomal-recessive inheritance has been suggested (15). Although the broad-specificity transporter of neutral amino acids is the major carrier for histidine, as suggested by in vitro studies as well as by fractional excretion rates above 50% for this amino acid in Hartnup disease (15), the case reports of histidinuria suggest that an isolated histidine carrier is operating in the renal tubule and the intestine.

Iminoaciduria and Glycinuria

Two distinct disorders belong to this group of aminoacidurias: iminoglycinuria and isolated glycinuria.

Iminoglycinuria: Iminoglycinuria is an autosomal-recessive membrane transport defect characterized by excretion of excessive amounts of proline, hydroxyproline, and glycine in the urine (260). Iminoglycinuria is a benign condition with an estimated incidence of 1:15,000 live births (260, 261). It is the shared, brush-border membrane-bound, group-specific transport pathway for imino acids and glycine (see earlier) that is most likely defective in this condition (148, 260). The selective glycine-specific and imino acid-specific transport systems are not affected. The activity of these selective transporters accounts for the normal plasma levels of glycine and imino acids observed in iminoglycinuria. As discussed earlier, the late maturation of these selective transporters in normal infants is responsible for neonatal physiologic iminoglycinuria (146, 148). The absence of the selective transporters during early life also explains why infants with iminoglycinuria who lack the shared transporter have fractional excretion values for glycine and proline approaching 100%, which decline after the first months of life (15). Defective intestinal transport of proline has been found in some patients with iminoglycinuria (260). As expected, proline transport is not defective in leukocytes or skin fibroblasts because these cells do not possess carriers corresponding to a brush-border membrane carrier (15).

Iminoglycinuria is a genetically and pathophysiogically heterogenous condition with several mutant alleles (15, 260). This view is supported by several observations. First, obligate heterozygotes may have hyperglycinuria (but not iminoaciduria) or may have normal urinary amino acid excretion (262). Second, some homozygous patients have an intestinal transport defect and others do not have such defect. Third, a variant exists with normal transport maximum (Tm) for proline and a defect affecting glycine transport more than proline transport, indicating a K_m defect (263) (see later).

The genetic defect responsible for iminoglycinuria is unknown. The transport characteristics of the amino acid transport system expected to be defective in this disorder best fit either the low affinity, proton gradient–driven PAT1 (SLC36A1) imino acid/glycine transporter expressed in the brush border membrane of the early proximal tubule or, alternatively, the high affinity, Na^+- and Cl-dependent SIT1 (IMINO; SLC6A20) imino acid transporter expressed in the brush border membrane throughout the length of the proximal tubule (3, 48, 97) (see Molecular Structure of Amino Acid Transporters). To date, no direct evidence exists that these transporters are implicated in iminoglycinuria.

Differential diagnosis of iminoglycinuria includes neonatal iminoglycinuria, Fanconi syndrome in which generalized aminoaciduria occurs, and hyperprolinemia,

an inborn error of proline metabolism that exhibits overflow prolinuria as well as hydroxyprolinuria and glycinuria secondary to a proline-induced inhibition or hydroxyproline and glycine transport. This inhibition occurs at the shared luminal carrier for these amino acids.

Isolated Glycinuria: Isolated glycinuria without iminoaciduria has been reported in two families (263, 264). It has been suggested that this condition is a manifestation of a mutation affecting the specific high-affinity carrier for glycine, the molecular identity of which remains to be established (15). It is possible, however, that hyperglycinuria represents a defect in the shared, iminoacid-glycine transport system affecting affinity rather than capacity of the system (K_m variant). Because the condition appears to be inherited as an autosomal-dominant trait, it also is possible that these cases represent hyperglycinuric heterozygotes for the iminoglycinuria allele. Glycinuria has also been reported in association with glycosuria, called glycoglycinuria (265). The Na^+- and Cl^--dependent XT2 (SLC6A18), which likely transports glycine and is expressed in the brush border membrane of the late proximal tubule (see Molecular Structure of Amino Acid Transporters) could potentially be involved in the pathogenesis of isolated glycinuria (48). However, the genetic defect underlying this autosomal dominant condition remains unknown.

Dicarboxylic Aminoaciduria

Selective urinary hyperexcretion of the acidic amino acids glutamate and aspartate was first reported in two children (266, 267). One of the children also had impaired intestinal absorption of these amino acids. The condition is inherited as an autosomal-recessive trait and appears to be benign; screening in a French-Canadian population revealed a large number of healthy probands with hyperdicarboxylic aminoaciduria and an incidence of 1:35,000 live births (268).

The pathogenic mechanism appears to be a defect in the dicarboxylic amino acid transport system in the brush-border membrane. The excretion of dicarboxylic amino acids in homozygous patients may greatly exceed the GFR, suggesting tubular secretion or backflux of dicarboxylic amino acids from cell to lumen (15). Treatment with glutamate and aspartate corrected the hypoglycemia observed in one patient (266). This hypoglycemia probably resulted from the absence of these gluconeogenic amino acids.

The Na^+/H^+ and K^+-dependent EAAC1 (SLC1A1; EAAT3) glutamate/aspartate transporter expressed in the luminal membrane of the late proximal tubule (see Molecular Structure of Amino Acid Transporters) is an obvious candidate for the transporter defective in dicarboxylic aminoaciduria (3, 9, 48). Interestingly, Peghini et al. (269) have shown that EAAC1 deficient mice develop dicarboxylic aminoaciduria, a finding that strongly suggests that the EAAC1 gene is a candidate gene for dicarboxylic aminoaciduria. No direct evidence exists, however, for the involvement of EAAC1 in this disease.

An interesting feature of dicarboxylic aminoaciduria is the decreased uptake of anionic amino acids by cultured skin fibroblasts from patients with this condition (270). It has been shown that anionic amino acid-preferring carriers in epithelial brush-border and parenchymal cells have similar characteristics (15, 270). As pointed out by Scriver (15), the apparent expression of the mutant gene in both the brush-border membrane of the epithelium and the plasma membrane of parenchymal cells is unique and contrary to the expected pattern of analogy between basolateral membrane-bound and plasma membrane-bound carriers (see Classic Cystinuria, Hartnup Disease, and Lysinuric Protein Intolerance). The possibility that other transporters of anionic amino acids expressed at the basolateral membrane of the proximal tubule could be implicated in dicarboxylic aminoaciduria awaits future investigation.

β-Aminoaciduria

No inborn error of the luminal membrane-bound, Na^+- and Cl^--dependent transport systems that carry taurine/β-alanine (TAUT; SLC6A6) and betaine/GABA (BGT1; SLC6A12) have been reported in humans. An impaired taurine transport, however, has been found in an inbred mouse strain (C57BL/6J) (271). Studies using kidney slices (271) and isolated basolateral membrane vesicles (149) have localized the transport defect to the antiluminal membrane of the proximal tubular epithelium. In analogy with the defect in LPI, an impaired taurine exit across the basolateral membrane and backflux from cell to lumen may underlie the hypertaurinuria observed in the C57BL/6J mouse (149, 271). A transporter mediating such an exit from cell to interstitium remains to be identified.

Glycosuria

General Characteristics of Renal Glucose Transport

Under normal conditions, the reabsorption of filtered glucose by the renal tubule is almost complete. Less than

0.05% of the renal glucose load is excreted in the human urine (272); 90% of the filtered glucose is reabsorbed in the proximal convoluted tubule and the rest is reclaimed in the proximal straight tubule, the loop of Henle and, to some extent, the collecting duct (273).

Reabsorption of glucose across the proximal tubular brush-border membrane occurs by an active, carrier-mediated, concentrative, Na^+-dependent transport process (272, 273). The Na^+ electrochemical gradient driving glucose transport across the brush-border membrane is maintained by the activity of the basolateral membrane-bound Na^+–K^+-ATPase that pumps Na^+ out and K^+ into the cell. Na^+-glucose cotransport across the luminal membrane is electrogenic positive and phlorizin inhibitable. Glucose exit from the cell occurs by an Na^+-independent, facilitated diffusion down the glucose concentration gradient (273). This diffusional exit of glucose is mediated by a carrier that is distinct from that found at the luminal membrane surface (see later).

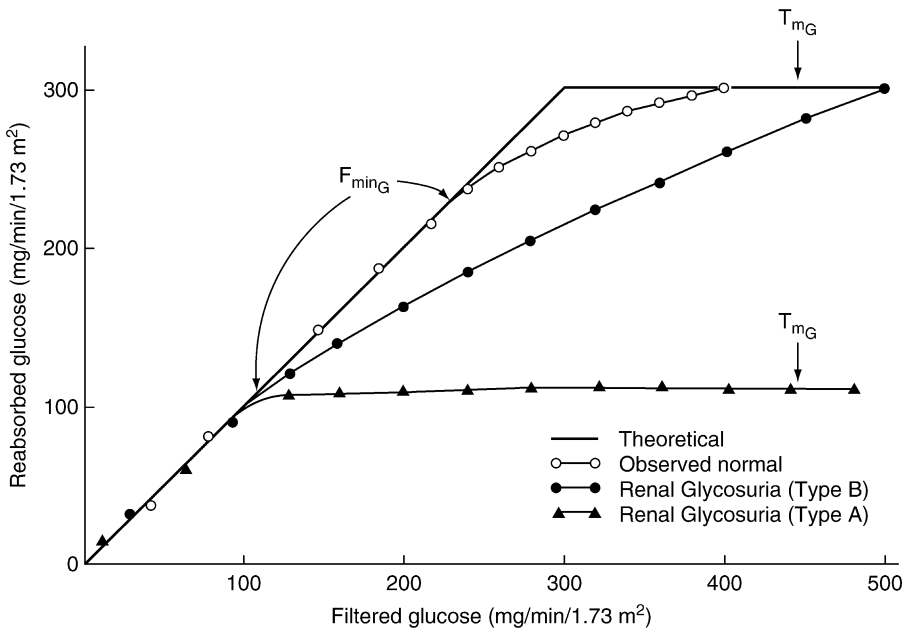
The mammalian kidney is characterized by a limited capacity to reabsorb D-glucose (272). As plasma glucose concentration is progressively elevated, the amount of glucose reabsorbed increases linearly until a maximum value is reached (*Fig. 37-7*). Beyond this maximum rate of glucose reabsorption, further increases of filtered D-glucose are excreted in the urine. As shown in *Fig. 37-7*, the glucose titration curve provides parameters of glucose transport such as minimum threshold (F_{minG}), defined as the filtered glucose load at which 1 mg of glucose per min appears in the urine, and the tubular maximum for glucose reabsorption (Tm_G). Reported Tm_G values for glucose in human adults and children have ranged between 260 and 350 mg/min/1.73 m^2 (275–277), with lower values in infants (277, 278). When corrected for the GFR (Tm/GFR), transport maximum value for glucose in infants, children, and adults is approximately 2.5 mg/mL (277).

The glucose titration curve (*Fig. 37-7*) is characterized by a splay, a rounding of the curve during the transition from virtually complete reabsorption of filtered D-glucose to complete excretion of excess glucose load. This deviation from the theoretical curve is explained by the nephron functional heterogeneity (the gradual progression from the first to the last nephron to saturate) as well as by the variation in affinity for glucose or K_m values between carriers (the magnitude of the splay is inversely proportional to the affinity of the transporter for the D-glucose) (272).

Studies in isolated perfused tubules (279) and later experiments using isolated BBMV from pars convoluta

Figure 37-7

Renal glucose titration curves. Theoretical and observed normal curves are compared to abnormal curves observed in type A and type B renal glycosuria. Tm_G, maximum rate for glucose reabsorption; F_{minG}, minimum threshold (reprinted with permission from (274)).



(S1 and S2 segments) and pars recta (S3 segment) of the proximal tubule (280–282) demonstrate that the kinetic properties and stoichiometric relationships of D-glucose reabsorption change along the length of the nephron. These studies provide evidence for two Na^+-dependent transport mechanisms, a low-affinity/high-capacity cotransport system in the early proximal tubule and a high-affinity/low-capacity system in the late proximal tubule. The Na^+-glucose coupling ratio was found to be 1:1 in the convoluted proximal tubule and 2:1 in the straight proximal tubule (281, 282).

The efficiency of a coupled carrier system increases as the power of the stoichiometry (273, 282). Whereas the early proximal tubule glucose transporter is responsible for the reabsorption of the bulk of filtered D-glucose from the tubular lumen, the late proximal tubule glucose transporter is responsible for the removal of the last traces of glucose from the urine (282). The arrangement of transporters in series along the proximal tubule enables the kidney to reabsorb glucose from the urine in a more energy-efficient mechanism than can be achieved by either of the cotransporters acting alone.

Molecular Biology of Na^+-Glucose Cotransporters

Various approaches have been used to identify and isolate the Na^+-glucose cotransporters. Earlier methods including solubilization and reconstitution techniques, semiselective and photoaffinity labeling, phlorizin affinity chromatography, radiation inactivation, and immunoaffinity absorption (273) have been inconclusive and yielded very limited biochemical data. However, considerable progress has been made in the past two decades in elucidating the molecular structure of membrane proteins that catalyze sugar transport processes, including the Na^+-glucose cotransporter (272, 283–285). Hediger et al., using expression cloning in *Xenopus* oocytes (see earlier), cloned and sequenced the first to be identified Na^+-dependent D-glucose transporter from rabbit (44) and human (286) intestine. Subsequently, molecular analysis revealed that rabbit intestinal and renal Na^+-glucose cotransporters are essentially identical (287). The transporter was later termed SGLT1 and the gene encoding it, SLC5A1 (284).

The Na^+-glucose cotransport proteins belong to the SLC5 family of sodium cotransport proteins, which includes more than 220 eukaryotic and prokaryotic homologues (284, 285). Included in the SLC5 family are 11 human genes most of which are plasma membrane, Na^+- substrate cotransporters for solutes such as glucose, *myo*-inositol and iodide. The group contains six Na^+-glucose cotransporters (SGLTs) all of which are expressed in the kidney among other organs (284, 285).

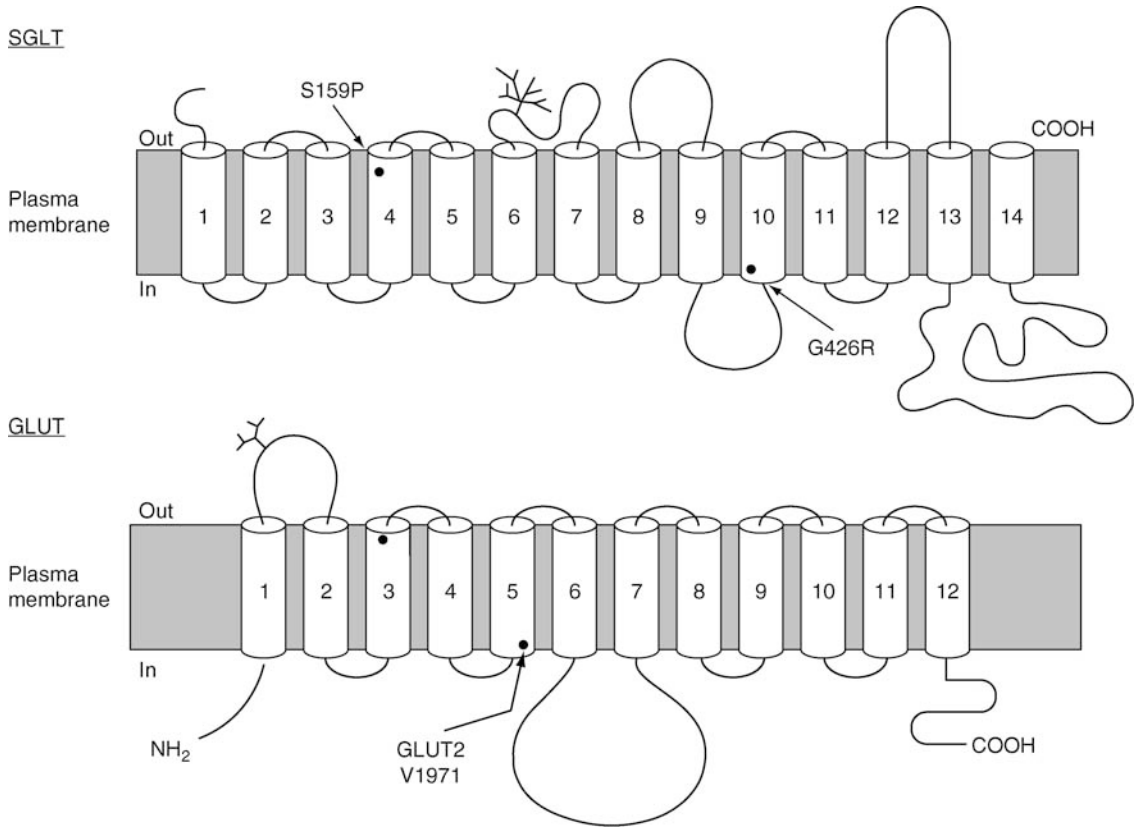
The SGLT1 (SLC5A1) transporter, the prototype of the SGLT group (*Fig. 37-8*), consists of 662–664 amino acids and has 14 membrane-spanning sequences that are presumed to be α-helical, with the NH_2 and COOH termini located on the cytoplasmic side of the membrane. A simple glycosylation site is found in the hydrophilic domain between transmembrane segments 5 and 6 (288). SGLT1 from rabbit (44), rat (289), pig (290), and human (286) show high homology in sequence and structure. The gene encoding the human intestinal SGLT1 has been localized to the q11.2lqter region of chromosome 22 (291). Studies using *Xenopus* oocytes (292) demonstrate that protein kinases (PKA and PKC) regulate SGLT1 activity by controlling the distribution of transporters between intracellular compartments and the plasma membrane and that this occurs by exocytosis and endocytosis.

Studies by Pajor et al. (293) and Lee et al. (289) using expression studies in *Xenopus* oocytes, Western and Northern analysis, and in situ hybridization and immunocytochemistry have provided evidence that the renal Na^+-glucose cotransporter SGLT1 is the high-affinity/low-capacity transporter found predominantly in the straight proximal tubule (S3 segment). Evaluation of the stoichiometry of SGLT1-mediated transport in *Xenopus* oocytes revealed a Na^+ to glucose coupling ratio of 2:1 (289). Recent structure – function studies, using functional analysis of mutated and truncated proteins as well as chimeras, have revealed that the COOH terminal domain containing the 5 terminal transmembrane helices is involved in sugar binding and translocation (294, 295). The SGLT1 (SLC5A1) gene has been implicated in hereditary glucose – galactose malabsorption (see Glycosuria).

A second low-affinity Na^+-glucose cotransporter named SGLT2 (SLC5A2) was isolated from rat (296) and human (297, 298) kidney. The amino acid sequence of SGLT2 is 59% identical to that of SGLT1 (297). SGLT2 has a Na^+ glucose coupling ratio of 1:1, does not recognize galactose (which is a substrate for SGLT1), and is strongly expressed in proximal tubule early (S1 and S2) segments (298). Human SGLT2 gene was mapped to chromosome 16p11.2 close to the centromere (299). SGLT2 is poorly expressed in cultured cells and oocytes which has precluded thorough investigation of its properties and function. It has been hypothesized that a second protein is required for the insertion of SLGT2 into the membrane (284). The accumulating clinical, physiologic and molecular data

Figure 37-8

Schematic structure of the Na^+/glucose cotransporter (SGLT) and the facilitative glucose transporter (GLUT). The two mutations shown in SGLT were identified in one of the original families with glucose-galactose malabsorption (adapted with permission from (308)).



made the SGLT2 gene, SLC5A2, a candidate for the affected gene in hereditary renal glycosuria (see Glycosuria).

A third Na^+-dependent glucose transporter, termed SGLT3 (SLC5A4), was isolated from a LLC-PK_1 pig renal cell line (300). It is a low-affinity, Na^+-glucose (not galactose) cotransporter with a very low expression level in renal tissue (272, 301). Human SGLT3 gene is located on chromosome 22 (284). It should be noted that human SGLT3 has been shown to function as a glucose – gated ion channel in muscles and neurons rather than a Na^+/glucose cotransporter (302). Two additional SGLT proteins expressed in the kidney are SGLT4 (SLC5A8) and SGLT5 (SLC5A10), the properties of which in general and in the kidney, in particular, have not been explored (283).

It is noteworthy that some of the SGLT proteins display unexpected properties in addition to their Na^+-glucose cotransport activity. SGLT1 (SLC5A1) has been shown to serve as a water channel, a urea channel and a cotransporter of both water and urea (303–305).

Facilitative Glucose Transporters

Glucose concentrated inside tubular epithelial cells flows to the interstitium down its concentration gradient through facilitative glucose transporters located in the basolateral membrane (272, 306). The basolateral Na^+-independent and the luminal Na^+-dependent glucose transport systems also differ with respect to inhibition and specificity (273). The basolateral glucose transporter is inhibited by the mold metabolite cytochalasin-B, but not by phlorizin. It also accepts D-glucose.

The basolateral membrane glucose transporters belong to the SLC2 family of Na^+-independent facilitative hexcose (and polyol) transporters which comprises 13 members including 12 facilitative glucose transporters GLUT1–12 (306–309). All these isoforms have extensive structural homologies but differ in their tissue distribution, specific function, insulin sensitivity, sugar specificity, and kinetic characteristics (309). The human erythrocyte

glucose transporter GLUT1 (SLC2A1) was the first glucose transporter to be cloned and sequenced (310). It is the most ubiquitously distributed of the transporter isoforms (307, 309). Isoforms GLUT2–5 were subsequently cloned by screening cDNA libraries from various tissues and species with a GLUT1 DNA probe and this was followed by cloning GLUT6–12 (306, 309). The facilitative glucose transporters (*Fig. 37-8*) have 12 membrane-spanning domains, with both the NH_2 and the COOH termini of the protein facing the cytoplasm (309). A single glycosylation site is located between transmembrane domains 1 and 2.

GLUT1 (SLC2A1) is ubiquitors with different levels of expression in different cell types (309). Studies using antipeptide antibodies specific for GLUT1 have detected this high-affinity transporter in the kidney (311–313). It was found in the basolateral membrane of cells forming the proximal straight tubule (S3 segment). GLUT2 (SLC2A2), a low-affinity transporter, is the predominant facilitative glucose transporter in hepatocytes and in the basolateral membrane of intestinal and renal tubular cells (311, 312). In the kidney, GLUT2 is present only in the basolateral membrane of cells in the proximal convoluted tubule (S1 and S2 segments) (311, 312). SLC2A2 has been identified as the mutated gene in Fanconi – Bickel syndrome (see Glucosuria). GLUT5 has also been identified in the kidney (314).

Thus, it appears that transepithelial glucose transport in the proximal tubule occurs by two different pairs of apical Na^+-dependent and basolateral Na^+-independent glucose transporters (272, 306) (*Fig. 37-9*). A luminal Na^+-dependent, low-affinity/high-capacity glucose transporter designated SGLT2/SLC5A2 and a basolateral Na^+-independent, low-affinity GLUT2/SLC2A2 are responsible for the bulk of glucose reabsorption in the early part of the proximal tubule. A luminal Na^+-dependent, high-affinity/low-capacity SGLT1/SLC5A1, coupled with the basolateral Na^+-independent, high-affinity GLUT1/SLC2A1, reabsorb the remaining low concentration of glucose in the late part of the proximal tubule.

Maturation of Glucose Transport

The immature renal tubule in animals (315, 316) and humans (277, 317, 318) is characterized by decreased ability to reabsorb glucose. Tubular reabsorption of glucose relates directly to postnatal age and glycosuria occurs commonly in infants less than 30 weeks' gestational age (317–319). There are few data on the development of renal glucose transport mechanisms. The fetal kidneys of various animal species (315, 320, 321) have been shown to reabsorb glucose. In the fetal rat kidney (320), glucose reabsorption appeared to be Na^+ dependent and phlorizin inhibitable. A decreased initial rate uptake of -methyl-D-glucoside was found in isolated renal tubules from neonatal rat (322) and dog (316). BBMV studies (323) demonstrated a concentrative, Na^+-dependent, electrogenic and phlorizine-sensitive glucose transport in the fetal rabbit kidney. The fetal glucose transport mechanism, however, had a significantly lower capacity than the adult glucose transport system. Experiments exploring the expression of SGLT1 and SGLT2 mRNAs in embryonic rat kidneys (296) revealed that the two Na^+-glucose cotransporters appeared early in gestation and that they were developmentally regulated. Evidence suggests that the increase in SGLT1 mRNA accompanying cell differentiation in the pig kidney cell line LLC-PK1 is regulated by PKA (324) and PKC (325).

Little is known about the maturation of the facilitative glucose transporters in the kidney. A study (326) examining the developmental pattern of these transporters in the rat kidney showed that renal GLUT1 and GLUT5 gene expression was unchanged throughout development, whereas GLUT2 was most abundant before weaning. The latter finding may be related to the fact that the kidney alone seems to be responsible for gluconeogenesis before expression of gluconeogenic enzymes by the liver.

Further studies are needed to explore the activity, expression, and distribution of various glucose transporters during kidney development and to elucidate the molecular mechanisms underlying the maturation of renal tubular glucose transport.

Glycosuria

Hereditary renal glycosuria is an abnormality in which variable amounts of glucose are excreted in the urine at normal concentrations of blood glucose (327). The renal defect is specific for glucose and there is no increase in the urinary excretion of other sugars. Renal glycosuria is a benign condition without symptoms or physical consequences except during pregnancy or prolonged starvation, when dehydration and ketosis may develop (327). The metabolism, storage, and use of carbohydrates as well as insulin secretion are normal. The condition exists from infancy throughout adult life, and diagnosis usually is done on routine urine analysis. The distinction between renal glycosuria and diabetes mellitus is made with a fasting blood glucose level and a glucose tolerance test.

The genetic pattern in renal glycosuria is autosomal recessive, although glycosuria in some heterozygotes has led some investigators to postulate a dominant inheritance (327, 328). Renal glycosuria is not associated with impaired D-glucose transport in the intestinal epithelium (329). The underlying pathogenic mechanism appears to be an isolated, selective defect in proximal tubular glucose transport.

Renal glycosuria is a heterogenous condition. Analysis of renal titration curves for glucose reabsorption reveals two types of renal glycosuria (274, 327) (*Fig. 37-7*). In type A, or classic renal glycosuria, minimal glucose threshold (F_{minG}) and maximum rate of glucose reabsorption (Tm_G) are reduced. In type B, F_{minG} is reduced, while Tm_G is normal but has an increased splay. It has been suggested that the type A mutation reflects reduction in the capacity of the glucose transport system, which might arise from a uniform defect in all nephrons, and type B reflects a decrease in the affinity of the transport system, which might also be a consequence of nephron heterogeneity (329, 330). A third type of glycosuria, termed type O, was described by Oemar et al. (331). In this rare condition, tubular reabsorption of glucose is virtually absent, and all glucose filtered is excreted in the urine.

In 2002, the SGLT2 gene, SLC5A2, was first established as the mutated gene in hereditary isolated glycosuria (332) (*Fig. 37-9*). Subsequent studies have confirmed this finding (333–337). To date, close to 30 mutations have been identified in patients with hereditary renal glycosuria. These include missense, nonsense and frame shift mutations.

Of note, some SLC5A2 mutations may result in renal glycosuria accompanied by generalized aminoaciduria (336). Renal glucose wasting associated with generalized aminoaciduria is also a feature of maturity-onset diabetes of the young type 3 (MODY 3) caused by a mutation in the hepatocyte nuclear factor – 1 alpha (HNF-1α) gene (338). HNF-1α acts as a regulator of both transcription of SLC5A2 ((339, 340) and expression of collectrin, a mediator of amino acid transport (341) (see Hartnup Disease). Hence, aberrant HNF-1α may constitute the link between the glycosuria and the aminoaciduria observed both in some cases of hereditary renal glucosuria and in MODY 3.

It is important to consider the relationships between renal glycosuria, a benign condition, and intestinal glucose-galactose malabsorption, a potentially lethal disease. Glucose-galactose malabsorption, an autosomal-recessive disease, is characterized in homozygotes by a neonatal onset of severe watery diarrhea that results in death unless glucose and galactose are removed from the diet (327). Studies using jejunal biopsy specimens from affected patients have demonstrated a defect in intestinal Na^+-dependent glucose transport (342). In 1991, molecular genetic studies in two sisters afflicted with glucose-galactose malabsorption have revealed a missense mutation that causes a change in residue 28 from aspartate to asparagine in the intestinal brush-border SGLT1 Na^+-glucose cotransporter (343). This made glucose-galactose malabsorption the first reported disease that is caused by a mutation in a membrane transport protein. To date, more than 40 mutations have been identified in patients with glucose-galactose malabsorption (284, 327). The mutant proteins, when expressed in *Xenopus* oocytes cause a marked reduction in Na^+-glucose transport activity (327), which, in most cases is due to missorting of the protein in the cell (284).

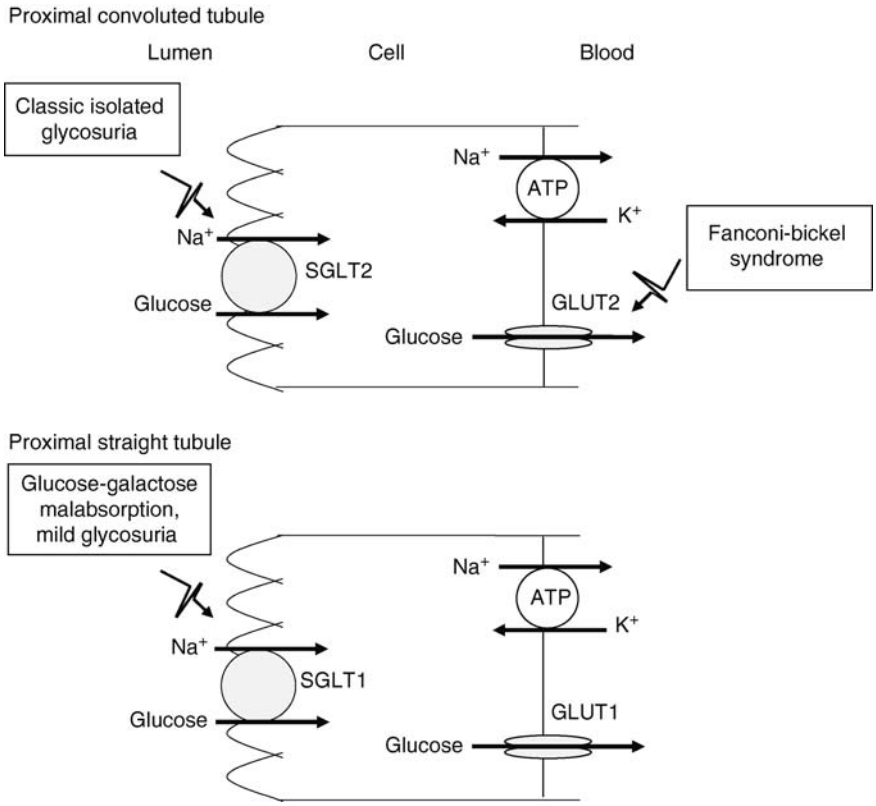
Patients with glucose-galactose malabsorption who have been studied show a mild defect in renal tubular reabsorption of glucose, with normal Tm for glucose but decreased minimal threshold (F_{minG}) (327, 342). In contrast, patients with renal glycosuria show no defect in intestinal D-glucose absorption. This has indicated that the SGLT1 Na^+-glucose cotransporter affected in glucose-galactose malabsorption is shared between the intestine and the kidney, as also suggested by the molecular studies of Pajor et al. (293) and Lee et al. (289) (see earlier). It has been evident that the glucose transporter impaired in glycosuria is not shared.

The accumulating clinical, physiologic, and molecular data on renal glucose transport have led to the following model of the pathogenesis of hereditary renal glycosuria (272, 274) (*Fig. 37-9*). A defect in the low-affinity/high-capacity, 1 Na^+:1 glucose cotransporter (SGLT2/SLC5A2) of the early proximal tubule, which reabsorbs most renal tubular glucose, would produce type A or type O, classic renal glycosuria but has no effect on glucose absorption in the intestine. By contrast, SGLT1/SLC5A1, the high-affinity/low-capacity, 2 Na^+:1 glucose cotransporter of the late proximal tubule (which also carries galactose) mediates residual glucose reabsorption in the renal tubule and, when defective as in glucose-galactose malabsorption, causes only mild type B renal glycosuria.

Mutations in the gene for GLUT2 (SLC2A2), the basolateral membrane – bound, facilitative glucose transporter of the proximal convoluted tubule, are also associated with glycosuria in the Fanconi-Bickel syndrome (344–346) (*Fig. 37-9*). This autosomal recessive disorder is characterized by hepatorenal glycogen accumulation, fasting hypoglycemia, impaired utilization of glucose and galactose. Fanconi syndrome, rickets and markedly stunted growth. The renal loss of glucose is

Figure 37-9

Schematic model for the distribution (luminal or basolateral) of cloned glucose transporters in renal tubular epithelium. Orientation permits net reabsorption from lumen to interstitium. Depicted are hereditary glycosurias caused by defects in these transporters. See text for details.



due to the transport defect for monosaccharides across the renal basolateral membrane. To date, more than 30 different mutations in SLC2A2 have been detected in patients with Fanconi-Bickel syndrome (347). Knockout mice lacking the GLUT2 gene exhibit extreme glycosuria (348). Therapy of patients with Fanconi Bickel syndrome is symptomatic and includes stabilization of glucose homeostasis and replacement of renal solute losses (346).

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38 Tubular Disorders of Electrolyte Regulation

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In this section, we will discuss the inherited disorders associated with defective tubular handling of NaCl, causing secondary aldosteronism and hypokalemia (Bartter-like syndromes), abnormal handling of calcium and magnesium, the states of low-renin hypertension with hypokalemia, and the two forms of pseudohypoaldosteronism (type I and type II). We will not address other types of inherited tubulopathies, such as renal Fanconi syndrome, diabetes insipidus, or renal tubular acidosis, which are detailed elsewhere.

Bartter-Like Syndromes

Introduction

In 1962, F. Bartter and co-workers described two African American patients with a new syndrome, characterized by hypokalemic metabolic alkalosis, renal K^+ wasting, hypertrophy and hyperplasia of the juxtaglomerular apparatus, and normotensive hyperaldosteronism (1). The disorder also featured increased urinary excretion of prostaglandins, high plasma renin activity, and a relative vascular resistance to the pressor effects of exogenous angiotensin II (1). For decades, many similar cases and several phenotypic variants have been progressively identified and included in a group of hypokalemic salt-losing tubulopathies, referred to as Bartter-like syndromes (2). All these disorders are recessively inherited and associated with hypokalemia and hypochloremic metabolic alkalosis due to stimulation of the renin-angiotensin-aldosterone system (RAAS). However, they markedly differ in terms of age of onset, severity of symptoms, presence of urinary concentrating defect, other electrolyte abnormalities (including hypomagnesemia), and magnitude of urinary calcium excretion. Over the years, it became apparent that these tubulopathies affect salt handling in distinct nephron segments, based on the analogy between patient's symptoms and the effects of loop and thiazide diuretics affecting the thick ascending limb (TAL) and the distal convoluted tubule (DCT), respectively.

In the normal nephron, the TAL reabsorbs approximately 25% of the filtered NaCl load. The apical Na^+–K^+–$2Cl^-$ cotransporter NKCC2 mediates the uptake of Na^+, K^+ and Cl^- from the lumen into the epithelial cells, driven by the electrochemical gradient for Na^+ established by the basolateral Na^+-K^+-ATPase. The K^+ channel ROMK recycles K^+ across the luminal membrane, whereas the ClC-Ka and ClC-Kb Cl^- channels coupled to their beta-subunit barttin are responsible for the basolateral exit of Cl^- (*Fig. 38-1*). These concerted transport processes are crucial for the vectorial NaCl transport in the TAL, and thus the urinary concentrating ability, and for generating the lumen-positive electrical charge that drives the paracellular reabsorption of Na^+, Ca^{2+} and Mg^{2+} in this segment (3). The importance of NKCC2 in the TAL transport is evidenced by the effects of loop diuretics, which, as pharmacologic NKCC2 inhibitors, induce a strong increase in urinary water, salt, and calcium excretion. The DCT is responsible for the reabsorption of 5–10% of the filtered NaCl (4). Driven by the activity of the basolateral Na^+-K^+-ATPase, Na^+ enters the DCT cells via the thiazide-sensitive Na^+-Cl^- cotransporter, NCCT (or TSC, for thiazide-sensitive cotransporter). Because it is coupled to Na^+, Cl^- moves into the cell against its electrochemical gradient and then passively exits through the ClC-Kb channel in the basolateral membrane. The DCT cells are also involved in K^+ secretion, through the K^+ channel ROMK and a K^+-Cl^- cotransporter located in the apical membrane, and the transcellular reabsorption of Ca_2^+ and Mg_2^+, via the TRPV5/6 and TRPM6 channels, respectively, which belong to the transient receptor potential (TRP) channel family (5). Thiazide diuretics, which specifically bind and inhibit NCCT, induce a milder diuretic response than loop diuretics, typically associated with magnesium wasting and hypocalciuria.

Based on clinical manifestations, the Bartter-like syndromes were grouped into two major groups: the antenatal Bartter syndrome (aBS) (also named hyperprostaglandin-E syndrome (HPS)), which can be associated or not with sensorineural deafness (SND); and the classic Bartter and Gitelman syndromes (cBS and GS, respectively). Despite

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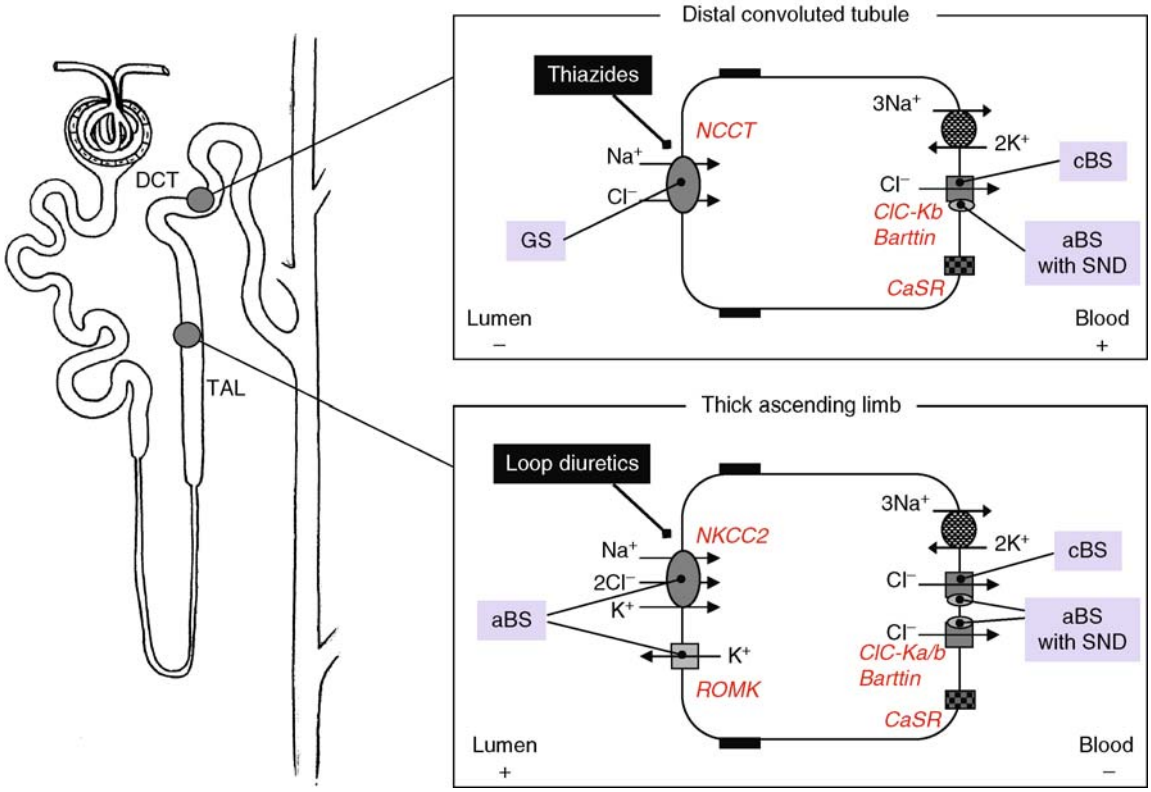
Figure 38-1

Molecular basis of Bartter-like syndromes. Approximately 25% of the filtered NaCl is reabsorbed in the thick ascending limb (TAL) via the apical Na^+–K^+–$2Cl^-$ cotransporter NKCC2 (inhibited by loop diuretics), organized in parallel with the apical K^+ channel ROMK to ensure K^+ recycling and the lumen-positive voltage. The Na^+–K^+-ATPase and the Cl^- channels ClC–Ka and ClC–Kb associated with the regulatory beta-subunit Barttin mediate the exit of Na^+ and Cl^- ions from the cells. The thiazide-sensitive Na^+–Cl^- cotransporter NCCT mediates 5–10% of the NaCl reabsorption in the distal convoluted tubule (DCT). Loss of function mutations in *SLC12A1* (coding for NKCC2) and *KCNJ1* (coding for ROMK) cause antenatal Bartter syndrome (aBS), whereas inactivating mutations of *BSND* encoding the beta-subunit barttin cause antenatal Bartter syndrome with sensorineural deafness (aBS with SND) and mutations in *CLCNKB* (ClC-Kb) cause classic Bartter syndrome (cBS). Inactivating mutations in *SLC12A3* (coding for NCCT) are associated with Gitelman syndrome (GS). It must be noted that a few patients with autosomal dominant hypocalcemia due to severe gain-of-function mutations of the CASR may present a salt-losing, Bartter-like tubulopathy.



some overlapping features, the aBS group included disorders affecting the TAL, with furosemide-like manifestations, whereas the second group—and GS in particular—was related to a defect in the DCT, with thiazide-like manifestations (2). From 1996, a series of seminal studies by Lifton and colleagues identified loss-of-function mutations in transporters and channels responsible for these inherited tubulopathies. The aBS was associated to inactivating mutations in the genes encoding the apical NKCC2 (6) or ROMK (7), whereas inactivating mutations in barttin, a regulatory beta-subunit of the basolateral ClC-Ka and ClC-Kb channels, were detected in aBS with SND (8). On the other hand, inactivating mutations of ClC-Kb, which is located both in the TAL and DCT, were associated with the cBS (9), whereas GS was found to be associated with mutations of NCCT (10) (*Fig. 38-1*).

A classification of these salt-losing tubulopathies, based on the clinical, physiological, and molecular insights discussed above, provides a basis to understand the distinct phenotypes of these disorders (*Table 38-1*). When discussing such patients, the clinical diagnosis of the BS subtype, based on relatively simple clinical criteria, should

Table 38-1

Inherited Bartter-like salt-losing tubulopathies

Disorder	OMIM #	Inheritance	Gene locus	Gene	Protein	Affected tubular segment
Antenatal Bartter syndrome (aBS), Hyperprostaglandin-E syndrome (HPS), Type I Bartter syndrome[a]	601678	AR	15q15-q21.1	*SLC12A1*	Na^+–K^+–$2Cl^-$ cotransporter NKCC2	TAL
Antenatal Bartter syndrome (aBS), Hyperprostaglandin-E syndrome (HPS), Type II Bartter syndrome[a]	241200	AR	11q24	*KCNJ1*	K^+ channel ROMK (Kir1.1)	TAL + CCD
Antenatal Bartter syndrome with sensorineural deafness (aBS with SND)[b], Type IV Bartter syndrome[a]	602522	AR	1p31	*BSND*	Barttin, beta-subunit of ClC-Ka/b	TAL + DCT
Classic Bartter syndrome (cBS) Type III Bartter syndrome[a]	607364	AR	1p36	*CLCNKB*	Cl^- channel ClC-Kb	TAL + DCT
Gitelman syndrome (GS)	263800	AR	16q13	*SLC12A3*	Na^+-Cl^- cotransporter NCCT	DCT

TAL thick ascending limb; *CCD* cortical collecting duct; *DCT* distal convoluted tubule

[a]This classification is based on the chronological order of gene discovery

[b]A digenic disorder with inactivating mutations of *CLCNKA* and *CLCNKB* has been associated with the aBS with SND phenotype

be completed whenever possible by the genotyping information since there is no direct genotype-phenotype correlation in these diseases (2, 11).

Antenatal Bartter Syndrome

Genetics

Antenatal Bartter syndrome (aBS, OMIM #601678, #241200) is a rare, life-threatening disorder characterized by massive polyuria that manifests *in utero* with the development of polyhydramnios and premature delivery in almost all cases. Affected neonates rapidly develop salt wasting, hypokalemic metabolic alkalosis, and profound polyuria (12–14). The disorder is accompanied by markedly elevated urinary PGE_2 excretion, and treatment with PG synthesis inhibitors effectively reduces clinical and biochemical manifestations, explaining why aBS is also designed as hyperprostaglandin-E syndrome (HPS) (15). As patients with aBS/HPS fail to respond to loop diuretics such as furosemide, a defective NaCl reabsorption in the TAL was suspected (16). By combining a candidate gene approach with linkage analysis, Simon et al. demonstrated that aBS/HPS is either due to mutations in NKCC2 (type I BS) or in ROMK (type II BS) (6, 7) (*Fig. 38-1*). The two forms of aBS are clinically and biochemically hardly distinguishable (17).

Type I BS is due to mutations in the *SLC12A1* gene located on 15q15-q21.1 and containing 26 exons (6, 14, 18). The *SLC12A1* gene codes for the bumetanide-sensitive NKCC2, a 121 kD protein with 12 putative membrane-spanning domains. NKCC2 is expressed in the apical membrane of epithelial cells lining the TAL and in the macula densa (19). Loop diuretics bind to portions of transmembrane domains 11 and 12, whereas portions of domains 2, 4, and 7 are involved in ion transport (20). At least 30 mutations, essentially missense or frameshift, have been described thus far (21). The 5 initial kindreds (6), and others reported subsequently (14, 18) were consanguineous, but compound heterozygotes and patients harboring only one heterozygous mutation have been reported (14, 18, 21, 22). Although a founder mutant allele (W625X) was reported in a cohort of Costa Rican patients (22), the aBS mutations are evenly distributed throughout the *SLC12A1* gene (21). Of note, alternative splicing of the NKCC2 pre-mRNA results in the formation of three full-length isoforms of NKCC2, which differ in their variable exon 4, their localization along the TAL, and their transport characteristics (23). Accordingly, one could speculate that mutations affecting low-capacity/high-affinity isoform might result in a milder phenotype (14).

Type II BS has been linked to mutations in the *KCNJ1* gene that is located on chromosome 11q24 and contains 5 exons (7). The *KCNJ1* gene encodes ROMK (also known as Kir1.1), an ATP-sensitive, inwardly-rectifying renal K^+ channel that is critical for K^+ recycling in the TAL and K^+ secretion in the distal nephron (24). ROMK channels are assembled from four subunits, each consisting of two transmembrane domains flanking a conserved loop that contribute to the pore and selectivity filter, and cytoplasmic N and C termini that contain regulatory and oligomerization domains (25). ROMK exists in three N-terminal splice variations that all behave as rectifying K^+ channels gated by intracellular pH (24). Through the recycling of reabsorbed K^+ back to the lumen, ROMK is believed to be a regulator of NKCC2 cotransporter activity. Therefore, loss of function in ROMK, as well as in NKCC2, disrupts NaCl reabsorption in the TAL. At least 40 mutations in *KCNJ1* that coseggregate with aBS have been reported, including missense, nonsense, frameshift and deletions (7, 17, 26–30). The first mutations were reported in exon 5, common to all ROMK isoforms (7, 17), but homozygous deletions in exons 1 and 2 have also been reported (26, 29).

Clinical Manifestations

Typical features of aBS type I (NKCC2) and type II (ROMK) include polyhydramnios (within the second trimester of gestation), premature delivery (around 32 weeks), severe polyuria, life-threatening episodes of dehydration, hypercalciuria, leading to nephrocalcinosis within the first months of life, and activation of the RAAS (*Table 38-2*). The polyuria can be massive (>20 mL/kg/h) despite adequate fluid replacement. Magnesium wasting is not common in aBS (31), although hypomagnesemia was evident in half of the Costa Rican patients (32). Failure to thrive and growth retardation are invariably observed (13, 32, 33). A peculiar facies, characterized by a triangularly shaped face, prominent forehead, large eyes, protruding ears and drooping mouth, has been reported but could reflect dystrophic premature babies (22, 32). Systemic manifestations including fever of unknown origin, diarrhea, vomiting, generalized convulsions, which have been attributed to enhanced systemic overproduction of PGE, as well as recurrent urinary tract infection may occur (31, 34). Osteopenia is common in aBS (13, 35), associated with high urinary excretion of bone resorption markers (36). Increased urinary PGE2 excretion is usually detected, although not invariably (2, 13). Hypophosphatemia with decreased tubular phosphate reabsorption has been described, possibly related to tubular damage and hypokalemic nephropathy (33, 36). High Cl^- and aldosterone concentrations in the amniotic fluid have been reported (13).

Although rare, phenotype variability among NKCC2-deficient patients has been reported, including absence of hypokalemia and/or metabolic alkalosis during the first years of life and persistent metabolic acidosis or hypernatremia (18). The Costa Rican cohort harboring the W625X founder allele showed a somewhat milder phenotype with a median age of diagnosis at 10 months of life, and no necessity of indomethacin treatment in most patients (22, 32). A late-onset presentation (age 13 and 15 years) with mild polyuria and borderline hypercalciuria has been reported in two brothers compound heterozygotes for NKCC2 mutations (37).

While renal function is generally well preserved in aBS (18, 32), progressive renal failure leading to ESRD has been reported (32, 38, 39). The potential mechanisms that could lead to kidney damage in aBS include consequences of early neonatal events and dehydration episodes, hypokalemic nephropathy, nephrocalcinosis, and nephrotoxicity of NSAID (38, 40, 41). Renal biopsies of children with aBS revealed a marked hypertrophy and hyperplasia of the juxtaglomerular apparatus, with stimulation of the renin-angiotensin system (42, 43). This feature is not specific for aBS and may be observed in all Bartter-like syndromes. Reinalter et al. (41) showed inflammatory infiltrates, with areas of interstitial fibrosis, focal tubular atrophy with thickening of basement membranes and degenerated tubular epithelia, and focal segmental mesangial matrix increase and hypercellularity in renal biopsies obtained in 10 aBS/HPS patients. Of interest, patients harboring ROMK mutations had only minimal histological lesions as compared with NKCC2 patients and cBS patients (41).

Because NKCC2 and ROMK are functionally coupled in the apical membrane of the TAL, patients with a defective ROMK have a very similar clinical picture than NKCC2 deficiency, with polyhydramnios, premature delivery, severe neonatal polyuria with isosthenuria, and hypercalciuria with secondary nephrocalcinosis (2, 31). However, there is an important difference in that ROMK-deficient patients show a transient hyperkalemia during the first days of life, correlated with gestational age (31, 44). The association of such hyperkalemia with hyponatremia and hyperreninemic hyperaldosteronism may erroneously suggest the diagnosis of pseudohypoaldosteronism type 1 (PHA1) (44). Renal K^+ wasting in these cases may not be apparent until 3–6 weeks postnatally, leading to modest hypokalemia in most patients. Typically,

Table 38-2

Clinical and biochemical features of Bartter-like syndromes

Feature	aBS (*SLC12A1*) Type I BS	aBS (*KCNJ1*) Type II BS	aBS with SND (*BSND*) Type IV BS	cBS (*CLCNKB*) Type III BS	GS (*SLC12A3*)
Age of onset	Antenatal	Antenatal	Antenatal	Variable	Childhood, adolescence
Maternal polyhydramnios	Present	Present	Present	Rare	Absent
Prematurity	Present	Present	Present	Rare	Absent
Polyuria	Present	Present	Present	Occasional	Absent
Failure to thrive	Present	Present	Present	Common	Absent
Growth retardation	Present	Present	Present	Common	Occasional
Spasm/tetany/ muscle weakness	Absent	Absent	Absent	Occasional	Present
Nephrocalcinosis	Present	Present	Absent	Rare	Absent
Sensorineural deafness	Absent	Absent	Present	Absent[a]	Absent
Dehydration episodes	Severe	Severe	Severe	Severe	Mild
Hypokalemic metabolic alkalosis	Present	Present (transient neonatal hyperkalemia)	Present	Present	Present
Plasma Mg^{2+}	Normal	Normal	Normal or low	Normal or low	Low
Urinary Ca^{2+} excretion	High	High	Moderate (transient) or normal	Usually normal	Low
Urinary NaCl excretion	High	High	Very high	Variable increase	Mild increase
Maximal urine osmolality	Hyposthenuria	Hyposthenuria	Iso-/hyposthenuria	Usually normal	Normal
High renin/ aldosteronism	Present	Present	Present	Present	Present
Urinary PGE2 excretion	High	High	High	Slightly elevated	Usually normal

PGE2 prostaglandin E2

[a]SND is present in case of digenic disorder with inactivating mutations of *CLCNKA* and *CLCNKB*

hypokalemia in ROMK-deficient patients is less severe than that observed in NKCC2-patients (29, 31).

Recently, Ji and Lifton reported that heterozygote carriers of inactivating mutations in NKCC2 and ROMK in the general population had significantly lower systolic and diastolic blood pressure, and a significant reduction in the risk of developing hypertension (45). Furthermore, Tobin et al. (46) showed that polymorphisms in *KCNJ1* were associated with blood pressure values in a cohort of 2,037 adults after adjusting for age, sex, and familial correlations. Taken together, these studies suggest that the transporters involved in NaCl handing in the TAL may exert a profound influence on blood pressure regulation (47).

Differential Diagnosis

Antenatal BS should always be suspected in face of polyhydramnios due to fetal polyuria. As discussed above, patients with type II aBS due to ROMK deficiency may show a transient hyperkalemia, which can mimick PHA1. However, PHA1 is characterized by permanent hyperkalemia with metabolic acidosis, whereas type II aBS patients

typically have metabolic alkalosis, as well as hypercalciuria and nephrocalcinosis. In some patients with aBS, the urinary concentrating defect is so severe that it can lead to hypernatremia, resembling nephrogenic diabetes insipidus (18). Some patients with aBS may lack metabolic alkalosis during the first year of life, or even present a transient metabolic acidosis with defective urinary acidification (48). This association, which probably results from medullary nephrocalcinosis, can mimick incomplete distal renal tubular acidosis (18). Other causes of pseudo-Bartter syndromes will be discussed in the section on cBS.

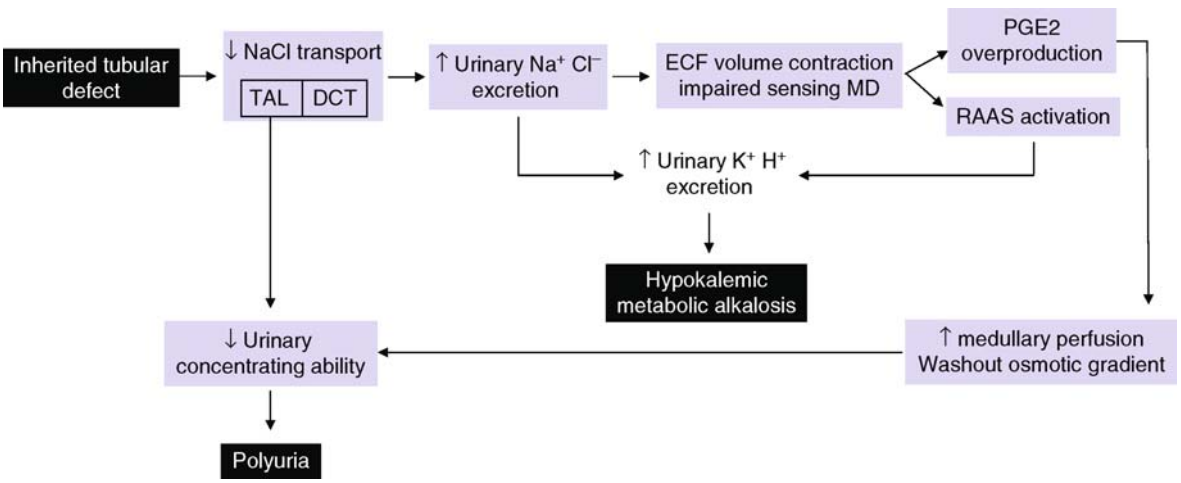
Pathophysiology

Functional investigations of pathogenic NKCC2 mutations in *Xenopus laevis* oocytes revealed a low expression of normally routed but functionally impaired transporters (49). A partial intrinsic transport defect was demonstrated in a peculiar mutant (F177Y), which may account for the attenuated phenotype of the affected patients (37). Similar functional studies provided insights into the role of ROMK in aBS. Two C-terminal mutations located nearby or inside the putative protein kinase A (PKA) phosphorylation site of ROMK showed a decreased open probability of the channel (50). Several missense mutations located in the intracellular N- and C-termini were shown to encode functional channels, but with altered pH gating (28). Starremans et al. investigated eight ROMK mutants and showed that loss-of-function may result from defective cellular routing to the plasma membrane, or impaired channel function (30). Peters et al. (51) identified defective membrane trafficking in 14/20 naturally occurring ROMK mutations, and showed that two early inframe stop mutations could be rescued by aminoglycosides, resulting in full-length ROMK and correct trafficking to the plasma membrane.

The functional data obtained in vitro suggest that loss-of-function mutations in NKCC2 and ROMK disrupt NaCl transport in the TAL, leading to salt wasting, volume contraction, and stimulation of the RAAS. In turn, the distal reabsorption of Na^+ via the epithelial Na^+ channel ENaC leads to increased urinary excretion of K^+ and H^+, causing hypokalemic metabolic alkalosis (*Fig. 38-2*). These changes are already observed prenatally, with polyhydramnios, high Cl^- concentrations and increased aldosterone levels in the amniotic fluid (52, 53). Chronic volume contraction, elevated levels of angiotensin II, and intracellular Cl^- depletion stimulate PGE2 production, which further inhibits NaCl transport in the TAL and contributes to the washout of the osmotic gradient through enhanced medullary perfusion (2). The inhibition of NKCC2 in the macula densa could impair the luminal Cl^- sensing of these cells, which may disrupt the tubuloglomerular feedback and further activate renin release from juxtaglomerular cells (54). It also appears that cyclooxygenase-2 (COX-2) is induced in the macula densa of children with aBS (55), which could further contribute to hyperreninemia (56). The early neonatal hyperkalemia harbored by some ROMK-deficient patients is explained by the involvement of ROMK in K^+ secretion in the cortical collecting duct, with subsequent

Figure 38-2

Physiopathology of hypokalemic metabolic alkalosis and polyuria in Bartter syndrome. See text for details.



activation of an alternative pathway for K^+ secretion in the CCD explaining the transient nature of this feature (44).

The impaired Cl^- transport in the TAL affects the lumen-positive electrical potential, causing persistent hypercalciuria and early-onset nephrocalcinosis in aBS. Renal Mg^{2+} wasting and overt hypomagnesemia do not reliably segregate with aBS, consistent with the observation that chronic furosemide treatment is not generally associated with hypomagnesemia (57). Possibly, the loop of Henle or more distal segments may adapt and compensate more efficiently for Mg^{2+} than Ca^{2+} in this syndrome. In the TAL, this adaptation might involve tight jnction structures (claudin16/claudin19), whereas increased PGE2 synthesis may contribute to increased Mg^{2+} reabsorption in the DCT (58). It has been suggested that a bone resorption process and a PGE2-mediated increase in calcitriol may contribute to hypercalciuria in aBS (35, 59). Recently, Schurman et al. (60) suggested that elevated levels of angiotensin II may stimulate the synthesis of basic-fibroblast growth factor (b-FGF), with a resulting increase in bone resorption via a prostaglandin-dependent mechanism.

NKCC2 knockout (KO) mice show massive perinatal fluid wasting and dehydration, leading to renal failure and death prior to weaning (61). Treatment of the NKCC2 KO mice with indomethacin from day 1 allowed 10% mice to survive until adulthood, despite polyuria, hydronephrosis, hypokalemia, and hyperalciuria. Similarly, the ROMK KO mice (62) manifest early death associated with polyuria, polydipsia, and impaired urinary concentrating ability. Approximately 5% of these mice survive the perinatal period, but show renal failure, hypernatremia and metabolic acidosis. Micropuncture analysis revealed that the absorption of NaCl in the TAL was reduced, with severe impairment of the tubuloglomerular feedback. Another strain of ROMK-null mouse with a BS-like phenotype showed increased survival to adulthood, due to compensatory mechanisms mostly active in the DCT (63). Recently, Lu et al. used CFTR-deficient mouse models to demonstrate that this Cl^- channel may regulate the ATP sensitivity of ROMK in the TAL, which could explain why patients with cystic fibrosis are prone to develop the pseudo-Bartter features of hypokalemic metabolic alkalosis (64).

Treatment

The initial treatment of preterm infants or neonates with aBS should focus on the correction of dehydration and electrolyte disorders, which often requires continuous saline infusion in a neonatal intensive care unit. Elevated levels of urinary PGE2 provided a rationale for NSAID, and indomethacin has been widely used (2, 13, 31, 33, 48). Typically, administration of indomethacin starting 4–6 weeks after birth, when massive urinary electrolyte losses have been controlled and hypokalemic metabolic alkalosis is established, corrects both the systemic and biochemical manifestations of aBS (31). Indomethacin (at doses ranging from 0.5 to 2.5 mg/kg/day) reduces polyuria, improves hypokalemia, normalizes plasma renin levels, and reduces hypercalciuria (13, 31, 33, 34, 48). However, the potential benefit of indomethacin administration in premature babies and neonates should be weighted against risks of severe gastrointestinal complications, such as ulcers, perforation and necrotizing enterocolitis (13, 33, 65). In particular, administration of indomethacin in newborn infants with defective ROMK may be complicated by oliguric renal failure and severe hyperkalemia. At any time, the ROMK-deficient patients are particularly sensitive to indomethacin, with doses well below 1 mg/kg/day sufficient to maintain normal plasma K^+ levels (66). Similarly, the potential benefit of prenatal treatment with indomethacin (66) should be weighed against the lack of evidence for hyperprostaglandinism in the fetus (13), and the negative effects of NSAID on the ductus arteriosus and the development of the kidney (65). Kleta et al. pointed that selective and nonselective cyclooxygenase inhibitors can be used for treatment (66). As an alternative to indomethacin, the COX-2 selective inhibitor rofecoxib ameliorated clinical and biological manifestations in aBS patients, with significant suppression of PGE2 and correction of hyperreninemia (56, 67). However, a case of reversible acute renal failure associated with rofecoxib in an 18-month-old girl with aBS has been reported (68).

Importantly, it should be remembered that HPS is secondary to volume depletion (13), and that the appropriate compensation of fluid and salt wasting remains the essential priority. Additional K^+ supplementation is required, more often for NKCC2-deficient than ROMK-deficient patients (2, 31). In some cases, a K^+-sparing diuretic (usually, spironolactone) is necessary to increase serum K^+ levels (13). Treatment with angiotensin converting enzyme (ACE) inhibitors has been reported effective in a few cases (33, 69) but should be used with caution as these drugs could block the distal compensatory Na^+ reabsorption. Thiazides should not be used to reduce hypercalciuria, since they interfere with compensatory mechanisms in the DCT and further aggravate dehydration.

The appropriate management of aBS with correction of fluid and electrolyte disorders, indomethacin and K^+ supplements results in catch-up growth and normal

pubertal and intellectual development (13, 33). However, most patients show a persistent deficiency in height and weight (32, 41). Correction of hypercalciuria is usually partial, with progression of nephrocalcinosis and a slow decrease in renal function evidenced in some cases (13, 33, 38, 48). Chaudhuri et al. (39) reported a case of severe aBS in whom pre-emptive nephrectomy followed by a living-related donor renal transplantation resulted in correction of metabolic abnormalities and excellent graft function.

Antenatal Bartter Syndrome with Sensorineural Deafness

Genetics

In 1995, Landau et al. (70) described a subtype of aBS/HPS associated with sensorineural deafness (SND) in 5 affected subjects from an inbred Bedouin kindred. These patients had a particularly severe salt wasting and fluid loss, with poor response to indomethacin and, most often, progressive renal failure (70, 71). By studying the large original kindred, Brennan et al. mapped the disease-causing gene to chromosome 1p31 (72). In 2001, Birkenhager et al. (8) identified a novel gene, *BSND*, within the critical interval, and detected inactivating mutations in affected individuals. This subtype of aBS was named aBS with SND, or type IV BS (OMIM #602522). The original mutations included a splicing mutant, a deletion of two exons, three missense mutations affecting a conserved residue close to the first putative membrane domain, and one mutation resulting in the loss of the start codon (8). The *BSND* gene consists of 4 exons. It encodes barttin, a 320 amino-acids protein that contains two putative transmembrane domains and is expressed in the thin limb and thick ascending limb of the loop of Henle and the DCT in the kidney (*Fig. 38-1*), and in the stria vascularis surrounding the cochlear duct in the inner ear (8, 73).

Clinical Manifestations

Typically, patients harboring mutations in *BSND* show the most severe form of aBS, with maternal polyhydramnios beginning at week 25 of gestation, severe prematurity, life-threatening neonatal episodes of dehydration, polyria with hypo- or isosthenuria, and increased urinary PGE2 excretion (2, 74) (*Table 38-2*). All patients are deaf and show a severe growth defect, with delayed motor development (71). They present multiple episodes of fever, vomiting, and bacterial infections. Jeck et al. (71) reported progressive renal failure in all patients, attributable to glomerular sclerosis and tubular atrophy. However, Shalev et al. reported that early renal failure is not a uniform finding (74). In contrast with patients harboring mutations in NKCC2 and ROMK, barttin-deficient patients exhibit only moderate and transient hypercalciuria and do not show nephrocalcinosis (2, 74). This could be due to defective NaCl transport in both the TAL and DCT, with divergent effects on urinary calcium excretion somehow similar to a combined action of a loop diuretic with a thiazide. Accordingly, barttin-deficient patients may show a severe Mg^{2+} wasting, caused by a defect in both the paracellular (TAL) and transcellular (DCT) pathways of Mg^{2+} reabsorption (2). Of note, a lack of diuretic response to furosemide and to hydrochlorothiazide was evidenced in one barttin-deficient patient, supporting a defect in both TAL and DCT (75).

Recent reports have suggested some degree of phenotype variability among patients with *BSND* mutations. Miyamura et al. (76) reported a patient harboring the loss-of-function G47R mutation of *BSND* who presented at age 28 years, with congenital deafness but without polyhydramnios, premature labor, or severe salt wasting in the neonatal period. In contrast, five patients from two unrelated Spanish families harboring the same G47R mutation presented with polyhydramnios, premature birth and salt loss (77). Kitana et al. (78) reported a patient harboring two mutations in *BSND* (Q32X and G47R) who presented with relatively mild perinatal clinical features but developed end-stage renal failure at age 15 years, requiring renal transplantation. The functional evaluation of the G210S mutation of *BSND* revealed only a very mild disturbance in current-voltage relationship (79), possibly accounting for a milder phenotype (74). Renal biopsies obtained in patient with *BSND* mutation showed variable features including hyperplasia of the juxtaglomerular apparatus, mild mesangial hypercellularity, mild to severe tubulointerstitial fibrosis, areas of tubular atrophy, and sclerosed glomeruli (71, 74).

Pathophysiology

Functional expression studies revealed that barttin is an essential beta-subunit for the Cl^- channels ClC-Ka and ClC-Kb, by stimulating Cl^- currents and enhancing surface expression of these channels (73). ClC-Ka and ClC-Kb are two members of the CLC gene family that are located on the basolateral membrane of the cells lining the thin ascending limb (ClC-Ka only), TAL and DCT, and the intercalated cells of the collecting duct. ClC-Ka

and ClC-Kb are also expressed in the inner ear, where they colocalize precisely with barttin in specialized, K^+-secreting cells of the stria vascularis and the vestibular organ (73). The co-expression of barttin with ClC-Ka/b channels is crucial for NaCl reabsorption in the TAL/DCT (*Fig. 38-1*) and K^+ recycling in the inner ear. Disease-causing mutations in barttin disrupt the Cl^- exit from the TAL and DCT, causing the severe salt-losing tubulopathy. Furthermore, the defective barttin-ClC-Ka/b complex impairs the basolateral recycling of Cl^- in the stria vascularis, decreasing the secretion of K^+ into the endolymph and causing SND (73, 80). The role of barttin as an essential beta-subunit for ClC-Ka and ClC-Kb has been substantiated by two recents reports of patients showing a typical aBS with SND phenotype indistinguishable from barttin-deficient patients, in association with a digenic disease caused by loss-of-function mutations in both *CLCNKB* and *CLCNKA* genes (81, 82).

Treatment

Barttin-deficient patients are managed primarily with intravenous fluids in neonatal intensive care units. In contrast with other forms of aBS, and despite high levels of urinary PGE2, the effect of indomethacin on growth and correction of electrolyte disorders is rather poor (71, 74). Hypokalemic metabolic alkalosis persists despite high doses of NaCl and KCl supplementation (71). Zaffanello et al. (75) reported that combined therapy with indomethacin and captopril was needed to discontinue intravenous fluids and improve weight gain in a single patient. A pre-emptive nephrectomy for refractory electrolyte and fluid losses and persistent failure to thrive, followed by peritoneal dialysis and succesful renal transplantation has been reported in a 1-year-old child with type IV BS (39).

Classic Bartter Syndrome

Genetics

Classic Bartter syndrome (cBS, or type III BS, OMIM #607364) usually presents during infancy or early childhood, with a phenotype similar to the original description given by Bartter et al. (1), i.e., without the prenatal onset and the nephrocalcinosis seen in the aBS variant. The cBS variant is caused by mutations in the *CLCNKB* gene located on 1p36 (9, 11). The gene, which contains 19 exons, encodes the basolaterally located renal chloride channel ClC-Kb, which mediates Cl^- efflux from epithelial cells lining the TAL and DCT (9, 80) (*Fig. 38-1*). There is a high rate of deletions encompassing a part of or the entire *CLCNKB* gene (9, 11, 83). It is hypothesized that the close vicinity of the almost identical *CLCNKA* and *CLCNKB* genes, which are separated by only 11 kb, predisposes to a high rate of rearrangements, for example, by unequal crossing over as demonstrated in two kindreds (9, 11). In addition, missense, nonsense, small insertions/deletions, frameshift and splice-site mutations have also been reported (http://www.hgmd.cf.ac.uk). A founder mutation (A204T) affecting a highly conserved residue has been reported at the homozygous state in ten patients from nine unrelated, non-consanguinous families in Spain (84). As expected for a recessively transmitted disorder, a significant number of subjects originates from consanguineous kindred (9, 11).

Pathophysiology

As mentioned earlier (see section on barttin), ClC-Kb is a plasma membrane channel that belongs to the CLC family of chloride channels/exchangers (80). ClC-Kb and the closely related ClC-Ka isoform are located on the basolateral membrane of the cells lining the thin ascending limb (ClC-Ka only), TAL and DCT cells, as well as in the intercalated cells of the collecting duct (*Fig. 38-1*). They both require the beta-subunit barttin to facilitate their insertion in the plasma membrane and to generate Cl^- currents (73, 79). Disease-causing missense mutations of ClC-Kb result in significant reductions or the loss of ClC-Kb/barttin currents (73). Thus, inactivating mutations in ClC-Kb affect the basolateral exit of Cl^-, which in turn reduces the reabsorption of NaCl in the TAL and DCT. The phenotypic variability of type III BS, which ranges from aBS/HPS in some cases to typical GS in others, may thus be explained by the wide distribution of ClC-Kb (11, 31). Alternative pathways for Cl^- exit could partially compensate for ClC-Kb inactivation in the kidney (11, 85). Importantly, none of the type III BS patients with ClC-Kb mutations is deaf, because the function of ClC-Kb/barttin channels in the inner ear can be replaced by ClC-Ka/barttin. Only the disruption of the common β subunit barttin (73) or the combined loss of ClC-Ka and ClC-Kb (81, 82) results in a Cl^--recycling defect that lowers K^+ secretion in the stria vascularis to a pathogenic level.

To date, there is no mouse model with targeted deletion of ClC-Kb. Mice lacking ClC-K1 (corresponding to ClC-Ka in humans) show a phenotype of nephrogenic diabetes insipidus, with no modification in the fractional

excetion of Na^+ and Cl^-, and no hypokalemic alkalosis (86). These features are caused by the loss of the Cl^- transport across the thin ascending limb, which is essential for generating a hypertonic interstitium (86). No corresponding human disease linked to loss-of-function mutations of *CLCNKA* has been described. A recent study supported the potential role of *CLCNKA* as a susceptibility gene for salt-sensitivity (87).

Clinical Manifestations

Patients harboring mutations of ClC-Kb present a broad spectrum of clinical features (*Table 38-2*) that range from the aBS/HPS phenotype, with polyhydramnios, isosthenuria, and hypercalciuria, over the classic BS phenotype, with less impaired concentrating ability and normal urinary calcium excretion, to a GS-like phenotype with hypocalciuria and hypomagnesemia (2, 11, 31, 84). Most patients have episodes of hypokalemic alkalosis and dehydration complicated with muscular hypotonia and lethargy during the first years of life. They are also characterized by increased urinary excretion of PGE2 (2, 11, 31). The median duration of pregnancy was 38 weeks in the series of Jeck et al. (2), and failure to thrive is common (31, 84). The diagnosis of Bartter syndrome is usually made during the first year of life, but prenatal (with history of mild maternal polyhydramnios) and late-onset cases are also reported. Most patients with classic BS show failure to thrive and growth retardation (36, 88, 89). Like in the antenatal variants, osteopenia with increased markers of bone resorption can be observed (36).

The electrolyte abnormalities are usually severe at presentation, with low plasma Cl^- and severe hypokalemic alkalosis. Increased plasma renin levels, with high or inappropriately normal (with respect to the hypokalemia) aldosterone levels are typically observed (11, 31). Polyuria is not uniformly found in classic BS. Iso/hyposthenuria was only evidenced in approximately one-third of patients, whereas some achieved urinary osmolality above 700 mOsm/kg (2). The persistance of such a concentrating ability suggests that patients lacking ClC-Kb have a residual TAL function. This is further supported by the fact that only ~ 20% of patients had sustained hypercalciuria (31). Nephrocalcinosis was reported in 4/36 affected children (11), but was not detected in three other series (84, 88, 89). The patients may show a mild hypophosphatemia, which could be related to tubular damage and hypokalemic nephropathy (33, 36). Isolated cases present with manifestations of renal Fanconi syndrome or distal renal tubular acidosis (84). About half of the patients lacking ClC-Kb have hypomagnesemia (11). Several patients harboring mutations in *CLCNKB* show overlapping features of cBS (presentation within the first year of life with episodes of dehydration) and GS (hypomagnesemia with hypocalciuria) (11, 85, 90, 91). Sun et al. (92) reported a patient with cBS who had bilateral sclerochoroidal calcification attributed to persistent hypomagnesemia for 26 years despite magnesium supplementation.

If the full phenotypic spectrum of the Bartter-like syndromes can result from mutations in *CLCNKB*, a significant clinical heterogeneity is observed among patients harboring the same mutation, and even between siblings (84). No correlations between a particular phenotype and *CLCNKB* genotype have been documented yet (11, 85). It has been suggested that ethnic differences may participate in the phenotype variability (2). Indeed, the two original patients described by Bartter were of African Americans origin (1), and early reports suggested that the course of BS may be more severe in African Americans (93). More recently, Schurman et al. (89) reported significant phenotype variability in the neonatal period in a series of 5 unrelated African American children with a homozygous deletion of the entire *CLCKNB*.

A vascular hyporeactivity to the infusion of angiotensin II was originally described by Bartter et al. (1). This feature is not consistently observed, probably because vascular hyporeactivity improves after correction of volume depletion or treatment with NSAID. Extensive studies (reviewed in 94) showed that this hyporeactivity could be due to various modifications in the angiotensin II-signaling, including downregulation of the alpha subunit of the heterotrimeric Gq protein, decreased intracellular Ca^{2+} and blunted protein kinase C activation, downregulation of the RhoA/Rho kinase pathway, and upregulation of endothelial NO synthase. In turn, these modifications may affect the upregulation of NAD(P)H oxidase and prevent the release of free radicals – offering increased protection against cardiovascular remodeling in these patients (94). Stoff et al. (95) evidenced a defect in platelet aggregation in four subjects with Bartter syndrome, but not in other hypokalmic patients. The platelet abnormality was exacerbated by restriction of dietary sodium and lessened by the administration of PG inhibitors. A circulating metabolite of prostacyclin, 6ketoPGE1 may be responsible for the defect (96).

Bartter syndrome is not classically associated with proteinuria, and renal biopsies consistently show hyperplasia of the juxtaglomerular apparatus, with minimal or no glomerular or tubular abnormalities (1). However, a few cases of cBS with proteinuria have been reported. Sardani et al. (97) described a 4-year-old African American child

with a homozygous deletion in *CLCNKB* and mild mesangial proliferative glomerulonephritis consistent with C1q nephropathy. The recent follow-up studies of Bettinelli et al. (88) revealed mild-to-moderate glomerular proteinuria in 6/13 ClC-Kb-deficient patients. It was associated with decreased GFR in four patients and microhematuria in two. Renal biopsy in two patients revealed diffuse or moderate mesangial hypertrophy (88). In addition, a few cases of clinical Bartter syndrome with unknown genetic defect presented with focal segmental glomerulosclerosis (FSGS) and renal failure (98, 99). One of the patients described by Bartter developed renal failure, with evidence of advanced nephrosclerosis, interstitial fibrosis, tubular atrophy, and glomerular hyalinization (100). Causes of renal failure in BS include complication of renal salt-wasting (including long-standing hypokalemia, hypovolemic episodes or nephrocalcinosis), chronic activation of the RAAS with ensuing stimulation of TGF-beta and/or TNF-alpha, and toxicity of NSAID (38, 97, 99). Renal dysfunction was temporally associated with NSAID therapy in two cBS patients, with biopsy-proven interstitial nephritis and resolution after NSAID withdrawal (38). However, the pathogenic role of NSAID in causing renal damage in Bartter syndrome has been questioned by the nature and topology of the histological lesions, the fact that renal lesions were identified in some patients before initiation of AINS, and the lack of progression of tubulointerstitial lesions over more than a decade under NSAID treatment (41, 101). Of note, renal cysts have been identified in patients with classic BS (33, 102), potentially linked to renal K^+ wasting and secondary aldosteronism (103).

Recently, Jeck et al. identified the common T481S variant in *CLCNKB*, which showed significantly increased currents when expressed in oocytes (104) and was associated with essential hypertension in a German cohort (105). The relevance of these findings has been discussed (106), and linkage of the T481S variant to high blood pressure was not confirmed in a Japanese cohort (107).

Differential Diagnosis, Unusual Associations, Pseudo-Bartter Syndromes

The differential diagnosis of cBS includes the surreptitious use of loop diuretics, laxative abuse (108), which are both unusual in children (109), and chronic vomiting (110). Measurement of urinary Cl^- and urine screen for diuretics are usually useful to diagnose these patients (111, 112).

The association of hypokalemic metabolic alkalosis with hyperreninemic secondary aldosteronism is also found in other familial disorders affecting the kidneys or the gastrointestinal tract, or can be acquired. Generalized dysfunction of the proximal tubule (renal Fanconi syndrome), for instance due to cystinosis (113), or Kearns-Sayre syndrome, a mitochondrial cytopathy caused by large deletions in mitochondrial DNA leading to cytochrome c oxidase deficiency (114), can be associated with biochemical features resembling BS. A case of familial renal dysplasia with hypokalemic alkalosis has been reported (115). Patients with cystic fibrosis are prone to develop episodes of hyponatraemic, hypochloraemic dehydration with metabolic alkalosis (116, 117). As mentioned earlier, the Cl^- channel CFTR, which is mutated in cystic fibrosis, may regulate the function of ROMK in the TAL (64). Gastrointestinal malformations which are associated with Cl^- deficiency (118), or Hirschprung disease (119) can also lead to pseudo-Bartter syndrome. Administration of protaglandins in neonates with a ductus-dependent congenital cardiopathy (120), aminoglycosides (121, 122) or combined chemotherapy (123) can also induce the biochemical features of BS. Bartter syndrome has also been reported in association with autoimmune diseases, for isntance with Sjögren syndrome (124, 125). Güllner et al. (126) described a syndrome of familial hypokalemic alkalosis in a sibship presenting with hyperreninemia, aldosteronism, high urinary prostaglandin E2 excretion, normal BP, and resistance to angiotensin II. At variance with BS, the patients had hypouricemia, indicative of proximal tubule dysfunction, and the fractional chloride reabsorption in the TAL was normal. The renal biopsy showed an extreme hypertrophy of the PT basement membranes, whereas the juxtaglomerular apparatus were of normal appearance. The molecular basis of this familial tubulopathy remains unknown. Finally, the association of BS with a partially empty sella detected by MRI of the brain has been reported in both adult and pediatric patients (127, 128).

Treatment

Patients with cBS are typically treated with PG synthetase inhibitors and escalating doses of KCl, complemented with K^+-sparing diuretics (most often, spironolactone) and NaCl in some of them (41, 88, 89). Indomethacin is the most frequently used drug, usually started within the first 4 years of life at doses ranging from 1 to 2.5 mg/kg/day. Doses above 3 mg/kg/day are considered nephrotoxic. Indomethacin is well tolerated, but one should remain cautious for gastrointestinal complications (33) or alteration of renal function (38, 88). Selective COX2 inhibitors, such as rofecoxib have been used instead of

indomethacin (33) and are currently evaluated on a larger scale. Potassium supplementation (usually KCl, 1–3 mmol/kg/day) is mandatory in cBS, as hypokalemia is often severe at presentation and is not fully corrected by indomethacin (89). If KCl alone fails to correct hypokalemia, then addition of spironolactone (1–1.5 mg/kg/day) is recommended. The use of ACE-inhibitors, which have been used for treating hypokalemia in adults with BS (129), should be cautious given the risk of hypotension. Magnesium supplementation should be added when hypomagnesemia is present, but the correction is typically difficult (31). Some patients with cBS require gastrostomy tube placement and enteral feeding (89).

The long-term efficacy of the standard treatment with indomethacin and KCl supplementation has been established in cBS. Most biochemical features improve with therapy, although K^+ levels are typically difficult to normalize in most patients despite NSAID, KCl and spironolactone. Treatment also results in improved height and weight, but catch-up growth is inadequate and there is persistent height retardation (33, 41, 88, 89). Recently, growth hormone deficiency has been demonstrated in some patients, with a positive effect of recombinant human hormone treatment (88). As discussed above, some cases of cBS are complicated by chronic renal failure. A few cases of living-related kidney transplantation have been reported, with improvement of biochemical and hormonal abnormalities after transplantation (98, 101, 130).

Peri-operative management of patients with cBS requires a particular care for volume repletion and correction of electrolyte abnormalities during anesthesia, and the continuation of anti-prostanglandin therapy to prevent the defective platelet aggregation (131, 132).

Gitelman Syndrome

Genetics

Gitelman syndrome (GS) (OMIM #263800) is generally considered as a milder disorder than BS and, with a prevalence of ~ 1 per 40,000, arguably the most frequent inherited tubulopathy detected in adults (40). The syndrome was first described in 1966 by Gitelman and co-workers as a familial disorder in which patients presented with hypokalemic alkalosis and a peculiar susceptibility to carpopedal spasm and tetany due to hypomagnesemia (133). For more than 20 years, GS was assimilated with BS. In 1992 Bettinelli and co-workers concluded that GS could be distinguished from BS, based on low urinary Ca^{2+} excretion (molar urinary calcium/creatinine ratio less than or equal to 0.20) (134). Also, BS patients were more often born after pregnancies complicated by polyhydramnios or premature delivery and had short stature, polyuria, polydipsia and tendency to dehydration during infancy and childhood, whereas GS patients presented tetanic episodes or short stature at school age (134). The dissociation of renal Ca^{2+} and Mg^{2+} handling in GS, together with the subnormal response of these patients to thiazides (135, 136), pointed to a primary defect in the DCT.

In 1996, Simon and colleagues demonstrated that presumable loss of function mutations in the *SLC12A3* gene were responsible for GS (10). The *SLC12A3* gene is located on 16q13 and comprises 26 exons. It encodes the thiazide-sensitive Na^+-Cl^- cotransporter (NCCT), a 1,021 amino-acids integral membrane protein expressed in the apical membrane of cells lining the DCT (*Fig. 38-1*). NCCT belongs to the nine-member family of electroneural cation-chloride coupled cotransporters (*SLC12*) that also includes the Na^+-K^+-$2Cl^-$ and the K^+-Cl^- cotransporters (137). NCCT contains a central hydrophobic region comprising 12 putative transmembrane (TM) domains flanked by a short N-terminal and a long C-terminal hydrophilic intracellular termini (138). A model suggests that the affinity-modifying residues for Cl^- are located within TM 1–7 and for thiazides between TM 8–12 and that both segments are implicated in defining Na^+ affinity of NCCT (139).

Gitelman syndrome is transmitted as an autosomal recessive trait, and the majority of patients are compound heterozygous for different mutations in *SLC12A3*. To date, more than 110 mutations scattered through *SLC12A3* have been identified in GS patients (http://www.hgmd.cf.ac.uk). Most (~ 75%) are missense mutations substituting conserved amino acid residues, whereas nonsense, frameshift and splice-site defects, and gene rearrangements are less frequent. A significant number of GS patients, up to 25% in some series, is found to carry only a single mutation in *SLC12A3*, instead of being compound heterozygous or homozygous (140–142). Because GS is recessively inherited, it is likely that there is a failure to identify the second mutation in regulatory fragments, 5′ or 3′ untranslated regions, or deeper intronic sequences of *SLC12A3*, or that there are large genomic rearrangements. As discussed above, mutations in *CLCNKB* have been detected in a few patients presenting simultaneous features of cBS and GS (85, 90, 91). The distribution of ClC-Kb in both the TAL and DCT, and potential compensation by other Cl^- transporters, may probably explain these overlaping syndromes (2). In any case, GS is indeed genetically heterogeneous, raising the possibility of a concurrent heterozygous mutation in a

gene other than *SLC12A3*. In addition to *CLCNKB*, other genes participating in the complex handling of Na^+, Ca^{2+} and Mg^{2+} in DCT, or its regulation, are potential candidates (143).

Pathophysiology

The functional effects of mutants NCCT were tested using *X. laevis* oocytes (142, 144–146). Functional analyses revealed that some mutant NCCT proteins were synthesized but not properly glycosylated, targeted for degradation, and not delivered to the plasma membrane (144). Another class of *SLC12A3* mutations results in normal glycosylated proteins partly impaired in their routing and insertion, that perform normal function once they reach the plasma membrane (145, 146). Some NCCT mutants affect the intrinsic activity of the cotransporter, with normal glycosylation and plasma membrane insertion (142). Finally, splicing mutations of *SLC12A3* result in truncated transcripts that trigger nonsense-mediated decay (NMD), a mRNA surveillance pathway that allows cells to degrade mRNA that contains premature translation stop codons (142). Taken together, these results imply that GS may arise from impaired protein synthesis (splicing mutants); defective processing; defective protein insertion of functional mutants; and defective intrinsic activity of the mutant NCCT in the DCT cells (142).

Schultheis and colleagues generated a mouse model with a null mutation in the *Slc12a3* gene on a mixed background (147). The NCCT null mice showed hypocalciuria and hypomagnesemia at baseline but, in marked contrast to GS patients, no hypokalemic metabolic alkalosis. The NCCT-deficient mice had no signs of hypovolemia on a standard Na^+ diet, but they showed a lower blood pressure than wild-type when fed a Na^+-depleted diet for 2 weeks suggesting a subtle hypovolemia compensated at baseline. Subsequent studies performed on a homogeneous C57BL/6 strain showed that NCCT null mice had a mild compensated alkalosis with increased levels of plasma aldosterone (148) and an increased sensitivity to develop hypokalemia when exposed to dietary K^+ reduction (149). More recently, Belge and co-workers showed that mice lacking parvalbumin, a cytosolic Ca^{2+}-binding protein that is selectively expressed in the DCT, had a phenotype resembling GS, with volume contraction, aldosteronism and renal K^+ loss at baseline, impaired response to hydrochlorothiazide, and higher bone mineral density (150). They demonstrated that these modifications were due to modifications in intracellular Ca^{2+} signaling and decreased expression of NCCT in the DCT (150).

Studies of inactivating *SLC12A3* mutations and mouse models indicate that the GS phenotype results from dysfunction of NCCT. The loss of NCCT in the DCT leads to salt wasting, volume contraction, stimulation of the RAAS, and increased excretion of K^+ and H^+ in the collecting duct resulting in hypokalemic metabolic alkalosis. By contrast, the pathogenesis of hypocalciuria and hypomagnesemia remains debated. Two hypotheses prevail respecting hypocalciuria. First, the volume contraction causes a compensatory increase in proximal Na^+ reabsorption, driving passive Ca^{2+} transport in the PT (151). Second, the epithelial cells of the DCT hyperpolarize, due to lower intracellular Cl^- activity, which opens the apical voltage dependent Ca^{2+} channels (TRPV5), resulting in increased Ca^{2+} influx and reabsorption (152). Such hyperpolarization could also stimulate the basolateral Na^+/Ca^{2+} exchanger, further increasing Ca^{2+} reabsorption (153). Studies performed in chronic hydrochlorothiazide-treated mice (154) favor the first hypothesis, as micropuncture experiments demonstrated increased reabsorption of Na^+ and Ca^{2+} in the proximal tubule, whereas Ca^{2+} reabsorption in the distal convolution was unaffected. Furthermore, micropuncture experiments performed in NCCT-deficient mice revealed an enhanced fractional reabsorption of Na^+ and Ca^{2+} upstream of the DCT to compensate the transport defect in that segment (148).

Hypomagnesemia is an essential feature of GS. Several mechanisms, including K^+ depletion, increased passive Mg^{2+} secretion, or defective active Mg^{2+} transport in the DCT, have been proposed to explain the Mg^{2+} wasting in GS (4, 155). The recent identification of TRPM6 as a Mg^{2+} permeable channel in the DCT and its involvement in the pathogenesis of autosomal recessive hypomagnesemia (*see below*), suggested that this channel constitutes the apical entry step in active renal Mg^{2+} reabsorption (156). Indeed, chronic thiazide administration increased Mg^{2+} excretion and reduced renal expression levels of TRPM6 in mice. In addition, TRPM6 expression was also drastically decreased in mice lacking NCCT (154). These results suggest that the pathogenesis of hypomagnesemia in chronic thiazide treatment as well as GS could involve TRPM6 downregulation. Structural variations in the epithelial cells lining DCT, with decreased absorptive surface area for Mg^{2+}, may also play a role (147, 157).

Clinical Manifestations

Classically, GS was considered as a benign variant of Bartter-like syndromes, usually detected during adolescence or adulthood. Since the disorder originates from

the DCT, the salt and water losses in GS patients are less pronounced than in aBS or cBS because urinary concentrating ability should not be affected (*Table 38-2*). The GS patients are often asymptomatic or presenting with mild symptoms such as weakness, fatigue, salt craving, thirst, nocturia, constipation, or cramps. They may also consult for growth retardation and short stature, reflecting an alteration in the growth hormone-insulin-like growth factor I axis or pleiotropic effects resulting from magnesium depletion (158). Typical manifestations include muscle weakness, carpopedal spasms, or tetanic episodes triggered by hypomagnesemia (57, 159). Blood pressure is reduced, particularly for patients with severe hypokalemia and hypomagnesemia (160). Since Mg^{2+} ions increase the solubility of calcium pyrophosphate crystals and are important for the activity of pyrophosphatases, hypomagnesemia may promote the formation of calcium pyrophosphate crystals in joints and sclera, leading to chondrocalcinosis (161) and sclerochoroidal calcifications (162). Patients with GS have higher bone mineral density, similar to chronic thiazide treatment, which likely arises from increased renal Ca^{2+} reabsorption and a decreased rate of bone remodeling (163). Potassium and Mg^{2+} depletion prolong the duration of the action potential in cardiomyocytes, resulting in prolonged QT interval in $\sim$ 50% of the patients, which could lead to an increased risk for ventricular arrythmias (164, 165). Cases of GS patient who presented with long runs of ventricular tachycardia (166) or ventricular fibrillation with favorable outcome after cardioversion and continuous supplementation (167) have been reported. Pregnancies in GS appear to have a favorable outcome, provided continuous K^+ and Mg^{2+} supplementation and monitoring for oligohydramnios (168). A summary of the manifestations associated with GS and their frequency is shown in *Table 38-3*.

The classical biochemical features of GS include hypokalemic metabolic alkalosis, hypomagnesemia, and hypocalciuria. The presence of both hypomagnesemia (< 0.75 mM) and hypocalciuria (molar urinary calcium/creatinine ratio < 0.2) is highly predictive for the diagnosis of GS (134). The criteria for hypocalciuria in infants or children with GS have recently been precised (169). However, there are inter- and intra-individual variations in the extent of hypocalciuria, and hypomagnesemia may be absent in some GS patients (2, 31, 170). In addition, the combination of hypocalciuria and hypomagnesemia is also detected in rare cases of cBS (90). Although the urinary PGE_2 excretion is classically normal in GS, increased values can also be detected in some patients (2).

No specific findings are observed at renal biopsy, apart from occasional hypertrophy of the juxta-glomerular apparatus and markedly reduced expression of NCCT by immunohistochemistry (171). Hanevold et al. (172) reported a case of focal segmental glomerulosclerosis and C1q nephropathy in an African American child with GS who subsequently developed nephrotic range proteinuria.

Phenotype Variability and Potential Severity of GS

The view that GS is a benign condition has been challenged by reports emphasizing the phenotype variability and the

Table 38-3
Clinical manifestations associated with Gitelman syndrome

Most common (>50% of patients)	Prominent (20 to 50% of patients)	Occasional (Less than 20%)	Rare (Case reports)
Salt carving	Fainting	Early onset (before age 6)	Seizure
Cramps, muscle weakness, pain	Polyuria	Failure to thrive	Ventricular tachycardia
Fatigue	Arthralgia	Growth retardation	Rhabdomyolysis
Dizziness	Chondrocalcinosis	Vertigo, ataxia	Blurred vision
Nocturia	Prolonged corrected QT interval	Carpopedal spasm, tetany	Pseudotumor cerebri
Thirst, polydipsia	Febrile episodes	Vomiting	Sclerochoroidal calcifications
Paresthesia, numbness		Constipation	
Palpitations		Enuresis	
Low blood pressure		Paralysis	

potential severity of the disease. A detailed evaluation of 50 adult GS patients with identified *SLC12A3* mutations revealed that GS was associated with a significant reduction in the quality of life – similar to that associated with congestive heart failure or diabetes (173). Manifestations such as early onset (before age 6 years), growth retardation, invalidating chondrocalcinosis, tetany, rhabdomyolysis, seizures, and ventricular arrhythmia have been described, although in a limited number of cases (31, 142, 158, 166, 173). Based on the large number of patients harboring *SLC12A3* mutations, the phenotype of GS is highly heterogeneous in terms of age at presentation, nature/severity of biochemical abnormalities, and nature/severity of the clinical manifestations (*Table 38-3*). The phenotype variability has been documented not only between patients carrying different *SLC12A3* mutations, but also for a common underlying mutation (174) and between affected family members (142, 175).

The mechanisms that could account for intrafamilial variability include gender (affected brothers are apparently more severely affected than their sisters carrying the same mutation), modifier genes (affecting the regulation or activity of NCCT), and environmental factors (dietary intake of NaCl, Ca^{2+}, or Mg^{2+}) (2, 142, 175, 176). Compensatory mechanisms operating in other nephron segments should also be considered, as evidenced in mice with defective NCCT (148, 150, 154). Finally, considering that most of patients with GS are compound heterozygous harboring various mutant *SLC12A3* alleles, the phenotype variability could be related to the nature and/or position of the underlying mutation(s). This hypothesis has been substantiated by the recent studies of Riveira-Munoz et al. (142) which showed that a specific combination of mutations was preferentially associated with a severe presentation of GS.

Blood Pressure in GS: Effect of the Carrier State

In 2001, Cruz et al. investigated a large Amish kindred to show that patients with GS had significantly lower age- and gender-adjusted diastolic and systolic blood pressure, a higher urinary Na^+ excretion, and a higher salt intake than their wild-type relatives (160). Additional support for the role of NCCT in blood pressure regulation was provided by the report that transplantation of a GS kidney into a non-Gitelman hypertensive recipient resulted in the correction of hypertension in the latter (177). Considering that the frequency of heterozygote carriers of *SLC12A3* mutations is approximately 1%, the question was thus raised whether single loss-of-function mutations in *SLC12A3* may affect blood pressure regulation in the general population (47). Recently, Lifton and colleagues screened 3,125 adult subjects from the Framingham Heart Study for mutations in *SLC12A3* (and by extension *SLC12A1*, and *KCNJ1*, responsible for aBS) and identified 30 different mutations (15 in *SLC12A3*, 10 in *SLC12A1*, and 5 in *KCNJ1*) in 49 subjects (45). Of these mutations, ten were biochemically proven loss of function (seven in NCCT alone) and 20 were inferred from the conservation and rarity criteria. Examination of long-term BP revealed that 80% of the mutation carriers were below the mean systolic BP values of the entire cohort. The mean BP reduction in carriers was similar to values obtained with chronic thiazide treatment (45). Thus, rare functional variants of three genes involved in Bartter-like syndromes, including GS, have a significant impact in the heritability of BP variation.

Differential Diagnosis

The differential diagnosis of GS includes other Bartter-like syndromes (*Table 38-1*), and particularly cBS due to mutations in *CLCNKB* (2, 170), as well as diuretic or laxative abuse, and chronic vomiting. As mentioned above, the clinical history and biochemical features, even hypocalciuria and hypomagnesemia, may not be fully reliable to distinguish GS from cBS. Although implementation of genetic testing should be promoted, such testing in the context the BS and GS bears a significant cost, considering the number of exons to be screened, the lack of hot-spots, and the large number of mutations described. Recently, Colussi et al. evaluated the response to a simple thiazide test in the diagnosis of GS (170). They monitored the chloride fractional clearance during the 3 h following the administration of hydrochlorothiazide (HCTZ, 1 mg/kg or 50 mg in adults) orally. More than 90% (38/41) of patients with GS showed a blunted response ($<2.3\%$) to HCTZ, a feature that was never observed in seven patients with BS (five with aBS and two with cBS) and three patients with diuretic abuse or vomiting. Thus, the HCTZ test offers a high sensitivity and specificity for the diagnosis of GS (170). However, it should not be recommended to diagnose aBS, in view of the specific clinical history and the potential danger of diuretic treatment in these patients. Whether this test has the power to distinguish between the overlapping features of GS and cBS due to *CLCNKB* mutations is also uncertain (178).

Gitelman syndrome-like manifestations including hypokalemic metabolic alkalosis with hypomagnesemia and

hypocalciuria, have been reported as a rare complication of the use of cisplatin (179). Although the mechanism remains uncertain, cisplatin is known to induce focal tubular necrosis lesions in the DCT (180). Autoimmune disorders cause acquired renal tubular disorders, potentially due to autoantibodies against tubular components (181). Typical features of acquired GS have been reported in association with various autoimmune disorders including iritis and arthritis (182), sialoadenitis (125), and Sjögren syndrome (183). Of note, there was no improvement of renal K^+ wasting after corticosteroid treatment in one such case (183).

Treatment

Magnesium and potassium supplementations are the main treatments in patients with GS. Magnesium supplementation should be considered first, since Mg^{2+} repletion will facilitate K^+ repletion and reduce the risk of tetany and other complications related to hypomagnesemia (159, 184). All types of magnesium salts are effective, but their bioavailability is variable. Magnesium chloride, magnesium lactate and magnesium aspartate show higher bioavailability (159). $MgCl_2$ is recommended since it will also correct the urinary loss of Cl^-. The dose of magnesium must be adjusted individually in 3–4 daily administrations, with diarrhea being the limiting factor. In addition to magnesium, high doses of oral KCl supplements (up to 10 mg/kg/day in children) may be required (185). Importantly, Mg^{2+} and K^+ supplementation results in a catchup growth (142, 158). Spironolactone or amiloride can be useful, both to increase serum K^+ levels in patients resistant to KCl supplements and to treat Mg^{2+} depletion that is worsened by elevated aldosterone levels (186). Both drugs should be started cautiously to avoid hypotension. Patients should not be refrained from their usual salt craving, particularly if they practice a regular physical activity. Prostaglandin inhibitors are less indicated in GS than in aBS, since urinary PGE_2 levels are usually normal. Liaw et al. reported an improvement in growth response following high-dose indometacin, but complicated by gastrointestinal haemorrhage (187). Refractory hypokalemia has also been treated with the specific COX-2 inhibitor Rofecoxib (188). Considering the occurrence of prolonged QT interval in up to half GS patients (164, 165), QT-prolonging medications should be used with caution.

Although GS adversely affects the quality of life (160), we lack informations about the long-term outcome of these patients. Renal function and growth appear to be normal, provided lifelong supplementation (189). Progression to renal failure is extremely rare in GS: only two GS patients who developed end-stage renal disease have been reported (190, 191).

Disorders of the Calcium-Sensing Receptor

The extracellular Ca^{2+}-sensing receptor (CaSR) is a G protein-coupled receptor belonging to the metabotropic glutamate receptor subfamily that was identified in 1993 by Brown, Hebert, and colleagues (192). The human *CASR* gene is located on chromosome 3q21 with a coding region of 3,234 bp and 6 exons (193). The CaSR is a ~120 kD protein forming homodimers through interactions of cysteine residues in the extracellular domain (194). The CaSR is predominantly expressed in the apical membrane of the parathyroid hormone (PTH)-secreting cells in the parathyroids and, in the kidney, in the apical membrane of PT cells and principal cells of the medullary CD and on the basolateral membrane of cells lining the TAL and DCT (195). The CaSR regulates the PTH secretion and modulates the renal tubular reabsorption of Ca^{2+} and Mg^{2+} in response to ionized serum Ca^{2+} and Mg^{2+} concentrations (195, 196).

The CaSR responds to physiologically relevant, millimolar concentrations of extracellular $Ca^{2+}[Ca^{2+}_o]$, with a half-maximal response (EC50) of 3 mM. It also shows a distinct affinity for various multivalent cations in vitro, including Mg^{2+} (EC50, 10 mM) (192). Activation of the CaSR mediates different, cell-specific signal transduction pathways (195). In bovine parathyroid cells, high levels of $[Ca^{2+}_o]$ activate phospholipase C (PLC) via a member of the Gq family, followed by the breakdown of phosphatidylinositol 4,5-bisphosphate with formation of 1,2-sn-diacylglycerol and of inositol 1,4,5-trisphosphate (IP3). The accumulation of IP3 leads to the release of intracellular pools of Ca^{2+} causing inhibition of PTH secretion through mechanisms that remain to be fully defined (195). Microperfusion studies in rat revealed that elevation of peritubular $[Ca^{2+}_o]$ and $[Mg^{2+}_o]$ markedly reduces the fractional absorption of Ca^{2+}, Mg^{2+}, and Na^+ in the TAL (197). As discussed above (see section on BS), the reabsorption of Ca^{2+} and Mg^{2+} in the TAL occurs mainly through a paracellular pathway driven by a lumen-positive, transepithelial potential generated by the combined activity of NKCC2 and ROMK (*Fig. 38-1*). High $[Ca^{2+}_o]$ in the TAL decreases hormone-dependent cAMP accumulation, reflecting a direct inhibition of the CaSR-dependent Galpha-adenylylate cyclase (AC) activity (195). In turn, the

reduced cAMP levels decrease NaCl transport, hence Ca^{2+} and Mg^{2+} reabsorption. In addition, Ca^{2+}-induced activation of the CaSR leads to production of arachidonic acid and its metabolites which inhibit the activity of ROMK and NKCC2 activity, further reducing Ca^{2+} and Mg^{2+} transport (198).

Additional evidence of the role of the CaSR in regulating tubular reabsorption of Ca^{2+} and Mg^{2+} was provided by the identification of different types of mutations in the *CASR* gene (199). Loss-of-function CaSR mutations result in familial hypocalciuric hypercalcaemia (FHH) and neonatal severe primary hyperparathyroidism (NSHPT) (200, 201), whereas gain-of-function CaSR mutations result in autosomal dominant hypocalcemia (ADH) (202, 203), which can be associated with a Bartter-like syndrome (204, 205) (*Table 38-4*; *Fig. 38-3*). The prevalence of FHH is up to one in 16,000, ADH one in 70,000, whereas NSHPT is very rare (206).

Familial Hypocalciuric Hypercalcemia, Neonatal Severe Primary Hyperparathyroidism

In 1972, Foley et al. described *familial hypocalciuric hypercalcemia (FHH)*, also named *familial benign hypercalcemia (FBH)*, (OMIM #145980), an autosomal dominant disorder characterized by a mild-to-moderate hypercalcemia, with mild hypermagnesemia, inappropriately normal or mildly elevated serum PTH levels, and hypocalciuria (207). Although patients with FHH are usually asymptomatic, complications such as chondrocalcinosis, acute pancreatitis and gallstones may occur with age (208, 209). A simple diagnostic test is a calcium over creatinine clearance ratio (CCCR) <0.01 (210). The finding of hypercalcemia in first-degree relatives supports the diagnosis, particularly when found in children under age 10 years.

Calcium infusion studies in FHH patients revealed a higher-than-usual set point for the release of PTH, suggesting an alteration in Ca^{2+} sensing (211). Genetic linkage studies mapped the gene for FHH to the region of chromosome 3 where the CaSR gene was located, and mutational analyses of the *CASR* gene revealed unique heterozygous mutations in approximately 90% of the FHH kindreds examined (199, 212, 213). Isolated cases of FHH with a de novo mutation in *CASR* have also been reported (214, 215). Many *CASR* mutations cluster in aspartate and glutamate-rich regions of the extracellular domain of the receptor, which may act as cationic binding sites (199). Expression studies confirmed that FHH-causing mutations induce a rightward shift of the set point for the Ca^{2+}-dependent responses, corresponding to a loss-of-function (201, 203). The defective extracellular CaSR likely leads to inappropriate absorption

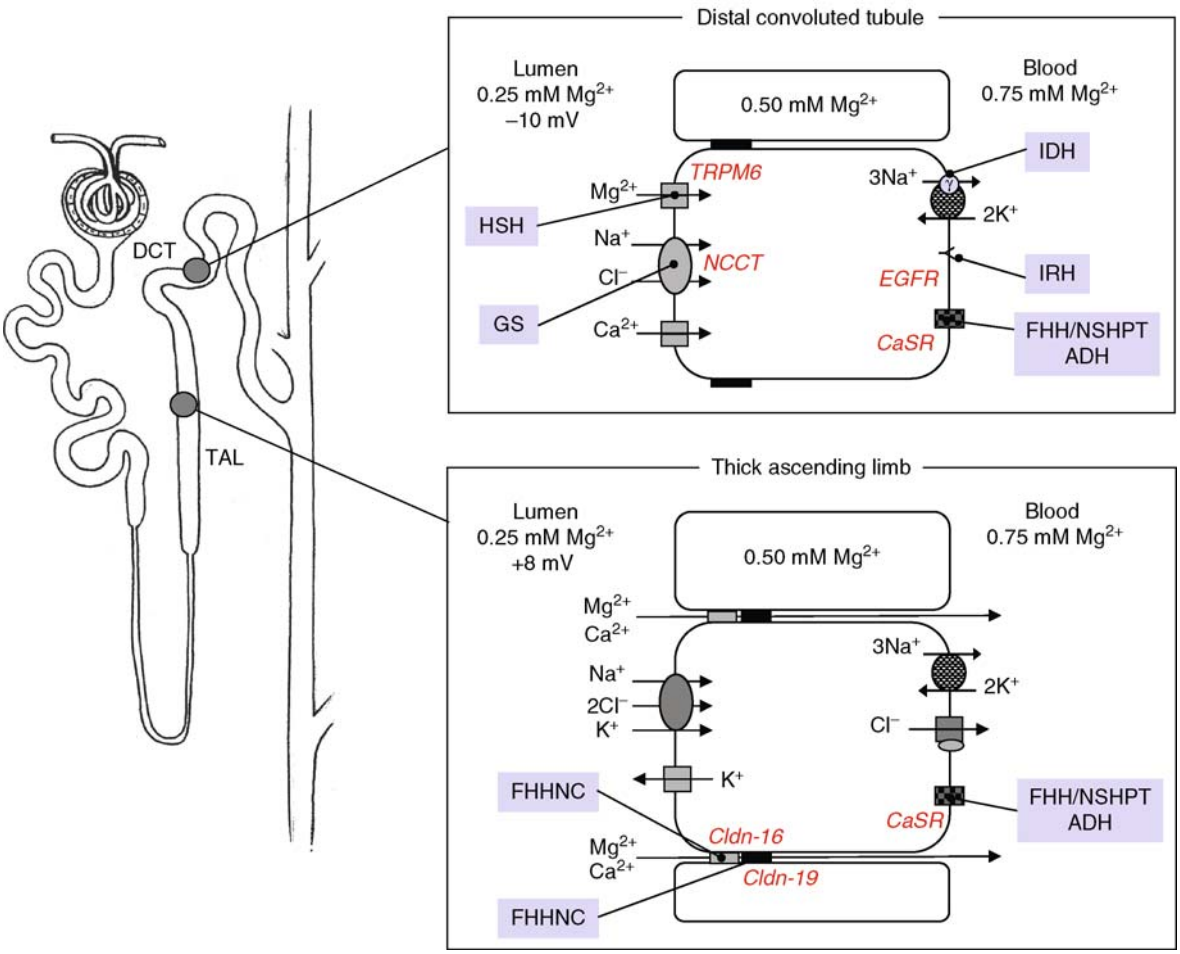
Table 38-4

Inherited disorders of the extracellular Ca^{2+}-sensing receptor

Disorder	OMIM #	Inheritance	Type of mutation	Age at onset	Serum Ca^{2+}	Serum Mg^{2+}	Serum PTH	Urine Ca^{2+}	Urine Mg^{2+}	Complications
Familial hypocalciuric hypercalcemia (FHH), Familial benign hypercalcemia (FBH)	145980	AD	Loss-of-function	Childhood	↑	N – ↑	N – ↑	↓	N – ↓	Pancreatitis, chondrocalcinosis, gallstones
Neonatal severe primary hyperparathyroidism (NSHPT)	239200	AR	Loss-of-function	Neonatal	↑↑	↑	↑↑	↓↓	↓	Life-threatening condition, failure to thrive, osteopenia, fractures
Autosomal dominant hypocalcemia (ADH), Autosomal dominant hypoparathyroidism	146200	AD	Gain-of-function	Infancy	↓	↓	↓	↑	↑ – ↑↑	Nephrocalcinosis and renal stones under vitamin D treatment, Bartter-like syndrome with the most severe activating mutations

Figure 38-3

Inherited disorders of magnesium reabsorption in the loop of Henle and distal convoluted tubule. In the thick ascending limb (TAL) of Henle's loop, Mg^{2+} is reabsorbed through a paracellular pathway, driven by the lumen-positive transcellular voltage generated by the transcellular reabsorption of NaCl. Mutations in the *CLDN16* and *CLDN19* genes that encode the tight junction proteins, claudin-16 and claudin-19 cause familial hypomagnesemia with hypercalciuria and nephrocalcinosis (FHHNC). In the distal convoluted tubule (DCT), Mg^{2+} is actively reabsorbed via the transcellular pathway involving an apical entry step through a Mg^{2+}-permeable ion channel (TRPM6) and a basolateral exit, presumably mediated by a Na^+–coupled exchange mechanism. The molecular identity of the basolateral exchange is unknown. Basolateral EGF stimulates the basolateral EGF receptor EGFR, which then increases the activity of TRPM6. Mutations of *SLC12A3* coding for NCCT are responsible for Gitelman syndrome (GS). Mutations in the apical TRPM6 channel (*TRPM6*) cause hypomagnesemia with secondary hypocalcemia (HSH), whereas mutations in the gamma-subunit of the Na^+-K^+-ATPase (*FXYD2*) cause isolated dominant hypomagnesemia (IDH) and mutations in the *EGF* gene coding for the epidermal growth factor EGF cause isolated recessive hypomagnesemia (IRH). Loss-of-function mutations in the *CASR* gene (CaSR) are associated with familial hypocalciuric hypercalcemia (FHH) and neonatal severe primary hyperparathyroidism (NSHPT), whereas activating mutations of the CaSR cause autosomal dominant hypocalcemia (ADH).



of Ca^{2+} and Mg^{2+} in the TAL (198) and Mg^{2+} transport in the DCT (196). Renal excretion of Ca^{2+} and Mg^{2+} is reduced, which leads to hypercalcemia and sometimes hypermagnesemia (216).

FHH is genetically heterogeneous, since no *CASR* mutation can be detected in ~ 10% of the probands. Two additional loci have been mapped on chromosome 19p13.3 and chromosome 19q13 (199). It must be noted

that patients with autoimmune manifestations may present circulating antibodies to the extracellular domain of the CaSR, which may interfere with the normal activation of the receptor by extracellular Ca^{2+}, leading to acquired FHH with hypocalciuria and hypercalcemia (217).

Neonatal severe primary hyperparathyroidism (NSHPT) (OMIM #239200) is a life-threatening, severe hyperparathyroidism characterized by hypercalcaemia, failure to thrive, osteopenia, multiple fractures, and rib cage deformities developing soon after birth (218). NSHPT is usually caused by homozygous *CASR* mutations in children born to consanguineous FBH parents (201, 202). Of note, a marked phenotypic heterogeneity has been observed amongst four members of a kindred harboring the homozygous (Q164X) mutation of *CASR* (219). Patients with sporadic NSHPT have been reported to be associated with de novo heterozygous inactivating mutations of *CASR* (199, 212). At least 60 mutations of the *CASR* gene, mostly missense, have been reported in FHH and NSHPT kindreds (http://www.hgmd.cf.ac.uk).

Ho et al. generated a CaSR knock-out mouse and showed that the heterozygous mice have modest elevations of serum calcium, magnesium and parathyroid hormone levels as well as hypocalciuria, thus mimicking FHH, whereas homozygous null mice show markedly elevated serum calcium and parathyroid hormone levels, parathyroid hyperplasia, bone abnormalities, retarded growth and premature death like humans with NSHPT (220). In order to remove the confounding effects of elevated PTH and assess the independent function of CaSR, double-homozygous mice lacking CaSR and Gcm2 were generated (221). Gcm2 is the mouse homologue of the *Drosophila* gcm (glial cell missing) gene which is specifically expressed in developing parathyroids, and its genetic ablation in mouse leads to a lack of parathyroid glands (222). The Gcm2 deficiency rescued the lethality of CaSR deficiency in this model. Furthermore, the lack of severe hyperparathyroidism prevented rickets and osteomalacia, but it did not rescue the hypocalciuria – indicating that hypocalciuria in FHH and NSHPT is mediated by the lack of CaSR in the kidney (221).

When treating FHH and NSHPT, one should consider that these disorders represent the mildest and severest variants of hyperparathyroidism, respectively. In most kindreds with FHH, the lifelong hypercalcemia is very mild, causing no specific symptoms, and requiring no treatment. In contrast, the severe hypercalcemia and hyperparathyroidism associated with NSHPT remains challenging and requires specific measures. The acute management of hypercalcemia classically relies on saline perfusion and careful use of loop diuretics. Pamidronate, a biphosphonate drug that could halt the bone resorption process mediated by uncontrolled hyperparathyroidism, has been successfully used in NSHPT patients to control severe hypercalcemia prior to parathyroidectomy (219). Radical subtotal parathyroidectomy is often the treatment of choice in NSHPT (223). Parathyroidectomy may also be appropriate in kindreds with FHH in which there is unusually severe hypercalcemia, particularly with musculoskeletal and neurobehavioral manifestations, or frankly elevated PTH levels (224). Calcimimetic CaSR activators, which potentiate the activation of the CaSR by extracellular Ca^{2+}, reset the Ca^{2+}-regulated PTH release in primary and secondary hyperparathyroidism toward normal. These drugs may be of interest in FHH and NSHPT, in which they could increase the sensitivity of the CaSR to extracellular Ca^{2+}, thereby reducing PTH secretion and serum calcium concentration (225). Such an effect has been documented in a single FHH patient due to a de novo inactivating mutation of the CaSR, in which a maintenance treatment with the calcimimetic drug Cinacalcet HCl resulted in a rapid decrease in PTH secretion and a sustained normalization of serum calcium (215).

Autosomal Dominant Hypocalcemia

Activating mutations of the *CASR* gene were first described in families affected with autosomal dominant hypocalcemia (ADH, also named autosomal dominant hypoparathyroidism, or autosomal dominant hypocalcemia with hypercalciuria, ADHH) (OMIM #146200) (202, 203). Affected individuals present with hypocalcemia, hypercalciuria, and polyuria, and about 50% of these patients have hypomagnesemia. The serum phosphate concentrations in patients with ADH are either elevated or in the upper-normal range (199, 203). Hypocalcemia in ADH is generally mild to moderate, and patients may present carpopedal spasms and/or seizures. Elevated urinary calcium may lead to nephrolithiasis despite increased magnesium excretion (57).

More than 20 different mutations of the *CASR* gene, mostly missense, have been identified in ADH patients. About half of these mutations are in the extracellular domain of the CaSR (199). Expression studies confirmed that these activating mutations induce a leftward shift in the dose-response curve of the mutant CaSR, corresponding to enhanced sensitivity for extracellular Ca^{2+} and Mg^{2+} (203). This results in inappropriately low serum PTH and decreased reabsorption of Ca^{2+} and Mg^{2+} in the TAL and DCT, leading to Ca^{2+} and Mg^{2+} wasting. The impaired reabsorption of Ca^{2+} and Mg^{2+} in the TAL is thought to

be due to a reduction of the paracellular permeability and/or to a decreased lumen-positive transepithelial voltage due to defective transcellular NaCl reabsorption (195). Hormone-stimulated Mg^{2+} reabsorption is also inhibited in the DCT, which probably contributes to the renal magnesium loss (155).

In treating ADH patients with an activating CaSR mutation, it is important to avoid vitamin D which can dramatically increase urinary calcium excretion, leading to nephrocalcinosis, nephrolithiasis, and even irreversible reduction of renal function in some patients (199, 203). Therefore, the treatment of hypocalcemia in ADH with vitamin D and calcium supplementation should be restricted to clearly symptomatic patients (57). Addition of hydrochlorothiazide may reduce urinary calcium excretion and maintain serum calcium concentrations near the lower limit of normal, allowing the reduction of vitamin D treatment (226).

It was shown recently that patients with ADH due to activating *CASR* mutations may have a clinical course complicated with a Bartter-like syndrome, i.e., development of a salt-losing tubulopathy associated with urinary concentrating defect and hypokalemic metabolic alkalosis (204, 205). All three patients described thus far had hypomagnesemia. Heterologous expression of the mutant CaSR revealed that the underlying mutations (L125P, C131W, A843E) are among the most severe gain-of-function *CASR* mutations, characterized by a leftward shift in the dose-response curve for the receptor and also a much lower EC50 than patients with ADH (204, 205). These mutations appear to be fully activated under normal serum Ca^{2+} concentrations and induce a significant salt-losing phenotype by inhibiting the reabsorption of NaCl in the TAL (*Fig. 38-1*). Accordingly, this subset of ADH patients presenting a Bartter-like syndrome was qualified as "Type 5 Bartter-like syndrome." The inclusion of these cases among the Bartter-like syndromes is debated, and it must be pointed that the Bartter-like phenotype may be very mild, as recently reported for another ADH-causing mutation (227).

Disorders of Magnesium Metabolism

Introduction

Magnesium is an important intracellular cation. As a cofactor, it is involved in energy metabolism and protein and nucleic acid synthesis. It is also critical for the modulation of membrane transporters and in signal transduction. Under physiologic conditions, serum Mg^{2+} levels are maintained at almost constant values. Mg^{2+} homeostasis depends on a balanced intestinal absorption and renal excretion. Mg^{2+} deficiency can result from reduced dietary intake, intestinal malabsorption or renal loss. The control of body Mg^{2+} homeostasis primarily resides in the kidney tubules.

The dietary intake of Mg^{2+} may vary substantially. The principal site of Mg^{2+} absorption is the small intestine, where Mg^{2+} absorption occurs via two different pathways: a saturable active transcellular transport and a nonsaturable paracellular passive transport (228, 229). In the kidney, approximately 80% of total serum Mg^{2+} is filtered in the glomeruli, of which more than 95% is reabsorbed along the nephron. Tubular Mg^{2+} reabsorption differs in quantity and kinetics depending on the different nephron segments. In the adult kidney, approximately 15–20% is reabsorbed in the PT, whereas the premature kidney of the newborn is able to reabsorb up to 70% of the filtered Mg^{2+} in this nephron segment (230). From early childhood on, roughly 70% of Mg^{2+} is reabsorbed in the cortical TAL of the loop of Henle. Transport in this segment is passive and paracellular, mediated by claudin-16 and claudin-19. The driving force for reabsorption against an unfavorable concentration gradient is the lumen-positive transepithelial voltage (*Fig. 38-3*). Only 5–10% of the filtered Mg^{2+} is reabsorbed in the DCT. However, in this part of the nephron the fine adjustment of renal excretion is accomplished. In the DCT, Mg^{2+} transport is an active transcellular process (*Fig. 38-3*). Physiologic studies indicate that apical entry into DCT cells is mediated by the specific and regulated Mg^{2+} channel TRPM6. The mechanism of basolateral transport into the interstitium is unknown. Here, Mg^{2+} has to be extruded against an unfavorable electrochemical gradient. Most physiologic studies favor a Na^{+}-dependent exchange mechanism (231). Mg^{2+} entry into DCT cells appears to be the rate-limiting step and the site of regulation. For details of Mg^{2+} transport in the distal tubule see Dai et al. (155). In the collecting duct, there is no significant Mg^{2+} uptake. Finally, 3–5% of the filtered Mg^{2+} is excreted in the urine.

Magnesium depletion is usually secondary to another disease process or to a therapeutic agent (e.g., loop diuretics, thiazides, aminoglycosides, cisplatine, calcineurin inhibitors). During infancy and childhood, a substantial proportion of patients receiving medical attention for signs of hypomagnesemia are affected by inherited renal disorders associated with Mg^{2+} wasting. In these disorders hypomagnesemia may either be a leading symptom or may be part of a complex phenotype resulting from tubular dysfunction, as will be detailed below. Recent advances

in molecular genetics of hereditary hypomagnesemia substantiated the role of a variety of genes and their encoded proteins in human epithelial Mg^{2+} transport, and helped to characterize different clinical subtypes of hereditary Mg^{2+}-wasting (Table 38-5). A careful clinical and biochemical assessment allows to distinguish the different disease entities in most cases, even when there is a considerable overlap in the phenotypic characteristics (Table 38-6).

Gitelman Syndrome

This primary salt-wasting disorder complicated by urinary Mg^{2+} wasting and hypomagnesemia is discussed in detail above.

Isolated Dominant Hypomagnesemia

Isolated dominant hypomagnesemia (IDH, OMIM #154020) results from a mutation in the *FXYD2* gene on chromosome 11q23 which encodes a γ-subunit of the Na^+-K^+-ATPase (232). Only two IDH families have been described so far (233, 234). In both families, the index patients presented with seizures during childhood (at 7 and 13 years) with serum Mg^{2+} levels of approximately 0.4 mmol/L. One patient was treated for seizures of unknown origin with antiepileptic drugs until serum Mg^{2+} levels were evaluated during adolescence. At that time mental retardation was evident. Serum Mg^{2+} measurements performed in members of both families revealed low serum Mg^{2+} levels (around 0.5 mmol/L) in numerous apparently healthy individuals. A ^{28}Mg-retention study in one of the patients indicated a primary renal defect (233). The intestinal absorption of Mg^{2+} was preserved and even stimulated in compensation for the increased renal losses. Urinary Mg^{2+} measurements in affected family members revealed significant renal Mg^{2+} loss (around 5 mmol per day) despite profound hypomagnesemia. Urinary Ca^{2+} excretion rates were low in all hypomagnesemic individuals, a finding reminiscent of patients presenting with GS. However, in contrast to GS patients, no other biochemical abnormalities were reported, especially no hypokalemic alkalosis. In the two families, hypomagnesemia was inherited as an autosomal dominant trait. A genome-wide linkage study could map IDH to chromosome 11q23 (235). Detailed haplotype analysis demonstrated a common

Table 38-5
Inherited disorders of renal magnesium handling

Disorder	OMIM #	Inheritance	Gene locus	Gene	Protein
Gitelman syndrome	263800	AR	16q13	*SLC12A3*	NCCT, Na^+-Cl^- cotransporter
Isolated dominant hypomagnesemia	154020	AD	11q23	*FXYD2*	γ-subunit of the Na^+-K^+-ATPase
Isolated recessive hypomagnesemia	611718	AR	4q25	*EGF*	Pro-EGF, epidermal growth factor
Autosomal dominant hypocalcemia, Autosomal dominant hypoparathyroidism	146200	AD	3q21	*CASR*	CaSR, Ca^{2+}/Mg^{2+}sensing receptor
Familial hypocalciuric hypercalcemia, Familial benign hypercalcemia	145980	AD	3q21	*CASR*	CaSR, Ca^{2+}/Mg^{2+} sensing receptor
Neonatal severe primary hyperparathyroidism	239200	AR	3q21	*CASR*	CaSR, Ca^{2+}/Mg^{2+} sensing receptor
Familial hypomagnesemia with hypercalciuria/ nephrocalcinosis	248250	AR	3q28	*CLDN16*	Claudin-16 (paracellin-1), tight junction protein
Familial hypomagnesemia with hypercalciuria/ nephrocalcinosis and severe ocular involvement	248190	AR	1p34	*CLDN19*	Claudin-19, tight junction protein
Hypomagnesemia with secondary hypocalcemia	602014	AR	9q22	*TRPM6*	TRPM6, Mg^{2+} channel
Hypomagnesemia/metabolic syndrome	500005	maternal	mtDNA	*MTTI*	Mitochondrial tRNA (Isoleucin)

Table 38-6

Clinical and biochemical characteristics of inherited hypomagnesemia

Disorder	Age at onset	Serum Mg^{2+}	Serum Ca^{2+}	Serum K^+	Blood pH	Urine Mg^{2+}	Urine Ca^{2+}	Nephro-calcinosis	Renal stones
Gitelman syndrome	Adolescence	↓	N	↓	↑	↑	↓	No	no
Isolated dominant hypomagnesemia	Childhood	↓	N	N	N	↑	↓	no	no
Isolated recessive hypomagnesemia	Childhood	↓	N	N	N	↑	N	no	no
Autosomal dominant hypocalcemia, Autosomal dominant hypoparathyroidism	Infancy	↓	↓	N	N or ↓	↑	↑ – ??	yes[a]	yes[a]
Familial hypocalciuric hypercalcemia, Familial benign hypercalcemia	Often asymptomatic	N to ↑	↑	N	N	↓	↓	no	?
Neonatal severe primary hyperparathyroidism	Infancy	N to ↑	↑↑↑	N	N	↓	↓	no	?
Familial hypomagnesemia with hypercalciuria/nephrocalcinosis	Childhood	↓	N	N	N or ↓	↑↑	↑↑	yes	yes
Hypomagnesemia with secondary hypocalcemia	Infancy	↓↓↓	↓	N	N	↑	N	no	no

[a]frequent complication during therapy with Ca^{2+} and vitamin D

haplotype segregating in the two families suggesting a common ancestor. Subsequent mutation analysis of the *FXYD2* gene demonstrated the identical mutation G41R in all affected individuals of both family branches (232).

The protein encoded by *FXYD2* is a member of a small single transmembrane protein family which share the common amino acid motif F-X-Y-D. FXYD proteins modulate the function of the ubiquitous Na^+-K^+-ATPase, a dimeric enzyme invariably consisting of one α- and one β-subunit. FXYD proteins constitute a third or γ-subunit that represents a tissue-specific regulator of the Na^+-K^+-ATPase. Two members of this family, FXYD2 and FXYD4, are highly expressed along the nephron displaying an alternating expression pattern (236). The FXYD2 γ-subunit comprises two isoforms (named γ-α and γ-β) that are differentially expressed in the kidney. The γ-α isoform is present predominantly in the proximal tubule and expression of the γ-β isoform predominates in the distal nephron, especially in the DCT and connecting tubule (237). The FXYD2 γ-subunit increases the apparent affinity of Na^+-K^+-ATPase for ATP while decreasing its Na^+ affinity (237). Thus, it might provide a mechanism for balancing energy utilization and maintaining appropriate salt gradients.

Expression studies of the mutant G41R-γ-subunit revealed a dominant-negative effect leading to a retention of the γ-subunit in the Golgi complex. The mechanism of a dominant negative effect is supported by the observation that individuals with a large heterozygous deletion of chromosome 11q including the *FXYD2* gene exhibit normal serum Mg^{2+} levels (234). Urinary Mg^{2+} wasting together with the expression pattern of the *FXYD2* gene indicate defective transcellular Mg^{2+} reabsorption in the DCT in IDH patients. The exact mechanism causing increased urinary Mg^{2+} excretion has yet to be determined. Meij and colleagues have suggested that diminished intracellular K^+ might depolarize the apical membrane resulting in a decreased Mg^{2+} uptake (232). Alternatively, an increase in intracellular Na^+ could impair basolateral Mg^{2+} transport which is presumably achieved by a Na^+-coupled exchange mechanism. Another explanation is that the γ-subunit is not only involved in Na^+-K^+-ATPase function but also an essential component of a yet unidentified ATP-dependent transport system specific for Mg^{2+}. Similar to Ca^{2+}, both, a specific Mg^{2+}-ATPase and a Na^+-coupled exchanger might exist. Further studies are needed to clarify this issue.

An interesting feature of IDH is the finding of hypocalciuria which is primarily observed in GS. Unfortunately, only one large family with IDH has been described and an animal model for IDH is still lacking. Mice lacking the γ-subunit (*Fxyd2*) do not demonstrate significant abnormalities in Mg^{2+} conservation or balance (238). One could speculate that, like in GS, a defect in Na^+-K^+-ATPase

function and energy metabolism might lead to an apoptotic breakdown of the early DCT responsible for Mg^{2+} reabsorption, while later parts of the distal nephron remain intact. In IDH, there is no evidence for renal salt wasting and no stimulation of the RAAS. The finding of hypocalciuria without apparent volume depletion apparently contradicts recent experimental data which favor an increase in proximal tubular Ca^{2+} reabsorption due to volume depletion in GS (154).

Isolated Recessive Hypomagnesemia

Geven and colleagues reported a form of isolated recessive hypomagnesemia (IRH, OMIM #611718) in a consanguineous family (239). Two affected girls presented with generalized seizures during infancy. Possibly related to late diagnosis, both patients also exhibited neurodevelopmental deficits. Clinical and laboratory workup at 4 and 8 years of age, respectively, revealed serum Mg^{2+} levels around 0.5–0.6 mmol/L with no other associated electrolyte abnormalities. A ^{28}Mg-retention study in one patient pointed to a primary renal defect while intestinal Mg^{2+} uptake was preserved (239). Both patients exhibited renal Mg^{2+} excretion of 3–6 mmol per day despite hypomagnesemia confirming renal Mg^{2+} wasting. In contrast to IDH, renal Ca^{2+} excretion rates in IRH are within the normal range.

The molecular defect for IRH was identified by Groenestege et al. who demonstrated a homozygous P1070L mutation in both affected siblings in the *EGF* gene encoding the epidermal growth factor EGF (240). The EGF protein is expressed in the DCT, and its binding to the receptor EGFR is essential for the function of the TRPM6 channel. The mutation is located in the cytosolic C-terminal terminus within a sorting motif (PXXP) which is necessary for the trafficking of EGF to the basolateral membrane. Expression studies demonstrated that mutant pro-EGF retains EGF secretion to the apical membrane but does not reach the EGF receptor in the basolateral membrane, resulting in dysfunction of TRPM6 (240). Even if IRH seems to be an extremely rare disease phenotype, the identification of EGF mutations is important because this is the first autocrine/paracrine magnesiotropic hormone known at the molecular level.

Familial Hypomagnesemia with Hypercalciuria and Nephrocalcinosis

Familial hypomagnesemia with hypercalciuria and nephrocalcinosis (FHHNC, OMIM #248250) is an autosomal recessive tubular disorder. Since its first description by Michelis et al. in 1972 (241), numerous kindreds have been reported, allowing a comprehensive characterization of the clinical spectrum of this disorder and discrimination from other Mg^{2+} losing tubular diseases (242–246). As a consequence of excessive renal Mg^{2+} and Ca^{2+} wasting, patients develop the characteristic triad of hypomagnesemia, hypercalciuria and nephrocalcinosis that gave the disease its name. Most FHHNC patients present during early childhood with recurrent urinary tract infections, polyuria/polydispsia, nephrolithiasis, and/or failure to thrive. Clinical signs of severe hypomagnesemia are less common. Extrarenal manifestations, especially ocular involvement (including severe myopia, nystagmus, or chorioretinitis) have been reported (244–246). Additional laboratory findings include elevated serum PTH levels before the onset of chronic renal failure, incomplete distal tubular acidosis, hypocitraturia, and hyperuricemia, which are present in most patients (247). The clinical course of FHHNC patients is often complicated by the development of renal failure during the first two decades of life, and about one third of patients develop ESRD during adolescence. Due to a reduction in filtered Mg^{2+} that limits urinary Mg^{2+} excretion, hypomagnesemia may completely disappear with the decline of GFR.

Beside continuous Mg^{2+} supplementation, therapy aims to reduce Ca^{2+} excretion by using thiazides to prevent the progression of nephrocalcinosis and stone formation. The degree of renal calcification has been correlated with progression of chronic renal failure (245). In a short term study, thiazides have been demonstrated to effectively reduce urinary calcium excretion in FHHNC patients (248). However, these therapeutic strategies have not been shown yet to significantly influence the progression of renal failure. Supportive therapy is important for protecting kidney function and should include provision of sufficient fluids and whenever possible, the prevention and/or effective therapy of urinary tract infections. Renal transplantation does not result in recurrence of the disease because the primary defect resides in the kidney.

By positional cloning, Simon et al. identified a new gene on 3q27 (*CLDN16*, formerly *PCLN1*), which is mutated in patients with FHHNC (249). *CLDN16* codes for claudin-16, a member of the claudin family. More than 20 claudins identified so far comprise a family of ~22 kD proteins with four transmembrane segments, two extracellular domains, and intracellular N- and C-termini. Claudins are important components of tight junctions. The individual composition of tight junction strands with different claudins confers the characteristic properties of

different epithelia for paracellular permeability and/or transepithelial resistance. In this context, a crucial role has been attributed to the first extracellular domain of the claudin protein which is extremely variable in number and position of charged amino acid residues. Most mutations reported so far in FHHNC are simple missense mutations affecting the transmembrane domains and the extracellular loops with a particular clustering in the first extracellular loop containing the putative ion selectivity filter. Within this domain, patients originating from Germany and Eastern European countries exhibit a common mutation (L151F) due to a founder effect (247). As this mutation is present in approximately 50% of mutant alleles, molecular diagnosis is greatly facilitated in patients originating from these countries.

Family analysis revealed that carriers of heterozygous *CLDN16* mutations may also present with clinical symptoms. Two independent studies describe a high incidence of hypercalciuria, nephrolithiasis and/or nephrocalcinosis in first degree relatives of FHHNC patients (245, 247). A subsequent study also reported a tendency towards mild hypomagnesemia in family members with heterozygous *CLDN16* mutations (250). Thus, one might speculate that *CLDN16* mutations could be involved in idiopathic hypercalciuric stone formation.

A homozygous *CLDN16* mutation (T303R) affecting the C-terminal PDZ domain has been identified in two families with isolated hypercalciuria and nephrocalcinosis without disturbances in renal Mg^{2+} handling (251). Interestingly, the hypercalciuria disappeared during follow-up and urinary Ca^{2+} levels reached normal values beyond puberty. Transient transfection of Madine Darby canine kidney (MDCK) cells with the *CLDN16* (T303R) mutant revealed a mistargeting into lysosomes whereas wildtype claudin-16 was correctly localized to tight junctions. It still remains to be determined why this type of misrouting is associated with transient isolated hypercalciuria without increased Mg^{2+} excretion.

The exact physiological role of claudin-16 is still not fully understood. From the FHHNC disease phenotype, it was concluded that claudin-16 might regulate the paracellular transport of Mg^{2+} and Ca^{2+} ions by contributing to a selective paracellular conductance by building a pore permitting paracellular fluxes of Mg^{2+} and Ca^{2+} down their electrochemical gradients (249, 252). However, recent functional studies in porcine renal tubule epithelial kidney cells (LLC-PK1) cells could show that the expression of claudin-16 selectively and significantly increased the permeability of Na^{+} with a far less-pronounced change of Mg^{2+} flux. From these observations, it was hypothesized that in the TAL claudin-16 probably contributes to the generation of the lumen-positive potential (allowing the passive reabsorption of divalent cations) rather than to the formation of a paracellular channel selective for Ca^{2+} and Mg^{2+} (253).

As mentioned above, many FHHNC patients develop chronic renal failure associated with progressive tubulointerstitial nephritis. The pathophysiology of this phenomenon, which is not usually observed in other tubular disorders is unclear. Traditionally, renal failure in FHHNC has been attributed to the concomitant hypercalciuria and nephrocalcinosis, but a true correlation has not been established. Therefore, it has been speculated that claudin-16 is not only involved in paracellular electrolyte reabsorption but also in tubular cell proliferation and differentiation (254). This hypothesis is supported by the bovine *CLDN16* knockout phenotype, which exhibit early onset renal failure due to interstitial nephritis with diffuse zonal fibrosis (255, 256). Tubular epithelial cells were reported as "immature" with loss of polarization and attachment to the basement membrane. A close association between fibrosis and abnormal tubules was noted, and the term "renal tubular dysplasia" was used to emphasize that the lesions develop first in the epithelial cells of the renal tubules (257). These cattle have large homozygous deletions whereas human FHHNC mutations are mainly missense mutations affecting the extracellular loops of claudin-16. From these observations it appears that the site and extent of the mutation determines the phenotypic manifestation ranging from isolated alterations in channel conductance to an alteration in cell proliferation and differentiation. This hypothesis is consistent with the results of a large retrospective study in which the clinical course of FHNNC in more than 70 patients was compared to the functional analysis of the underlying *CLDN16* mutations (258). This study could demonstrate that patients carrying complete loss of function mutations on both alleles are younger at disease onset and have a much more rapid decline of GFR than those patients with at least one mutant allele which displays residual function (258).

FHHNC is genetically heterogenous, since mutations in another tight junction gene encoding claudin-19 have also been demonstrated to cause this disease (259). The identification of *CLDN19* mutations could explain the variable ocular phenotype, because *CLDN19* defects seem to be invariably associated with severe ocular abnormalities (including severe myopia, nystagmus, or macular coloboma) (244–246). The latter association has been named FHHNC with severe ocular involvement (OMIM #248190). In contrast, only a small subset of FHHNC patients with *CLDN16* defects display severe myopia whereas nystagmus or colobomata have not been described (247).

The renal phenotype is very similar between these two FHHNC subtypes. Expression studies revealed that claudin-16 and claudin-19 perfectly colocalize at tight junctions of the TAL (259). It could further be demonstrated that claudin-16 and claudin-19 functionally interact, which could increase the cation selectivity of tight junctions above that of claudin-16 alone (260). This is most likely due to anion-blocking properties of claudin-19 preventing back diffusion of Cl^- anions to the tubular lumen.

Hypomagnesemia with Secondary Hypocalcemia

Hypomagnesemia with secondary hypocalcemia (HSH, OMIM #602014) is a rare autosomal recessive disorder first described in 1968 (261). It manifests in early infancy with generalized seizures or other symptoms of increased neuromuscular excitability. Delayed diagnosis or noncompliance with treatment can be fatal or result in permanent neurological damage. Biochemical abnormalities include extremely low serum Mg^{2+} (about 0.2 mmol/L) and low serum Ca^{2+} levels. The mechanism leading to hypocalcemia is still not completely understood. Severe hypomagnesemia results in an impaired synthesis and/or release of PTH (262). Consistently, PTH levels in HSH patients were found to be inappropriately low. The hypocalcemia observed in HSH does not respond to therapy with Ca^{2+} or vitamin D. Relief of clinical symptoms, normocalcemia, and normalization of PTH levels can only be achieved by administration of high doses of Mg^{2+} (263). Transport studies in HSH patients indicated a primary defect in intestinal Mg^{2+} absorption (264). However, in some patients an additional renal leak for Mg^{2+} was suspected (265).

A gene locus (*HOMG1*) for HSH had been mapped to chromosome 9q22 in 1997 (266). Later, two independent groups identified *TRPM6* at this locus and reported loss of function mutations, mainly truncating mutations, as the underlying cause of HSH (156, 267). Subsequently, additional HSH mutations in *TRPM6* have been identified (268, 269). *TRPM6* encodes a member of the transient receptor potential (TRP) family of cation channels. The TRPM6 protein is homologous to TRPM7, a Ca^{2+} and Mg^{2+} permeable ion channel regulated by Mg-ATP (270). TRPM6 is expressed along the entire small intestine and colon but also in the kidney in distal tubule cells. Immunofluorescence studies localized TRPM6 to the apical membrane of the DCT (271) confirming that renal Mg^{2+} wasting could play a role in the pathogenesis of HSH (272). This was also supported by intravenous Mg^{2+} loading tests in HSH patients, which disclosed a considerable renal Mg^{2+} leak (267).

TRPM6 is closely related to TRPM7 and represents the second TRP protein being fused to a C-terminal α-kinase domain. The *TRPM6* gene encodes a large protein with 2,022 amino acid residues. *TRPM6*-mRNA shows a more restricted expression pattern than TRPM7 with highest levels along the intestine and the DCT of the kidney (156). Immunohistochemistry shows a complete colocalization with the Na^+-Cl^- cotransporter NCCT (also serving as a DCT marker) but also with parvalbumin and calbindin-D_{28K}, two cytosolic proteins that putatively act as intracellular (Ca^{2+} and) Mg^{2+} buffers (271). As yet, the biophysical characterization of TRPM6 remains controversial. Voets et al. could demonstrate striking parallels between TRPM6 and TRPM7 with respect to gating mechanisms and ion selectivity profiles, since TRPM6 was shown to be regulated by intracellular Mg^{2+} levels, and to be permeable for Mg^{2+} and Ca^{2+} (271). Permeation characteristics with currents almost exclusively carried by divalent cations with a higher affinity for Mg^{2+} than Ca^{2+} support the role of TRPM6 as the apical Mg^{2+} influx pathway. Furthermore, TRPM6 -analogous to TRPM7- exhibits a marked sensitivity to intracellular Mg^{2+}. Thus one might speculate about an inhibition of TRPM6-mediated Mg^{2+} uptake by rising intracellular Mg^{2+} concentrations, as a possible mechanism for regulation of intestinal and renal Mg^{2+} (re-)absorption. This inhibition might in part be mediated by intracellular Mg-ATP as shown for TRPM7 (270). Chubanov et al. reported that TRPM6 is only present at the cell surface when associating with TRPM7 (273). Furthermore, FRET (fluorescence resonance energy transfer) analyses showed a specific direct protein-protein interaction between both proteins. Electrophysiological data in a *Xenopus* oocyte expression system indicated that coexpression of TRPM6 results in a significant amplification of TRPM7-induced currents (273). Schmitz et al. (274) demonstrated that TRPM6 and TRPM7 are not functionally redundant and that both proteins can influence each other's biological activity. In particular, TRPM6 can phosphorylate TRPM7 and TRPM6 might modulate TRPM7 function in a Mg^{2+}-dependant manner (274).

TRPM6 has been shown to be regulated by the first magnesiotropic hormone identified so far, namely the epithelial growth factor (EGF) which is expressed in the DCT. In a cell culture model, Groenstege et al. could show that EGF increases the activity of TRPM6 expressing cells (240). This is in line with the clinical observation that cancer patients treated with cetuximab, a monoclonal antibody directed against the EGF receptor, develop

hypomagnesemia secondary to increased Mg^{2+}-wasting. These findings suggest that EGF acts in an autocrine or a paracrine manner to stimulate TRPM6 activity leading to increased reabsorption of Mg^{2+} in the DCT. TRPM6 is also regulated by changes in body magnesium content, with hypomagnesemia resulting in the upregulation of TRPM6 expression not only in the DCT but also in the gastrointestinal tract (275). Similarly, 17-beta-Estradiol induces an upregulation of TRPM6, as shown in ovariectomized rats (275). From a clinical point of view it is important to note that the well-known hypomagnesemia in patients receiving calcineurin inhibitors (cyclosporin A, FK506) is at least in part mediated by downregulation of TRPM6 (276, 277).

Mitochondrial Hypomagnesemia

A mutation in the mitochondrial-coded isoleucine tRNA gene, $tRNA^{Ile}$ or MTTI, related to hypomagnesemia has been discovered in a large Caucasian kindred (278). An extensive clinical evaluation of this family was prompted after the discovery of hypomagnesemia in the index patient, leading to the characterization of mitochondrial hypomagnesemia (OMIM #500005). Indeed, pedigree analysis was compatible with mitochondrial inheritance as the phenotype was exclusively transmitted by affected females. The phenotype includes hypomagnesemia, hypercholesterolemia, and hypertension. Of the adults on the maternal lineage, the majority of offspring exhibited at least one of the mentioned symptoms, approximately half of the individuals showed a combination of two or more symptoms, and around 1/6 had all three features. Serum Mg^{2+} levels of family members on the maternal lineage varied greatly from ~0.3 to ~1.0 mmol/L with approximately 50% of individuals being hypomagnesemic. The hypomagnesemic individuals (serum Mg^{2+} <0.9 mmol/L) showed higher fractional excretions (median around 7.5%) than their normomagnesemic relatives (median around 3%) clearly pointing to renal Mg^{2+} wasting as causative for hypomagnesemia. Interestingly, hypomagnesemia was accompanied by decreased urinary Ca^{2+} levels, a finding pointing to the DCT as the affected tubular segment.

The mitochondrial mutation observed in the affected family involves the $tRNA^{Ile}$ gene *MTTI*. The observed nucleotide exchange occurs at the T-nucleotide directly adjacent to the anticodon triplet. This position is highly conserved among species and critical for codon-anticodon recognition. The functional consequences of the tRNA defect for mitochondrial function remain to be elucidated in detail. As ATP consumption along the tubule is highest in the DCT, the authors speculate about an impaired energy metabolism of DCT cells as a consequence of the mitochondrial defect which in turn could lead to disturbed transcellular Mg^{2+} reabsorption (278). Further studies in these patients might help to better understand the mechanism of distal tubular Mg^{2+} wasting in this disease.

Management of Hypomagnesemia/ Magnesium Deficiency

The main goal of Mg^{2+} substitution in hypomagnesemic patients is the relief of clinical symptoms. In most cases, especially in primary Mg^{2+} wasting diseases, normal levels cannot be achieved by oral substitution without considerable gastrointestinal side effects. The route of administration depends on the severity of clinical symptoms. Acute intravenous infusions should be reserved for patients with severe symptoms, i.e., with cerebral seizures (279). Especially in children, painful intramuscular injections should be avoided. In infants and children, the starting dose is 20–50 mg Mg^{2+} sulfate (0.1–0.2 mmol Mg^{2+}) per kilogram body weight. Mg^{2+} sulfate should be given slowly intravenously (over 20 min). The maximum dose for adults is 2 g of Mg^{2+} sulfate. Single doses can be repeated every 6–8 h or followed by continous infusion of 100–200 mg Mg^{2+} sulfate (0.4–0.8 mmol Mg^{2+}) per kilogram body weight per day (280). During Mg^{2+} infusion, close monitoring of cardiorespiratory function is important and Ca^{2+} gluconate should be available as an antidote. The assessment of renal function is also mandatory.

Asymptomatic hypomagnesemia or chronic Mg^{2+} deficiency should be treated with oral Mg^{2+} substitution. In children, 10–20 mg Mg^{2+} (0.4–0.8 mmol) per kg body weight given three to four times a day has been recommended to correct hypomagnesemia (281). Of note, the solubility, intestinal absorption, and side effects considerably differ depending on the Mg^{2+} salt used for oral therapy. The bioavailability and pharmacokinetics of different Mg^{2+} salts have been reviewed recently (282). With respect to solubility, intestinal absorption and bioavailability, organic Mg^{2+} salts (e.g., citrate or aspartate) appear most suitable for oral substitution. Moreover, the laxative effect of organic Mg^{2+} salts seems to be less pronounced compared to inorganic salts.

The use of certain diuretics has been proposed for the reduction of renal Mg^{2+} excretion. Both, K^+-sparing diuretics and aldosterone antagonists, exert Mg^{2+}-sparing effects (283, 284). Their beneficial effect on renal Mg^{2+} excretion, serum Mg^{2+} levels and clinical symptoms is well documented in hereditary Mg^{2+}-wasting diseases (186, 285).

Low Renin Hypertension with Hypokaliemia

Glucocorticoid-Remediable Aldosteronism

Glucocorticoid-remediable aldosteronism (GRA, OMIM #103900), also called dexamethasone-suppressible hyperaldosteronism (DSH) or familial hyperaldosteronism type I (FH-I) is a rare but fascinating disease. It was first individualized by Sutherland and coworkers in 1966 (286) and since then has been reported in less than 100 unrelated cases (287, 288).

Clinical Features

Individuals with GRA are usually hypertensive in the youth and demonstrate rapidly a severe form of hypertension, despite the fact that few families with a moderate phenotype have been described (289). Since the disease is transmitted as an autosomal dominant trait with a high penetrance, there is often a strong family history of hypertension and/or stroke. Indeed, analysis of affected kindreds has shown a high prevalence of hemorrhagic stroke and ruptured intracranial aneurysms, usually before the age of 40 years (290). The biological profile of affected subjects suggests a primary aldosteronism but GRA patients can be normokaliemic. A specific feature of the disease is the aldosterone hyperresponsiveness to maneuvers stimulating or inhibiting the cortisol hypopituitary axis (291). Acute or chronic administration of ACTH induces a strong increase in plasma aldosterone level, whereas it has little or small effect on patients with other forms of primary aldosteronism. Conversely, aldosterone is suppressed by the administration of glucocorticoids, the acute dexamethasone suppression test being recognized as a diagnostic test for the pathology (292, 293). The second specific feature is the abundant urinary excretion of 18-hydroxycortisol and 18-oxocortisol (294). However, dosages of these steroids require sophisticated methods and antibodies and are not performed routinely.

Genetics

In 1992, Lifton and colleagues (295) showed that GRA was linked to an abnormal aldosterone synthase gene. They studied a large affected kindred and found a gene duplication arising from an unequal crossing over, resulting in a fusion of the 11β-hydroxylase (CYP11B1) promoter with the coding sequence of aldosterone synthase. In all families reported so far, the chimaeric gene derives from unequal homologous recombination between intron 1 and intron 4 of the *CYP11B1* and *CYP11B2* genes, respectively. This recombination takes always place upstream of exon 5, since this exon contains two residues that differ between the two homologous enzymes (296) and that are critical to confer the aldosterone synthase specificity. Thus, it encodes a protein that can hydroxylate cortisol (the steroid substrate present in the zona fasciculata) in the 18-position. This gene is under the control of the 11 β-hydroxylase gene regulatory region, which expression is under ACTH control and can be down-regulated by exogenous glucocorticoid administration (297). Therefore, aldosterone hypersecretion seems to mainly derive from the zona fasciculata. The genetic screening for this condition is easy to perform and is based on Southern-blotting or on a long-range PCR looking for the existence of an hybrid gene containing the 5′ part of the CYP11B1 gene and the 3′ part of the CYP11B2 gene (298). Even if the condition is rare, clinicians should not hesitate to prescribe this genetic test since it is 100% sensitive and specific in reference laboratories and since a positive finding strongly influences the medical care of the patient and possibly his family. An early-onset of hypertension (before 30 years of age) associated to biological features compatible with an hyperaldosteronism and a positive familial history of early hypertension and/or stroke, should encourage to perform this genetic test.

Therapy

Taking into account this mechanism, the treatment of GRA is based on the administration of dexamethasone at low doses (0.5 mg/day), which only partially suppresses ACTH but lowers the possible side effects from long-term exogenous glucocorticoid treatment. A complementary treatment based on amiloride or spironolactone at low dose as well other classical antihypertensive agents is often required (299).

Other Forms of Familial Aldosteronism

GRA is probably very rare. In our experience, we only detected six unrelated cases amongst 700 patients with primary aldosteronism (X. Jeunemaitre, unpublished data).

Gordon and Stowasser described another form of familial primary aldosteronism, called type II (FH-II). It was initially detected in few families with about one-third

of the affected patients presenting an aldosterone-producing tumor (300). The clinical and biological characteristics of these patients do not differ from those with usual primary aldosteronism, except that the trait seems inherited according to an autosomal dominant transmission with partial penetrance (301). The screening of the entire genome in a large family with FH-II showed linkage with chromosome 7p22 (302). In addition to two Australian kindreds, two other Italian families with FH-II were found to be linked to this locus (303), but no causal gene has been identified yet.

Very recently, a new familial form of aldosteronism has been reported in one single family (304) that could be due to a new gene regulating adrenal steroid biosynthesis. A father and his two daughters had very early (by the age of 7) and severe hypertension with hyperaldosteronism. Very high levels of 18-oxocortisol and 18-hydroxycortisol were not influenced by dexamethasone and GRA was excluded.

Liddle Syndrome

In 1963, Liddle and colleagues described a family with hypertension and an abnormality of Na^+ reabsorption at the level of the renal distal tubule which simulated primary aldosteronism but had negligible basal and stimulated aldosterone secretion (305) (OMIM #177200). Although blood pressure and hypokaliemia were not influenced by spironolactone treatment, triamterene, a specific inhibitor of the distal renal epithelial Na^+ channel, corrected these abnormalities. The authors proposed that the primary abnormality was a constitutive activation of the epithelial Na^+ channel. Some 30 years later, this hypothesis was reinvestigated in the originally described pedigree. The index case developed renal failure and renal transplantation corrected the aldosterone and renin responses to salt restriction. These features demonstrated the involvement of the kidney in the disease (306), making the epithelial amiloride-sensitive Na^+ channel (ENaC) located in the cortical CD an attractive candidate gene for Liddle syndrome.

Genetics

Analyzing the original Liddle's pedigree, Shimkets et al. (307) showed complete linkage of the gene encoding the β subunit of ENaC, located at chromosome 16p13-12. In this pedigree and in other unrelated kindreds, a premature stop codon, a frameshift mutation and other deleterious mutations were found, all located in the last exon of the *SCNN1B* gene encoding for the intracellular carboxy-terminal domain of the β subunit. These mutations were showed to be gain of function mutations, with an increased amiloride-sensitive Na^+ current after transfection of the corresponding mutant subunits together with α and γ wild-type subunits. In a Portuguese family affected with this syndrome, we found a 32 base pair deletion leading to a premature termination of the carboxy-end of the same subunit (308). Measurement of transnasal potential difference, as an alternative to transepithelial transport in the kidney, showed the presence of an increased amiloride-sensitive conductance in the three affected boys but not in their unaffected sister (309). Other point mutations affecting the same region of the *SCNN1G* gene coding for the γ subunit of ENaC have also been found to cause Liddle's syndrome (310). No mutation of αENaC has been associated with Liddle syndrome, yet.

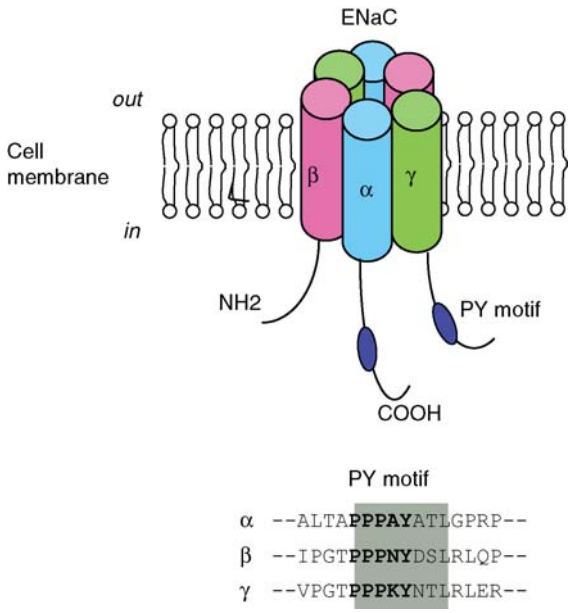
Pathophysiology

ENaC is constituted of at least three homologous subunits, α, β and γ which act together to confer its low Na^+ conductance, and its high selectivity for Na^+ and amiloride (311). The stochiometry of the channel can be deduced from the crystal structure of a chicken acid-sensing ion channel which belongs to the same family (312). It is an heterotrimeric protein composed of one α, β and γ subunit (*Fig. 38-4*). Each subunit is composed of short amino and carboxy termini, two transmembrane helices, and a multidomain extracellular region. ENaC is located in the apical membrane of epithelial cells and plays a major role in the reabsorption of Na^+ ions, especially in kidney, colon and lung. In the kidney, it is mainly expressed in the distal part of the DCT and in the cortical CD. It is finely tuned by changes in salt regimen, thus allowing appropriate changes in Na^+ reabsorption (313).

Comprehensive studies have shown that the mechanism by which the truncation of the C terminus of the β and γ subunits alters the ENaC function corresponds to an alteration of a conserved motif (PPxxY) in the C-terminus of all three subunits of ENaC (314, 315). Normally, a specific interaction between this PY motif and cytosolic proteins (Nedd4 isoforms 1 and 2, and other related WW proteins) leads to ubiquitylation and then degradation of part of the newly synthetized subunits (316). Thus, cell surface expression of ENaC is in part controlled via ubiquitylation which it itself regulated by aldosterone-induced proteins and glucocorticoid induced

Figure 38-4

Membrane topology of the epithelial Na^+ channel ENaC. The proposed model is a heterotrimeric structure by one α subunit, one β subunit, and one γ subunit. Each subunit is formed by two transmembrane domains, a large extracellular loop and cytoplasmic amino – and carboxy termini. The C-terminus contains a PY motif with at least three proline and a tyrosine residues (PPPXYXXL), highly conserved between the α, β, and γ subunits and between species. This motif is essential for the interaction with intracytoplasmic proteins (among them Nedd4–2) and the regulation of the number of channels at the plasma membrane.



kinase 1 (317). Both truncation or punctual mutation of the C-terminal PY motif increased surface expression of the mutant proteins and thus increased the number of Na^+ channels in the apical membrane (318), favoring renal Na^+ absorption and hypertension. This results in expanded plasma volume which in turns inhibits the renin aldosterone secretion. The fact that only one heterozygous mutation of either the β or γ ENaC subunit is sufficient to lead to the pathology is probably due in part to the multimeric arrangement of the channel.

It has been suggested that polymorphisms at each *SCNN1A*, *SCNN1B* and *SCNN1G* genes could be related to essential hypertension (319). However, the in vitro demonstration of their functionality has proven to be difficult (320). A special emphasis has been put on the Thr574Met polymorphism at the βENaC which frequency is higher (around 8%) in the African populations. A higher frequency of the 574Met allele has been suspected in black hypertensives living in London compared to normotensives (321), this allele being associated with lower plasma renin values, and possibly with an enhanced sensitivity to amiloride (322). Other polymorphisms at the γENaC have also been associated to essential hypertension (323, 324).

Diagnosis

Recognition of Liddle syndrome is important because it is potentially cured by the administration of amiloride. It is a form of pseudoaldosteronism, i.e., hypertension associated with hypokalemia, metabolic alkalosis, and suppression of plasma renin but with very low levels of aldosterone in plasma and/or urine. As a consequence of the high penetrance of this genetic defect and its autosomal dominant transmission, one can usually find the presence of hypertensive individuals in successive generations. As well, affected individuals are diagnosed at a relatively young age, most often between the age of 10 and 30 years (325). These features are clearly different from the more severe and recessively transmitted apparent mineralocorticoid excess (see below). It resembles to the very rare (only one family described) form of hypertension secondary to activated mineralocorticoid receptor, both forms being not sensitive to spironolactone (see also below). In any case the peculiar sensitivity to amiloride and the possibility of genetic testing allow a sure and rapid diagnosis. The genetic screening is based on the sequence analysis of the last exon 13 of the *SCNN1B* and *SCNN1G* genes.

Therapy

It is interesting to consider that a specific drug therapy for Liddle syndrome was developed in 1967 as a K^+-sparing diuretic (326), a long time before ENaC was cloned and was demonstrated to be responsible for the disease. Due to its very potent inhibiting properties on ENaC, amiloride is very effective in Liddle syndrome at doses comprised between 10 and 20 mg/day (327). Surprisingly for such a chronic and often severe condition, a change in blood pressure and in the biological profile can be observed as soon as after 2–4 weeks of treatment (308). In our experience, chronic therapy with amiloride alone is

sufficient to control blood pressure. Spironolactone has no effect which is expected since renin and alosterone are completely suppressed.

Early-Onset Hypertension Secondary to a Gain of Function Mutation in the Mineralocorticoid Receptor

One unique case of a new monogenic form of hypertension, that could be called pseudoaldosteronism type II, has been reported by Geller and colleagues (328). It is characterized by an early-onset and severe form of hypertension, associated with low plasma levels of renin and aldosterone, with severe exacerbation in pregnancy (OMIM #605115). It is caused by an activating missense mutation (Ser810Leu) of the *MR* (or *NR3C2*) gene that encodes the mineralocorticoid receptor. This mutation is located within the hormone binding domain and results in a constitutive mineralocorticoid receptor activity and in an alteration of the receptor specificity. The receptor becomes abnormally activated by progesterone and other steroids lacking 21-hydroxyl groups which act normally as antagonists. Thus, spironolactone acts an angonist on the mutated receptor instead of an antagonist and could be detrimental. In the family described by Geller and coworkers, all women bearing the mutation had severe pregnancy-induced hypertension with hypoaldosteronism which was caused by the massive increased production of progesterone during pregnancy (328). These original findings open the possibility of discovering other cases of similar type of mutations in pregnancy-induced hypertension. However, several groups including ours (unpublished) failed to find such activating mutation in large series of pre-eclamptic women.

Apparent Mineralocorticoid Excess

The syndrome of apparent mineralocorticoid excess (AME) is a rare autosomal recessive form of hypertension (OMIM #218030). It was first described by New (329) and Ulick (330) in two subjects: one was a 3-year-old Native American girl and the other one was a boy from Middle Eastern who had suffered a stroke at age 7 and was severely hypertensive. For both of them, clinical and biochemical evaluation failed to reveal overproduction of aldosterone or any other known steroid, establishing a new syndrome. AME is usually diagnosed within the first years of life and is characterized by polyuria and polydipsia, a failure to thrive, a severe hypertension associated with hyporeninism and hypoaldosteronism, a profound hypokalemia with alkalosis and most often nephrocalcinosis (331). The syndrome is rare, since less than 100 cases have been reported in the last 30 years. The clinical and biochemical characteristics of AME, mimicking a very strong hyperaldosteronism, together with the frequent consanguinity between parents make the diagnosis relatively easy. A few patients with a mild form of AME, also called AME type 2 (OMIM # 207765), have also been reported, with less caricatural hypertension and only mild abnormalities of cortisol metabolism. It was first described by Ulick (332) and later in an extensive consanguineous Sardinian pedigree in whom Li et al. found a novel homozygous mutation in the *HSD11B2* gene (333). Affected homozygous individuals were >30 years of age and had both mineralocorticoid hypertension and evidence of impaired metabolism of cortisol to cortisone, whereas heterozygous subjects were phenotypically normal with only subtle biochemical defects.

Pathophysiology and Genetics

The 11-beta-hydroxysteroid dehydrogenase is a microsomal enzyme complex responsible for the interconversion of cortisol and cortisone. Whereas the type I isoform (HSD11B1) is capable to have both the dehydrogenase and reductase activities, the type II isoform (HSD11B2) has only the 11-beta-dehydrogenase activity and thus only catalyzes the cortisol to cortisone reaction (334). Edwards and colleagues (335) showed that the HSD11B2 isoform is highly concentrated in aldosterone-responsive tissues – particularly in the distal nephron -, and actually protects the mineralocorticoid receptor from a stimulation by the cortisol which plasma concentration is about 100-fold higher than aldosterone. Because of the defect in the HSD11B2 isoform, AME patients are characterized by high values of the cortisol/cortisone ratio in plasma (F/E) and urine (THF/THE), and by arterial hypertension, mimicking a primary aldosteronism (336).

The group of White first showed that AME is due to mutations in the *HSD11B2* gene that encodes the HSD11B2 isoform (337). They screened the five exons of *HSD11B2* in nine affected individuals and found missense and frameshift mutations which markedly affected enzymatic activity in vitro and were associated with increased urinary (THF/THE) ratios, the more severe mutations resulting in the higher precursor/product ratios. Subsequently, other loss-of-function mutations have been found in affected patients being either homozygous for the mutation – especially in consanguineous families – or

composite heterozygous (331, 338–340). HSD11B2-null mice provide a good model for the pathology (341). They show signs of hypertension, hypotonic polyuria, hypokalemia and hypochloremia, a phenotype directly comparable to AME patients. They confirm the crucial importance of the HSD11B2 isoform in metabolizing cortisol to cortisone in the renal tubule, thus protecting the mineralocorticoid receptor from the influence of cortisol.

Differential Diagnosis

Consumption of natural licorice can mimick an AME in adults but is exceptionally observed in children. Indeed, it requires either a sustained and chronic or a high acute exposure to cause this adverse effect. The mechanism behind this effect is the fact that licorice contains glycyrrhetinic acid and glycyrrhizic acid, the latter being a potent inhibitor of HSD11B2 (342). These molecules are mostly excreted in the bile together with their metabolites, with very little excretion in urine, explaining the need of an important and chronic consumption to lead to a mineralocorticoid form of hypertension. Carbenoxolone, an antiulcer drug, is also a competitive inhibitor of HSD11B2, and cause sodium retention and hypertension (343).

Treatment

Two main strategies can be used to treat AME. The first is the blockade of the mineralocorticoid receptor by spironolactone, thus acting as a competitive antagonist of the endogeneous cortisol. Daily doses of spironolactone between 2 and 10 mg/kg are usually sufficient to correct hypertension and increase natriuresis and renin levels (331). The addition of thiazides can help to normalize blood pressure and lower hypercalciuria and nephrocalcinosis. The second, complementary strategy consists in administering exogenous corticoids to block ACTH and suppress the endogeneous secretion of cortisol. This strategy has shown its efficiency on blood pressure, renin and aldosterone but has little effect on urinary concentrations of the metabolites of cortisol, cortisone and corticosterone (344). Interestingly, a 31-years-old women with AME was cured by renal transplantation while receiving also dexamethasone. The curative effect of renal transplantation on the cortisol/cortisone ratio confirmed that AME as a renal disorder and that the transplanted kidney had functional HSD11B2 activity (345). In addition to these two strategies, the use of non-specific antihypertensive agents such as calcium antagonists is often required in AME, due to the severity of hypertension (346).

Pseudohypoaldosteronism Type II, Gordon Syndrome

Pseudohypoaldosteronism type II (PHA2) (OMIM #145260), also known as Gordon syndrome, or familial hyperkalemic hypertension, is an autosomal dominant form of volume-dependent hypertension characterized by hyperkaliemia and hyperchloremic acidosis despite normal renal function (347). Since the first description of the disease by Paver and Pauline in 1964 (348), about 100 other cases and families have been reported. Gordon and colleagues reported their first case in 1970 and helped to demonstrate the existence of a unifying syndrome (347). The original case was a 15-year-old boy with severe hypertension (180/120 mmHg) and very high potassium levels (7.0–8.2 mmol/L). Detailed analyses showed that the kidney was probably involved but that the renal tubule reacted normally to an acid load and to carbonic anhydrase inhibitor. Sensitivity to thiazide diuretics was reported a few years later in unrelated affected subjects (349). A high variability in the age at diagnosis, which may range from the first few weeks of life until late in adulthood, has been reported in sporadic and familial cases. Usually, the biochemical abnormalities precede the increase in blood pressure which seems to depend primarily on age in affected individuals (350). This phenotypic variability, associated with sensitivity to thiazides — which are widely used in hypertension — may have led to an underestimation of PHA2 frequency.

The low renin levels in PHA2 are thought to be the consequence of volume expansion whereas plasma aldosterone levels are variable depending on the opposite influences of low renin and high potassium levels. At least two arguments suggest a primary renal tubular defect along the DCT: affected patients are highly sensitive to thiazide diuretics (349) and the clinical and biochemical features of PHA2 are the mirror image of Gitelman syndrome in which inactivating mutations in NCCT have been demonstrated (see above). However, the pathophysiology of PHA2I is certainly more complex as it has been associated in some cases with defective proximal reabsorption, impaired chloride reabsorption, and altered sensitivity to mineralocorticoids (351).

Genetics

The first genome scan was reported by Lifton's group (352). Using eight affected but limited families, they identified two loci on chromosome 1 (1q31-q42) and chromosome 17 (17q21-q22), respectively named PHA2A and PHA2B

(*Fig. 38-5*). The selection and genetic analysis of a large French pedigree led us to identify a third chromosomal region on chromosome 12p13, called PHA2C (353). Exclusion of linkage for these 3 chromosomal regions in two additional affected pedigrees suggests further genetic heterogeneity (354). Thus, it is expected that molecular genetics of this syndrome will lead to a variety of molecular defects, revealing the role of either several new components of the same pathway, or direct or indirect partners of the sodium-chloride cotransporter.

The two genes, *WNK1* and *WNK4*, corresponding to the PHA2B and PHA2C loci were identified in 2001 (355) (*Fig. 38-6*). Both genes are members of a particular family of serine-threonine kinases, called With No Lysine (WNK) kinase (356). Disease-causing mutations in the *WNK1* gene are large deletions in the first intron which lead to increased gene expression of the kidney isoform. Mutations in the *WNK4* gene are missense mutations clustering in a highly conserved domain among this type of kinases. Altogether, these findings implicate these kinases in a previously unrecognized signaling pathway likely to be involved in the control of Na^+ reabsorption in the distal nephron and blood pressure regulation (357).

Pathophysiology

The mechanism of PHA2 has been debated for many years. The two main suggested mechanisms – excessive Na^+ reabsorption via the thiazide-sensitive cotransporter NCCT in the DCT, or a "chloride shunt" that would favor Na^+ reabsorption via ENaC (351) – have been revisited according to the recent identification of *WNK1* and *WNK4* genes as causing the disease (358).

WNK4 seems to be a major player in regulating Na^+, K^+, and Cl^- reabsorption in the distal nephron by regulating regulation of a number of renal ion transporters and channels. In vitro experiments showed that it inhibits NCC activity in *Xenopus* oocytes by decreasing its surface expression and that mutated WNK4 has lost this ability to regulate NCCT (359). It could also inhibit the activity of the renal apical K^+ channel ROMK, the basolateral isoform of the Na^+-K^+-$2Cl^-$ cotransporter (NKCC1), the apical $Cl^-/HCO3^-$ exchanger CFEX and TRPV4 (360), again by decreasing their surface expression. Finally, WNK4 was shown to stimulate the paracellular Cl^- transport, via phosphorylation of members of the claudin family that encode tight junction proteins. Transgenic

Figure 38-5

Genetic heterogeneity of Pseudohypoaldosteronism type II. Three loci have been identified on chromosome 1 (PHA2-A), chromosome 17 (PHA2-B) and latest on chromosome 12 (PHA2-C). Genes corresponding to two of these loci have been identified, *WNK4* for the PHA2-B and *WNK1* for the PHA2-C locus, respectively. Further genetic heterogeneity exists since several affected families do not show linkage at these loci (for details see text).

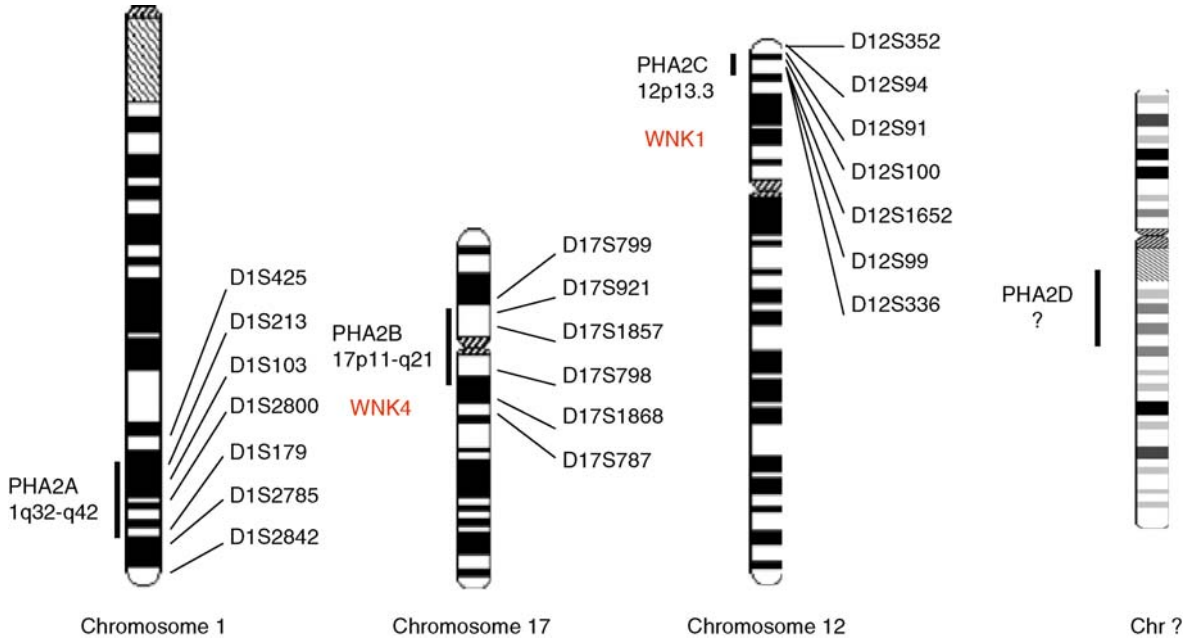
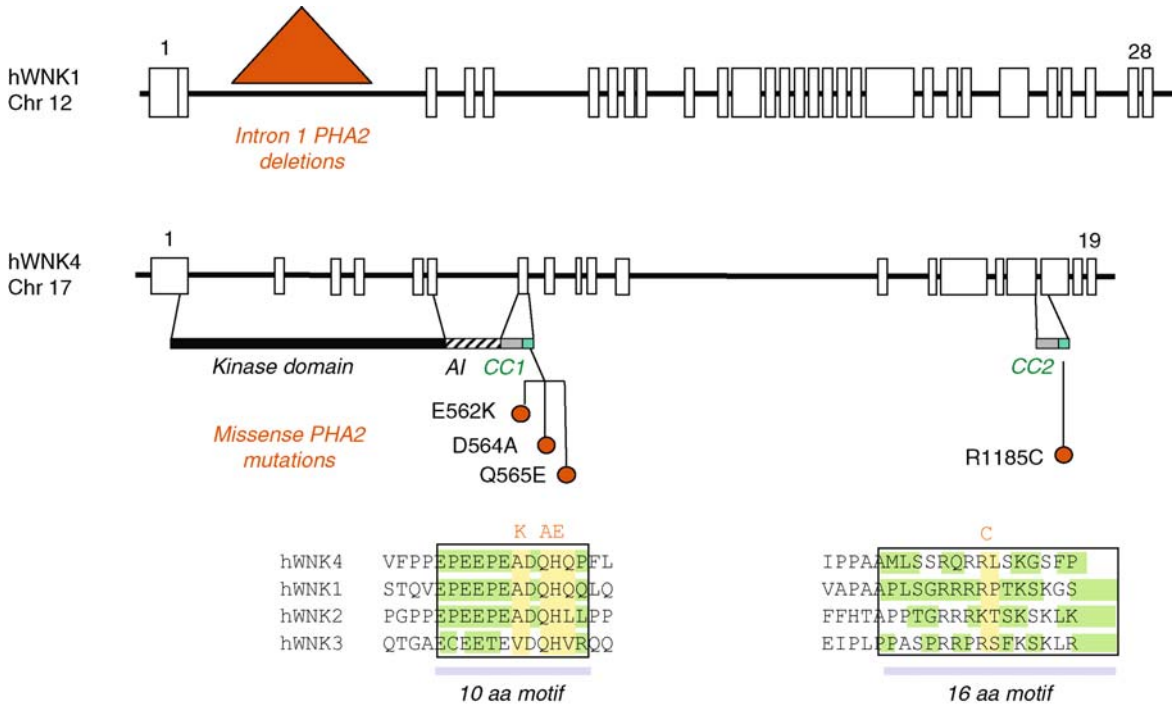


Figure 38-6

WNK1 and WNK4 mutations in pseudohypoaldosteronism type II. PHA2 causing mutations at the *WNK1* gene correspond to large (41 and 22 Kb) deletions in the intron 1. As a consequence of the suppression of important regulatory elements, the expression of the L-WNK1 and KS-WNK1 are increased in the renal tubule. PHA2 causing mutations at the *WNK4* gene correspond to missense mutations located into two conserved negatively and positively charged regions downstream the two coiled-coil domains.



mouse models showed that the main effect of the *WNK4* PHA2 mutations is an increased NCCT activity associated to a marked hyperplasia of the DCT (361, 362). Thus, WNK4 could be a determinant of the choice faced by the kidney between maximal NaCl reabsorption and K^+ secretion in response to aldosterone secretion and this determination could be mediated mainly by modulating NCCT activity.

The role of WNK1 is more complex to analyze since multiple isoforms are produced from the *WNK1* gene due to the existence of three promoters, two polyadenylation sites and several alternatively spliced exons (363). The short kidney-specific isoform (KS-WNK1) lacks any kinase activity and is produced at a high level, exclusively in the DCT. The full-length (long) isoform (L-WNK1) is produced ubiquitously and at a low level all along the nephron. In vitro, L-WNK1 and KS-WNK1 have been shown to interact together and with other partners such as WNK4, the Serum Glucocorticod Kinase SGK1 and ENaC. Several studies in vitro have shown that WNK1 may act as an osmotic sensor in various cells (364). It is supposed that the ratio between L- and KS-WNK1 is probably important in the distal nephron, adding a supplementary level of fine regulation of the ionic transport (358).

It is not clear yet whether the two genetic defects on *WNK1* and *WNK4* give a similar phenotype in terms of biochemical and clinical severity, because of the very low number of families detected up to now. The only biochemical difference that has been shown concerns calcium excretion. PHA2 families with *WNK4* mutation have been reported as having hypercalciuria whereas normocalciuria was observed in the only *WNK1*-linked PHA2 family analysed for this parameter (350). In the WNK4 pedigree analysed by Farfel'group (365), affected members had hypercalciuria with normomagnesemia and decreased bone mineral density. No susceptibility to kidney stones was observed. Transgenic mice overexpressing a mutant WNK4 also display increased blood pressure, hyperkalemia, hypercalciuria together with marked

hyperplasia of the DCT (362). The effet on the calcium balance could be due to the interaction between WNK4 and TRPV4 which are coexpressed in the distal nephron (366).

Therapy

Low salt regimen can be in part effective in PHA2 as also evidenced in other forms of volume-dependent hypertension (367). Low-salt diet can be sufficient, especially in childhood, since blood pressure might be normal and chronic hyperkaliemia is usually well tolerated. Thiazides diuretics are the treatment of choice since they interfere with the mechanism of the disease. Low doses of hydrochlorothiazide (i.e., 12.5–25 mg daily) are very efficient to correct both biochemical abnormalities and blood pressure in a few weeks. Thiazide diuretics also correct the hypercalciuria that is observed in WNK4-linked PHA2 (365). Furosemide is also effective but less logical since it increases hypercalciuria and could enhance the risk of nephrolithiasis. From our experience, there is no loss of efficiency of thiazides along the years. However, their potential metabolic side effects constitute a rationale to develop new antihypertensive agents controlling the WNK pathway.

Pseudohypoaldosteronism Type I

Pseudohypoaldosteronism type I (PHA1) (OMIM#177735, OMIM #264350) is a rare form of mineralocorticoid resistance characterized by neonatal renal salt wasting, failure to thrive and dehydration. It is associated with hyponatriemia, hyperkalemia and metabolic acidosis, despite extremely high values of plasma renin and aldosterone (368). It was first reported by Cheek and Perry, who described a male infant with severe salt wasting in the absence of any renal or adrenal defect (369). There exist two different clinical forms of PHA1: (1) a renal form, in which mineralocorticoid resistance is restricted to the kidney, and (2) a generalized form, where mineralocorticoid resistance is systemic and salt loss occurs in multiple organs (370) (*Table 38-7*).

Clinical and Biochemical Features

Renal PHA1. Renal PHA1 (also called autosomal dominant PHA1) (371) is a mild and most frequent form of the disease. Clinical expression is variable: in general, patients show a neonatal salt losing syndrome, with weight loss, failure to thrive, vomiting and dehydration. Biological findings are hyponatremia, hyperkalemia and inappropriately high urinary Na^+ excretion, with occasional hypercalciuria. Urinary K^+ excretion is low, with reduced fractional K^+ excretion and transtubular K^+ gradient (372, 373). The diagnosis is confirmed in the presence of high plasma and urinary aldosterone and high plasma renin levels. Symptoms of renal PHA1 usually improve in early childhood. The mechanisms which restore Na^+ homeostasis in these patients are not clear; most likely, kidney maturation, access to dietary salt, compensatory increase in proximal Na^+ reabsorption as well as the up-regulation of the mineralocorticoid axis all play a role to compensate for the distal salt loss. Indeed, high plasma aldosterone levels persist into adulthood, while plasma renin activity decreases into normal range (370, 374, 375).

Generalized PHA1. In contrast to the renal form, patients affected by generalized PHA1 (also called autosomal recessive PHA1) (371) present with severe salt wasting from kidney, colon, sweat and salivary glands (376). In addition to severe dehydration, vomiting and failure to thrive, the clinical picture may be complicated by cardiac dysrhythmias, collapse, shock or cardiac arrest (374). Severe hyperkalemia and high aldosterone and plasma renin levels orient the diagnosis that can be completed by a positive salivary or sweat test. The prognosis of this form of PHA1 is poor: no remission has been reported and patients suffer from recurrent, life-threatening episodes of salt loss. In addition to the renal phenotype, frequent respiratory tract illnesses have been observed, evoking cystic fibrosis, that are caused by an increase in the volume of airway surface liquid (377). Also, an eczematoid rush of the skin is frequent in these patients, due to the severe salt loss from sweat glands (374, 378, and unpublished observations).

Transient pseudohypoaldosteronism has been observed in infants less than 7 months of age suffering from urinary tract malformations or urinary tract infections (379–381). In these patients, medical or surgical care of the primary disease restores the normal response to aldosterone. The possibility that a transient tubular mineralocorticoid resistance can arise in infants with urinary tract malformations or urinary tract infections strongly supports an indication for renal ultrasonograpy and urine cultures in all children presenting with salt wasting and hyperkalemia (379). Secondary PHA1 may also develop in the adult following resection of the ileum and colon (382) or after renal transplantation (383).

Table 38-7

Distinctive clinical and biological features of renal and generalized PHA1

Feature	Renal PHA1	Generalized PHA1
OMIM #	177735	264350
Inheritance	AD	AR
Age of onset	Neonatal	Neonatal
Phenotype	Mild	Severe
Failure to thrive	Present	Present
Vomiting	Present	Present
Dehydration episodes	Mild	Severe
Cardiac dysrhythmias, collapse, shock or cardiac arrest	No	Sometimes
Respiratory tract illnesses	No	Frequent
Eczematoid rush of the skin	No	Sometimes
Hyperkalemic metabolic acidosis	Present	Present
Serum Na^+	↓	↓
Serum K^+	↑	↑
Urinary Na^+ excretion	↑	↑
Urinary Ka^+ excretion	↓	↓
Plasma aldosterone	↑	↑
Plasma renin	↑	↑
Urinary aldosterone	↑	↑
Sweat Na^+	N	↑
Salivary Na^+	N	↑
Type of mutation	Loss-of-function	Loss-of-function
Gene locus	4q31.1	12p13, 16p13-p12, 16p13-p12
Genes	*NR3C2*	*SCNN1A*, *SCNN1B*, *SCNN1G*
Proteins	MR, mineralocorticoid receptor	α, β, γ Subunits of the epithelial Na^+ channel ENaC

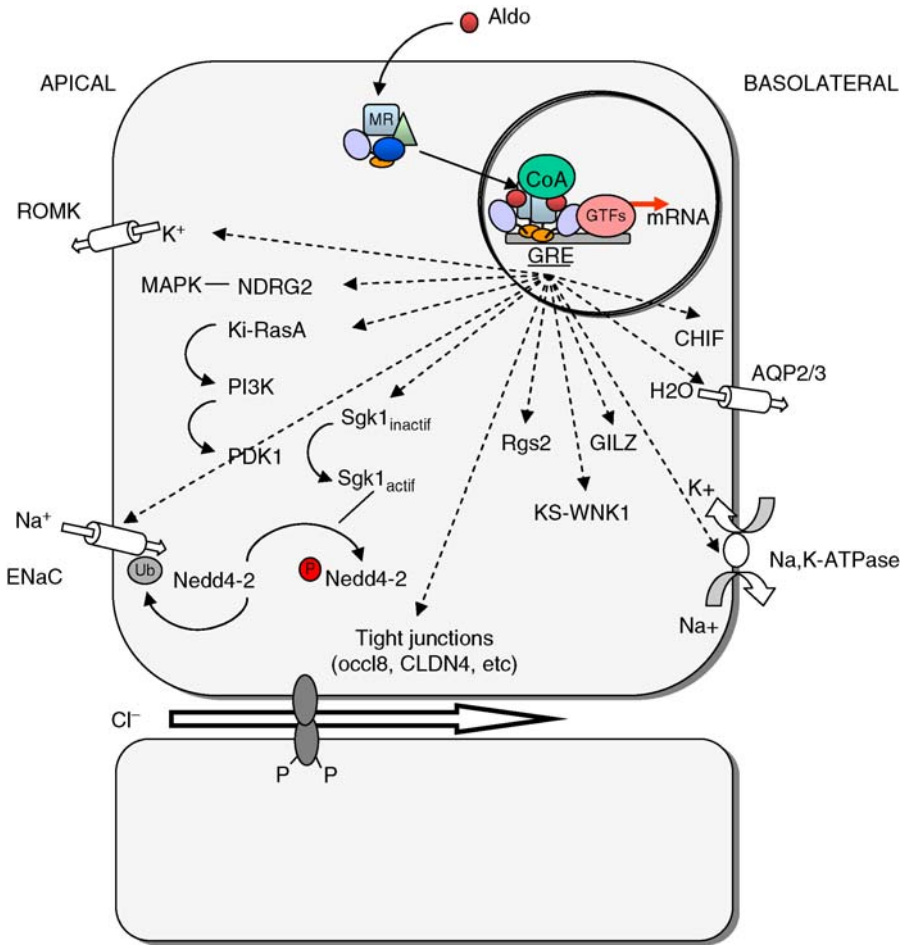
Pathophysiology and Genetics

Since the first description of PHA1, a defect of the tubular response to aldosterone had been suggested as the underlying cause. The mineralocorticoid receptor (MR) classically mediates aldosterone effects on electrolyte balance and blood pressure by regulating trans-epithelial sodium transport through tight epithelia (*Fig. 38-7*). The MR belongs to the nuclear receptor superfamily and acts as a ligand-activated transcription factor regulating expression of a coordinate set of genes involved in the physiologic response to aldosterone, including ENaC (384, 385). Armanini et al. confirmed the hypothesis of an abnormal MR-aldosterone axis by showing that ^{3}H-aldosterone binding was absent or very low in mononuclear leukocytes of affected patients (386). In a detailed study of 8 families, a dual pattern of inheritance was observed that correlated with receptor binding abnormalities. In some families an autosomal recessive inheritance was observed, and binding studies and aldosterone levels were normal in both parents. In contrast, some families presented with an autosomal dominant inheritance, and low receptor number and elevated plasma aldosterone were always found in one of the parents (387).

Subsequently it was shown that the two clinical forms of PHA1 are caused by different genetic defects. Inactivating mutations in the *MR* (or *NR3C2*) gene coding for the MR are found in renal PHA1 (368, 388, 389). Patients are

Figure 38-7

Molecular basis of pseudohypoaldosteronism type I. Aldosterone binds to its intracellular receptor, the mineralocorticoid receptor (MR). Hormone binding induces dissociation of MR-associated proteins and translocation of the aldosterone-receptor complex into the nucleus, where it binds to specific DNA sequences in regulatory regions of hormone-responsive genes. These genes code for proteins involved in transepithelial sodium transport (the epithelial sodium channel, ENaC; the sodium-potassium pump Na,K-ATPase), for regulatory proteins (serum- and glucocorticoid- regulated kinase 1 (sgk1), channel-inducing factor (CHIF), the proto-oncogene K-Ras2, N-myc down-regulated gene 2 (NDRG2)) and others. Aldo, Aldosterone; apical and basolateral indicate the two poles of the epithelial cell.



always heterozygous for the mutations, which occur at high frequency both in patients with familial autosomal dominant PHA1 and patients with a sporadic renal presentation (390). In the latter group, only one third are de novo mutations, implying that carriers develop clinically evident disease only in a small proportion of kindreds. Although the exact causes for the variable phenotypic expression of renal PHA1 are unknown, possible reasons include the occurrence of events that trigger neonatal salt loss, such as intercurrent volume-depleting events or infections. Also, naturally occurring hypomorphic or hyperfunctioning alleles of other genes, coding for proteins involved in distal sodium reabsorption, may aggravate or attenuate the phenotype.

The severe and generalized, recessive form of PHA1 is due to mutations in the genes coding for the α, β and γ subunits of the sodium channel ENaC, *SCNN1A*, *SCNN1B*, and *SCNN1G*, respectively. Deleterious mutations have been found in affected patients being either homozygous – in consanguineous families – or composite heterozygous (385, 391–393). They include missense and nonsense mutations, deletions, insertions and splice site

junction mutations leading to abnormal mRNA splicing. Mutations appear in all ENaC subunits, but are more frequent in the α subunit, consistent with its determinant role in channel function. None of these mutations occur in the cytoplasmic C-terminus of ENaC subunits, where mutations result in a hyperfunctioning channel and a clinical phenotype, Liddle's syndrome, which is a mirror image of PHA1.

The pathogenic mechanism of PHA1 in patients with heterozygous MR mutations depends on the mutation. Although haploinsufficiency is sufficient to cause autosomal dominant PHA1 (371, 394), mutated receptors may also exert dominant negative effects on the wild type receptor (394), since the MR regulates transcription by binding as receptor dimer to regulatory regions of target genes. In this case, effects of mutated receptors are strongly promoter-dependent and may differentially affect MR function in a gene-specific manner (395). In generalized PHA1, inactivating mutations affect one of the subunits of the amiloride-sensitive sodium channel ENaC. Absence of amiloride-sensitive Na^+ transport across airway epithelia has been evidenced in a neonate with generalized PHA1 by measuring transepithelial voltage across the nasal epithelium (396). The mutations causing generalized PHA1 are distributed all along the sequence of ENaC subunits. It is conceivable that nonsense, frameshift mutations, as well as mutations leading to abnormal splicing, cause a channel decrease or a loss of function. Missense mutations are found in critically important domains of the protein and affect functions like intracellular trafficking of the channel, channel gating, or the ion-selectivity filter (385).

Treatment and Prognosis

Treatment of PHA1 consists in the replacement of salt loss and rehydration, as well as correction of hyperkalemia and acidosis in the acute phase of the disease. Since the main differential diagnosis is congenital adrenal hyperplasia or isolated deficiency in aldosterone synthase (CMOI and CMOII) (397, 398), replacement therapy with fludrocortisone and hydrocortisone may be undertaken while confirming the diagnosis by hormonal measurements. Early postnatal hyperkalemia may sometimes complicate aBS, due to mutation in the potassium channel ROMK (44). Its association with hyponatremia and hyperreninemic hyperaldosteronism may erroneously suggest the diagnosis of PHA1. However, hyperkalemia appears usually very early and normalizes by the end of the first postnatal week, whereas PHA1 is characterized by permanent hyperkalemia. Other distinctive features of aBS patients are metabolic alkalosis as well as hypercalciuria and nephrocalcinosis. Also maternal hydramnios, present in aBS, is a rare event in generalized PHA1.

After the acute period, treatment consists in salt supplementation. The doses vary depending on the severity of the disease. In renal PHA1, 3–20 mEq/kg/day of NaCl, given as NaCl and $NaHCO_3^-$, are sufficient to compensate for the salt loss and are followed by rapid clinical and biochemical improvement. The expansion of extracellular volume results in increased tubular flow and Na^+ delivery to the distal nephron, stimulating K^+ secretion. Nevertheless, ion exchange resins are often included to the treatment to normalize plasma K^+ levels. The amount of Na^+ required is deduced from the normalization of plasma K^+ concentration and plasma renin. Since renal PHA1 improves with age, treatment can be discontinued after a variable period of time in most patients, generally around age 18–24 months. Older children are generally asymptomatic on a normal salt intake and show a normal growth and psycho-motor development.

In contrast to renal PHA1, generalized PHA1 represents a therapeutic challenge. No evidence-based treatment has been described, and therapeutic intervention is patient-specific. Generally, high doses of sodium (between 20 and 50 mEq/kg/day) are used, together with ion exchange resins and dietary manipulations to reduce K^+ levels. Corticoid treatment is sometimes associated and seems to provide some additional benefit. Administration of indomethacin may be useful in occasional patients (399). Symptomatic treatment is necessary for the respiratory tract illnesses and to correct the skin phenotype. Only few cases of generalized PHA1 followed up for several years or into adulthood have been described: treatment is necessary throughout life, consisting of salt supplementation (8–20 g NaCl/day) and ion exchange resins (374, 375).

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39 Renal Tubular Acidosis

Raymond Quigley

Introduction

Renal tubular acidosis (RTA) is *a condition in which there is a defect in renal excretion of hydrogen ion, or reabsorption of bicarbonate, or both, which occurs in the absence of or out of proportion to an impairment in the glomerular filtration rate* (1). Thus, RTA is distinguished from the renal acidosis that develops as a result of advanced chronic kidney disease (2–4). Albright originally described the disease as "renal acidosis resulting from tubular insufficiency without glomerular insufficiency" to emphasize this distinction (5). The term was reduced to "renal tubular acidosis" by Pines and Mudge in their studies published in 1951 (6). These renal tubular abnormalities can occur as an inherited disease or can result from other disorders or toxins that affect the renal tubules.

Historical Development of Classification of RTA

The historical development of renal tubular acidosis parallels the historical development of our understanding of renal physiology. As with many complex diseases, investigations into disease processes improve our understanding of normal physiology and, in turn, the advances in basic physiologic research shed light on pathophysiology and mechanisms of diseases. This is apparent in the historical development of renal tubular acidosis which began in the early twentieth century and is now extending into the molecular biologic era as medicine enters the twenty-first century. In addition, some of the confusion with the classification scheme of RTA stems from its historical development.

At the British Pediatric Association meeting in 1935, Lightwood described six infants out of an autopsy series of 850 that had "calcium infarction" of the kidneys (7). This would later be recognized as the first report of infants with nephrocalcinosis from renal tubular acidosis. Butler et al. described a series of four infants with similar findings in 1936 (8). In addition to nephrocalcinosis, these infants were also found to have hyperchloremia and acidosis, suggesting that there was a relationship between the biochemical findings and nephrocalcinosis. It was not clear from these first reports if the biochemical findings were the cause of the calcium deposits in the kidneys or were the result of damage to the renal tubules from the calcinosis.

The first description of the potential pathophysiologic explanation for these findings was put forward by Albright, et al. in 1946 (5). In this classic description of various forms of osteomalacia, the authors also outlined the treatment of these patients with a solution of citric acid and sodium citrate that was advocated by Dr Shohl. Albright described this form of acidosis as "renal acidosis resulting from tubular insufficiency without glomerular insufficiency" to distinguish this form of acidosis from the acidosis that occurs in renal failure.

The entity of "infantile renal acidosis" was then described by Lightwood in 1953 in a series of 35 infants (9). This was a larger series of infants than his first description and they had similar clinical histories and biochemical findings as the series by Butler (8). The first description of an adult with similar findings was made in 1945 by Baines, et al. (10).

During the 1940s and 1950s, a number of cases of renal tubular acidosis were described and led to investigations of the renal acidification defect (4, 11). The primary feature in these patients was the inability to lower their urine pH despite having mild to moderate acidosis. This became the defining characteristic of this disease as reported in a series of studies by Elkinton (12, 13). In the classic report by Pines and Mudge, the term "renal tubular acidosis" was used to replace the previously more cumbersome term of "renal acidosis resulting from tubular insufficiency without glomerular insufficiency" (6). This new term was emphasized in an editorial review by Elkinton and has remained the term for this disease ever since (12). Thus, at the end of the 1950s, renal tubular acidosis was thought to be a disease process that limited the ability of the kidneys to lower the urine pH, despite the fact that the patient had mild to moderate acidosis.

Although the concept of glomerular filtration had been well established in the early twentieth century, the measurement of the rate of glomerular filtration in humans had not been performed. This was accomplished

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by the pioneering work of Homer Smith. He was one of the first to conceive of the idea of a renal excretion system in which there was a high glomerular filtration rate which required tubular reabsorption of solutes (14). The fact that the glomerular filtration rate was very high and was followed by tubular modifications of the urine had profound effects on the ideas of bicarbonate handling and acid secretion.

The disorder of renal tubular acidosis was initially thought to be due to the inability of the kidney to maintain the steep pH gradient in the distal nephron segment. The idea that this disorder could arise from the inability of the proximal tubule to recover the filtered bicarbonate was first suggested in 1949 by Stapleton (15). He reported a patient that had significant amounts of bicarbonate in the urine at low concentrations of serum bicarbonate. This idea was further advanced by Soriano in a report of two patients that demonstrated an abnormally low threshold for bicarbonate excretion (16, 17). Based on their findings in these patients, Soriano and Edelmann proposed classifying patients with RTA as having either distal or proximal tubule defects. This was the initial description of the need for a classification scheme for this disease, suggesting that there could be multiple causes for this disease process.

The dichotomy of proximal and distal RTA was firmly established in the classic review by Rodriguez-Soriano and Edelmann which summarized the understanding of the pathophysiology at that time (1). The nomenclature of type I and type II RTA was established by the end of the 1960s in a review by Morris (18). In this review, distal RTA was referred to as type I (or classic) and proximal RTA as type II. The author also described a type III RTA as those patients that displayed features consistent with both forms of RTA. In 1972, McSherry, et al. described several patients that displayed characteristics of classic type I RTA but in addition had a reduced threshold for bicarbonate reabsorption. These patients seemed to fit the description of type III RTA. Subsequently, the reabsorption of bicarbonate in these patients normalized so that they were thought to have classic type I RTA with a developmental immaturity of the proximal tubule. Since that time, type III RTA has been essentially dropped from the classification scheme of RTA. It is interesting to note that the review by Genarri and Cohen did not mention type III RTA (19).

In the middle of the twentieth century, the discovery of aldosterone revolutionized our understanding of the physiology of sodium and potassium metabolism (20). Subsequently, it was found that patients with aldosterone deficiency had a form of RTA that resembled that of distal RTA, but the patients had hyperkalemia and not hypokalemia (21, 22). This form of RTA was then referred to as type IV RTA. More recently, other defects in distal nephron transporters have also been characterized and resemble the findings of type IV RTA. Although they are not true aldosterone deficient syndromes, they also are described as type IV RTA since these patients also have hyperkalemia. To add to the confusion, a review published in 1986 classified RTAs as type I (distal), type II (proximal) and type III (aldosterone deficient RTA) (23).

In recent years, there have been suggestions to clarify the classification of RTAs in a scheme that is based more on the pathophysiologic mechanism of the disease (24, 25). While this might eventually be the preferred nomenclature, most practicing nephrologists continue to use the historical classification. The other schemes will be discussed as part of the pathophysiology of RTA.

Over the past century, advances in renal physiology, acid-base chemistry, and molecular genetics have greatly improved our understanding of the various forms of renal tubular acidosis. Currently, the diagnosis and classification of the various types of renal tubular acidosis continue to rely on biochemical measurements of blood and urine. During the twenty-first century, however, the diagnosis of renal tubular acidosis may eventually be made by a molecular genetic approach and not by extensive biochemical testing.

Physiology of Acid Secretion

The kidney is the primary organ for long term acid base regulation. Thus, an understanding of the normal renal excretion of acid is necessary to understand the defects present in patients with RTA.

The typical Western diet generates approximately 1 mmol of H^+ per kilogram of body weight in adults (26). In addition, children generate acid from the production of hydroxyapatite in growing bone and thus generate a total of approximately 2–3 mmol of H^+ per kilogram of body weight (27–29). The acid generated from the diet and bone growth necessitates the excretion of acid by the kidneys.

The amount of acid excreted by the kidneys is referred to as Net Acid Excretion (NAE) and is expressed quantitatively as:

$$NAE = \left(U_{NH_4^+} + U_{TA} - U_{HCO_3}\right) \times V,$$

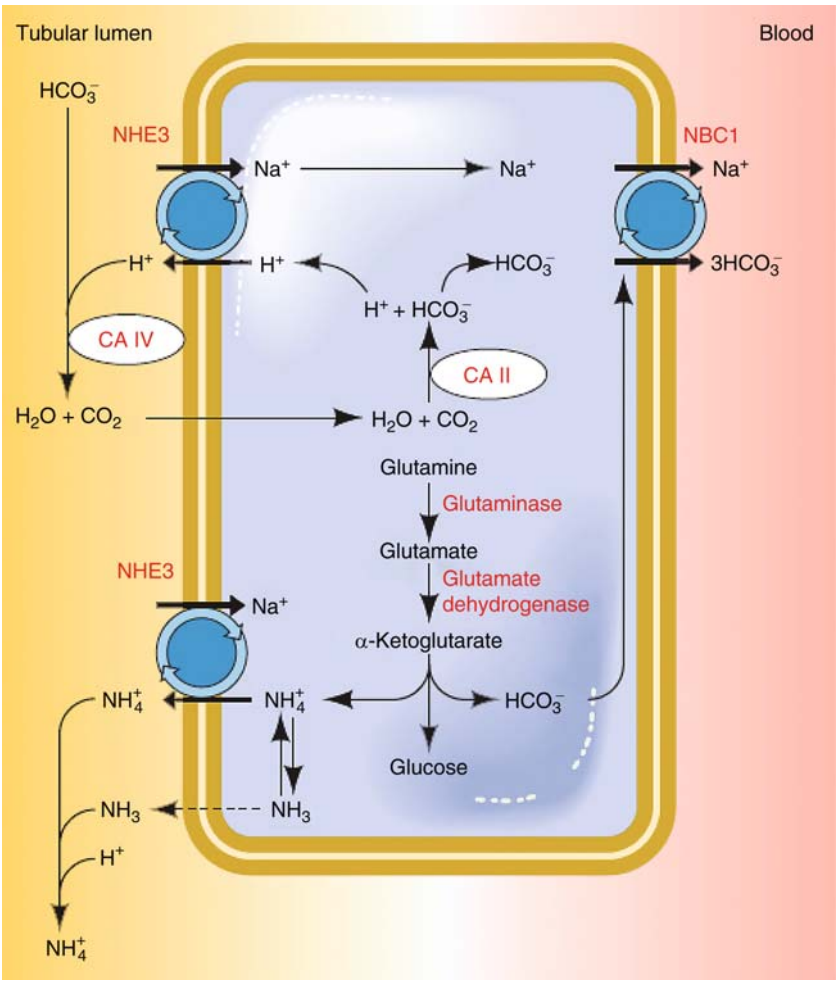
where V is the urine flow rate, $U_{NH_4^+}$ is the urine ammonium concentration, U_{TA} is the urine titratable acid concentration and U_{HCO_3} is the urine bicarbonate

concentration. Thus, the components of acid secretion can be thought of as bicarbonate reclamation to prevent bicarbonate loss, ammonium excretion and titratable acid excretion. The processes for maintaining acid base balance are quite complex, but the basic concepts will be reviewed so that the pathophysiologic changes in RTA can be described.

The kidneys are responsible for the excretion of nitrogenous waste products, principally urea, that are generated from our diet. In mammalian kidneys, urea is excreted primarily by filtration which requires having a high filtration rate so this can be accomplished. The average adult will filter about 150–180 L of blood per day. Because bicarbonate is freely filtered in the glomerulus, a large amount of bicarbonate (about 4,000 mEq per day in an adult) must be reabsorbed by the tubules each day to prevent loss of base. The bulk of the filtered bicarbonate is reabsorbed in the proximal tubule by mechanisms that are illustrated in *Fig. 39-1*. A number of proteins, both transporters and enzymes, work in

Figure 39-1

Model of bicarbonate reabsorption by a proximal tubule cell. The Na-K-ATPase located in the basolateral membrane generates and maintains the low intracellular sodium concentration. Protons are excreted into the tubule lumen by the sodium-proton exchanger (NHE3) where they combine with bicarbonate to form carbonic acid. In the presence of carbonic anhydrase IV (CAIV) the carbonic acid is hydrolyzed to water and carbon dioxide which enter the cell and recombine to form carbonic acid by the action of intracellular carbonic acid II (CAII). The carbonic acid ionizes into a proton which is then excreted into the lumen and bicarbonate which is transported by the sodium-bicarbonate symporter (NBC1) into the blood stream (reprinted with permission from (30)).



concert to reclaim approximately 80% of the filtered bicarbonate in this tubule segment (31–33).

The initial step in the reabsorption of bicarbonate is the secretion of protons into the tubular lumen. About two thirds of the proton secretory rate is provided by the sodium-proton antiporter (34–36). The isoform that is present on the luminal membrane of the proximal tubule has been termed NHE3 (sodium hydrogen exchanger 3). The energy for proton secretion by the antiporter is derived from the low intracellular sodium concentration that is maintained by the basolaterally located sodium-potassium ATPase. There is evidence that approximately one third of the proton secretory rate is provided by a proton ATPase located in the luminal membrane (36, 37). This transporter derives its energy directly from ATP.

Once the hydrogen ion is in the lumen of the proximal tubule, it combines with bicarbonate to form carbonic acid which will then form carbon dioxide and water as shown in the following equation:

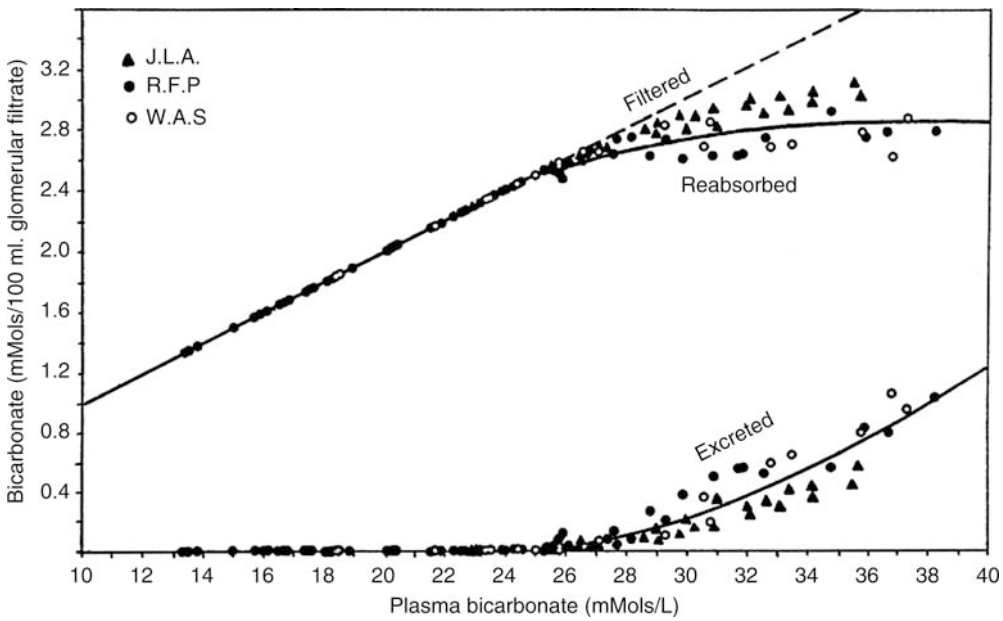
$$H^+ + HCO_3^- \longleftrightarrow H_2CO_3 \xleftrightarrow{\text{carbonic anhydrase}} H_2O + CO_2$$

The enzyme, carbonic anhydrase, is critical for catalyzing this process (38–40). One isoform of this enzyme (carbonic anhydrase IV) is located in the brush border membrane of the proximal tubule and serves to catalyze the forward reaction while another isoform of the enzyme (carbonic anhydrase II) is located inside the tubule cell for catalyzing the reverse reaction (38). Thus, carbon dioxide and water can move rapidly into the proximal tubule cell and recombine to form carbonic acid which will ionize to form bicarbonate and a hydrogen ion. The hydrogen ion is then available for secretion into the tubule lumen while the bicarbonate ion is then transported through the basolateral membrane by the sodium-bicarbonate cotransporter, NBC (41–43).

The overall process for reabsorbing bicarbonate in the proximal tubule is saturable (44). This is illustrated in *Fig. 39-2*. When the serum bicarbonate concentration is within the normal range, the filtered load of bicarbonate can be almost completely reabsorbed. If the serum bicarbonate concentration begins to rise, the filtered load of bicarbonate will then exceed the reabsorption rate of the kidney and bicarbonate will then be excreted into the urine. This has been studied in humans who were administered bicarbonate to determine the point at which bicarbonate would appear in the urine (44). The data from these experiments form a titration curve (see *Fig. 39-2*). The normal serum concentration of bicarbonate is thus determined by the threshold at which bicarbonate is excreted.

Figure 39-2

Bicarbonate titration curves for normal humans. At low concentrations of serum bicarbonate, all of the filtered load can be reabsorbed. The process of bicarbonate reabsorption is saturable, so once the delivered bicarbonate rate exceeds the transport maximum, bicarbonate will be excreted in the urine (reprinted with permission from (45)).



An additional task in maintaining acid base balance for the proximal tubule is the generation of ammonia to serve as a buffer to efficiently excrete the bulk of the acid that is generated from our diet. It has long been recognized that the excretion of ammonium is critical to the overall excretion of acid by the kidneys (46). This is primarily due to ammonium's ability to buffer hydrogen ions. To excrete 100 mmol of unbuffered H^+ at a pH of 4.0 ($[H^+] = 10^{-4}$ mol/L) would require a volume of 1,000 L of urine. The reaction of ammonia and H^+ to form ammonium has a pKa of approximately 9.0 (47). Thus, at a pH of 7.0, 99% of all the ammonia in the urine is in the form of ammonium ion and is excreted as ammonium chloride, limiting the amount of free hydrogen ions in the urine. Thus, the ammonium excretion rate is a quantitatively more important factor for the excretion of acid than the urine pH.

This can also create confusion in the assessment of a patient's ability to excrete acid. The equation that defines net acid excretion (see above) does not include information about the urine pH. Since the pKa of the ammonia/ammonium equilibrium is nine, if the patient is excreting a large amount of protons as ammonium, the pH will tend to rise even though the amount of acid being excreted has increased.

The tubular handling of ammonia and ammonium is complex (48–50). Briefly, ammonia is generated in the proximal tubule by the metabolism of glutamine and is secreted into the tubule lumen by the sodium-proton exchanger as the ammonium ion (see *Fig. 39-1*). The diffusion of ammonia gas across the proximal tubule apical membrane accounts for a small fraction of the total excretion of ammonia. The ammonium ions are then reabsorbed into the interstitium by the thick ascending limb of Henle to be secreted again by the collecting ducts (50, 51). The generation of ammonia by the proximal tubule can be upregulated in the presence of acidosis by 5- to 10-fold over baseline in adults (46, 52, 53). The ability of the neonatal kidney to upregulate ammonium excretion is somewhat limited and can prolong the recovery phase of acidosis in infants. The upregulation of ammonium production and secretion serves as the principal means of correcting acidosis that is due to non-renal causes. As will be seen below, the inability of the kidney to secrete acid as ammonium is a key feature of RTA.

The thick ascending limb of Henle is responsible for continued reabsorption of bicarbonate as well as ammonium (54, 55). The transporters involved include the sodium-hydrogen exchanger (NHE3), the sodium-potassium-2 chloride cotransporter, NKCC2 and the sodium-potassium ATPase (54). The thick ascending limb of Henle reabsorbs approximately 10% of the filtered bicarbonate.

The distal nephron is responsible for the secretion of protons which are then buffered by ammonia and titratable acid. The cell type in the collecting duct that is responsible for this is the alpha intercalated cell that is depicted in *Fig. 39-3*. The luminal membrane has a proton ATPase that utilizes ATP directly to secrete protons into the lumen of the tubule (56–59). This generates a bicarbonate ion that is then excreted through the basolateral membrane by the anion exchanger AE1 in exchange for a chloride ion (60, 61). The chloride can then exit the cell by the potassium chloride cotransporter (KCC) or the chloride channel, CLC-Kb (62, 63). Carbonic anhydrase II is critical for the formation of the carbonic acid in the cell that ionizes into the proton and bicarbonate ion (39).

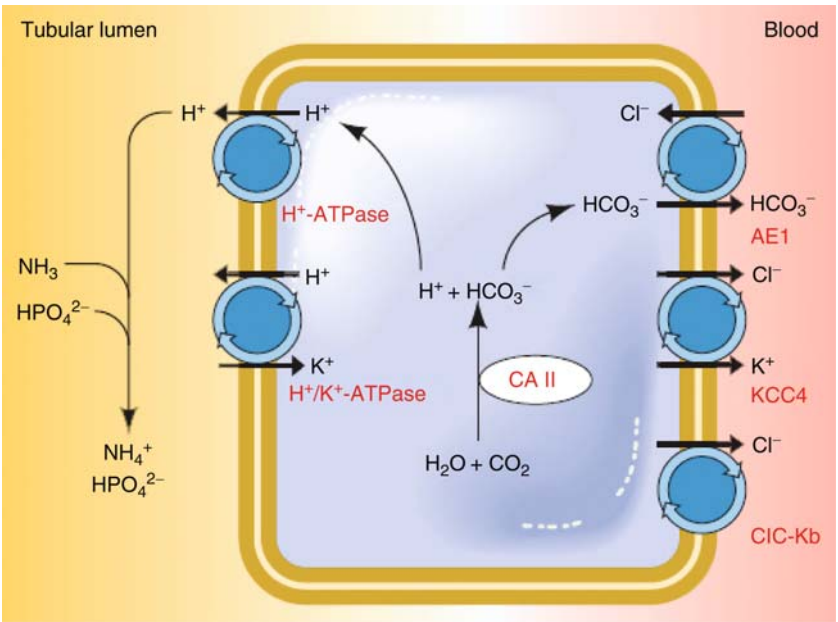
The principal cells of the collecting duct are responsible for the reabsorption of sodium and the secretion of potassium and thus do not directly secrete protons into the tubular fluid. However, these processes influence the rate of acid secretion indirectly by affecting the electrical potential difference across the epithelium. Thus, disease processes or drugs that have a primary effect on sodium or potassium transport in the collecting duct can eventually lead to acid base disturbances.

As discussed above, the proximal tubule generates ammonia that is eventually excreted as ammonium as a mechanism for acid excretion. The other major buffers in the urine are referred to as titratable acids and include phosphate, sulfate and many other anions. Of the many buffers available, the quantitatively most significant is phosphate. Phosphate exists in the blood as several different ionic species (H_3PO_4, $H_2PO_4^{-1}$, HPO_4^{-2} and PO_4^{-3}) with $H_2PO_4^{-1}$ and HPO_4^{-2} being the most abundant at physiologic pHs. The pK for the equilibrium between $H_2PO_4^{-1}$ and HPO_4^{-2} is 6.8, thus at a normal blood pH of 7.4, the ratio of $H_2PO_4^{-1}$: HPO_4^{-2} is approximately 4:1. As the urine passes through the collecting duct where the pH is lower, HPO_4^{-2} can accept protons and be converted to $H_2PO_4^{-1}$ and will aid in the buffering of excreted acid.

In addition to bicarbonate reabsorption and ammonia generation, the proximal tubule reabsorbs almost the entire filtered load of glucose and amino acids as well as approximately 85% of the filtered load of phosphate. These processes are coupled to the apical membrane sodium electrochemical gradient and are thus driven by the low intracellular sodium concentration and the negative electric potential inside the cell. Diseases that affect

Figure 39-3

Model of acid excretion in an alpha-intercalated cell in the distal nephrons. Protons are excreted into the tubule lumen by the proton-ATPase and are buffered by ammonia or titratable acid (mostly phosphate). Inside the cell, carbonic anhydrase II (CAII) provides the protons and bicarbonate through the hydration of carbon dioxide to form carbonic acid. Bicarbonate is excreted into the blood stream by action of the chloride bicarbonate exchanger (AE1) on the basolateral membrane. Chloride homeostasis is maintained by the potassium-chloride cotransporter (KCC4) and the chloride channel (ClC-Kb) (reprinted with permission from (30)).



the ability of the proximal tubule cell to maintain this gradient result in a condition known as the Fanconi syndrome (64). This is a form of proximal tubule dysfunction that includes proximal RTA, glucosuria, amino aciduria and phosphaturia. As will be discussed below, most forms of proximal RTA are associated with the Fanconi syndrome.

Proximal Renal Tubular Acidosis (Type II RTA)

Pathophysiology

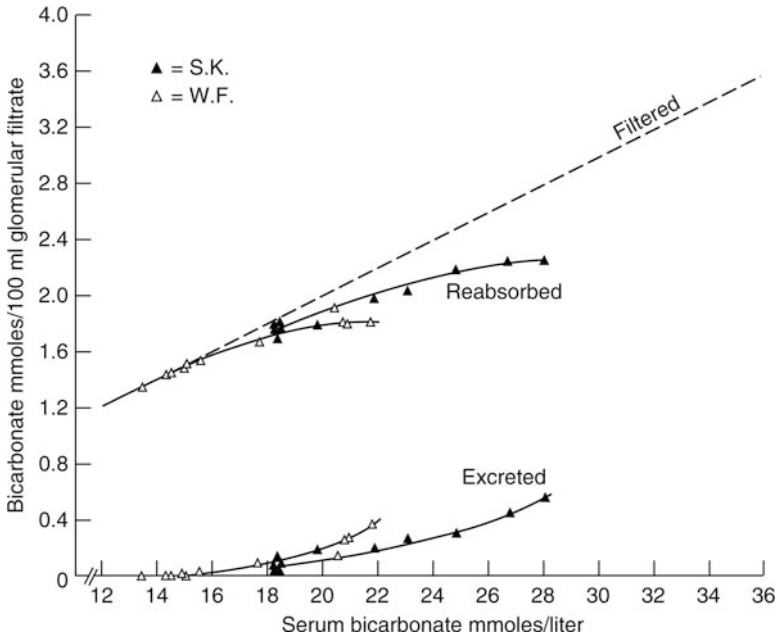
As discussed above, the transport of bicarbonate in the proximal tubule is a saturable process. Thus, the transport of bicarbonate exhibits the typical titration curve which has a threshold for bicarbonate reabsorption as illustrated in *Fig. 39-2* (44). This threshold for the reabsorption of bicarbonate is the main factor determining the serum bicarbonate concentration. If the serum bicarbonate concentration rises above the threshold, the filtered load will exceed the transport maximum for reabsorption and bicarbonate will be excreted. This will bring the serum concentration down until it matches the threshold and then all of the filtered bicarbonate is again reabsorbed.

The hallmark of proximal RTA is a reduced threshold for the reabsorption of bicarbonate as illustrated in *Fig. 39-4* and thus, these patients will have a low serum bicarbonate concentration (17, 66). When the serum bicarbonate concentration increases and approaches the normal range, patients with proximal RTA will develop bicarbonaturia. Their bicarbonate titration curve is similar to that of normal patients, but it is shifted to the left (see *Fig. 39-4*). It is important to note that the threshold for bicarbonate excretion is generally in the 14–18 mEq/L range and remains stable (1, 17).

This reduction in the capacity for reabsorption of bicarbonate makes the treatment of patients with proximal RTA difficult. Most patients require well over 6 mEq/kg/day of bicarbonate therapy to make an improvement in their serum bicarbonate concentration (67, 68).

Figure 39-4

Bicarbonate titration curves for patients with proximal renal tubular acidosis. Patients with proximal RTA have a reduced threshold for bicarbonate reabsorption and will thus excrete significant amounts of bicarbonate in their urine at lower serum bicarbonate concentrations. Thus, their titration curves are shifted to the left (reprinted with permission from (65)).



As the patient is treated with bicarbonate and the serum bicarbonate rises, bicarbonate excretion will increase dramatically with little increase in the serum bicarbonate concentration. In addition, the distal delivery of the non-reabsorbable anion will obligate the excretion of sodium and potassium. This leads to volume depletion and an increase in the serum aldosterone concentration (69). The combination of the increased distal delivery of sodium and the elevated aldosterone concentration leads to a marked excretion of potassium. Thus, many patients with proximal RTA become hypokalemic during the treatment of the disease.

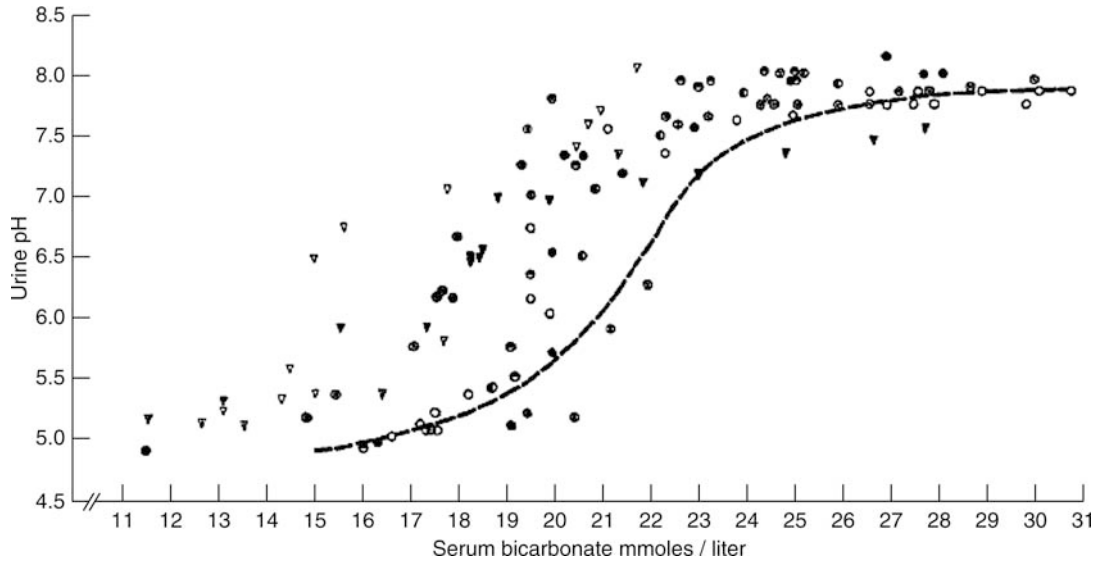
Although the treatment of these patients can be difficult, their overall acid base balance is generally good. In patients with proximal RTA, when the serum bicarbonate remains at or below the threshold for bicarbonate excretion, the patient can reclaim the filtered load of bicarbonate and will remain in relative acid base balance (16, 17, 67). This is due to the fact that the patient's distal nephron remains intact and is able to excrete the acid generated from their diet and will help prevent the patient from developing a large base deficit. This is reflected in the fact that their urine pH can decrease to less than five (*Fig. 39-5*) (1). Thus, while most patients with proximal RTA have a low serum bicarbonate, it will remain constant because the patient remains in acid base balance.

The acid base balance of patients with pure proximal RTA has been extensively studied (67, 68). At baseline, the patients were found to be in acid base balance with normal ammonium and titratable acid excretion and did not develop a base deficit. When challenged with an ammonium chloride load, they were able to increase the excretion of acid in the form of ammonia as well as titratable acid (67).

While the excretion of ammonium increased in this study, it is unclear if patients with proximal RTA have the same capacity to increase ammonia excretion as normal individuals. Because the proximal tubule is the site of ammoniagenesis, there could conceivably be a defect in the ammonia generation rate. When these patients were loaded with ammonium chloride, their excretion of acid was increased; however, the ratio of ammonia excretion to titratable acid excretion remained constant (16, 17, 67). This brought into question their ability to increase ammonia excretion in the face of an acid load and thus would probably not be able to recover from acidosis as well as a normal patient would. It was also thought that the

Figure 39-5

Urine pH of patients with proximal RTA. When patients with proximal RTA become acidotic, their serum bicarbonate concentration falls below the threshold for excretion. Because their distal nephron is intact, they can lower their urinary pH to values less than 6.0 (reprinted with permission from (70)).



level of ammonium excretion could be considered low for the chronic acidotic state (67).

A more recent study has indicated that while patients with proximal RTA are in balance at baseline, when their acidosis worsens, they cannot fully compensate (71). In this study, patients with proximal RTA were loaded with ammonium chloride for 3 days. Previous studies had been performed with an acute ammonium chloride load. The chronic loading demonstrated that the patients with proximal RTA indeed had an inability to increase their ammonium excretion as compared to the normal control subjects (71). Interestingly, the patients with proximal RTA were able to lower their urine pH to a value below the control subjects' urine pH (4.66 vs. 5.00). This was thought to be due to the fact that the normal subjects had higher amounts of ammonium in their urine to buffer the protons.

The mechanism for the ability to maintain acid base balance is due in part to an increase in titratable acid excretion (67). Because of their intact distal nephrons, these patients can also lower their urine pH to the 4.5–5 range. However, this usually occurs at very low serum pH values and is depicted in *Fig. 39-5* (1).

Calcium excretion rates in patients with proximal RTA were found to be within the normal range, indicating that there was no loss of calcium from their bones (67, 68). There was also no evidence of rickets or osteomalacia in these patients with isolated proximal RTA (67).

Fanconi Syndrome (See also Chapter 42)

As discussed above, the proximal tubule is also responsible for the reabsorption of glucose, amino acids and phosphate by sodium dependent transport systems. Many of the processes that interfere with the reclamation of bicarbonate are due to a defect in maintaining a low intracellular concentration of sodium and will thus affect the reabsorption of all of these solutes. This condition is known as the Fanconi syndrome which can be thought of as a global dysfunction of the proximal tubule (64). Thus, proximal RTA can be divided into isolated proximal RTA, which is relatively rare, and Fanconi syndrome, which is actually a more common cause of proximal RTA. This will be an important point in the clinical presentation and work up of these patients.

In addition to the problems with bicarbonate wasting, patients with Fanconi syndrome have additional pathophysiologic changes. The original definition of Fanconi syndrome consisted of skeletal findings secondary to hypophosphatemia (i.e., rickets), generalized

aminoaciduria and glucosuria (64). Later, it was found that the tubular reabsorption of bicarbonate was impaired and the definition then included proximal RTA (1). Recent reports indicate that severe osteomalacia can develop in adult patients with Fanconi syndrome (72, 73). Hypokalemia also develops in most patients with this disorder (30).

There are numerous diseases that present with Fanconi syndrome, but they appear to have a final common pathway for the proximal tubule dysfunction. A number of studies have indicated that depletion of the intracellular ATP store is responsible for the loss of the transmembrane sodium gradient (74, 75). This then leads to the inability to secrete protons and reabsorb glucose, phosphate and amino acids.

Etiology

As with most clinical disease processes, isolated proximal RTA and Fanconi syndrome can occur as an inherited defect or as an acquired disease. We will first discuss the congenital causes of this syndrome, and then review the acquired causes.

Congenital Isolated Proximal RTA

As mentioned above, isolated proximal RTA is rare (76). The initial descriptions of isolated proximal RTA were of infants that had a transient form of the disease (1, 68, 77). This form was found predominately in males and appeared to improve after several years of life. Patients presented with failure to grow and repeated bouts of vomiting and dehydration. This form follows a sporadic inheritance pattern and has no known cause.

There is a well described kindred of patients from Puerto Rico that have isolated proximal RTA that follows an autosomal dominant pattern of inheritance (67). To date, there are no reports of a gene defect in this family. Interestingly, the patients are more severely affected as infants, but tend to have less of a problem when they are older. This suggests that either the defect is attributable to a developmental transporter or to compensation with age by other transport processes in the more distal nephron segments. Children in these families have moderate acidosis and do not grow at normal rates unless they receive treatment (67). As discussed above, treatment with alkali therapy does not fully correct their acidosis because of the increased excretion of the administered base, but treatment will allow them to grow at near normal rates.

In recent years, another family with isolated proximal RTA that has an autosomal dominant inheritance pattern has been reported (78). The clinical features of this family were very similar to the previous report (67). A candidate gene approach was taken in an attempt to determine the genetic defect in this family. Extensive sequencing was done on many of the genes known to be involved in the proximal tubule reabsorption of bicarbonate; carbonic anhydrase II and IV as well as carbonic anhydrase XIV; NBC1; NHE2, NHE3 and NHE8 as well as the sodium proton exchanger regulatory proteins NHRF1 and NHRF2; and the chloride bicarbonate exchanger, SLC26A6. However, no defects were found. The authors concluded that either additional proteins are involved in the regulation of bicarbonate reabsorption or that there might have been defects in transcription factors that could regulate the expression of these genes (78).

A rare cause of isolated proximal RTA is a mutation in the sodium bicarbonate cotransporter, NBC1, which is inherited in an autosomal recessive pattern (79–81). The initial patients described were two brothers that had proximal RTA as well as eye and dental abnormalities (82). Since then, only a few other patients have been described with these features (82, 83). These patients were found to have a defect in the sodium bicarbonate cotransporter, NBC1, that is responsible for transporting bicarbonate out of the proximal tubule cell and into the blood stream (80, 83–85). Although these cases are very rare causes of isolated proximal RTA, they have demonstrated the critical function of NBC1 in the proximal tubule reabsorption of bicarbonate.

The sodium bicarbonate cotransporter, NBC1, is in the class of transporters that are critical in the membrane transport of bicarbonate known as SCLA4 (60, 61, 86–88). This class also includes the chloride bicarbonate exchanger that will be discussed in the section on distal RTA. The sodium coupled bicarbonate transporter in the proximal tubule cotransporter is designated SCLA4A4 (NBC1) and is also found in other tissues such as the eyes as well as the heart (80). This kidney specific isoform is determined by alternate splicing of the gene. Defects in this transporter result in proximal RTA due to the inhibition of bicarbonate transport in the proximal tubule. Because of the distribution of the protein in the eye, patients also develop ocular defects (80). Recently it was found that the defect might be in trafficking of the protein and not the actual function of the protein (89).

Defects in carbonic anhydrase cause dysfunction of the proximal tubule, but because of its distribution in the distal nephron, these defects cause combined proximal

and distal RTA (43, 90). These will be discussed in detail below in the section on Type III RTA.

The sodium hydrogen exchangers have been considered candidate genes for the cause of isolated proximal RTA, however, to date there have been no defects found in these genes. To determine the role of these exchangers in overall acid base balance, knockout mouse models have been generated. The primary sodium hydrogen exchanger in the apical membrane of the proximal tubule is NHE3 (34). Mice that have had NHE3 knocked out have a modest metabolic acidosis (91). They have an elevated serum aldosterone level as well as upregulation of colonic sodium transporters indicating that these animals have evidence of volume contraction (91). Perfusion of the proximal tubules in vitro shows a reduced ability to acidify the urine (92). As discussed above, a recent study in patients with isolated proximal RTA failed to detect a defect in any known gene for bicarbonate transport including NHE3 (78).

Another mouse model of proximal RTA was developed recently (93). The TASK K + channel is located in the proximal tubule and appears to regulate bicarbonate transport. When this channel was knocked out, the animals developed acidosis which was due to renal bicarbonate wasting (93).

Congenital Fanconi Syndrome

There are a number of genetic defects that result in Fanconi syndrome. These are listed in *Table 39-1* and will be described briefly.

The most common cause of congenital Fanconi syndrome is cystinosis which is an autosomal recessive disorder (94, 95). This disease results from a defect in the gene CTNS which encodes for the lysosomal membrane transporter, cystinosin (96, 97). Lysosomes are organelles responsible for degradation of proteins within the cell. Cystinosin is responsible for the transport of cystine out of the lysosome so that the organelle can continue to function. In the disease cystinosis, cystine accumulates within the lysosome of the cells throughout the body (95). It is not clear how this leads to the Fanconi syndrome, but it appears to be related to depletion of intracellular ATP (74, 75).

The other diseases that result in the Fanconi syndrome are much rarer. One in particular is worth mentioning because it is thought to be the cause of the syndrome first described by Fanconi (98–102). This is a defect in the facilitative glucose transporter GLUT2. This transporter is responsible for transporting glucose out of the proximal tubule cell and into the blood stream. Thus, a mutation in this protein would lead to accumulation of glucose within the proximal tubule. It is unclear how this would cause the Fanconi syndrome, but could be due to the consumption of intracellular phosphate by the accumulated glucose.

Table 39-1
Inherited causes of Fanconi syndrome

Disease	Gene defect	OMIM
Cystinosis	Cystinosin (CTNS)	219,800
Tyrosinemia	Fumarylacetoacetase	276,700
Fanconi-Bickel syndrome	Glut 2	138,160
Hereditary fructose intolerance	Fructose-1-phosphate aldolase	229,600
Dent's disease	ClCN5	300,009
Lowes syndrome	Phosphatidylinositol 4,5-bisphosphate 5-phosphatase deficiency (OCRL1)	309,000
Galactosemia	Galactose-1-phosphate uridylyltransferase	230,400
Wilson's disease	ATPase, Cu^{2+}-transporting, beta polypeptide	277,900

Hereditary fructose intolerance is of interest because this served as a useful model for the study of Fanconi syndrome (103, 104). The cause of the Fanconi syndrome in this disorder is thought to be due to the depletion of intracellular phosphate that occurs when the cell is presented with a load of fructose. Patients with this disorder tend to have normal renal function and no acid-base disturbance when they remain on a fructose restricted diet.

The oculo-cerebro-renal syndrome of Lowe is due to a mutation in the OCRL1 gene which encodes for the enzyme, phosphatidylinositol 4,5-bisphosphate 5-phosphatase (105). This causes an accumulation of phosphatidylinositol 4,5-bisphosphate in the cells which presumably leads to the Fanconi syndrome because it interferes with actin polymerization (106). The syndrome is inherited in an X-linked pattern.

Dent disease is caused by mutations in the chloride channel encoded by the gene, CLCN5 (107–110). The original term for this disorder was X-linked hypercalciuric nephrolithiasis. The chloride channel that the gene encodes for is found in intracellular organelles and appears to be critical for maintaining pH gradients. It is not clear how this defect results in the Fanconi syndrome.

Other diseases that lead to the Fanconi syndrome include galactosemia and tyrosinemia (111–114). These disease processes can also be controlled by diet. Rarely, other forms of glycogen storage disease can result in the Fanconi syndrome (115–117). Mitochondrial defects can also rarely be associated with the Fanconi syndrome (118–121).

Recently, mutations in the transcription factor HNF1 alpha have been associated with dysfunction of the proximal tubule (122). In addition, these defects result in maturity onset diabetes of the young type 3 (MODY3) (123). This syndrome has been reproduced in a mouse model (124). Thus, it appears that this transcription factor is a key regulator of glucose metabolism and could impact the function of the proximal tubule.

Acquired Isolated Proximal RTA

Most diseases and toxins that affect the proximal tubule result in the Fanconi syndrome, thus it is rare for isolated proximal RTA to be acquired. The primary cause of isolated proximal RTA is inhibition of carbonic anhydrase (CA) (125). Acetazolamide is given to treat pseudotumor cerebri and some forms of glaucoma. One side effect of this treatment is the development of proximal RTA. Indeed, this is often used as a marker of treatment adequacy. A number of other medicines can also cause CA inhibition, e.g., hydrochlorothiazide and topiramate (126–129).

Acquired Fanconi Syndrome

There are many toxins and medications including heavy metals that are now known to affect the proximal tubule and result in the Fanconi syndrome (130–133). In particular, a number of well documented cases of Fanconi have been reported with valproic acid (134, 135). These appear to be reversible processes, but the time of resolution can be significant. Chinese herbs containing aristolochic acid have also been associated with Fanconi syndrome (136, 137). Other agents that have been associated with Fanconi syndrome include aminoglycosides, ifosfamide, the antiviral agent tenofovir and salicylate (138–145).

Disease processes that cause the Fanconi syndrome are either immune mediated diseases or paraproteinemia syndromes. For example, Sjogren's disease will typically cause distal RTA but has been reported to cause Fanconi syndrome (146). The classic paraproteinemia that results in Fanconi syndrome is multiple myeloma (147–149).

Other conditions that are associated with Fanconi syndrome include vitamin D deficiency (150, 151). The mechanism of action for this process is not well understood. In addition, proximal RTA has been reported in pregnancy and with paroxysmal nocturnal hemaglobinuria (152, 153).

Distal Renal Tubular Acidosis (Type 1 RTA)

Pathophysiology

The hallmark of distal RTA is the inability to lower the urine pH maximally in the face of moderate to severe systemic acidosis (1). This is clearly shown in *Fig. 39-5* where the urine pH is graphed against the serum bicarbonate concentration. As can be seen in the normal individuals, the urine pH decreases to a value of approximately 4.5–5.0, but the patients with distal RTA fail to reduce their urine pH below 6.5. While this feature has been known for many years and was the initial defining characteristic of RTA, the causes of this dysfunction have only recently been elucidated (*Fig. 39-6*).

The primary function of the distal nephron in acid base homeostasis is excretion of the acid generated by the metabolism of our diet. As described earlier, the typical western diet generates approximately 1 mmol of acid per kilogram of body weight (26). Children have an additional 1–2 mmol of acid per kilogram body weight that is generated from the formation of hydroxyapatite in growing bone. Thus, the distal nephron in the growing child has the task of excreting between 1 and 3 mmol of acid per kilogram (27–29). If the distal nephron is not capable of performing this function, the patient will use the existing buffers in the body to buffer this acid. Most of the pathophysiologic consequences of distal RTA are due to accumulation of acid. Even though the proximal tubule is functioning normally to reabsorb the filtered load of bicarbonate, the patient will continue to accumulate acid and develop an ever increasing base deficit.

After the bicarbonate buffers in the extracellular fluid space are depleted, the bones begin to serve as the buffer source for the accumulated acid. Hydroxyapatite can be dissolved to liberate hydroxyl ions to help in the neutralization of the acid. Studies in patients with distal RTA have shown that they are in negative calcium balance due to the reabsorption of bone (1). This will lead to nehrocalcinosis and nephrolithiasis.

Another contributing factor to the development of nephrocalcinosis is the fact that citrate reabsorption in the

Figure 39-6

Urine pH of patients with distal RTA. Because patients with distal RTA cannot excrete hydrogen ions against a gradient in the distal nephron, they are unable to significantly lower their urine pH, even when they become very acidotic (reprinted with permission from (70)).

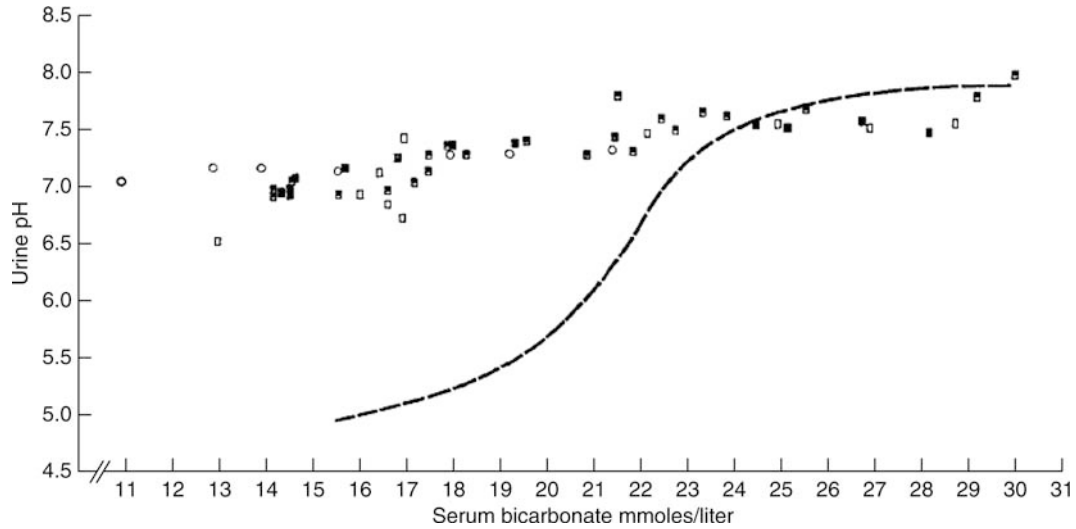
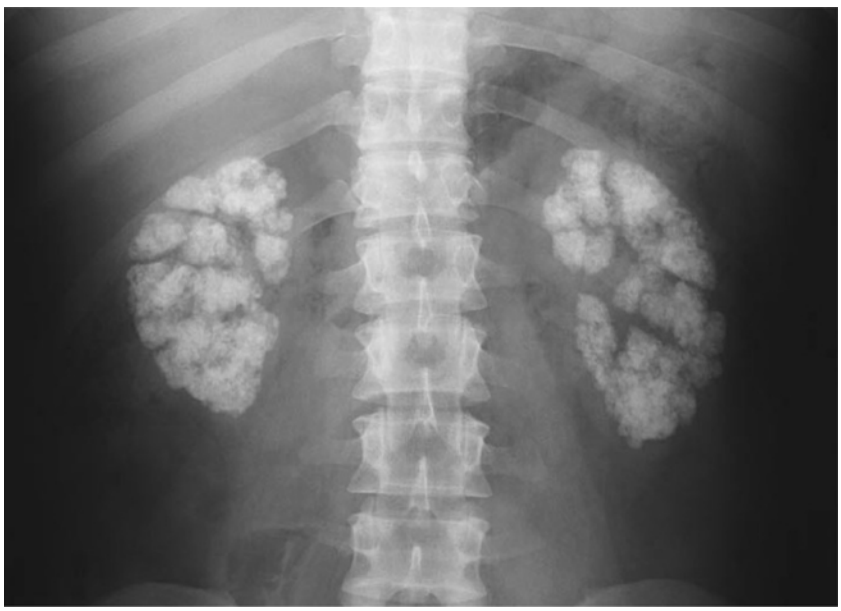


Figure 39-7

Nephrocalcinosis in a patient with distal RTA (reprinted with permission from (156)).



proximal tubule will be increased to help provide for base equivalents (154, 155). The resulting hypocitraturia will contribute to the development of nehrocalcinosis and nephrolithiasis. This can be used to help differentiate distal RTA from proximal RTA as seen in *Fig. 39-7* (157). In growing bones, the acid base disturbance will lead to rickets whereas in the older patient, they will develop osteomalacia. The description of this was provided by Albright (5).

Nephrocalcinosis has also been associated with increased production of red cells (158, 159). It is not clear what the mechanism is in these patients. Erythrocytosis has been observed in some patients with distal RTA, presumably as a result of the nephrocalcinosis (158, 159).

The proximal tubule provides ammonia that is delivered to the distal nephrons to serve as a buffer. Recently, it has been appreciated that the rate of ammonium excretion in patients with distal RTA is less than that of normal subjects (24, 25, 160). This is presumably due to the fact that ammonia that is not converted to ammonium ion by the secretion of protons can then diffuse back into the blood stream and is subsequently not excreted. There have been a number of reports of patients with distal RTA that have hyperammonemia at the time of presentation when they are extremely acidotic (161–163). They do not have liver dysfunction but they have an inability to excrete the ammonia generated in the proximal tubule.

This phenomenon has lead some investigators to postulate that the excretion of ammonium be used as a new classification scheme of RTA (160). While this could result in a more physiologic scheme for the classification of RTA, this is probably not practical at the present time. The measurement of ammonium in the urine is not a routine laboratory test. Methods for estimating ammonium excretion will be discussed in the section on clinical aspects.

Another pathophysiologic finding in patients with classical distal RTA is hypokalemia (164, 165). The exact mechanism for this is not entirely clear but is at least partially due to elevated aldosterone concentrations in these patients (45). Careful studies have indicated that the aldosterone concentration is routinely elevated in patients with distal RTA. A few patients had aldosterone concentrations in the normal range, but were inappropriately normal for the degree of hypokalemia. It was thought that the patients were mildly volume depleted because of mild proximal tubule dysfunction. The hypokalemia can be severe and cause muscle paralysis (166). This has occasionally been the presenting sign of RTA (167).

Etiology

Congenital

Congenital forms of distal RTA are divided into autosomal dominant (type Ia) and autosomal recessive with (type Ib) and without (type Ic) hearing loss. The molecular basis for these forms of inherited distal RTA have become clear over the past few years and have greatly improved our understanding of the molecular basis of renal acid base metabolism.

Autosomal dominant distal RTA is caused by mutations in the anion exchanger (AE1) that is located in the basolateral membrane of the alpha intercalated cells of the collecting duct. This exchanger is responsible for the basolateral exit of bicarbonate into the blood stream. Thus, if the protein is not functioning, acid secretion into the tubule lumen will be limited.

The biology of AE1 has proven to be very interesting (60, 168, 169). The exchanger is also located in the red cell membrane where it was first discovered and was termed "band 3 protein" (169). While it serves to function in the red blood cell as an anion exchanger, it also binds to other membrane proteins and contributes to the stability of the red cell membrane. Thus, defects in AE1 have been associated with hereditary spherocytosis and south-east Asian ovalocytosis (SAO) (168). In general, patients with these disorders do not have RTA.

The mutations in AE1 that result in autosomal dominant distal RTA are located in a different area of the molecule than the mutations causing the red cell membrane defects (170, 171). Patients with autosomal dominant distal RTA tend to develop a less severe form of RTA than patients with the autosomal recessive forms (172, 173). Most of the patients do not have red cell membrane defects. However, there have been recently described patients with both RTA and SAO. These patients were found to be compound heterozygotes for mutations that cause the two different disorders or they were homozygous for a mutation that could cause both RTA and SAO (174–176).

The autosomal recessive distal RTA with hearing loss (type Ib) was found to be due to mutations in a subunit (ATP6V1B1) of the proton pump located on the apical membrane of the alpha intercalated cell of the collecting duct (177). This led to the discovery of the proton pump location in the inner ear (178, 179). The proton pump is a key transporter in the secretion of hydrogen ions (56–59). It is a complex molecule with multiple subunits that are specific to the location in the body.

Subsequent to the initial discovery, a number of other mutations have been discovered that are responsible for autosomal recessive distal RTA with hearing loss (70, 180). Most recently, a large family with this form of RTA and hearing loss had been reported (181). The defect has been recently determined to be a truncating mutation of the ATP6V1B1 which prevented the subunit from organizing with the rest of the proton pump for complete function (182).

As families were characterized for mutations in the proton pump, it was clear that some of the families did not have hearing loss and did not have defects in the ATP6V1B1 subunit. This led to the designation of autosomal recessive distal RTA without hearing loss (type Ic). Defects in a separate subunit (ATP6N1B) were found to be the cause in the initial families studied (183). Subsequently, a number of patients developed hearing loss later in life. These patients were found to have a defect in subunits that were found in the inner ear (180).

Mouse models of distal RTA have also been developed. A mouse model that lacks AE1 (slc4a1) has been produced and found to have many of the same features as the human disease (184). The importance of the potassium chloride transporter KCC4 for function of the alpha intercalated cells was shown in a knock out model (62). These mice had features of distal RTA. A mouse that lacked the transcription factor Foxi1 was shown to have distal RTA (185). This transcription factor is evidently important in the development of the alpha intercalated cells. There are no known human mutations in this factor, but the mouse model raises the possibility of this being another gene to consider in human disease.

Acquired

The most common cause of acquired distal RTA is immunologic destruction of the alpha intercalated cells. This occurs most frequently with Sjogren's syndrome (186, 187). Distal RTA in Sjogren's has been reported to occur in about one third of the patients and after a duration of 10 years (187, 188). It can also occur in patients with systemic lupus erythematosis and has been reported in a patient with Graves' disease (189–192). Distal RTA has also been reported in renal transplant patients, however it is not clear if this is immune mediated or secondary to the medications (193).

A number of medications have been found to cause distal RTA. The classic example is amphotericin (194). This model has been used to study the pathogenesis of RTA in the laboratory (195, 196). The primary defect in acid secretion due to amphotericin appears to be an increase in the permeability of the collecting duct cells to hydrogen ions. This would then prevent the formation of the gradient that is necessary to secrete protons into the urine. While these results helped explain the pathophysiology of the backleak and is important clinically, this probably does not apply to patients with inherited defects that result in distal RTA.

Other medications that are known to cause distal RTA include lithium, foscarnet and melphalan (197–199). The mechanisms for these effects are not clear.

Acquired distal RTA can also result from the treatment of hypophosphatemic rickets (200). This is probably a result of the nephrocalcinosis that develops form the high dose of vitamin D these patients receive. Examination of patients with idiopathic hypercalciuria also demonstrated some defects in renal acidification (201).

An interesting association of distal RTA and ingestion of vanadate has been proposed as a mechanism for the high endemic rate of RTA in northeastern Thailand (202). These patients develop severe hypokalemia and it is thought that this could be due to inhibition of the H-K-ATPase by vanadate. There is a high level of vanadate in the soil in this area and experiments with rats have shown that administration of vanadate can lead to renal tubular acidosis (203).

Glue sniffing has been listed as a cause of distal RTA; however careful examination of a patient with acidosis form glue sniffing suggests a different cause of the acidosis (204). The toluene in the glue is rapidly metabolized to hippuric acid which is promptly excreted by the kidneys. When measurements were made of ammonium excretion rates, they were found to be normal. Thus, the conclusion is that while there might be some renal tubule damage from the glue sniffing, the bulk of the acidosis results from hippuric acid production. The prompt excretion of the hippurate prevents the development of an increase in the anion gap (204).

Type III Renal Tubular Acidosis

Type III RTA refers to a form of renal tubular acidosis that has features of both proximal RTA and distal RTA. During the middle of the twentieth century, a number of patients were found to have features of both forms of RTA and the third type of RTA was suggested. It was subsequently found that these patients had distal RTA with a transient form of proximal RTA. Thus, the term fell out of favor and had not been used.

More recently, a form of RTA that occurs with some forms of osteopetrosis has been characterized that seems to meet the criteria for the designation of Type III RTA. This association was originally described in 1972 (205). Subsequently, the defect was found to be a mutation in the gene for carbonic anhydrase II (43, 90). After the initial finding of the genetic defect, a number of other patients have been described with similar clinical findings

(156, 206, 207). These patients have other extrarenal findings such as cerebral calcifications as well as the bone problems associated with osteopetrosis (207). It should be pointed out that osteopetrosis can be caused by a defect in a number of different genes that affect the osteoclast (208). Thus, the finding of osteopetrosis does not imply that the patient will have a defect in carbonic anhydrase II and will develop RTA. The form of osteopetrosis associated with the carbonic anhydrase deficiency is the syndrome known as Guibaud-Vainsel syndrome or marble brain disease (208).

Type IV Renal Tubular Acidosis

The effects of aldosterone on electrolyte balance have been extensively studied since the discovery of aldosterone in the 1950s (20). The initial findings demonstrated dramatic effects of aldosterone on sodium reabsorption and potassium secretion. In the latter half of the twentieth century it became clear that aldosterone also had effects on acid base balance. With the recent advances in molecular biology, the mechanisms involved in the genetic causes of type IV RTA have been elucidated.

Type IV RTA was initially used to describe patients that developed acidosis from aldosterone deficiency. This could occur as an inherited defect, such as congenital adrenal hyperplasia, or could be acquired as in Addison's disease. The principal feature that distinguished type IV RTA from classic type I RTA was the finding of hyperkalemia. Patients with type IV RTA are hyperkalemic while many of the patients presenting with classic type I RTA were hypokalemic. This led investigators to believe that the cause of this form of RTA was aldosterone deficiency. Later it became apparent that many of the patients were not aldosterone deficient, but had a decreased responsiveness of the renal tubules to aldosterone and hence developed hyperkalemic RTA. Currently the term type IV RTA is applied to all forms of hyperkalemic RTA, regardless of the serum aldosterone concentration.

Pathophysiology

The primary effect of aldosterone on the collecting duct is to stimulate sodium reabsorption and potassium secretion in the principle cells (209). This results in an enhancement of the lumen negative electrical potential which can then help promote proton secretion. Aldosterone also has direct effects on the alpha intercalated cells to promote proton secretion by upregulating expression of the proton ATPase as well as carbonic anhydrase (209). The effect of aldosterone on ammonia excretion is not clear. There is evidence that aldosterone deficiency could directly inhibit the production of ammonia while other studies indicate that the effect could be secondary to hyperkalemia (210–212). Ammonia secretion in patients with aldosterone deficiency was low and was shown to increase after administration of mineralocorticoid; however, it was still not clear if the effect could be secondary to changes in potassium concentration.

Patients that are aldosterone deficient or resistant to the actions of aldosterone have increased excretion of sodium which leads to volume depletion and potentially a decrease in the glomerular filtration rate (213, 214). Thus, many of the symptoms of this process are secondary to the volume depletion.

The acidosis in most patients with type IV RTA is not as severe as in other forms of RTA (213). Thus, the main clinical problem with most of these patients is the hyperkalemia. Treatment often relies on restricting the intake of potassium but will ultimately depend on the cause of the RTA.

Etiology

As discussed above, type IV RTA can result from a deficiency of aldosterone or from a resistance of the renal tubules to the actions of aldosterone.

Aldosterone Deficiency

Aldosterone deficiency can be the result of a global dysfunction of the adrenal gland, referred to as Addison's syndrome, or it can be the result of isolated aldosterone or mineralocorticoid deficiency. The most common inherited form of mineralocorticoid deficiency is congenital adrenal hyperplasia (CAH) which is due to 21-hydroxylase deficiency (65, 215). Other infants can present with isolated aldosterone synthase deficiency which is not as severe a disease process since the glucocorticoid pathway remains intact (216).

Aldosterone Resistance

There are a number of inherited and acquired conditions that result in resistance of the tubules to the action of aldosterone. The pathway for aldosterone action includes the mineralocorticoid receptor and the epithelial sodium

channel (ENaC). Defects in both of these components results in type IV RTA. Because of the renal tubular resistance to aldosterone, aldosterone concentrations in the blood are quite elevated. Thus, this is referred to as pseudohypoaldosteronism (PHA).

Defects in the mineralocorticoid receptor lead to an autosomal dominant form of PHA (217). This form is the least severe of the PHAs and patients tend to improve as they get older. This is presumably due to compensation by other pathways to reabsorb sodium and secrete potassium and hydrogen ions.

An autosomal recessive form of PHA is due to defects in ENaC (218). Patients with this form can be severely affected since the final pathway for sodium regulation in the collecting duct involves ENaC. In addition, they have severe pulmonary problems at birth because ENaC is present in the lungs and is a key factor in the reabsorption of fluid from the lung space after birth.

Both of these forms of PHA lead to salt loss and volume depletion. Patients tend to be hypotensive and dehydrated. Additionally, plasma concentrations of renin and aldosterone are quite elevated because of the volume depletion.

A form of PHA that occurs in patients that are hypertensive was originally thought to be due to a "chloride shunt" in the collecting duct and was referred to as PHA type 2 or Gordon's syndrome (219). These patients are characterized by having hyperkalemia and acidosis, but have a low concentration of renin and aldosterone in their plasma. This led investigators to hypothesize that the paracellular pathway in the collecting duct was allowing chloride to be reabsorbed at a higher rate than was needed (220). This would cause the electrical potential difference in the tubule to decrease and would thus decrease the excretion of potassium and protons.

Recent discoveries have shown that PHA type 2 is due to defects in WNKs (with no lysine kinases) (221). Specifically, there are families with the syndrome that have mutations in WNK1 and some with mutations in WNK4. The biology of the WNKs has turned out to be very complicated and is beyond the scope of this chapter. However, they seem to be key players in the regulation of potassium and blood pressure.

Acquired

Addison's disease can be an autoimmune disease or can be the result of damage to the adrenal gland from infection or infarction. Treatment involves replacing the adrenal hormones as needed as well as treating the underlying infection. In adult patients, diabetes is a leading cause of type IV RTA as a result of hyporeninemic hypoaldosteronism (222). There are other disease processes that also lead to a decrease in production of renin which would then lead to a decrease in aldosterone secretion. If the patient has type IV RTA from acquired hypoaldosteronism, treatment with mineralocorticoids will correct the defect (223).

Tubular resistance to aldosterone can occur as a result of a number of different processes. Autoimmune diseases can lead to interstitial nephritis that decreases the tubule responsiveness to aldosterone (224). Patients with systemic lupus erythematosis classically develop type 1 RTA, but have been reported to present with type IV RTA (192). Infections such as acute pyelonephritis can also cause a resistance to aldosterone action.

Probably the most common cause of acquired type IV RTA in the pediatric age range is obstruction of the urinary tract. The mechanism by which obstruction causes resistance of the tubule to aldosterone is not clear, but this is commonly seen in patients with posterior urethral valve or with prune belly syndrome.

Type IV RTA can also been seen in patients with a renal transplant (225). This could be due to either an immune mediated mechanism or it could be related to medications used for the treatment of rejection. In particular, calcineurin inhibitors are known to cause a type IV RTA (193, 226).

Other medications that are known to interfere with the action of aldosterone include angiotensin converting enzyme inhibitors (ACE inhibitors), heparin, prostaglandin inhibitors (NSAIDs) and a number of potassium sparing diuretics. These would include amiloride which blocks the epithelial sodium channel and spirinolactone which blocks the mineralocorticoid receptor.

Clinical Aspects of Renal Tubular Acidosis

The diagnosis of renal tubular acidosis represents a challenge to the clinician for a number of reasons. Depending on the severity of the disease presentation, the patient could present with findings consistent with proximal and distal RTA. The patients are also many times quite volume depleted at presentation and it is not clear how much this impacts the serum chemistries. In addition, patients with infections can be septic and in shock. Thus, the complete evaluation of a patient for renal tubular acidosis might have to occur after the acute illness has subsided.

As with any complex disease, the diagnosis of RTA begins with clinical suspicion. If the disease is not being considered in the differential diagnosis, then a definitive diagnosis will not be made. There have been a number of recent reviews that outline practical guidelines for the diagnosis and management of RTA (227–230). This section of the chapter will focus on the reasoning behind the laboratory testing that is recommended for the work up of patients with suspected RTA.

The inherited forms of renal tubular acidosis present almost uniformly with failure to grow and repeated episodes of vomiting and dehydration (1, 68). It should be emphasized that most of these patients are very ill appearing at the time of presentation. The patient with failure to grow that otherwise appears healthy ahs a much lower probability of having RTA. A recent study examined patients referred for failure to thrive that had serum chemistries indicating the possibility of RTA (231). Simply performing a venous blood gas analysis in the patients demonstrated the absence of acidosis.

The first step in the evaluation of patients with an acidosis is to determine the serum anion gap (232–234). Patients with RTA are characterized by having a normal anion gap. This is also referred to as a hyperchloremic metabolic acidosis. Interpretation of the anion gap can occasionally be misleading. Other factors can affect the anion gap such as serum protein concentrations, calcium and other anions such as phosphate (233). Thus, the determination of a normal anion gap acidosis can only be correctly made when these factors are taken into account.

Although renal tubular acidosis should be suspected in these patients with metabolic acidosis with a normal anion gap, there are other disorders to consider in the differential diagnosis such as gastrointestinal loss of bicarbonate. The workup of these patients is therefore designed to differentiate whether the acidosis is of renal or extrarenal origin. Thus, it is necessary to examine the response of the kidney to the metabolic acidosis. As discussed above, the normal renal response to metabolic acidosis is to increase ammonium chloride excretion as a way to enhance hydrogen ion excretion to correct the acidosis. Unfortunately, measuring ammonium in the urine is not a routine function in most hospital laboratories. Over time, several approaches have been taken to estimate the urinary excretion of ammonium to determine if the kidney is responding normally (235–239).

The measurement of the urine pH can be helpful but also can be misleading in the diagnosis of RTA (240). Where it tends to be helpful is in determining whether or not there is bicarbonate in the urine (see *Fig. 39-8*).

The simplest test that was devised is to measure the urine sodium, potassium and chloride concentrations and calculate the urinary anion gap using the following equation:

$$Urinary\ anion\ gap = U_{Na} + U_K - U_{Cl},$$

where U_{Na} is the urinary sodium concentration, U_K the urinary potassium concentration and U_{Cl} the urinary chloride concentration. This approach is based on the fact that the unmeasured cations and anions are constant and that ammonium would be the primary cation other than sodium and potassium that would be excreted with chloride. The amount of ammonium in the urine when the anion gap is zero turned out to be 80 mmol/L. The other assumptions in this approach are that there is no appreciable bicarbonate in the urine and the patient is not receiving medications that are excreted in the urine in ionic form such as penicillins. If the urine pH is less than seven, the urinary bicarbonate will be less than 10 mmol/L (see *Fig. 39-8*). This simple approach has been verified in normal controls as well as patients with RTA and gastrointestinal causes of acidosis (235, 237).

Modifications to the urinary anion gap calculation have been made to expand its application to conditions that could yield misleading results. If patients are excreting other anions, ammonium would be excreted with the unmeasured anion instead of chloride. Thus, the urinary anion gap would underestimate the amount of ammonium in the urine. The osmolal gap was developed to take this into account (238). The osmolal gap is calculated by the following equation:

$$Urine\ osmolal\ gap = measured\ urine\ osmolality - calculated\ osmolality.$$

The calculated osmolality is determined by the following equation:

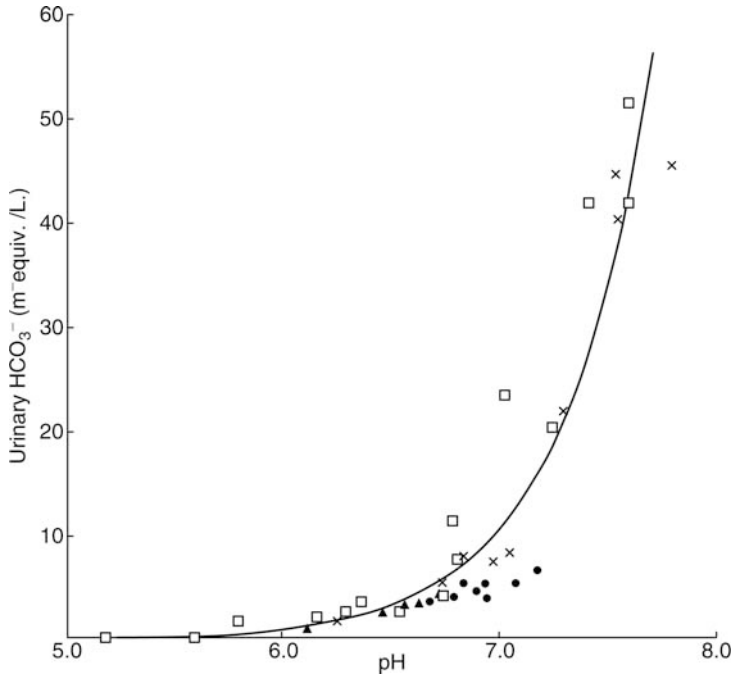
$$\text{Calculated osmolality} = \text{Na} + \text{K} + \text{HCO}_3 + \text{urea nitrogen}/2.4 + \text{glucose}/18.$$

This was shown to correctly account for the unmeasured anions in patients with ketoacidosis (238). An additional modification was then developed because of the difficulty in measuring the urine bicarbonate concentration. This method replaces the urine bicarbonate measurement by multiplying the sum of the sodium and potassium concentrations by two (236).

The above approaches are designed to estimate the amount of ammonium in the urine. Normal controls have about 80 mmol/L of ammonium in the urine (237). What makes the test work well in the evaluation of acidosis is

Figure 39-8

Urinary bicarbonate concentration as a function of urinary pH. As can be seen, once the urine pH becomes less than 6.5, the concentration of bicarbonate is less than 10 mEq/L. This might have an impact in determining the urinary anion gap (reprinted with permission from (241)).



the fact that normal individuals will have an increase in their ammonium excretion but the patients with RTA will not. A recent study examined the correlation of these techniques with actual measurement of urinary ammonium (242). This study concluded that the correlation many times was not good and that direct measurement of the urinary ammonium would be a better method. Another problem with this approach is that neonates were found to have a poor correlation between urinary anion gap and urinary ammonium concentration (243).

Another approach to examine the urine for proton secretory rate is to measure the urine and blood pCO_2 during bicarbonate loading (244–246). The idea is to take advantage of the low level of carbonic anhydrase activity in the distal nephron. When the patient is loaded with bicarbonate, the delivery to the proximal tubule will exceed the transport maximum and significant amounts of bicarbonate will be delivered to the distal nephron. If the patient has a normal proton secretory rate, hydrogen ions will be secreted into the tubule lumen. Although there is CA II in the distal nephron, the rate of reaction is slow enough that the carbon dioxide will be excreted in the urine and not reabsorbed. Under these conditions, normal individuals will have a urinary pCO_2 of greater than 70 mm Hg or a blood-urine pCO_2 of greater than 30 mm Hg. Patients with a defect in hydrogen ion secretion will have a urinary pCO_2 of less than 70 mm Hg or a blood-urine pCO_2 of less than 30 mm Hg. This method has been shown to be useful in neonates as well as adults (246).

Other tests might be indicated if the results of the above remain indeterminate. Traditionally, the patient's ability to acidify the urine is tested using acute or chronic loading with ammonium chloride (228). Because of the unpalatable nature of the ammonium loading, urinary acidification can be evaluated using a combination of a mineralocorticoid and furosemide (247).

Differentiating Proximal and Distal RTA

Once it has been determined that the patient has RTA, it is necessary to determine if it is a proximal or distal defect. Usually this can be determined by the associated findings in the patient. As outlined above, most patients with proximal RTA have the Fanconi syndrome. Thus, it is

very helpful to evaluate the urine for glucosuria and phosphaturia. If these are normal but the patient is suspected of having a proximal tubule defect, it might be necessary to perform a bicarbonate titration to find the threshold for bicarbonate excretion (228). The serum potassium concentration will also help determine if the patient has a type IV RTA.

Another useful determination is a renal sonogram or X-ray to determine if the patient has nephrocalcinosis (see *Fig. 39-7*). Patients with distal RTA have hypocitraturia and therefore are much more likely to have nephrocalcinosis and form renal stones. Patients with proximal RTA are in relative acid base balance so that they have normal amounts of citrate in their urine and they do not excrete large amounts of calcium.

Treatment

The treatment of RTA will of course be determined by the type and cause of RTA. Fanconi syndrome due to cystinosis should be treated with cysteamine (241, 248, 249). This will prevent further damage to the renal tubular cells by preventing the accumulation of cystine. However, these patients continue to have Fanconi syndrome and require large amounts of alkali therapy as well as phosphate and vitamin D.

The sporadic forms of proximal RTA are also difficult to correct completely, but mild improvements in their acid base status allows them to grow normally (67). These patients tend to improve with age and will need less alkali as the grow.

The treatment of distal RTA is somewhat more straight forward. The amount of alkali needed to correct the acidosis and maintain normal acid base balance is much less than that needed in patients with proximal RTA. The dosage of alkali necessary has been recently studied. Using potassium citrate, investigators have found that 3–4 mEq/kg/day was necessary to normalize the urinary citrate excretion (250, 251). A previous study had also indicated that the dosage of alkali needed to be higher in younger children and decreased to about 3 mEq/kg/day after the age of 6 years (69).

The importance of continued therapy in these children has been a recent concern (252). It appears that subclinical acidosis could have long term effects on the bone, resulting in osteoporosis. The loss of calcium from the bones would also lead to nephrocalcinosis and renal stone formation.

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40 Nephrogenic Diabetes Insipidus

Nine V. A. M. Knoers · Elena N. Levtchenko

History

First familial cases with diabetes insipidus were described by McIlraith in 1892, however, he did not distinguish between renal and neurohormonal forms of the disorder (1). The renal type of diabetes insipidus was appreciated as a separate entity more than 50 years ago, when it was described independently by two investigators: Forssman (2) in Sweden and Waring et al. (3) in the United States. In 1947, Williams and Henry (4) noticed that injection of antidiuretic hormone (ADH) in doses sufficient to induce systemic side effects could not correct the renal concentrating defect. They coined the term *nephrogenic diabetes insipidus*. Subsequent studies revealed active hormone in the serum and urine of affected persons and lent further support to the theory of renal unresponsiveness to vasopressin. Nephrogenic diabetes insipidus is synonymous with the terms *vasopressin-* or *ADH-resistant diabetes insipidus* and *diabetes insipidus renalis*.

Definition and Clinical Manifestations

Congenital nephrogenic diabetes insipidus (NDI) is a rare inherited disorder, characterized by insensitivity of the distal nephron to the antidiuretic effects of the neurohypophyseal hormone arginine vasopressin (AVP). As a consequence, the kidney loses its ability to concentrate urine, which may lead to severe dehydration and electrolyte imbalance (hypernatremia and hyperchloremia). Patients with NDI have normal birth weight and pregnancies are not complicated by polyhydramnios. Urine concentrating defect in NDI is present from birth, and manifestations of the disorder generally emerge within the first weeks of life. With breast milk feedings, infants usually thrive and do not develop signs of dehydration. This is because human milk has a low salt and protein content, and therefore a low renal osmolar load. With cows' milk formula feedings, the osmolar load to the kidney increases, resulting in an increased demand for free water. This is not provided by oral feeding and, therefore hypernatremic dehydration appears. Irritability, poor feeding, and poor weight gain are usually the initial symptoms (5). Patients are eager to suck but may vomit during or shortly after the feeding. Dehydration is evidenced by dryness of the skin, loss of normal skin turgor, recessed eyeballs, increased periorbital folding, depression of the anterior fontanelle, and a scaphoid abdomen. Intermittent high fever is a common complication of the dehydrated state, particularly in very young children. Body temperature can be normalized by rehydration. Seizures can occur but are rare and most often seen during therapy, particularly if rehydration proceeds too rapidly. Constipation is a common symptom in children with NDI. Nocturia and nocturnal enuresis are common complaints later in childhood.

Untreated, most patients fail to grow normally. In a retrospective study of 30 male NDI patients, most children grew below the 50th percentile, most of them having standard deviation (SD) scores lower than −1 (6). Some well-treated patients, however, may achieve normal adult height. Catch-up growth occurs at least in some patients after normalization of water and electrolyte balance, especially in those with adherence to treatment. Bone age is generally not delayed (7). Weight for height SD scores are initially low, followed by global normalization at school age (6). Initial feeding problems and the ingestion of large amounts of low-caloric fluid resulting in a decreased appetite may play roles in failure to thrive seen in NDI (8, 9). Furthermore, it is possible that repeated episodes of dehydration have some as yet undetermined negative effects on growth.

Mental retardation has long been considered an important complication of untreated NDI and assumed to be a sequel of recurrent episodes of severe brain dehydration and cerebral edema caused by overzealous attempts at rehydration (10–12). Additional evidence underscoring the assumption that NDI has adverse effects on the cerebrum is provided by several reports describing intracranial calcifications in NDI patients (13, 14). Such lesions are generally considered to be the result of hemorrhage or necrosis. Most of the reported patients with cerebral calcifications were mentally retarded. Nowadays mental retardation is rare due to earlier recognition and treatment of NDI. Exact estimates of the current frequency of mental retardation under modern treatment are unknown,

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but in the largest psychometric study ever reported only 2 of the 17 male NDI patients (aged 3–30 years) tested had a total intelligence quotient more than 2 SD below the norm. Fourteen patients had an intelligence score within or above the normal range and one patient had a general index score between −1 and −2 SD. (15)

The psychological development of NDI patients is influenced by a persistent desire for drinking and the need for frequent voiding, which compete with playing and learning. Therefore many NDI patients are characterized by hyperactivity, distractibility, short attention span, and restlessness. In the psychometric study mentioned earlier, the criteria for attention deficit hyperactivity disorder were met in 8 of 17 tested NDI patients (15).

Persistent polyuria can result in the development of megacystis, trabeculated bladder wall, hydroureter, and hydronephrosis (6, 16, 17). Large capacity hypotonic bladder dysfunction might require clean intermittent catheterization (17). Patients should be trained to void regularly in order to assure that maximal urinary bladder capacity remains within normal range. Both patient's groups with AVPR2 and AQP2 mutations can develop urinary tract dilatation and bladder dysfunction (16, 18).

Diagnostic Procedures

The observation of polyuria in a dehydrated infant, together with the finding of a high serum sodium concentration, provides presumptive evidence for a renal concentrating defect. To confirm the concentrating defect and to distinguish the renal form of diabetes insipidus from the central form, a vasopressin test is performed with 1-desamino-8-D-arginine vasopressin (DDAVP), a synthetic analogue of the natural arginine vasopressin that produces a high and prolonged antidiuretic effect. In the test, DDAVP (10 µg for infants, 20 μg for children) is administered intranasally. Urine is collected during the subsequent 5.5 h. The first collected portion of the urine should be discarded. The maximal urine osmolality in any collected aliquot is chosen as a measure of the concentrating capacity (19). After DDAVP administration, NDI patients are unable to increase urinary osmolality, which remains below 200 mOsm/kg H_2O (normal $\geq$807 mOsm/kg H_2O) and cannot reduce urine volume or free-water clearance.

Plasma vasopressin levels are normal or only slightly increased in affected children. Other laboratory findings have been described, which mainly result from chronic dehydration. Serum sodium concentration is generally elevated and may be above 170 mmol/L. There is also an increase in serum chloride concentration and retention of urea and creatinine. All values are normalized by adequate rehydration. In addition, reduced glomerular filtration rate (GFR) and renal blood flow return to normal when a normal hydration state has been achieved.

The primary congenital form of NDI has to be differentiated from central diabetes insipidus (due to lack of AVP) and from the secondary or acquired forms, which are much more common. In our experience, the urinary osmolality obtained after DDAVP administration in secondary disorders is always higher than in NDI. Several secondary causes, some of which will be discussed later, are listed in *Table 40-1*.

Cellular Physiology of Arginine Vasopressin's Antidiuretic Action in the Distal Nephron

The physiologic action of vasopressin on the renal collecting duct has been one of the most intensively studied processes in the kidney. Arginine vasopressin (AVP, ADH) is synthesized on the ribosomes of the magnocellular neurons of the supraoptic and paraventricular nuclei of the hypothalamus as a large biologically-inactive bound form. Within storage granules, the hormone is cleaved into the biologically active form and transported down the neuronal axons to the posterior pituitary and stored there. Following appropriate stimuli, AVP is secreted from the posterior pituitary into the circulation as biologically active hormone. AVP release is primarily regulated by changes in

Table 40-1

Common causes of acquired or secondary nephrogenic diabetes insipidus

Amyloidosis
Analgesic nephropathy
Chronic pyelonephritis
Chronic renal failure
Drug-induced
Lithium
Tetracyclines
Hypercalcemia/nephrocalcinosis
Hypokalemia
Juvenile nephronophthisis
Obstructive uropathy
Renal dysplasia
Sarcoidosis
Sickle cell anemia and trait

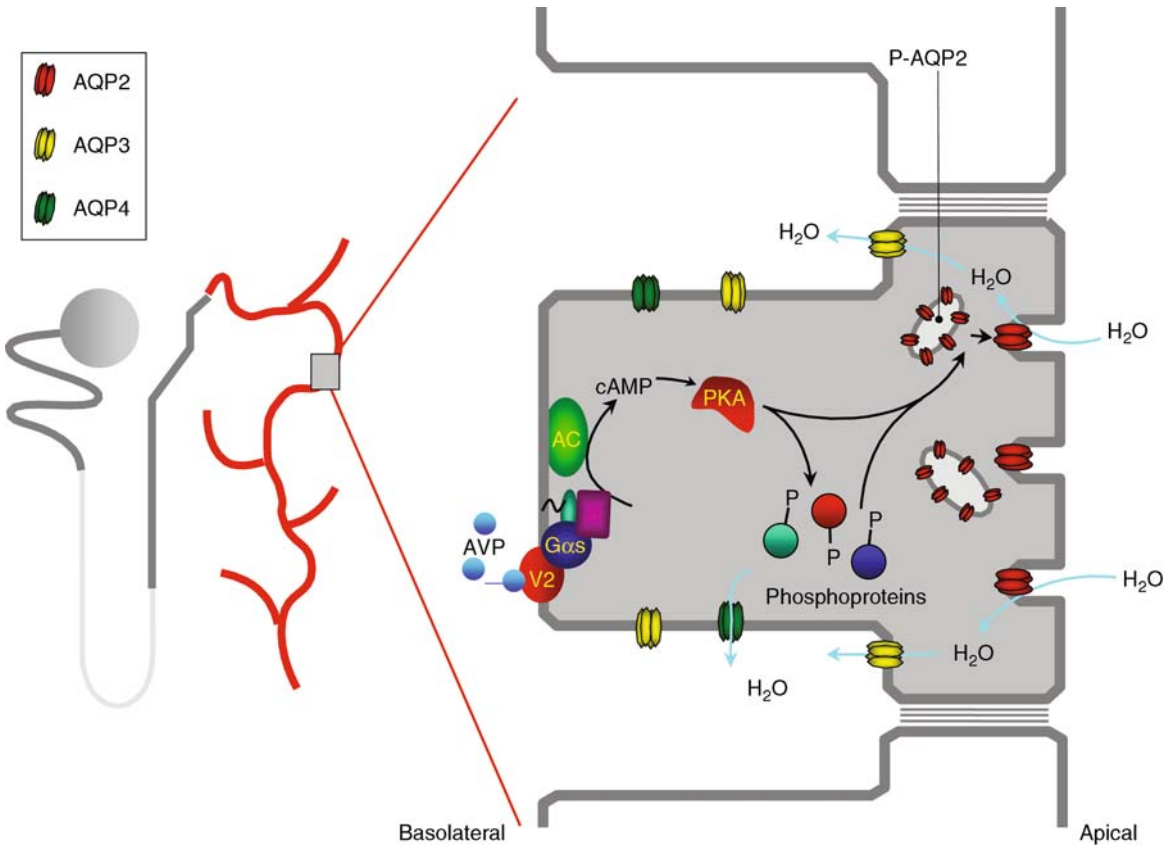
plasma osmolality (by >2%), but can also occur in response to nonosmotic stimuli. These nonosmotic stimuli are generally related to changes in either total blood volume or the distribution of extracellular fluid. In addition, physical pain, emotional stress, and certain drugs (e.g., nicotine) influence the release of AVP. In its effector organ, the kidney, AVP binds to vasopressin type-2 (V_2) receptors on the basolateral membrane of the principal inner medullary collecting duct cells and of the arcade cells (*Fig. 40-1*). The arcades are long, highly branched renal tubule segments that connect distal convoluted tubules of several deep and midcortical nephrons to the origin of cortical collecting ducts. V_2 receptor occupancy results, via the intermediacy of a stimulatory G-protein (G_s), in activation of adenylate cyclase and an increase in intracellular cyclic adenosine monophosphate (cAMP) from adenosine triphosphate (ATP). The elevated cAMP levels stimulate protein kinase A (PKA), which in turn initiates a redistribution of aquaporin-2 (AQP2) water channels from intracellular vesicles to the apical plasma membrane, rendering this membrane water permeable.

The increase in apical membrane permeability allows water to flow from the tubule lumen to the hypertonic medullary interstitium, via AQP2 in the apical membrane and via AQP3 and AQP4, constitutive water channels in the basolateral membrane (*Fig. 40-1*). This then leads to the formation of concentrated urine. Upon fluid intake, AVP release into the blood decreases, AQP2 is redistributed into intracellular vesicles, and water reabsorption is reduced. Katsura et al. have shown that the AVP-regulated recycling of AQP2 can occur at least six times with the same molecules (20).

In recent years, our knowledge of the AQP2 dynamics in the cell has increased significantly. We will describe the

Figure 40-1

Simplified scheme of the antidiuretic arginine vasopressin (AVP) signaling pathway in collecting duct cells *V2*, Vasopressin type-2 receptor; *Gαs*, stimulatory guanine nucleotidebinding protein; *AC*, adenylate cyclase; *cAMP*, cyclic AMP, *PKA*, protein kinase A; *AQP2*, aquaporin-2 water channel; *pAQP2*, phosphorylated AQP2, *AQP3*, aquaporin3 water channel; *AQP4*, aquaporin-4 water channel. (See color plate 23)



most important regulators here. For further details the reader is referred to several excellent reviews on this subject (21–23).

Phosphorylation of a PKA-consensus site in AQP2, the serine at position 256 in the cytoplasmic carboxy terminus, is absolutely essential for AQP2 delivery to the apical membrane (24, 25). In addition, it has been shown that anchoring of PKA to PKA-anchoring proteins (AKAPs) which ensures targeting of PKA to AQP2- bearing vesicles, is another prerequisite for AVP-mediated AQP2 translocation. (26). AQP2 is expressed at the apical membrane as a homotetramer and studies using oocytes as a model system indicated that for plasma membrane localization 3 out of 4 monomers in an AQP2 tetramer need to be phosphorylated. (27).

PKA is the main kinase for AQP2 phosphorylation, but other kinases may potentially participate in the regulation of AQP2 trafficking. Besides PKA sites, putative phosphorylation sites for PKG, PKC, and casein kinase II are also present in the AQP2 sequence.

There is evidence that a cGMP-dependent pathway can induce AQP2 apical accumulation. Thus, Bouley et al. have shown that nitric oxide and atrial natriuretic factor stimulate the insertion of AQP2 in renal epithelial cells via a c-GMP dependent pathway (28).

Intracellular calcium mobilization also has an important role in vasopressin-mediated AQP-2 trafficking. Vasopressin binding to the V_2 receptor was demonstrated to trigger a rapid increase of intracellular calcium, which in turn stimulates apical exocytosis of AQP2 and increase of osmotic water permeability across the collecting duct (29). A recent study indicated that Epac (Exchange Protein directly Activated by cAMP) rather than PKA is the signaling cascade that transmits the vasopressin signal to calcium mobilization (30).

Reorganization of the actin cytoskeleton is an another important mechanism in the translocation of AQP2-containing vesicles to the apical membrane. The actin cytoskeleton most likely provides a network that anchors the AQP2-bearing vesicles in the unstimulated cell. Vasopressin has been shown to depolymerize apical F-actin in rat inner medullary collecting duct, resulting in the fusion of AQP2-carrying vesicles with the apical membrane (31), indicating that reorganization of the apical actin network may be critical in promoting the trafficking of AQP2-bearing vesicles. Both forskolin and okadaic acid stimulate AQP2 translocation by inducing a reorganization of the apical actin network (32). Furthermore, RhoA inhibition through PKA-mediated phosphorylation of RhoA is shown to be a key event for actin dynamics inducing AQP2 translocation (33).

The molecular machinery for the docking and fusion of AQP2-containing vesicles with the apical membrane is similar to the process of synaptic vesicle fusion with the presynaptic membrane and involves vesicle (v) SNAREs (soluble NSF attachment protein receptors) and target membrane (t) SNAREs. Thus, in subcellular fractions from rat kidney, enriched for AQP2-containing vesicles, the v-SNARE synaptobrevin (VAMP-2) was found, as well as the fusion mediating protein SNAP23 (34–37). A proteomic analysis of AQP2-bearing vesicles also indicated the presence of a series of SNAREs, confirming their importance in AQP2 trafficking (38).

During the endocytotic process, AQP2 accumulates in clathrin-coated pits and is internalized via a clathrin-mediated process (39–41). Thus, AQP2 on the apical membrane is dependent on the balance between exocytosis and endocytosis, and inhibition of endocytosis is another way to increase the water permeability of collecting ducts. Prostaglandin E_2 stimulated removal of AQP2 from the surface of the cells when added after vasopressin treatment, but did not alter the phosphorylated state of AQP2 in MDCK cells, indicating that dephosphorylation of AQP2 may not be necessary for its subsequent internalization (42).

AQP2 on the plasma membrane is retrieved to early endosomes through phosphatiylinositol 3-kinase-dependent mechanism, and then is transferred to Rab11-positive storage vesicles (43). AQP2 in the storage vesicles reappears on the apical membrane upon vasopressin stimulation and the importance of Rab11 in such an AQP2-specific recycling was confirmed by a knockdown study using siRNA (44).

Evidence is emerging for ubiquitination as a signal for endocytosis and subsequent transport to multivesicular bodies (MVB) or proteasomes (45). AVP removal induces a short-chain ubiquitination of AQP2 at amino-acid 270 (Lysine = K), and the K270R (R = arginine) mutant was endocytosed with a much slower rate (46).

Long-term adaptation to circulating AVP- levels, for instance in a dehydrated state, is accomplished by increasing the expression of AQP2 mRNA and protein. Transcriptional regulation of the AQP2 gene seems to be the main action of AVP and is mediated by phosphorylation of a cAMP-response element-binding protein (CREB) and binding of the phosphorylated cAMP-response-element-binding protein to the cAMP-response element in the promoter region of the AQP2 gene (47).

Genetics

Three different inheritance patterns of NDI have been recognized. In most cases (about 90%) NDI is transmitted

as an X-linked recessive trait (MIM304800). In these families, female carriers who are usually unaffected transmit the disease to sons, who display the complete clinical picture (2, 4, 48). In 1988, the major NDI locus was mapped to the distal region of the long arm of the X-chromosome (Xq28) (49), and in 1992 mutations in the V_2 receptor gene were shown to underlie X-linked NDI (50–52). In a minority of families (about 10%), the transmission and phenotypic characteristics of NDI are not compatible with an X-linked trait. In these families, females display the complete clinical picture of NDI and are clinically undistinguishable from affected male family members (53–55). In addition, linkage analysis in these families has excluded linkage between NDI and polymorphic DNA markers from the Xq28 region. Family pedigrees suggested the existence of both an autosomal recessive (MIM 222,000) and an autosomal dominant form (MIM 125,800) of NDI. It was subsequently demonstrated that both autosomal forms of NDI are caused by mutations in the AVP-sensitive aquaporin-2 water channel (56, 57). The prevalence of NDI is not exactly known, but the disease is assumed to be rare. The most recent estimate of the prevalence of NDI in Quebec, Canada is 8.8:1,000,000 males (58). In the Dutch population of about 16 million, 40 different families are known.

X-Linked Nephrogenic Diabetes Insipidus: Mutations in the Vasopressin V_2 Receptor Gene

The vasopressin type-2 receptor had long been considered a prime candidate for the defective step in the AVP-mediated response in X-linked NDI. The reason for this belief was the observation that in patients with X-linked NDI, not only the antidiuretic but also the vasodilatory coagulation and fibrinolytic responses to the V_2 receptor-specific agonist DDAVP were lacking (59). This finding suggested a general V_2 receptor defect in these patients. Independent support for the V_2 receptor being involved in X-linked NDI was provided by the finding that a gene conferring V_2-like binding activity colocalized with the NDI locus in the subterminal region of the X-chromosome long arm (Xq28) (60). Soon after the identification of the human V_2 receptor gene and complementary DNA (cDNA) in 1992 (41), the role of the V_2 receptor in the pathogenesis of NDI was finally proven by the demonstration of mutations in the encoding gene in affected individuals (50–52; reviews in 12, 62, 63). The V_2 receptor gene (AVPR2; Genbank association number L22206) is relatively small and consists of three exons separated by two short intervening sequences (introns). The mRNA has been found exclusively in the kidney, specifically in the cortical and medullary collecting ducts. The cDNA encodes a receptor protein of 371 amino acids, has a predicted molecular mass of approximately 41 kDa and shares the general structure of a G protein-coupled receptor consisting of seven hydrophobic transmembrane helices, connected by extracellular and intracellular loops. The receptor contains one unique consensus sequence site for N-linked glycosylation in the extracellular aminoterminus (64) and phosphorylation sites for G-protein-coupled receptor kinases (GRK) represented by a serine cluster in the carboxy-terminus (65, 66). The amino-terminal part of the protein including the first transmembrane domain and the positively-charged first intracellular loop are important for proper insertion and orientation in the membrane (67). A conserved glutamate-dileucine motif in the intracellular carboxy-terminal part of the receptor is essential for receptor transport from the endoplasmic reticulum (ER) to the Golgi-apparatus (68). Two conserved adjacent cysteines in the C-terminus are palmitoylated, thereby anchoring the carboxy-tail to the plasma membrane and controlling the tertiary structure of this region of the receptor (69).

To date, more than 190 distinct putative disease-causing mutations in the V_2 receptor gene have been detected in families with X-linked NDI (see: http://www.medcor.mcgill.ca/nephros). The mutations are not clustered in one domain of the V_2 receptor but are scattered throughout the protein, except for the part coding for the N- and C-terminal tails of the receptor. Approximately 50% of the mutations are missense mutations. Nucleotide deletions and insertions causing frame-shifts (27%), nonsense mutations (12%), large deletions (5%), in-frame deletions or insertions (4%), and splice site mutations (2%) account for the remainder of mutations (12, 62). Several mutations are recurrent as evidenced by the fact that these mutations were found on different haplotypes in ancestrally independent families. The most frequent of these recurrent mutations (D85N, V88M, R113W, R137H, S167L, R181C, and R202C) occur at potential mutational hotspots.

The molecular mechanism underlying the renal insensitivity for AVP differs between mutants. As upcoming pharmacological treatments for NDI likely depend on the underlying mechanism, GPCR mutations in general and V_2R mutations in particular have been divided in five different classes according to their cellular fate (70, 71).

Class I comprises all mutations that lead to improperly processed or unstable mRNA, like promoter alterations, exon skipping or aberrant splicing. This class also holds

frame shift and non-sense mutations, which result in truncated proteins like V_2R-Q119X, -W293X and -R337X and -C358X.

Class II mutations are missense or insertions/deletions of 1 or more nucleotide triplets, resulting in fully translated proteins. Due to the mutation, however, mutant receptors are misfolded and retained in the endoplasmic reticulum (ER), as the ER is the organelle that has the cellular quality control over proper folding and maturation of synthesized proteins. Misfolded proteins are subsequently mostly targeted for proteasomal degradation (72). Intracellular entrapment of missense V_2R mutants and their rapid degradation likely represents the most important cause of NDI, as more than 50% of the mutations in V2R are missense mutations and cellular expression revealed that most of these result in ER-retained proteins. The extent of ER retention, however, may differ between mutants, and may represent differences in their folding state. Hermosilla et al. recently reported that of eight V_2R mutants that are retained, only three were strictly kept in the ER, whereas the five other mutants were transported to the ER-Golgi intermediate compartment, followed by retrograde transport to the ER (73).

Class III comprises similar mutations as of class II, but the resulting mutants are not considered misfolded by the ER and can continue their itinerary to the plasma membrane. However, these mutations disturb binding of the stimulatory Gs protein, leading to a reduced activation of adenylate cyclase and thus formation of cAMP.

Class IV mutations also result in full-length receptors expressed at the cell surface, but here the mutation interferes with, or reduces, AVP binding. These mutations especially involve residues thought to be in or close to the AVP binding pocket, of which V_2R-ΔR202 is a clear example (74).

Finally, class V mutations allow normal protein synthesis and maturation, but they cause misrouting to different organelles in the cell. The NDI R137H mutation, located in the well conserved DRY/H motif of GPCRs, is a member of this class, as V_2R-R137H is constitutively internalized from the plasma membrane, and therefore only briefly available to bind AVP (75, 76).

Sometimes, mutants do not exert a full phenotype of a particular class and then often also show features of another class. For example, some V_2R missense mutants are partially ER-retained (class II), but are also partially expressed in the plasma membrane, where they might show a reduced G protein coupling (class III) or AVP binding (class IV). As such, it provides an explanation for the observed small anti-diuretic response to high doses of dDAVP in NDI patients harbouring such mutations (77).

Genotype-Phenotype Correlations in X-Linked NDI?

Almost all mutations in the V_2 receptor gene result in a uniform clinical NDI phenotype with polyuric manifestations in the first weeks of life and poor growth. There are, however, a few exceptions to this rule. Several mutations (D85N, G201D, P322S, S329R) are associated with a milder form of NDI, characterized by a later manifestation, not at birth but later in childhood, and without growth retardation (74, 78). Functional studies of some of these mutations by in vitro expression systems have confirmed the partial phenotype of the NDI. P322S is the most remarkable of these three mutations, since another mutation substituting proline 322, namely P322H, is associated with a severe phenotype. By in vitro expression of both P322H and P322S in COS-7 cells, Ala et al. (74), have shown that the P322H mutant had totally lost the ability to stimulate the Gs/adenylate-cyclase system whereas the P322S mutant was able to stimulate adenylate-cyclase, albeit less than the wild-type receptor. Thus, the in vitro experiments closely correspond to the clinical phenotype. On the basis of three-dimensional modeling of the P322H and P322S mutant receptors, a plausible hypothesis to explain the molecular basis for the mild phenotype of the P322S has been proposed. Based on this modeling it is suggested that complete loss of function of the P322H receptor could be due, in part, to hydrogen bond formation between the His322 side chain and the carboxyl group of Asp85, which does not occur in the P322S receptor (74).

Recently, a family was reported in which the R137H mutation was associated with severe NDI in the proband but with very mild NDI in his affected brother (79). Genetic and/or environmental modifying factors are likely to account for this intrafamilial phenotype variability.

The Autosomal-Recessive and Autosomal Dominant Forms of Nephrogenic Diabetes Insipidus: Mutations in the Aquaporin-2 Water Channel

Both the autosomal recessive and the autosomal dominant types of NDI are caused by mutations in the AQP2 water channel gene (Genbank accession number z29491). The human AQP2 gene is a small gene consisting of 4 exons, comprising 5 kb genomic DNA. The 1.5 kb mRNA encodes a protein of 271 amino-acids which has a predicted molecular weight of 29 kDa (80). AQP2

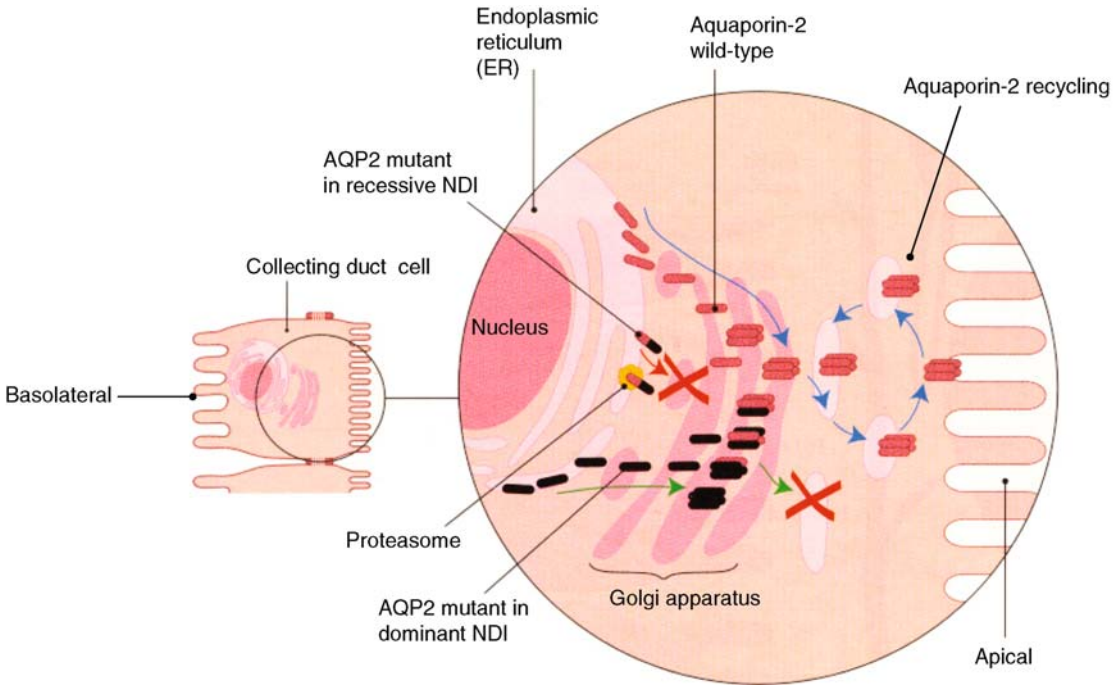
belongs to a family of membrane integral proteins, aquaporins, which function as selective water transporters throughout the plant and animal kingdom. In mammals, 13 different aquaporins have been identified to date, eight of which (aquaporins 1–4, 6–8, and 11) are highly expressed in the kidney. Like other aquaporins, AQP2 is assembled in the membrane as a homotetramer in which each 29 kDa monomer, consisting of six membrane spanning α-helical domains and intracellular N- and C-termini, is a functional water channel. The six transmembrane domains are connected by five loops (A through E). In the hourglass hypothesis, loops B and E are assumed to fold back into the membrane and to interact via their highly conserved motifs asparagine-proline-alanine (NPA boxes) to form the water pore (81). Structural characterization of AQP2 crystals by atomic force and electron crystallography revealed that this was indeed the case (82). AQP2 is exclusively localized in the apical membrane and a subapical compartment of collecting duct cells. It is upregulated by dehydration or AVP, indicating that it is the AVP-regulated water channel.

To date, 40 putative disease-causing mutations in AQP2 have been identified in families with autosomal recessive NDI (reviews in 62, 63, and 83–86). Most are missense mutations, but in addition some nonsense mutations, small deletions, and splice-site mutations have been detected. About one-half of these mutations are found in the B and E loops of the protein where it is likely that they will destroy the pore function of the protein. Expression studies in *Xenopus leavis* oocytes have revealed that all but one AQP2 missense mutations that cause recessive NDI are class II mutations. Thus, these mutations lead to misfolding of the mutant protein and retention in the endoplasmic reticulum (ER) (84, 87, 88; *Fig. 40-2*). At high expression levels in oocytes and Chinese hamster (CHO) cells, six of these AQP2 mutants (A147T, T126M, G64R, L22V, A47V and T125M) confer water permeability (84, 87, 89, 90). This indicates that at high expression levels these AQP2 mutant proteins escape from the ER and are routed to the plasma membrane, where they are functional. Recently one AQP2 missense mutation, P262L, located in the AQP2 C-terminal tail, a region until then believed to result in dominant NDI, surprisingly was found to be involved in recessive NDI (91). In cell biological experiments it was shown that the P262L mutant is a functional water channel that forms hetero-oligomers with wt-AQP2s. These wtAQP2/AQP2-P262L are located in the apical membrane, indicating that the apical sorting of wild type AQP2 is dominant over the mis-sorting signal of AQP2-P262L. This is different from dominant NDI, because in this form mutants retain wtAQP2 in intracellular locations (see below). The recessive inheritance in the two patients encountered (patients were heterozygous for a R187C or A190T mutation on one allele, combined with a P262L mutation on the other allele) can be explained as follows: AQP2-R187C and AQP2-A190T are retained in the ER and do not interact with AQP2-P262L. AQP2-P262L folds properly and assembles in homotetramers, but will be retained mainly in intracellular vesicles. The consequent lack of sufficient AQP2 proteins in the apical membrane of the patients'collecting duct cells explains their NDI phenotype. In the parents coding for wt-AQP2 and AQP2-R187C or AQP2-A190T, wt AQP2 will not interact with either mutant but will form homotetrameric complexes, of which the insertion into the apical membrane will be regulated properly by vasopressin and will give a healthy phenotype. In the parents coding for wt-AQP2 and AQP2-P262L both proteins likely assemble into heterotetramers. The dominancy of wt-AQP2 sorting on the localization of AQP2-P262L will result in proper AVP-regulated trafficking of the heterotetremic complexes to the apical membrane and will also give a healthy phenotype (91).

At present eight families have been described with autosomal dominant NDI, initially uncovered due to father -to -son transmission of the disease. In these families subsequent sequencing of the AQP2 gene revealed putative disease-causing mutations of one AQP2 allele. The identified mutations in AQP2 comprise deletions, insertions and missense mutations (review in 83). All mutations causing dominant NDI are located in coding region of the C-terminal tail of AQP2, which is not part of the pore-forming segment, but, as shown by the role of phosphorylation of S256 in AQP2, has an important role in AQP2 trafficking. Indeed, all mutants AQP2 proteins found in dominant NDI appeared to be folded functional water channels that were sorted to other subcellular locations in the cell than wt-AQP2. Because none of these mutants was misfolded, they were, in contrast to AQP2 mutants in recessive NDI, able to interact and form heterotetramers with wt-AQP2. Due to this wt-mutant interaction and the dominancy of the mis-sorting signals in the mutant protein, the wt-mutant complexes are also mis-sorted. For instance, expression studies in polarized cell lines have revealed that the dominant AQP2- E258K mutant is routed to the Golgi-complex or late endosomes/lysosomes (57; *Fig. 40-2*). In co-expression studies with wild-type AQP2, a dominant-negative effect was observed, caused by impaired routing of wild-type AQP2 to the plasma membrane after hetero-oligomerization with the E258K mutant. (92). Mistargeting to the basolateral membrane has been reported for the AQP2-721delG, AQP2-763-772del, AQP2-812-818del, and AQP2-779-780insA

Figure 40-2

Schematic representation of the postulated mechanisms in a collecting duct cell to explain recessive and dominant forms of NDI causes by AQP2 mutations. Normal AQP2 proteins are transported from the endoplasmic reticulum (ER) via the Golgi apparatus to the luminal side of the cell. In case of a recessive NDI mutation AQP2 is retained in the ER due to misfolding and subsequently broken down by proteasomes. In case of a dominant NDI mutation there is a change in the transport signal of the AQP2 protein and consequent misrouting to i.e., the Golgi network or late endosomes/lysosomes (adapted from 134).



mutants (93, 94). The AQP2-727delG mutant was shown to interfere with the routing of wild-type AQP2 to the apical membrane by its mistargeting to the basolateral membrane en late endosomes/lysosomes (94).

Differential Diagnosis Between the X-Linked and the Autosomal Forms of NDI

With a few exceptions, no differences in clinical symptoms between X-linked and autosomal-recessive forms of NDI can be observed, nor in the time of onset of the disease. Only in a minority of patients with the X-linked form of NDI, namely those individuals carrying V_2R mutations with partial insensititvity to AVP, the disease onset is not directly after birth but later in childhood. In general the initial symptoms in most autosomal-dominant cases also appear later in childhood.

Male patients with X-linked NDI can de discriminated from patients with autosomal recessive NDI on the basis of their extrarenal reaction to administration of the synthetic V_2 -vasopressin analogue 1-desamino-8-D-arginine vasopressin (DDAVP). Patients with autosomal-recessive NDI show a decrease in blood pressure, accelerated heart rate and an increases in von Willebrand factor, factor VIII, and tissue-type plasminogen activator levels, whereas in all studies patients with X-linked NDI these extra-renal responses are absent as a result of an extrarenal mutant V_2R (95). In female patients, the interpretation of this intravenous DDAVP test is more complicated. Although absence of the extrarenal responses to intravenous administration of DDAVP in females clearly points to the presence of a V_2R defect, a normal response cannot be interpreted as indicative of a defect beyond the V_2R, and thus an AQP2 defect. For instance, a symptomatic female patient described by Moses et al., who was shown to be hetereozygous for a V_2R mutation, showed a twofold increase in factor VIII activity after administration of DDAVP. (96). The discrepancy between the renal and extrarenal response to DDAVP in these female V_2R

mutation carriers might be explained by variability in the pattern of X-inactivation between different tissues.

Nephrogenic Diabetes Insipidus in Females

Several families have been described in which females show classical clinical and laboratory features of NDI. After the identification of AQP2 mutations as a cause for autosomal-recessive NDI, and in some cases for autosomal-dominant NDI, a satisfying explanation for the complete manifestation of the disease in some females had been found. However, several families have been reported in which symptomatic females do not have an AQP2 defect, but are heterozygous for a V_2 receptor defect (97–99). In some of these women maximal urinary osmolality after DDAVP administration does not exceed 200 mOsmol/L. Of interest, in some of the reported families, asymptomatic female family members shared the same V_2 receptor mutation with the manifesting females (97, 100). The most likely explanation for the existence of different phenotypes in carriers of a V_2 receptor mutation, varying from no symptoms to complete manifestation of the disorder, is skewed X-inactivation. This hypothesis was underlined by studies investigating the X-inactivation patterns in peripheral blood leukocytes of female carriers via the detection of a methylated trinucleotide repeat in the human androgen receptor gene (101). In asymptomatic females random X-inactivation was found, while in most female carriers who showed clinical NDI symptoms, skewed X-inactivation patterns occurring preferentially to normal X alleles were recognized. In a few females with overt clinical NDI, however, random X-inactivation was identified.

In conclusion, clinical NDI phenotypes may correlate with the X-inactivation patterns in females with heterozygote V_2R mutations. In some female carriers, however, the clinical phenotype cannot be predicted by evaluation of X-inactivation patterns in peripheral blood cells, probably due to the fact that X-inactivation ratios within an individual may vary between different tissues.

Acquired Nephrogenic Diabetes Insipidus

Although the hereditary forms of NDI are relatively rare, a wide range of pathologic conditions and drug treatments can lead to acquired NDI (*Table 40-1*). In our experience, the urine osmolality obtained after dDAVP administration in these acquired disorders is always higher than in congenital NDI. All these disorders have been shown to coincide with decreased AQP2 expression in the collecting duct and in its apical membrane. (102–108). For instance, prolonged treatment with lithium, the drug of choice for treating bipolar disorders and prescribed to 1 in 1,000 of the population, leads to AQP2- downregulation and development of NDI. It has been shown that lithium-NDI is associated with decreased mRNA levels, likely through reduced AQP2 transcription, with attenuated apical targeting of AQP2 in rats (102, 109), and with decreased urine AQP2 excretion in humans (110). The onset of lithium-induced AQP2 downregulation and NDI development occurs independent from the activity of principal cell adenylyl cyclase activity (111). Since lithium enters the cells via epithelial sodium channel (ENaC), lithium-induced NDI can be modified by the administration of ENaC channel blocker amiloride. In rats treated with lithium it was demonstrated that amiloride restored urinary concentrating mechanisms via upregulation of AQP2, AQP3 and urea transporter UT-A1 expression and an increase of renal medullary osmolarity (112).

Treatment

Symptomatic treatment of NDI is aimed to achieve normovolemia by replacing urinary water losses and reducing urinary volume. Adequate supply of fluid to prevent dehydration is the most important component of the therapy. For reducing urine output a low-solute diet is applied to diminish the renal osmolar load and decrease obligatory water excretion (113). Initially, a diet low in sodium (1 mmol/kg per day) as well as protein (2 g/kg per day) was recommended. However, severe limitations of dietary protein may introduce serious nutritional deficiencies. Therefore, it is preferable to prescribe dietary restriction of sodium only.

Diuretics such as hydrochlorothiazide (2–4 mg/kg per 24 h) were the first class of drugs shown to be effective in lowering the urine volume in NDI (114). When combined with a reduction of salt intake, hydrochlorothiazide reduces urine volume by 20–50% of baseline values. However, thiazide-induced hypokalemia may cause further impairment or urine concentrating ability in patients with NDI. Another possible risk associated with hypokalemia is cardiac arrhythmia. Simultaneous administration of potassium salt is therefore advised in most cases. Very low daily sodium intake in combination with thiazide diuretics should be avoided to prevent the development of hyponatremia.

There is ample evidence that the combined administration of hydrochlorothiazide with either a prostaglandin-synthesis inhibitor such as indomethacin (2 mg/kg per 24 h), or the potassium-sparing diuretic amiloride, is much more effective in reducing urine volume than the thiazide-diuretic alone (115–119). Prolonged use of prostaglandin-synthesis inhibitors, however, is often complicated by gastrointestinal and hematopoietic side effects. Gastrointestinal complaints and complications include anorexia, nausea, vomiting, abdominal pain, ulceration, perforation, and hemorrhage. Hematopoietic reactions include neutropenia, thrombocytopenia, and rarely, aplastic anemia. In addition, renal dysfunction has been described during indomethacin therapy, most often consisting of a slight reduction in GFR.

In patients, who are not tolerating indomethacin, selective inhibitors of cyclooxygenase-2 (COX-2) might be helpful (120). Caution in using indomethacin and selective COX-2 inhibitors in NDI is warranted as their administration can potentially lead to the acute deterioration of renal function in dehydrated patients.

Amiloride counterbalances the potassium loss from prolonged use of thiazides and thus prevents hypokalemia. Since amiloride appears to have only minor long-term side effects, the combination of hydrochlorothiazide (2–4 mg/kg/24 h) with amiloride (0.3 mg/kg/24 h) is the first choice of treatment. Our personal experience of more than 17 years with the amiloride-hydrochlorothiazide combination, however, indicates that amiloride is less well tolerated in young children below the age of 4–6 years because of persistent nausea. Therefore we advise the temporary use of the combination of indomethacin-hydrochlorothiaizde in these young children.

For a long time the following mechanism for the antidiuretic effect of thiazides in NDI has been proposed: thiazides reduce sodium reabsorption in the distal tubule by inhibition of the NaCl co-transporter (NCC). This subsequently results in increased sodium excretion, extracellular volume contraction, decreased glomerular filtration rate, and increased proximal sodium and water reabsorption. Consequently, less water and sodium reach the collecting tubules and less water is excreted (121, 122). This hypothesis, however, has been challenged by Magaldi, who reported new insights into the possible mechanism of action, based on microperfusion studies in rat inner medullary collecting duct (IMCD) (123, 124). In these studies it was shown that in the absence of vasopressin, hydrochlorothiazide, when added to the luminal side, increased osmotic and diffusional water permeabilities, thus, decreasing water excretion. When prostaglandins were added, the effect of thiazides decreased. This finding may offer one explanation why indomethacin potentiates the effect of thiazides in NDI (124). Antidiuretic effect of thiazides is associated with an increase in AQP2 expression in collecting duct cells (125). Long-term side effects of chronic thiazide administration such as hyperuricemia, alterations in serum lipid spectrum and glucose intolerance should be monitored.

Based on the fact that the majority of V_2R mutations found in X-linked NDI and all AQP2 mutations found in autosomal recessive NDI are retained within the endoplasmic reticulum (*class II* mutations), a treatment aimed at restoring the plasma membrane routing of ER-retained, but otherwise functional V_2R or AQP2 mutants, becomes an interesting potential. Indeed, Morello et al. have shown in vitro that selective, nonpeptide cell-permeant V_2R-antagonists increased cell-surface expression and rescued the function of V_2R mutants by promoting their maturation and targeting to the plasma membrane (126). Thus, these V_2R antagonists function as pharmacological chaperones.

A recent study in MDCK cells showed that four nonpeptide V_2R antagonists induced receptor maturation and rescued the basolateral membrane expression of eight from nine V_2R mutants. At clinically relevant concentrations only high affinity antagonists (OPC31260 and OPC41061) induced functional rescue in vitro (127). Long-term efficiency and extra-renal side affects of these compounds in vivo should be further evaluated.

Another study demonstrated that V1a receptor antagonist SR49059, having moderate affinity for VR2, had beneficial effect on urine volume and osmolality in five patients with X-linked NDI harboring three different AVPR2 mutations (128). However, the clinical development of this drug was interrupted because of the possible interference with P450 metabolic pathway. Similarly to V_2R mutants, it has been shown that treatment of cells, expressing ER-retarded AQP2 mutants with chemical chaperones, such as glycerol, facilitated the translocation of these mutants to the plasma membrane (90). Another potential therapeutic strategy for treating autosomal NDI could be an activation of cGMP signaling pathway (129), or inhibition of AQP2 endocytosis (130). Both approaches would induce AVP independent apical AQP2 accumulation.

Although in vitro studies have suggested that in future gene-therapy for NDI may become technically feasible (131), there are questions as to whether gene-therapy would be the treatment of choice for this disorder, and even if so, it will certainly take a long time before the therapeutic potential of this form of therapy can be assessed. Therefore, developments of gene-transfer vectors

and gene-delivery techniques and analysis of gene-therapy safety need to be pursued and facilitated in order to determine the feasibility of gene-therapy in renal diseases, such as NDI. In this respect, the developed transgenic mice carrying a functionally inactive V_2R receptor protein (132) and the renal collecting duct-selective conditional AQP2 knock-out mice (133) are of important value.

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41 Cystinosis

William A. Gahl

Introduction

Overview

Nephropathic cystinosis (1–3) deserves a special place in the annals of clinical medicine as the first treatable lysosomal storage disease. The pathophysiology itself, based upon the formation of cystine crystals within the lysosomes of cells, is remarkable. The presence of cystine crystals provides a clue to the basic defect in cystinosis, i.e., failure to transport of cystine out of lysosomes (4–6). This created a new area of biomedical investigation, explained the lysosome's function in salvaging small molecules for reutilization by the cell, and revealed a new category of lysosomal storage disorders due to transport defects rather than enzyme deficiencies. Even more striking, a rational therapy of cystine depletion (i.e., cysteamine) emerged (7–9), transforming nephropathic cystinosis from a universally fatal disease to a treatable chronic disorder with a decent quality of life. Today, physicians can even observe the gradual dissolution of cystine crystals by cysteamine eyedrops bathing the corneas of patients' eyes (10–12).

History

Cystinosis was first described by Abderhalden in 1903 (13), when understanding of its renal disease remained rudimentary. Fanconi, de Toni and Dubre recognized the renal tubular defect of cystinosis in the 1930s (3), and this complication retains the appellation Fanconi syndrome today. Generalized aminoaciduria was noted as a concomitant of nephropathic cystinosis in the late 1940s (14), and cystine storage within cellular lysosomes was proven in the late 1960s (15). By 1982, the basic defect of impaired lysosomal membrane transport of cystine was reported (4–6), and therapy with the cystine depleting aminothiol cysteamine was shown to be safe and effective by 1987 (8). Over the past two decades, numerous non-renal complications of cystinosis have been described, and oral cysteamine therapy has been shown to prevent virtually all of them (16, 17).

The Basic Defect

Cystine has a molecular weight of 240 daltons and consists of two molecules of cysteine ($HS\text{-}CH_2\text{-}CH(NH3^+)COO^-$) joined by a disulfide bond. The equilibrium between cystine and cysteine depends upon their redox potentials and the pH of the milieu; if a high enough concentration of cystine obtains, it may fall out of solution because of its poor solubility in aqueous, i.e., less than 2 mM (18). Cysteine, which is very soluble, is produced by the hydrolysis of proteins; this occurs within lysosomes by the action of acidic hydrolases. Cysteine is then oxidized to cystine within lysosomes, where it accumulates if the cell is cystinotic (15, 19). For decades, scientists investigated the cause of lysosomal cystine accumulation in cystinosis (3).

One possibility was a defective enzyme responsible for the reduction of cystine to cysteine, or for catalyzing disulfide interchange reactions between cystine and other free thiols. This hypothesis was examined, but no deficiency in a cystine-catabolizing enzyme was found (20). Another possibility was that, in cystinosis, cystine could not exit lysosomes because a transporting system present in normal lysosomal membranes was defective in cystinosis. Indeed, normal polymorphonuclear leucocytes were able to clear themselves (i.e., their lysosomes) of cystine, but cystinotic cells could not (21). Studies of isolated granular fractions, i.e., lysosomes, gave similar results (5). In fact, normal, cystine-loaded lysosomes could transport cystine in either direction across their membranes, while cystinotic lysosomes could neither take up nor release cystine (4, 22). Cystine transport in neutrophil lysosomes was subsequently shown to be ligand specific, stereospecific, and ATP-dependent (6, 22). It displayed classical saturation kinetics (*Fig. 41-1*) and counter-transport. Similar findings were observed in cultured lymphoblasts (6). In composite, these discoveries proved that the process of lysosomal cystine transport is carrier-mediated and deficient in cystinosis. Indeed, heterozygotes for cystinosis displayed half the maximum velocity of cystine transport (23), consistent with having half the normal number of cystine carriers (*Fig. 41-1*).

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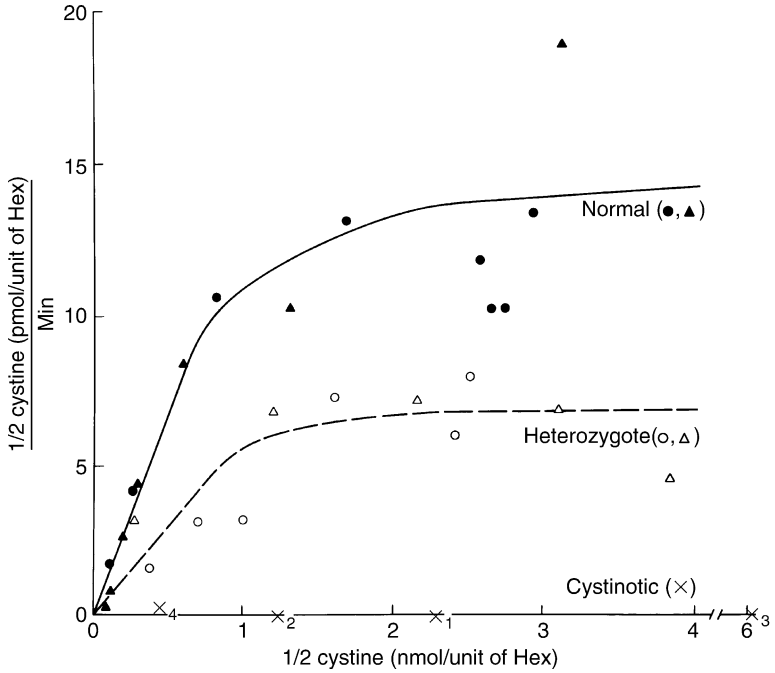
Figure 41-1

Lysosomal cystine transport in leucocyte granular fractions. Normal lysosomes were loaded with cystine by exposure of whole leucocytes to cystine dimethylester, which is hydrolyzed to cystine within the acidic lysosome. Cystinotic lysosomes contain endogenously produced cystine. The abscissa gives the level of cystine loading per unit of hexosaminidase, i.e., per lysosome. The ordinate gives the rate of cystine egress in picomoles per minute per unit of hexosaminidase. Normal lysosomal cystine egress exhibits saturation kinetics, while cystinotic lysosomes show virtually no cystine egress. Heterozygotes for cystinosis, with half the number of lysosomal cystine transporters, display half the normal maximal velocity of cystine egress. Reprinted from (4).



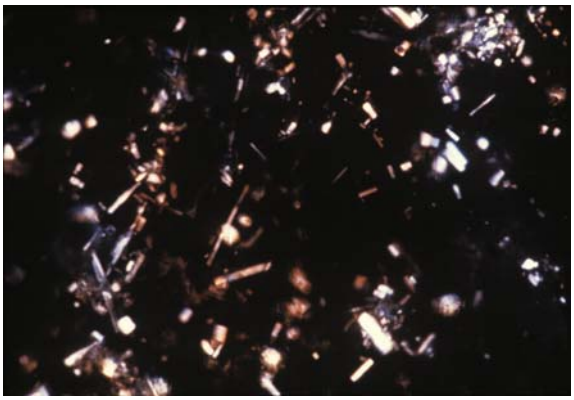
The later discovery of the cystinosis gene, *CTNS*, and studies of its gene product, cystinosin, demonstrated that this protein does indeed transport cystine, in a process driven by the hydrogen ion gradient across the lysosomal membrane (24, 25).

Pathology

Patients who have not received significant cystine-depleting therapy exhibit specific pathological features. As a consequence of excessive cellular cystine storage (to 10–1,000 times normal levels), patients with cystinosis develop microscopic crystals of cystine, apparent within lysosomes by electron microscopy (19). Subcellular fractionation using sucrose density gradients (15) verified the lysosomal location of cystine. Tissues containing cystine crystals include the cornea, conjunctiva, liver, spleen, kidneys, intestines, rectal mucosa, pancreas, testes, lymph nodes, bone marrow, macrophages, thyroid, muscle, and choroid plexus (3, 26). Crystals form in macrophages but not in cultured fibroblasts or lymphoblasts. The crystals are generally hexagonal or rectangular, and appear birefringent under polarizing light (*Fig. 41-2*). To preserve crystals during histological processing, tissues should be fixed in absolute alcohol rather than in aqueous solutions.

Tissue damage accompanies the intracellular cystine accumulation of cystinosis, with the greatest effects seen in the kidney. Renal tubules show a characteristic narrowing called a "swan-neck deformity" (27), followed by interstitial nephritis and endothelial glomerular proliferation, necrosis and hyalinization. Crystals are occasionally seen within glomeruli. In the eye, the retina exhibits patchy hypopigmentation (28); crystals appear occasionally in the iris and rarely in the retina (29). In older patients, the thyroid and testes appear fibrotic. Muscle histopathology involves a late vacuolar myopathy with

Figure 41-2
Birefringent cystine crystals in cystinosis tissue under light microscopy. Note rectangular and needle-like shapes. Some crystals are undergoing dissolution due to the aqueous fixative. (See color plate 24)



variation in fiber size, atrophy of type I fibers, and ring fibers (30, 31). In the liver, a nodular regeneration can occur in adults (32).

The pathogenesis of tissue damage in cystinosis is thought to involve cell death, followed by replacement with fibrous tissue. Crystal enlargement could disrupt lysosomal integrity, releasing hydrolytic enzymes that might destroy the cell. However, there has been no direct evidence for this. There are data supporting a more ordered loss of cells through the process of *apoptosis,* which is putatively triggered by cystine accumulation (33).

Genetics

Cystinosis is an autosomal recessive disorder, and heterozygotes are always clinically normal. The disease occurs with an incidence of approximately one in 100,000–200,000 live births, although there are genetic clusters of cystinosis, including one with a frequency of 1 in 26,000 in Brittany (34) and another among French Canadians. Within the United States population, one in 150–200 individuals carry a cystinosis (i.e., *CTNS*) mutation. This number provides the basis for appropriate genetic counseling for individuals, who are at risk for having a child with cystinosis because a family member has been diagnosed.

Approximately 500–600 cystinosis patients reside in the United States, and approximately half of them have undergone renal transplantation. It is estimated that 20–40 children with cystinosis are born annually in the United States. Our NIH patient cohort includes individuals from all over the world, including Mexico, Brazil, India, Iran, Egypt, Australia, and a variety of European countries. The pan-ethnic distribution of the disease, along with the scarcity of reports of cystinosis in underdeveloped countries, suggests that there are large numbers of undiagnosed patients with disease around the world. In general, cystinosis breeds true within families; siblings manifest very similar clinical phenotypes.

The *CTNS* Gene

The cystinosis gene was mapped to chromosome 17p13 in 1995 (35) and identified in 1998 (24). It contains 12 exons within 23 kb of genomic DNA, and codes for a 367-amino acid protein, cystinosin, with 7 transmembrane domains. The function of cystinosin as a cystine transporter has been confirmed (25). More than 60 different *CTNS* mutations have been reported, including deletions, insertions, and nonsense, missense and splice site mutations (1, 36, 37). The promoter (38), leader sequence, transmembrane regions, and non-transmembrane regions are affected by different mutations. The most common mutation is a 57,257 deletion removing the first 10 exons of *CTNS* (39, 40). This deletion arose in Germany ~500 A.D. and is present in the homozygous or heterozygous state in more than half of the cystinosis patients of European descent (36). Homozygosity for this deletion is associated with a slightly greater frequency of non-renal complications of cystinosis in adulthood (17). Otherwise, there is only a mild correlation of genotype and phenotype within nephropathic cystinosis patients. Two other founder mutations, W138X and G339R, have been reported among French Canadians (41) and Amish Mennonites of southwestern Ontario (42), respectively.

Cystinosis Variants

Traditionally, cystinosis has been divided into three subtypes (3). Infantile nephropathic cystinosis is the classic disease, described below and comprising 95% of cases. Affected individuals have two severe *CTNS* mutations. Patients with intermediate (formerly juvenile or adolescent) cystinosis have milder disease, with diagnosis in adolescence or early adulthood; they eventually develop renal failure. Intermediate patients number fewer than 20 reported cases, and carry one severe and one mild *CTNS* mutation (43). Patients with ocular (formerly adult or benign) cystinosis never exhibit renal failure or

retinal hypopigmentation, but have cystine crystals in their bone marrow and corneas (3). They do have measurable residual cystine-transporting capacity in their polymorphonuclear leucocytes' lysosomes (44). The only clinical manifestation of ocular cystinosis is photophobia, and patients are generally diagnosed incidentally on eye examination that includes the use of a slit lamp. There exist approximately 20 ocular cystinosis patients, who have either one mild and one severe or two mild *CTNS* mutations (45). Several ocular cystinosis patients from different families have at least one allele with a 928 G > A mutation in *CTNS*. The distinction among the three types of cystinosis is artificial, since a continuum of disease severity, rather than discrete categories, exists.

A $Ctns^{-/-}$ mouse accumulates cystine in its tissues, but does not manifest renal disease (46).

Early Clinical Manifestations

As for patients with other lysosomal storage disorders, individuals with cystinosis appear entirely normal at birth. Nevertheless, the disease eventually affects nearly every tissue of the body, with variable times of onset. The signs and symptoms can be described as early and late findings, demarcated roughly by adolescence. The earliest manifestations of cystinosis involve complications of renal Fanconi syndrome and growth retardation.

Renal Tubular Fanconi Syndrome

Cystinosis, the most common identifiable cause of renal Fanconi syndrome in childhood, is also one of the most treatable causes. It should be considered first when renal tubular solute wasting is recognized. In cystinosis, Fanconi syndrome is not present at birth but generally appears at 6–12 months of age, with variable severity. Undoubtedly, some infants die of the dehydration and electrolyte imbalance associated with the Fanconi syndrome, without the benefit of a diagnosis.

The Fanconi syndrome of cystinosis (Table 41-1) is primarily a proximal tubular defect. It includes failure to reabsorb water, bicarbonate (acidosis), electrolytes (hypokalemia and occasional hyponatremia), minerals (phosphaturia, hypocalcaemia, and hypomagnesaemia), amino acids, carnitine, glucose, and small molecular weight proteins (less than 50,000 daltons). Clinical manifestations include polyuria, dehydration (sometimes with consequent fevers) and polydipsia, ranging from 2 to 3 L/day in small children, up to 5–6 L/day in older children (47). Urine osmolality can be 200–300 mOsm/L. Large volumes of fluid intake fill the stomach and reduce appetite.

Table 41-1
Characteristics of renal tubular Fanconi syndrome in cystinosis

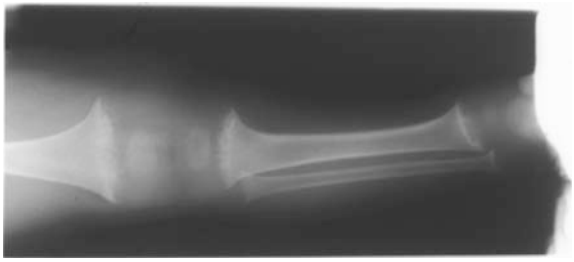
Polyuria
Polydipsia
Dehydration (fever)
Proteinuria
Glucosuria
Aminoaciduria
Acidosis
Hypokalemia
Hyponatremia (salt craving)
Hypophosphatemia
Hypocalcemia
Hypomagnesemia
Hypocarnitinemia
Increase serum alkaline phosphatase
Rickets
Tetany
Growth failure

Acidosis typically lowers the serum carbon dioxide level to below 20 mEq/L, and chronically untreated children can have levels below 5 mEq/L. Serum potassium concentrations below 2.0 mEq/L are not rare, and values below 3 mEq/L are common. Phosphate wasting causes hypophosphatemic rickets, with low serum phosphate and elevated heat-labile alkaline phosphatase levels (2,000–3,000 U/L in florid cases). Children may fail to walk because of the pain involved, and they exhibit tender, swollen wrists and ankles due to metaphyseal widening. In severe cases, frontal bossing, a rachitic rosary, and genu valgum develop. Osteoporosis and epiphyseal fraying are visible on radiographs (*Fig. 41-3*). The combination of hyperphosphaturia and hypercalciuria often results in medullary nephrocalcinosis (48). Hypocalcaemia can cause painful episodes of tetany, especially 20–30 min after a dose of an alkalinizing medication that lowers circulating concentrations of ionized calcium. Magnesium is lost commensurately with calcium, and serum magnesium levels are often low.

The poor health of infants and small children with cystinosis makes them irritable and picky eaters. Carnitine

Figure 41-3

Rickets in a 16-month-old boy with nephropathic cystinosis. Note widening of the metaphysis, fraying of the epiphysis, and osteoporosis.



is only ~70% reabsorbed in cystinosis (normal, 97%), leading to chronically low levels of free carnitine, typically 11 μM (normal, ~40 μM) (49). Since carnitine is essential for fatty acid transport into mitochondria, carnitine deficiency may contribute to poor muscle development, although this has not been proven. The aminoaciduria of cystinosis can be quantified using the Fanconi Syndrome Index (FSI), a measurement of the daily urinary excretion of 21 specific amino acids, expressed per kg of body weight (50). For children with cystinosis, the FSI is always above normal (94 ± 45 μmole/kg/day), and is often ~1 mmol/kg/day. Urine organic acids have been reported elevated in children with cystinosis, but without apparent clinical consequences (3).

The Fanconi syndrome of cystinosis can mislead physicians in several ways. The combination of polyuria and glucosuria has led to the incorrect diagnosis of diabetes mellitus, which can be readily dismissed by the finding of a normal serum glucose. Other patients have carried the diagnosis of diabetes insipidus or Bartter's syndrome for years before being correctly ascertained as having cystinosis (2, 3). The tubular proteinuria of cystinosis can reach nephrotic levels; some children excrete 3–4 g of protein per day. This can be taken to reflect glomerular damage, which may be present to a certain extent, but the bulk of the protein is generally of low molecular weight, reflecting tubular dysfunction. Urine protein electrophoresis can distinguish tubular from glomerular proteinuria. Finally, as cystinosis patients approach renal failure, their reduced filtration function creates the expectation that oliguria, hyperkalemia, and hyperphosphatemia will occur. In cystinosis, however, the tubular defect consistently overrides the glomerular damage. Patients with creatinine clearances less than 30 ml/min/1.73m^2 can still have urine volumes of 3 L and, if not supplemented, profound dehydration, hypokalemia and phosphate wasting.

Glomerular Damage

Cystinosis accounts for ~5% of chronic renal failure in children (51). By the time a typical cystinosis infant is diagnosed at approximately 1 year of age, significant renal glomerular damage has already occurred. A reasonable estimate would place the creatinine clearance at the time of diagnosis at approximately 70% of normal, and this level of function slowly but inexorably decreases. By age 10, most children with cystinosis have reached renal failure and require transplantation or dialysis. In a European study of 205 cystinosis children, the mean age for end-stage renal disease was 9.2 years (52). However, rates of decline are somewhat variable, with milder patients maintaining function until age 12, and severely affected children losing function by age 6. The substantial reserve of human kidneys means that serum creatinine seldom rises above normal until 5 years of age, especially if growth retardation creates a reduced creatinine load on the kidneys. However, measurement of glomerular filtration rate using a 24-urine collection and calculation of creatinine clearance generally reveals a significant deficit at the time of diagnosis even at a year of age.

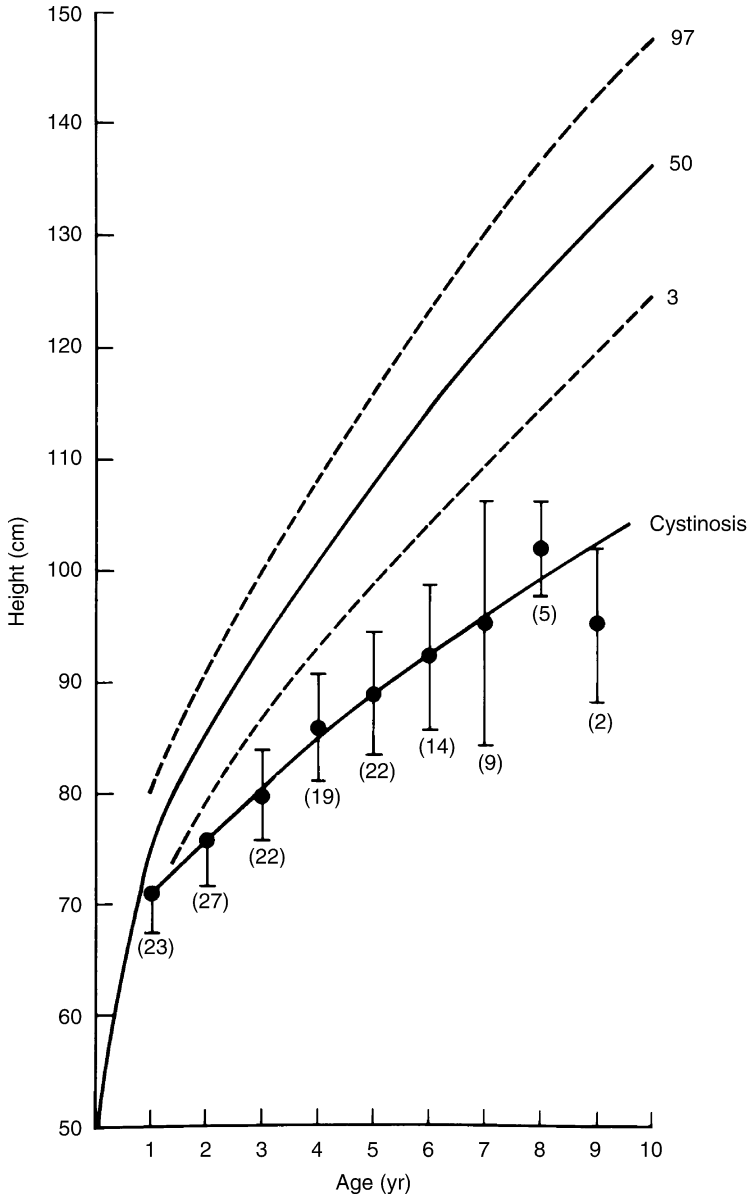
The uremia of cystinosis resembles that of other renal disorders, except that the growth retardation and anemia may be somewhat exaggerated. Hypertension can accompany chronic renal failure or arise in the post-transplant period, but cystinosis itself does not predispose to this complication.

Growth Impairment

Newborns with cystinosis are normal in height, weight, and head circumference. While head circumference is maintained, height and weight percentiles generally fall by 6–12 months of age; this is often the first indication of the diagnosis. By 1 year of age, the average infant with cystinosis has a height at the third percentile (8). Without treatment, growth continues at 50–60% of the normal rate. By age 8, the average untreated child with cystinosis has the height of a 4-year old (*Fig. 41-4*). In the past, some poorly treated, post-transplant adults achieved less than 4 ft in height. Weight usually follows height, but with greater variability. The normal head circumference,

Figure 41-4

Natural history of growth in children with cystinosis. Mean values for cystinosis children are superimposed upon a normal growth chart. On average, a child with cystinosis falls from normal height to the third percentile at 1 year of age, and continues to grow slowly so that the height age is 4 years when the chronological age is 8 years. Reprinted from (8).



combined with reduced height and weight, give the impression of macrocephaly, but this is relative, not absolute.

In patients not receiving growth hormone, bone age usually lags behind chronological age by 1–3 years. Children with retarded bone ages retain growth potential past the usual age of epiphyseal closure, but height is never gained past age 20, regardless of the bone age.

The cause of impaired growth has not been definitively determined. Growth hormone is normal (53), although patients respond to supraphysiologic doses of growth hormone (54). Hypophosphatemia, acidosis, poor nutrition, and cystine storage in the bone may contribute to poor growth in cystinosis. However, with adequate nutrition, replacement of renal losses, and

cystine depleting therapy, a normal growth rate can be achieved. (See below.)

Ocular Involvement

A patchy retinal depigmentation has been described early in infancy in cystinosis (28), but the primary ophthalmic manifestation of cystinosis is photophobia. This occurs due to corneal crystals that first appear in the anterior third of the cornea. Corneal crystals are always present by 16 months of age on slit lamp examination (12). Prior to then, the crystals may not be apparent. The number of crystals increases with age, reaching a maximum discernible density by ~8 years of age. An atlas of corneal crystal density at different ages has been published (12). Crystal density among ocular cystinosis patients appears less than that of patients with classical disease.

Children with cystinosis complain of sensitivity to light at variable ages, but usually not until 5–10 years of age. They squint to the point that, without treatment, they can eventually develop blepharospasm refractory to all modes of therapy. Often, patients wear dark glasses outside and turn down the lights inside. Occasionally, a child with cystinosis may experience a corneal ulceration as a crystal breaks through the corneal epithelium. This complication occurs much more frequently in adolescence and adulthood, when haziness of the cornea also appears.

Hypothyroidism

In the natural history of cystinosis, approximately half of patients are hypothyroid by age 10 (55) and 90% by age 30 (56). Thyroxine and free T4 are low and TSH is high, pointing to primary hypothyroidism, although partial pituitary resistance has also been reported (57). In cystinosis, the thyroid tissue appears fibrotic with occasional crystals present.

Cognition and Psychological Aspects

Children with cystinosis learn normally and have low normal full-scale IQs (58, 59). However, recent evidence indicates isolated deficits in visual processing and tactile recognition (60, 61). Short-term visual memory can be impaired (62), and several children have exhibited behavioral and social problems (58, 63), undoubtedly related in part to their chronic disease, renal failure, and growth retardation.

Other Clinical Findings

Cystinosis children are notoriously poor eaters, with understandable craving for salty foods such as pickles, ketchup and potato chips (47). In addition, many children exhibit a pica for hot foods such as jalapeno peppers and tabasco sauce, without an obvious explanation. Young children and infants exhibit an increased tendency toward vomiting, worse in the morning and on an empty stomach. Some of this may be related to medications, but there appears an intrinsic element as well. The nausea and vomiting often decrease with age, and generally cease by 7–8 years of age. Rarely, a patient may suffer from gastrointestinal immotility and have projectile vomiting upon eating or drinking slight amounts of food or water.

One-third to one-half of cystinosis children 10–18 years of age have mild hepatomegaly on physical examination (55), with no identifiable cause. One 9-year-old boy had hepatic veno-occlusive disease and underwent a liver transplantation (64). Patients from lightly pigmented backgrounds sometimes appear less pigmented than other family members. This may reflect dysfunction of melanosomes, which are lysosome-related organelles, or formation of excessive cysteinyl-dopaquinone, the precursor of pheomelanin, a blond-red pigment. Alternatively, the blond pigmentation characteristic of cystinosis may reflect the high frequency of Germanic and Nordic heritage among cystinosis patients. African American and hispanic patients have pigmentation indistinguishable from that of their siblings. Most cystinosis patients manifest decreased sweat production, causing flushing, heat avoidance and occasional hyperthermia (65). In addition, tear and saliva production is often reduced in cystinosis.

Enuresis in children with cystinosis may reflect the large volume of urine produced daily. Patients combat infections in a normal fashion, although gastroenteritis in children with Fanconi syndrome will cause dehydration much more rapidly than in normal individuals.

Idiopathic intracranial hypertension, or pseudotumor cerebri, has been reported in several patients and has been attributed, in part, to cystinosis itself (66). It has caused blindness in at least two children with cystinosis.

Laboratory Abnormalities

In addition to laboratory aberrations related to the Fanconi syndrome, children with cystinosis frequently exhibit mild microscopic hematuria, an elevated sedimentation rate, increased platelet counts, and anemia that is excessive for the degree of renal failure (3). Cholesterol levels

are usually elevated, with each of the lipoprotein fractions proportionally increased. The hypercholesterolemia persists after renal transplantation (16, 17).

Diagnosis

Because a safe and efficacious treatment exists for cystinosis, physicians should be anxious to make this diagnosis and should maintain a high index of suspicion. Unfortunately, the average age of diagnosis for cystinosis remains just over 1 year (3), and even today several patients escape detection for years, despite manifesting typical signs and symptoms.

Postnatal Diagnosis

A family history of cystinosis will naturally point to this disease in a child with suggestive findings. Even without a previously affected sibling, though, evidence of renal tubular Fanconi syndrome (polyuria, polydipsia, proteinuria, glucosuria, acidosis, dehydration, electrolyte imbalance, salt craving, and tetany) should prompt investigation of cystinosis. Other signs include poor growth, failure to walk at an appropriate age, and other evidence of rickets. The presence of typical corneal crystals on slit lamp examination by an experienced ophthalmologist will make the diagnosis. Such crystals are usually not apparent within the first several months of life, but a few crystals are always present by 16 months of age (12).

Elevated intracellular free (nonprotein) cystine concentrations also permit definitive diagnosis. Cystinosis patients have high concentrations of cystine in a variety of cell types, but polymorphonuclear leucocytes are the preferred cells in which to assay cystine (1–3). While cystinotic lymphocytes have three to fivefold normal cystine concentrations, neutrophils have 50–100 times the normal levels, i.e., 3–23 nmol half-cystine/mg protein (normal, <0.2) (67). Units of half cystine are employed by convention, because early assays did not distinguish cystine from cysteine. Patients with intermediate cystinosis generally have values at the lower end of the cystinotic range, and ocular cystinosis patients have values of 1–5 nmol half-cystine/mg protein (3). Heterozygous values are less than 1 nmol half-cystine/mg protein.

Several methods of cystine analysis have been employed. Ion exchange chromatography, used for amino acid analysis, can be insensitive if a very small amount of cell protein is available. The cystine binding protein assay is sensitive and specific (68), but is being supplanted by tandem mass spectrometric analysis. In general, approximately 3–10 ml of heparinized blood is used to isolate polymorphonuclear leukocyte-rich white cells, using dextran sedimentation and hypotonic lysis of erythrocytes. Red cell contamination will increase the protein denominator and underestimate the cystine concentration. Similarly, lymphocyte contamination will lower the value of cystine/mg protein. Keeping blood at room temperature for long durations (e.g., a day) can cause the cystine to leach out of leucocytes, giving spuriously low values. Relatively little sensitivity is needed to simply make a diagnosis of cystinosis, but high sensitivity is necessary when following cystine depleting treatment. (See below.)

In the past, biopsies of kidney, bone marrow, rectal mucosa, or conjunctiva were performed, seeking the presence of crystals to make or confirm the diagnosis of cystinosis (3). This outmoded method of diagnosis is not indicated.

CTNS mutation analysis can confirm the diagnosis of nephropathic cystinosis, and a multiplex PCR methodology is available for the common 57,257-bp deletion (40). However, molecular methods play little or no role in postnatal diagnosis of cystinosis. Currently, newborn screening for cystinosis does not exist.

Prenatal Diagnosis

When one child has been diagnosed with cystinosis, the disorder can be identified in subsequent pregnancies by several methods. At 8–10 weeks of gestation, chorionic villus samples can be directly assayed for cystine, as long as enough tissue (5 mg wet weight) is available (69). At 14–16 weeks' gestation, amniotic fluid cells can be cultured for approximately 4 weeks to obtain enough cells to measure the cystine content (70). Measurement of cystine in a placenta will make the diagnosis at birth (71). In addition, any of these cell sources can be used for molecular diagnosis, as long as both mutations have been previously identified in the affected sibling.

Heterozygote Detection

Carrier (heterozygote) detection based upon leukocyte cystine measurement is problematic. While carrier levels can be as high as 1 nmol half cystine/mg protein, they can also be within the normal range (<0.2). Hence, carrier status can be ruled in but sometimes cannot be ruled out. If both *CTNS* mutations are known in a proband, the

carrier status of other family members can be ascertained using molecular diagnostic techniques.

Differential Diagnosis

As noted above, the polyuria/hypokalemia/glucosuria associated with renal Fanconi syndrome can lead to the mistaken diagnosis of nephrogenic diabetes insipidus, Bartter syndrome, or diabetes mellitus. Once true Fanconi syndrome is diagnosed, cystinosis should be considered first, but the differential includes tyrosinemia type I, Wilson disease, Oculocerebrorenal Syndrome of Lowe, glucose-6-phosphatase deficiency, fructosemia, galactosemia, and heavy metal toxicity. The vitamin D-resistant, hypophosphatemic rickets of cystinosis has been mistaken for vitamin D deficiency. We know of patients who have carried the diagnosis of glomerulosclerosis or simply renal insufficiency until crystals were discovered upon removal of the native kidneys at transplant. Conversely, cystinosis was mistakenly diagnosed in a patient with idiopathic nephrocalcinosis but without intracellular cystine storage.

Cystinosis, in which free cystine accumulates within the lysosomes of cells, should not be confused with cystinuria, in which cystine, arginine, ornithine and lysine fail to be properly reabsorbed by renal tubular cells (72). In cystinuria, massively elevated urinary cystine concentrations result in renal stone formation, often beginning in adolescence. In cystinosis, generalized aminoaciduria occurs because of the Fanconi syndrome, but there is no particular elevation in urinary cystine, and no diathesis toward renal stone formation.

Therapy

Treatment of nephropathic cystinosis can be divided into symptomatic modalities for particular organ complications and cystine-depleting therapy directed specifically at reducing the cystine content of all cells of the body.

Replacement of Renal Losses

Renal tubular Fanconi syndrome results in wasting of small molecules, which need to be replaced. First, there must be free access to water (and bathroom privileges); dehydration can destroy glomeruli and hasten renal failure. At the same time, electrolytes must be supplied. Sodium should not be restricted, and affected children should be permitted to indulge their natural cravings for salty foods. Potassium generally needs to be supplemented, either as the chloride, gluconate or citrate salts. Oral doses can reach several times the normal daily maintenance requirements; excess potassium will be excreted. We are often unable to keep the serum potassium above 3.0 mEq/L, and remain content with that concentration. For alkalinization, solutions containing 2 mEq/ml of citrate can be administered. These include Polycitra (1 mEq/ml of sodium and 1 MEq/ml of potassium), Polycitra K (2 mEq/ml potassium), and Bicitra (2 mEq/ml sodium). A typical child's dose is 5–10 mL q6h, and we are satisfied to achieve a serum carbon dioxide of $\geq$20 mEq/l, especially in infants (47).

Phosphate is provided as Neutraphos or Neutraphos-K, one-half to one packet q6h. Vitamin D, as calcitriol, fosters gastrointestinal absorption of the phosphate, although patients are not naturally deficient in vitamin D. With adequate phosphate replacement, the rickets should resolve in 3–6 months, although osteoporosis may persist on radiography. Some children require calcium to prevent tetany. If so, the calcium administration must be separated from phosphate ingestion by at least an hour to prevent precipitation of calcium phosphate in the gut. An occasional patient requires magnesium supplementation, and many patients receive oral carnitine.

Doses of replacement medications are often greater than necessary at first, and requirements do not change significantly with growth. However, one consideration is that, after the adolescent growth spurt, phosphate supplementation could be reduced to minimize the occurrence or progression of nephrocalcinosis (48).

Physicians should be aware of the huge amounts of fluid and electrolytes required to replace cystinosis children who are dehydrated due to gastroenteritis. Amounts needed for a 10 kg infant who is 10% dehydrated include 1 L/day for maintenance, 1 L for replacement, and perhaps 2.5 L for ongoing, constitutive losses. At 4,500 mL/day, the intravenous line must run at nearly 200 mL/h, a rate which will challenge most pediatricians and 23 gauge i.v. catheters.

Another special case warrants mention. As cystinosis patients approach renal glomerular failure, they are expected to become oliguric, hyperkalemic and hyperphosphatemic. In fact, however, tubular losses mitigate against these conditions, as renal wasting persists despite a low glomerular filtration rate. Hence, when a uremic child with cystinosis develops gastroenteritis, large amounts of fluids and electrolytes, especially potassium, are still required.

Other Symptomatic Treatments

The poor eating habits, nausea, vomiting, and early satiety due to excessive fluid ingestion all contribute to poor nutrition, especially around the time of diagnosis. Nutritional consultations can prove critically important to ensure, for example, that ingested fluids contain calories whenever possible. Once mineral, electrolyte, and acid-base balance are established, children can begin to eat normally. Most children eat well by 6–10 years of age and do not require feeding tubes. An occasional child requires gastric tube access for medications or calories, but maintenance of the masticatory process is critical. When tube feeding bypasses the swallowing mechanism early, completely, and chronically, the swallowing reflex may have to be re-learned later.

Some nephrologists prescribe acetylcholinesterase inhibitors to reduce proteinuria, based upon the benefit of this therapy in maintaining renal function in diabetic nephropathy. The efficacy of this practice with respect to glomerular function has not been investigated in cystinosis. Some cystinosis patients, especially those in Europe, receive indomethacin to reduce urine volume (3). This medication causes a transient increase in serum creatinine, reversible upon cessation of the drug. Indomethacin administration is generally reserved for patients with huge urine volumes.

In the past, most patients with cystinosis required thyroid hormone replacement, but treatment with cystine-depleting therapy has reduced the number of patients who need L-thyroxine supplementation (56). Some children benefit from judicious use of anti-emetics, which are most often taken just before the breakfast meal. The impairment of sweating predisposes to heat prostration, so children should avoid prolonged periods (i.e., greater than 20 min) in the sun. Exercise helps to maintain muscle strength in the face of nutritional challenges. Occasionally, patients with severe rickets or renal osteodystrophy require corrective orthopedic surgery such as an osteotomy.

Although cystinosis patients possess normal amounts of growth hormone, they still benefit from growth hormone supplementation under the care of an endocrinologist (54). After the diagnosis of cystinosis, children should be stabilized, supplemented for their renal losses, and placed on an adequate dose of the cystine-depleting agent, cysteamine. (See below.) This regimen can achieve a normal growth rate within 6–12 months of initiation, although it will not usually allow for catch-up growth. Hence, if children remain below the third percentile for height a year after their medications have been adequately received, growth hormone should be considered. Initiating cysteamine and growth hormone therapy at the same time will not allow for attribution of benefit to either intervention. Some physicians think that the optimal time for growth hormone treatment is between 4 and 10 years of age. Growth hormone therapy is often truncated once a child reaches the 10th–25th percentile for height; after this milestone is reached, children are expected to continue to grow along their centiles without growth hormone, as long as their other medications remain adequate. The added growth of children receiving growth hormone increases creatinine production and its load on the kidney. Consequently, serum creatinine can rise even though renal function remains unaltered by the supplemental growth hormone.

Renal Replacement Therapy

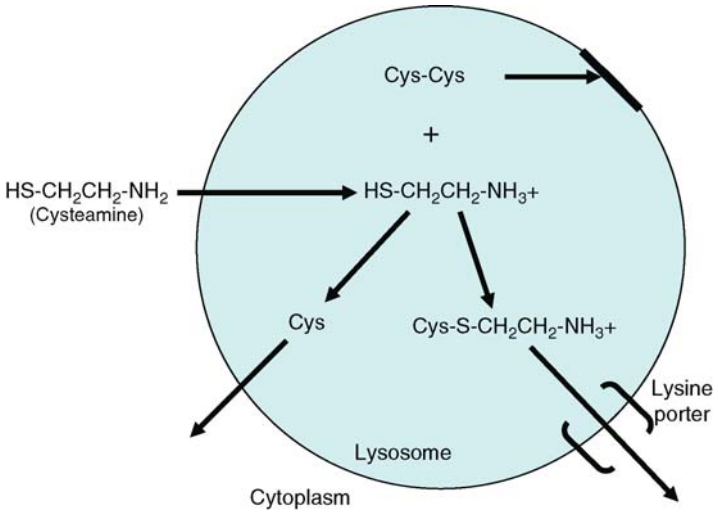
Either hemodialysis or peritoneal dialysis serves as a temporizing measure once a cystinosis patient approaches renal failure. The loss of renal function occurs slowly but inexorably, so that end-stage renal disease can be anticipated, as for other renal disorders, by plotting reciprocal serum creatinine against age. Prolonged dialysis carries significant risks; we have seen a patient who underwent peritoneal dialysis for approximately 10 years and had widespread calcification of his abdominal cavity.

Cystinosis patients respond very well to kidney transplantation, and no particular findings distinguish cystinosis patients from other candidates for renal replacement therapy. A living donor is preferred, and current anti-rejection therapy has obviated the requirement for a related donor. Carriers of a *CTNS* mutation, e.g., parents of an affected child, are suitable donors because no symptoms ever occur in heterozygotes for cystinosis; kidney function, in particular, is normal. When a living donor is available, families and physicians should consider a pre-emptive transplant. Even cadaver allografts, however, perform extremely well. Some transplants into cystinosis patients have lasted 25 years. Cystinosis does not recur in the transplanted kidney, because the lysosomal cystine transport defect is intrinsic to the host cell. Nevertheless, a graft biopsy can sometimes contain cystine crystals due to infiltration of the donor kidney by host macrophages (3).

Cystinosis patients receive standard anti-rejection therapy, which sometimes involves a steroid-free regimen. Current practice involves retention of host kidneys. When this occurs, especially in pre-emptive transplants when some renal function remains, blood flow to the host

Figure 41-5

Mechanism of action of cysteamine. The free thiol enters the lysosome and reacts with cystine (Cys-Cys), which cannot exit the cystinotic lysosome. The two products, cysteine and cysteine-cysteamine mixed disulfide, can readily leave the cystinotic lysosome; the mixed disulfide leaves through the lysine transporter. This process lowers lysosomal cystine levels by over 90%.



kidneys may result in persistent Fanconi syndrome post-transplant. Polyuria may occasionally be problematic, and potassium supplementation may be needed after renal transplantation.

After a renal allograft, cystinosis patients virtually always improve in activity, appetite, and feeling of well being. Patients grow significantly after receiving a transplant, at rates that depend upon chronological age and bone age.

Oral Cysteamine Therapy

Many putative cystine-depleting regimens have proven unsuccessful in cystinosis patients, including restriction of sulfur-containing amino acids, and the administration of ascorbic acid, dithiothreitol, and penicillamine (3, 47). One specific radioprotective agent, however, has proven efficacious. In 1976, the aminothiol cysteamine, or beta-mercaptoethylamine (7), was shown to deplete cystinotic fibroblasts in vitro, and polymorphonuclear leucocytes in vivo, of cystine. Cysteamine passes through the plasma and lysosomal membranes into the lysosome, where the acidic environment puts a charge on the amine group and traps the molecule. Within the lysosome, cysteamine interacts with cystine in a disulfide interchange reaction to produce cysteine and cysteine-cysteamine mixed disulfide, both of which readily exit the lysosome without requiring a functional cystine transporter (*Fig. 41-5*). In fact, cysteine leaves via its own carrier, and the mixed disulfide leaves via a lysine carrier (73), since it structurally resembles lysine. Once outside the lysosome, cysteine participates in normal cytoplasmic reactions and can be eliminated from cells and eventually excreted as sulfate.

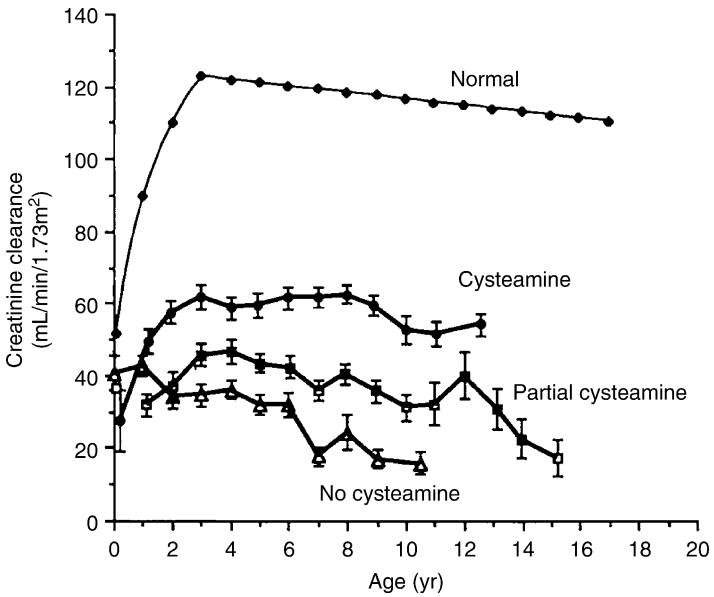
When taken orally, cysteamine removes up to 95% of leukocyte cystine within an hour of ingestion. Parenchymal cells are thought to respond in a similar fashion, although the extent and time course of depletion remains unknown for other tissues.

A clinical trial of oral cysteamine therapy was initiated in 1978, after a randomized, placebo-controlled trial of ascorbic acid failed to demonstrate efficacy in preserving renal function (74). The cysteamine trial was open-label, and 93 treated patients were compared to the 55 historical control patients of the ascorbic acid protocol (8). By the end of the study in 1985, the cysteamine-treated group had significantly better renal function than the untreated group (38.5 vs. 29.7 ml/min/1.73 m^2) despite being 1.4 years older, on average. Growth velocity was also better in the cysteamine-treated group. Based on this report, oral cysteamine became the treatment of choice for pre-transplant patients with nephropathic cystinosis.

Subsequent studies, including a seminal intent-to-treat analysis in 1993 (9), verified the safety and efficacy of chronic oral cysteamine therapy. In the 1993 study, 17 patients treated early and well (before 2 years of age,

Figure 41-6

Cysteamine preserves renal glomerular function in cystinosis and allows for growth of kidneys in the first 3 years of life. Creatinine clearance, based upon 24-h urine collections, was used as a measure of glomerular filtration rate. The normal curve indicates rapid growth of renal function early in life as the kidneys enlarge. The other curves were constructed using values from children with cystinosis. The "No Cysteamine" curve reflects the natural history of cystinosis, i.e., a monotonic decline in creatinine clearance leading to end-stage renal disease at 10 years of age. The "Cysteamine" curve represents mean values for 17 children treated early and well with oral cysteamine. Renal growth and acquisition of increased renal function were preserved. The "Partial Cysteamine" curve was for patients treated less well, and the results fall between those in the other two groups. Reprinted from (9).



achieving leukocyte cystines less than 2 nmol half-cystine/mg protein, and treated for a mean duration of 7.1 years) had a mean creatinine clearance of 57 ± 20 ml/min/1.73 m^2 at a mean age of 8.3 years. Of 27 children with no cysteamine treatment, 16 had gone to renal failure with a mean creatinine clearance of 8.0 ± 4.5 ml/min/1.73 m^2 at a mean age of 8.3 years. Children with poor treatment had outcomes between these extremes (*Fig. 41-6*). In this retrospective study, oral cysteamine not only slowed the deterioration of glomerular function, it also allowed for normal kidney growth in the first 3 years of life. Consequently, early initiation of cysteamine was deemed critical; every month of treatment in the first 2 years of life telescopes into 14 months of preservation of renal function later in life. The optimally treated patients also grew at a normal rate, parallel to normal height curves, while remaining below the third percentile on average (75). Cysteamine bitartrate was approved as Cystagon^R by the Food and Drug Administration on 15 August, 1994 for preservation of renal function and enhancement of growth in cystinosis patients.

Cysteamine is given at a dose of 60–90 mg/kg/day or 1.3–1.95 g/m^2/day, in divided doses every 6 h. Blood levels peak at 1 h and fall off over the next few hours. A high blood level must be achieved, since a large portion of the free thiol will bind to circulating proteins, preventing entry into parenchymal cells. Hence, the full recommended dose should be taken within a few minutes, and a dose should be repeated once if vomiting occurs within 15 min of ingestion. When initiating cysteamine therapy, dosing should begin at approximately one-fourth the target dose, with incremental increases every ~3–7 days. Doses are titrated to maintain leukocyte cystine levels less than 1 nmol half-cystine/mg protein. However, since a value of 0.2 is preferable to a value of 0.9, some patients receive the maximum tolerated dose, as long as it is below 90 mg/kg/day. The recommended adult dose of oral cysteamine is 500 mg q6h, although some patients receive as much as 750 mg q6h.

One child with intestinal immotility received cysteamine intravenously, through a central line, for years (76). The blood levels achieved with this regimen resembled those of other children given similar doses orally.

There was hope that early initiation of cysteamine therapy could allow patients with cystinosis to avoid the need for renal transplantation entirely (9). However, a significant amount of glomerular damage is often present by the time of diagnosis, and cystine depletion may not completely stop the progressive loss of renal function. Hence, even our early-treated, compliant patients reach end-stage renal disease by their late teens or early twenties. Furthermore, although cysteamine therapy can occasionally be associated with improved renal tubular function (77), the Fanconi syndrome of cystinosis is generally irreversible and refractory to cystine-depleting therapy. In patients receiving oral cysteamine beginning prior to 2 months of age because an affected sibling allowed early diagnosis, the Fanconi syndrome was delayed and attenuated but not entirely prevented.

In approximately 10–15% of patients, the side effects of cysteamine prevent this treatment (8), and in another fraction of patients the adverse effects limit either dosage or compliance. Because cysteamine is a free thiol, it smells like rotten eggs, and this odor sometimes emanates from the skin. Cysteamine also tastes terrible and can cause nausea and vomiting. Breath fresheners and a variety of over-the-counter remedies are variably effective in combating the smell. Anti-emetics can be useful for the nausea and vomiting. Some children and adults clearly do better than others taking oral cysteamine, and for children, parental persistence and diligence is critical for compliance. Several adolescents and young adults, upon achieving a measure of independence or upon leaving home, have eschewed cysteamine as a medication and have lost their kidneys within approximately 6 months.

Three decades ago, one uremic patient receiving oral cysteamine at high doses experienced a seizure, another had reversible neutropenia, and another experienced an apparent drug rash (78). These and other side effects are distinctly unusual and appear related to excessive dosing, sometimes initiated by families or patients themselves. We have noted lethargy or somnolence in two patients on very high doses of cysteamine. In addition, apparent vascular fragility, noted especially on the elbows, has been observed, largely among children on very high doses of cysteamine. To date, this later complication has been seen only among European patients. The free thiol group of cysteamine has the potential to interact with disulfides or other free thiols, including cysteine residues on circulating proteins (79).

Alternative methods of cysteamine delivery have been employed. On a molar basis, phosphocysteamine has efficacy equal to that of cysteamine in terms of leukocyte cystine depletion (80). Cystamine, the disulfide of cysteamine, is considered effective as well. A delayed release preparation of cysteamine is under development (81), with the hope that twice daily dosing can replace q6h dosing.

Cystinosis patients who are pregnant should be aware that the teratogenic potential of cysteamine has not been assessed in humans. It is recommended that females planning pregnancy discontinue cysteamine at the time of conception, throughout their pregnancies, and while nursing. Since all pregnant females to date have been post-transplant, adherence to this advice has not had repercussions on the patients' renal function. This issue will become acute and problematic as more young women reach child-bearing age with their native kidneys, which require constant cysteamine therapy for maintenance of function. Because cysteamine is not known to be safe in pregnancy, it has not been used for in utero treatment.

Chronic oral cysteamine treatment has been shown to deplete muscle and liver of cystine (82). The inference that other tissues became similarly depleted is bolstered by evidence that treated patients escape the need for thyroid hormone replacement (56). Recent evidence indicates that long-term oral cysteamine can prevent most, if not all, of the late complications of cystinosis (17) (see below).

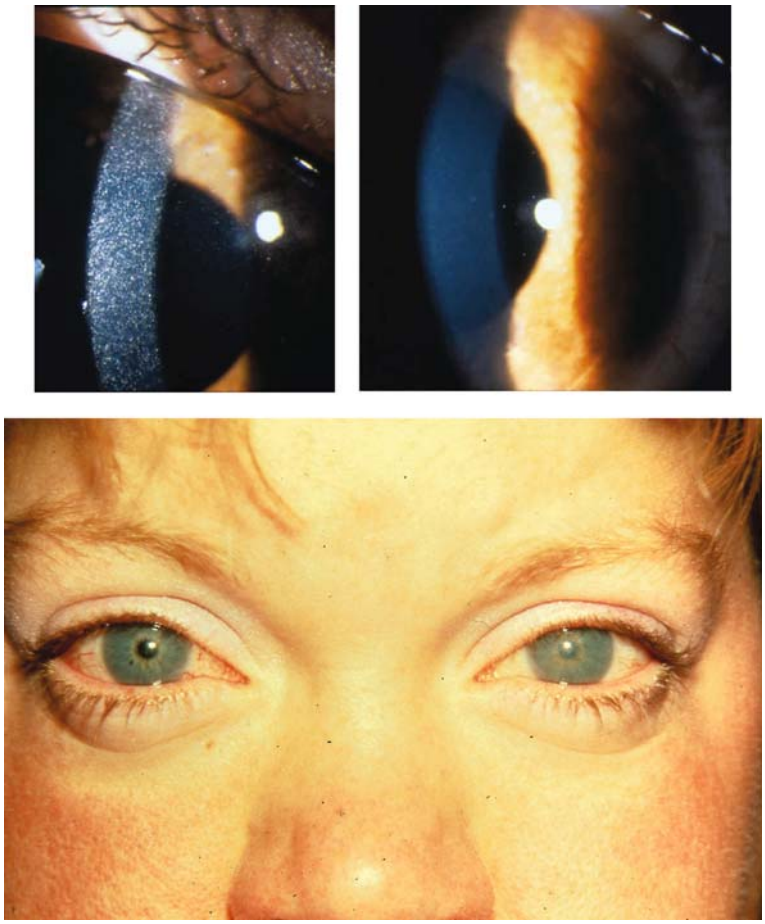
Patients diagnosed with ocular cystinosis may, in fact, have a mild form of nephropathic cystinosis, and may eventually develop renal glomerular dysfunction. Hence, such individuals should be monitored for any decrement in renal function and, if it occurs, consideration should be given to instituting cysteamine therapy, depending upon the age of the patient and the rate of deterioration.

Cysteamine Eyedrops

Oral cyteamine fails to reach the avascular cornea, so cystine crystals continue to accumulate there despite Cystagon^{R} treatment. Delivery of cysteamine directly to the cornea, through cysteamine eyedrops, has enormously salutary effects. In general, within approximately 3 weeks, patients' eyes feel better, and the photophobia is at least partially relieved (10). The eyedrops, containing 0.55% cysteamine hydrochloride in sterile normal saline plus 0.01% benzalkonium chloride as preservative, can dissolve corneal crystals within 1–3 years when given 8–10 times per day (*Fig. 41-7*). The beneficial effects of cysteamine eyedrops can occur at any age (12), and older patients can observe a clarification of the haziness in their corneas. The eyedrops, which can sting to various degrees in different patients, remain an investigational

Figure 41-7

Corneal cystine crystals and the effects of cysteamine eyedrops. Slit lamp examination of a 21-year-old cystinosis patient showing the cornea packed with crystals. (a) Same cornea 3 years later, nearly devoid of crystals subsequent to application of topical cysteamine therapy. (b) An adult post-transplant patient with cystinosis treated her right eye with cysteamine eyedrops, and the cornea became clarified. Her untreated left eye remained hazy, filled with cystine crystals.



drug, but this issue is being addressed. The disulfide cystamine is not as effective as the free thiol cysteamine in dissolving corneal cystine crystals (83). Some partial relief of corneal discomfort can be achieved by over-the-counter eyedrops not containing cysteamine, probably by virtue of their lubricating ability.

Other Therapeutic Considerations

Families must recognize that oral cysteamine therapy is life-saving, and counseling in this regard should be part of the therapeutic plan. By the time children with cystinosis enter school, they have interacted closely with health professionals for years and may appear sophisticated. However, they are still children who need limits as well as understanding. Conversely, some adults with cystinosis are small in stature, but should be treated as adults.

Cystinosis in Adults

The success of renal transplantation has created a population of adult patients with nephropathic cystinosis, and this situation has revealed the consequences of prolonged cystine accumulation (16, 17). Eventually, cystinosis damages or destroys nearly every tissue and organ of the body, with variable frequencies (*Table 41-2*). This multisystemic involvement presents a significant

Table 41-2

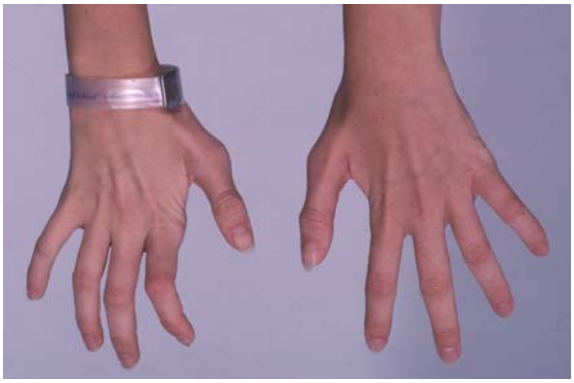
Frequency of complications in 100 adults with cystinosis*

Finding	Frequency (%)
Hypothyroidism	75
Male hypogonadism	74
Pulmonary dysfunction	69
Swallowing abnormality	60
Myopathy	50
Retinopathy	32
Vascular calcifications	31
Diabetes mellitus	24
Cerebral calcifications	22

*92 patients had received a renal allograft

Figure 41-8

Myopathic hands of an adult with cystinosis, untreated with oral cysteamine. Note muscle wasting and claw-hand posture of flexion.



challenge to the effective transitioning of patients from pediatrics to internal medicine.

The following is a description of the natural history of late cystinosis, without cystine depleting therapy.

Growth and Appearance

Untreated or poorly treated adult cystinosis patients (males and females) average 144 cm in height (~13 cm below the mean third centile for males and females) and 45 kg in weight (~5 kg below the third centile for males and females) (16, 17). Head circumference is normal. Because nearly all transplanted adults are receiving prednisone, they have the facial and body stigmata of steroid use, including a pudgy, aged visage and, occasionally, verrucal warts on the hands.

Myopathy

A large proportion of untreated adults with nephropathic cystinosis exhibit a distal vacuolar myopathy, beginning in the hands with loss of the interosseous muscles and the thenar and hypothenar eminences, and progressing to the arms, legs, shoulders, neck, and chest (30, 31). This complication does not occur before adolescence, and is attributable to cystine accumulation within muscle tissue, not to carnitine deficiency, which resolves with renal transplantation. Affected patients hold their fingers in partial flexion, giving the appearance of a claw hand (*Fig. 41-8*); they have difficulty opening jars. Without cysteamine treatment, the muscle weakness and atrophy progress inexorably to include the head and neck, causing poor tongue and lip strength and hypophonic speech. Nerve conduction velocities are normal, and electromyography shows a myopathic pattern (84).

Eventually, swallowing difficulty supervenes in most myopathic patients. Symptoms include slow eating, choking, pain on swallowing, and heartburn (85). The impairment results from deterioration of swallowing muscles rather than from nerve involvement, and it poses a significant risk of aspiration. The first patient ascertained with the myopathy of cystinosis died of aspiration (30). The swallowing problem involves pooling in the valleculae and pyriform sinuses and, rarely, a double bolus. Other issues of oromotor dysfunction include a hyperactive gag reflex, hoarse voice, and inability to elevate the palate and move the jaw normally.

Another concomitant of severe myopathy in cystinosis is impaired lung function, due to weakness of the thoracic musculature rather than parenchymal lung damage (86). Pulmonary function tests are often significantly reduced, with volumes approximating 50% of predicted for size and age. Early rickets might also confer a conical shape to the chest cavity, potentially reducing maximum achievable volumes.

Pancreatic Involvement

A significant number of post-transplant cystinosis patients have insulin-dependent diabetes mellitus (87), presumably due in part to destruction of pancreatic beta cells. The frequency and severity of diabetes in cystinosis cannot be attributed entirely to the effects of steroids given to prevent

rejection of renal allografts. Rarely, pancreatic exocrine insufficiency has occurred in cystinosis, as evidenced by steatorrhea that is ameliorated by institution of pancreatic enzyme supplementation (88).

Hypogonadism

In the natural history of cystinosis, puberty is delayed by approximately 1–3 years, and some males never completely develop secondary sexual characteristics, including muscular development, facial hair, and a deep voice. Adult males with untreated cystinosis have low levels of testosterone and elevated levels of luteinizing hormone and follicle stimulating hormone, reflecting damage to testicular function (89). Sperm counts, measured in three individuals, were zero in all of them. No male patient has been reported to sire a child.

Females have no apparent impairment of ovulatory function and have normal sex hormone levels. Several post-transplant patients have given birth to entirely normal children (90). Of course, having an allograft by itself makes a pregnancy high-risk.

Central Nervous System Involvement

Brain cystine levels were originally considered normal in cystinosis, and the brain was thought to be spared. This notion was discarded, however, as patients survived to adulthood by virtue of renal replacement therapy. In fact, many adults with cystinosis have cerebral atrophy and periventricular and basal ganglia calcifications on CT scans (91). These findings have not correlated directly or immediately with functional deficits. Serious involvement of the central nervous system rarely occurs, but destruction of the parenchyma, with bradykinesia, inability to walk, and loss of speech and short term memory have been reported (92). In addition, cerebrovascular accidents have occurred, most likely as complications of uremia, dialysis, or transplantation.

Ocular Findings

Untreated, the corneas of cystinosis patients continue to accumulate crystals, and can develop a vision-impairing band keratopathy. Chronic photophobia can result in irreversible blepharospasm and posterior synechiae (scar tissue binding the iris to the posterior lens), impairing accommodation and causing angle closure glaucoma (29). Loss of retinal pigment epithelium can damage the rods and cones, initially impairing night and color vision and eventually reducing visual acuity. The retinopathy can be confirmed by electroretinography (93). An estimated 3–5% of cystinosis adults who have not received oral cysteamine therapy have retinal blindness.

Other Complications

Vascular calcifications occur with increased frequency among all individuals who have received dialysis, but the prevalence among cystinosis patients appears greater than expected (94). Involved vessels include the internal carotids, coronary arteries, aortic arch, and abdominal aorta. One 25-year-old man required stent placement for occluded coronary arteries (94).

Two young men with cystinosis died of severe liver dysfunction with portal hypertension; the pathologic diagnosis was nodular regenerating hyperplasia (32).

Death

In a study of 100 adults age 18–45 years seen at the NIH Clinical Center between 1985 and 2006, 33 had died at an average age of 28.5 years (17). The most common causes of death were sepsis, uremia, and respiratory complications. A few patients died of intestinal perforations. The oldest possible living patient was born in 1958, since he would reach renal failure (age 10) at the time of the first renal replacement therapy for cystinosis, i.e., 1968.

Occupations

The physical impairments of cystinosis adults not treated with cysteamine (i.e., growth retardation, muscle weakness, reduced vision) restrict most patients to sedentary occupations. Similarly, preservation of intellectual capacity directs patients toward professions involving computers, music, health care, and teaching. The physical normalcy that accompanies good cystine-depleting therapy will broaden the spectrum of jobs held by cystinosis patients in the future.

Treatment of Adults

Symptomatic Therapy

The various late complications of cystinosis require specific, symptomatic treatments. Up to 90% of adult patients who never received oral cysteamine require

L-thyroxine replacement by 30 years of age (56). Some males with hypogonadism receive testosterone injections every 2–4 weeks; this enhances secondary sexual characteristics as well as libido. The diabetes mellitus of long-standing cystinosis often requires insulin therapy.

Lost muscle mass cannot be recovered, but maximum retention of muscle can be achieved by consistent exercising. For patients with swallowing difficulty, specialists recommend maneuvers to reduce the risk of aspiration. Decreased chest expansion, causing reduced lung volumes, prompts yearly administration of the influenza vaccine as prophylaxis against pneumonia. For severe hand weakness, tendon transplantation has improved function in selected individuals.

Cystine Depleting Therapy

Virtually all the late, non-renal complications of nephropathic cystinosis are preventable with chronic oral cysteamine therapy. Of 100 adult patients seen at the NIH, the 39 who received cysteamine for at least 8 years had better growth, later onset of renal failure, fewer deaths, lower cholesterol levels, and fewer overall complications of the disease (17). Diabetes mellitus, myopathy, pulmonary dysfunction, hypothyroidism and death increased in frequency as the number of years *without* cysteamine increased; these complications decreased in frequency as the number of years *with* cysteamine increased. Other studies showed that the frequencies of retinopathy (93), swallowing difficulty (95), and vascular calcifications (94) were reduced by chronic oral cysteamine therapy. All these findings make Cystagon^{R} the treatment of choice in post-transplant as well as pre-transplant cystinosis patients. In addition, topical cysteamine eyedrops can dissolve corneal cystine crystals in the fourth decade of life as well as in the first decade.

Cystinosis Advocacy Groups

Patient advocacy groups include the Cystinosis Research Network (www.cystinosis.org/), the Cystinosis Foundation (www.cystinosisfoundation.org/), and the Cystinosis Research Foundation (www.natalieswish.org/).

Summary

Nephropathic cystinosis serves as a model for lysosomal storage diseases due to membrane transport defects, which currently include only sialic acid storage disorders (96) and cobalamin F disease (97). The discovery of the defect in cystinosis revealed that small molecules (amino acids, sugars, minerals, and nucleotides), produced by lysosomal hydrolysis of macromolecules, are salvaged by the cell. Cystinosis is the first treatable lysosomal storage disease and, as the most common cause of Fanconi syndrome in childhood, must be the first disease considered when tubular wasting is ascertained. Recent evidence of preservation of non-renal organs by oral cysteamine treatment reinforces the need for diligent, life-long cystine-depleting therapy. The overall success of this treatment should prompt renewed pursuit of a method for newborn screening. When gene therapy can be safely delivered to the many tissues involved in cystinosis, that treatment modality may supplant small molecule therapy. Until then, we consider ourselves blessed with an incredibly effective, albeit imperfect, method of treatment for what used to be a universally fatal malady.

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42 Fanconi Syndrome

Takashi Igarashi

Fanconi syndrome (FS) is a generalized dysfunction of the renal proximal tubules leading to excessive urinary wasting of amino acids, glucose, phosphate, uric acid, bicarbonate, and other solutes. The patients develop failure to thrive, polyuria, polydipsia, dehydration, and rickets in children, and osteoporosis and osteomalacia in adults. The patients also manifest renal salt wasting, hypokalemia, metabolic acidosis, hypercalciuria, and low-molecular-weight (LMW) proteinuria. De Toni, Debré, and Fanconi described children with renal rickets and glucosuria in the 1930s (1, 2, 3). FS is named after Guido Fanconi, a Swiss pediatrician or it is called as de Toni-Debré-Fanconi syndrome.

Pathophysiology

The renal proximal tubules reabsorb almost all of the physiologically filtered load of proteins including of albumin, LMW proteins, amino acids, glucose, bicarbonate, sodium, chloride, phosphate, and uric acid. The transport processes in the proximal tubule can be characterized broadly as megalin/cubilin-mediated endocytic pathways and sodium (Na^+) gradient-dependent transport systems.

The proximal tubule cells have extensive luminal receptors and endocytic apparatus such as megalin and cubilin that are critical for the reabsorption and degradation of proteins that traverse the glomerular filtration barrier (4) (*Fig. 42-1*) as well as for the extensive recycling of many functionally important membrane proteins (5). Numerous filtered proteins including albumin, LMW proteins, polypeptide hormones, vitamin-binding proteins, and polybasic drugs such as aminoglycosides from glomerulus are bound to megalin and cubilin in the luminal membrane of proximal tubules. Then, the protein-receptor complex is incorporated into the endosome. The ligand and receptor are disassociated in the endosome; the receptor is recycled back to the luminal membrane and the reabsorbed proteins go into lysosome for further processing (*Fig. 42-2*). This disassociation is dependent on acidification of the endosome by increased concentration of H^+ and Cl^- due to the function of H^+-ATPase (proton pump) and ClC-5 chloride channel. An abnormal endocytosis pathway may affect the recycling of transport proteins (megalin and cubilin), the back to the luminal membrane, and the expression of megalin and cubilin in the luminal membrane, leading to decreased solute reabsorption. Perturbation of endosomal acidification in proximal tubule cells leads to diminished reabsorption and increased urinary wasting of albumin, LMW proteins, electrolytes, and solutes. Cadmium inhibits H^+-ATPase and mitochondria, which results in a Fanconi-like syndrome (6). Folimycin, a H^+-ATPase inhibitor, abolishes albumin uptake by proximal tubules (7). Moreover, a defect of ClC-5 chloride channel in patients with Dent disease manifests Fanconi syndrome (8). Acidification defect in the endosome in Dent disease leads to recycling from intracellular endosome into luminal membrane resulting in megalin deficiency in the luminal membrane of the proximal tubule. Analysis of normal human urine samples identified megalin as a physiologically excreted protein. The presence of megalin in normal human urine is due to shedding from the proximal tubule cells into the lumen. Patients with Dent disease demonstrate an almost complete absence of urinary megalin (9). This megalin-shedding deficiency in the urine is also observed in patients with Lowe syndrome (9).

Reabsorption of filtered solutes including glucose, phosphate, amino acids, and bicarbonate by proximal tubule cells is accomplished by transport system at the brush border membrane that are directly or indirectly coupled to Na^+ movement, by energy production and transport from the mitochondria, and by the Na^+, K^+-ATPase at the basolateral membrane. The Na^+, K^+-ATPase lowers intracellular Na^+ concentration and provides the electrochemical gradient that allows Na^+-coupled solute entry into the cell. Disturbances in energy generation could impair net transepithelial transport in the proximal tubule. Energy is necessary for the operation of Na^+, K^+-ATPase and other membrane carriers that are involved with solute reabsorption of amino acid, glucose, phosphate, uric acid, and bicarbonate. Although the weight of bilateral kidneys is less than 1% of total body weight, kidneys consume about 10% of the total energy consumed by the whole body in a static condition. Moreover, most of the energy is consumed in the proximal tubule cells to operate multiple

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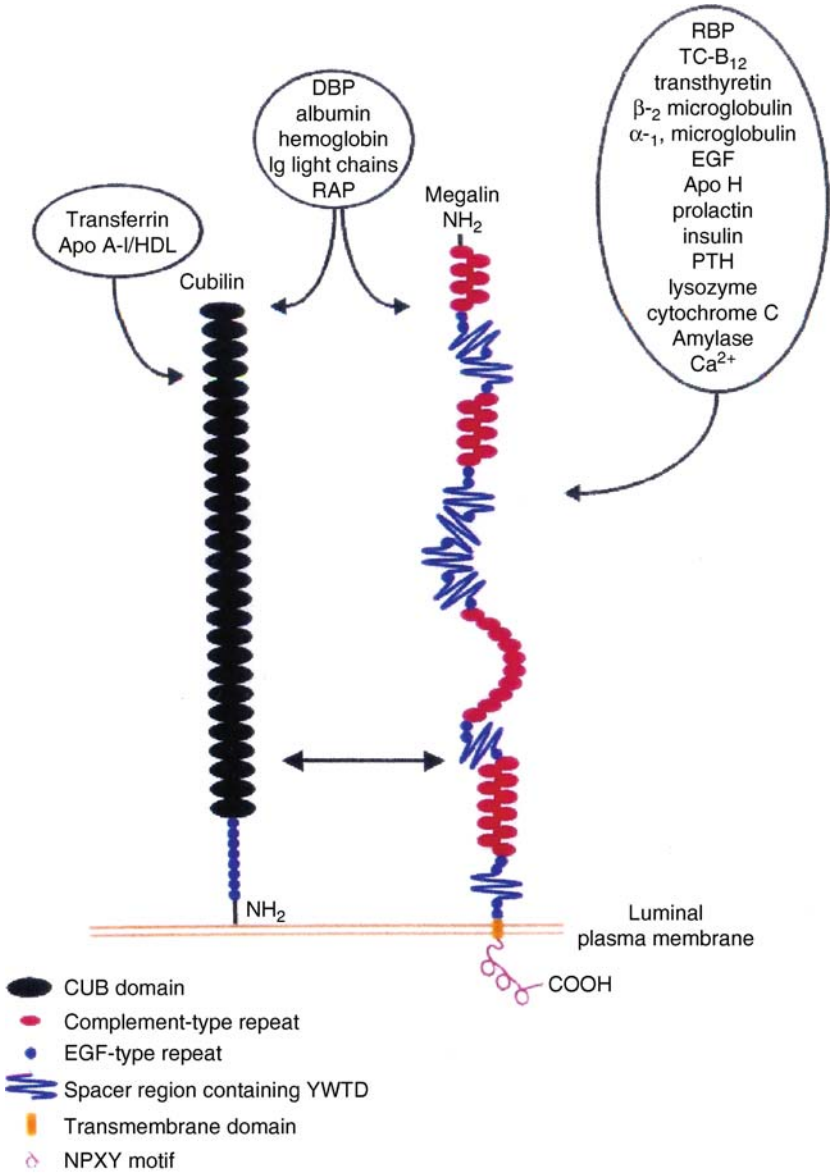
Figure 42-1

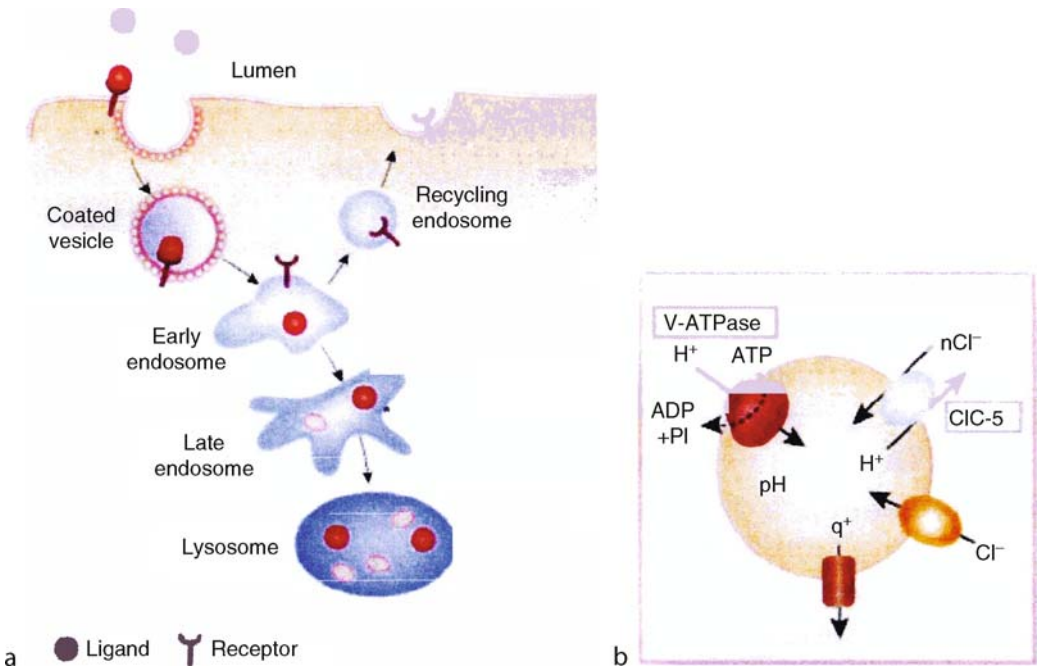
Structure of megalin and cubilin. (Veroust PJ, Birn H, Nielsen R et al. The tandem endocytic receptors megalin and cubilin are important proteins in renal pathology. *Kidney Int* 2002;62:745–756).



membrane and intracellular transporter proteins. A defect in energy generation in the proximal tubule cells produces multiple transport anomalies of the proximal tubule that characterize the FS. The ATP production is severely compromised in cystine-loaded tubule in cystinosis (10). Thiol-containing enzymes are critical for renal energy metabolism. Cystine inhibits in vivo and in vitro the thiol-containing enzyme activity resulting in multiple renal transport anomalies (11). The mitochondrial respiratory chain has a major role in ATP production during aerobic respiration. Genetic defects of enzyme complexes of the oxidative phosphorylation system or toxic substances including of drugs in the proximal tubule cells can produce mitochondrial respiratory chain defect leading to multiple renal transport anomalies (12, 13). In contrast, isolated dysfunction of transporter proteins in proximal tubule cells results in the selective wasting of amino acids, glucose, phosphate, bicarbonate, or uric

Figure 42-2

Schematic model of endocytosis in the proximal tubule cells. Albumin and low molecular weight proteins are filtered into the primary urine and endocytosed by proximal tubule cells via the megalin-cubilin receptor pathway. (a) The receptor-ligand complexes progress along the endocytic pathway. The endosomes undergo a progressive, ATP-dependent acidification that results in the dissociation of the receptor-ligand complexes, with megalin and cubilin being recycled in the luminal membrane, whereas the ligand is directed to lysosomes for degradation. (b) Vesicular acidification is mediated by the vacuolar H^+-ATPase, which requires a net Cl^- conductance to functions as an electrogenic nCl^-/H^+ exchanger, which is predicted to facilitate acidification and to play a role in keeping high vesicular Cl^- concentration. (Devuyst O, Pirson Y. Genetics of hypercalciuric stone forming disease. *Kidney Int* 2007:72:1065–1092).



acids. However, glucosuria and aminoaciduria are seen in some patients with defective isolated proteins. They are familial renal glucosuria resulting from the mutations in the kidney-specific low affinity/high capacity Na^+/glucose cotransporter gene (*SLC5A2*) and maturity-onset diabetes of young age type 3 (MODY3) resulting from the mutations in hepatocyte nuclear factor-1 alpha gene that acts as a regulator of transcription for *SLC5A2* (14, 15). Generalized aminoaciduria seen in these patients is considered as a consequence of the impairment in tubular glucose reabsorption, whereas the precise mechanism is not known.

Signs and Symptoms

Growth Retardation (Failure to Thrive)

Growth retardation (failure to thrive) is a common feature of FS in children (16). Patients with FS present severe growth failure at the time of diagnosis that persists into adult life. The pathomechanism of growth failure in FS is complex. Malnutrition, hypokalemia, hypophosphatemia, and metabolic acidosis can lead to growth retardation in patients with FS (17). Potassium deficiency induces growth retardation through reduced circulating levels of growth hormone (GH) and insulin-like growth factor I (IGF-I) (18, 19). Hypokalemia can induce appetite decrease leading to malnutrition and extracellular volume contraction. Metabolic acidosis inhibits growth hormone secretion, the expression of IGF-I and GH receptor (20). Hypophosphatemia is related to severe bone changes leading to rickets and growth retardation in children with FS (21). In patients with adult onset FS, osteomalacia is thought to result from hypophosphatemia due to renal phosphate loss and relative 1,25-dihydroxyvitamin D3 deficiency (22, 23). Metabolic acidosis impairs the conversion of 25-vitamin D3 to 1,25-dihydroxy vitamin D3. The patients present bone and joint pain in the hips,

shoulders, and trunk and difficulty of walking due to multiple bone fracture. Hypomineralization of dentin structure and immature formation of craniofacial bones are seen in patients with FS (24). Specific forms of FS are associated with endocytotosis pathway dysfunction; disruption of megalin-mediated uptake vitamin D-binding protein/25-vitamin D3 complex produces metabolic bone disease in affected individuals (25).

Earlier diagnosis and efficient correction of acidosis and electrolyte balances by supportive therapy can contribute to improve growth and final height in patients with FS (17, 21). However, supportive therapy is frequently unable to prevent further loss of relative height in patients with FS, especially those with cystinosis.

Polyuria, Polydipsia, and Dehydration

Polyuria, polydipsia, and dehydration are frequently seen in patients with FS. Polyuria is secondary to the osmotic diuresis from the excessive urinary solute losses and urine concentration defect in the collecting ducts due to chronic hypokalemia. Recurrent acute fever due to dehydration is a frequent manifestation in infants with FS. In the most common type of cystinosis, Fanconi syndrome occurs at 6–12 months of age. Recurrent febrile episodes are often the first sign of FS in infantile patients with cystinosis (26).

Generalized Aminoaciduria (Generalized Hyperaminoaciduria)

Molecular weight (MW) of 20 different amino acids is small; the largest amino acid is tryptophan [MW = 204 D (daltons)]. Amino acids are not bound to proteins in the plasma. Thus, amino acids are freely filtered from glomerulus. Then, 95–99% of filtered load of amino acids are reabsorbed in the proximal tubules. More than one transporter in the proximal tubule cells absorbs amino acids. Fractional excretion of amino acid is usually less than 3% in the controls except for neonate or premature babies. However, only histidine has a fractional excretion of 5% in the controls.

$$\text{Fractional excretion of amino acid } (\%) = [(\text{Uaa}/\text{Paa})/(\text{Ucr}/\text{Pcr})] \times 100$$

(aa; amino acid, cr; creatinine, U; urine, P; plasma)

Therefore, the excretion more than 5% of the filtered load of amino acid is termed aminoaciduria or hyperaminoaciduria. Every amino acid is highly excreted in patients with FS, and this phenomenon is called as *generalized aminoaciduria*.

Glucosuria

Filtered load of glucose (D-glucose, MW = 180 D) is almost completely absorbed by a sodium-coupled active transport located in the brush border membrane of the proximal tubule in the normal condition. Glucose reabsorption involves a couple of transporters at the luminal and basolateral membranes of the proximal tubules. The driving force for glucose reabsorption is provided by Na^+, K^+-ATPase in the plasma membrane. Thus, very small amount of glucose are present in the urine in the normal condition. Glucosuria is a common manifestation in FS. It is derived from impaired reabsorption of glucose when serum glucose is normal. Renal threshold of glucose is reduced in FS. Glucosuria is one of the originally described clinical features of FS (1, 2, 3). 0.5–20 g of glucose a day is lost in the urine in patients with FS.

Hypophosphatemia

Most of the patients with FS manifest a low tubular reabsorption of phosphate (percent tubular reabsorption of phosphate: %TRP, >80–85% in the control) and decreased serum phosphate. Rickets and osteomalacia are produced by the increased urinary wasting of phosphate as well as by impaired 1α-hydroxylation of 25-hydroxy vitamin D3 by proximal tubule cells (27).

$$\%\text{TRP} = [1-(\text{Up}/\text{Sp})/(\text{Ucr}/\text{Scr})] \times 100$$

(p; phosphate, cr; creatinine, U; urine, S; serum)

The maximal threshold of phosphate (TmP/GFR) is a very sensitive indicator that reflects the reabsorption of phosphate in the renal tubules.

$$\text{TmP}/\text{GFR} = \text{TRP} \times \text{Sp}$$

(GFR; glomerular filtration rate, p; phosphate, S; serum)

The Tm/GFR is usually very low (2.3–4.3 in the control) in patients with FS. Rickets manifests bowing deformity of the lower limbs, distal femur, the ulna, and the radius.

Phosphate handling in the kidney is affected by a couple of factors including parathyroid hormone (PTH) and vitamin D. PTH level is normal or elevated in patients with FS. Serum 1, 25-dihydroxy vitamin D3 is variable in patients with FS (28, 29).

Metabolic Acidosis

More than 85% of filtered load of bicarbonate ($HCO3^-$) is reabsorbed by the proximal tubule cells. This is accomplished by the coordinated function of luminal membrane Na^+/H^+ exchanger, luminal membrane carbonic anhydrase IV and XIV, and basolateral membrane $Na^+/HCO3^-$ cotransporter (30). Hyperchloremic metabolic acidosis is a common feature of FS resulting from defective bicarbonate reabsorption in the proximal tubules. Anion gap is normal. More than 30% of filtered load of $HCO3^-$ is not reabsorbed in patients with FS, and they manifest low plasma $HCO3^-$ levels between 12–18 mEq L^{-1}. Fractional excretion of $HCO3^-$ ($FEHCO3^-$) under the alkali treatment to increase plasma $HCO3^-$ to the normal ranges is >15% in patients with FS.

$$\text{Fractional excretion of } HCO_{3^-}\% = [(UHCO_{3^-}/PHCO_{3^-})/(Ucr/Pcr)] \times 100$$

($HCO3^-$; bicarbonate, cr; creatinine, U; urine, P; plasma)

Acidification in the distal tubule is usually normal or impaired in association with chronic hypokalemia or toxic effect on distal tubules due to the original disorder in patients with FS.

Sodium and Potassium Losses

60–80% of filtered load of Na^+ is reabsorbed in the proximal tubules in the normal condition. Renal Na^+ reabsorption in the proximal tubules decreased in patients with FS. It leads to hyponatremia, hypotension, and dehydration. Hypokalemia is a secondary phenomenon. Increased delivery of Na^+ into the distal tubules and activation of the renin-angiotensin system secondary to hypovolemia cause potassium (K^+) wasting in the distal tubules. Severe hypokalemia can cause sudden death.

Hypercalciuria

Hypercalciuria is a common finding in patients with FS due to several original diseases. Defective endocytosis of parathyroid hormone (PTH) in patients with Dent disease resulting in its persistence in the lumen of the proximal tubule stimulates 25-hyroxyvitamin D3 1-hydroxylase to produce more 1,25-dihydroxyvitamin D3, raising serum levels of this vitamin. 25-hydroxyvitamin D3 is presented to 25-hydroxyvitamin D3 1-hydroxylase in the form of a complex with the vitamin D3-binding protein. As this complex is lost in the urine as a result of defective endocytosis leading to LMW proteinuria, the precursor 25-hydroxyvitamin D3 could be in short supply. The overall outcome of increased 1, 25-dihydroxyvitamin D3 levels may depend on the delicate balance between these processes. The slightly elevated serum levels of 1, 25-dihydroxyvitamin D3 in patients with FS can lead to increased intestinal Ca^{2+} reabsorption which will lead to hypercalciuria (*absorptive hypercalciuria*) (29). Hypercalciuria is rarely associated with nephrolithiasis in FS, possibly because of the polyuria and alkalized urine. However, patients with Dent disease manifest hypercalciuria and nephrolithiasis.

Hyperuricosuria (Uricosuria)

Uric acid (urate) is the end product of purine metabolism in humans. Because of its small molecular size (MW = 126 D), uric acid is freely filtered from the glomerulus. Then, 90–95% of filtered load of uric acid is eventually reabsorbed in the proximal tubules. A four-component hypothesis has been proposed to explain the renal uric acid transport mechanism; it includes glomerular filtration, presecretory reabsorption, secretion, and postsecretory reabsorption (31). Hyperuricosuria is often present in FS, leading to secondary hypouricemia (<2 mg dL^{-1}) (32). A voltage-sensitive uric acid pathway and uric acid exchangers are located at both luminal and basolateral membranes of proximal tubule cells (33). Uric acid-anion exchanger (URAT1) that reabsorbs uric acid from the lumen of the proximal tubules in the luminal membrane of proximal tubules regulates serum uric acid levels. This uric acid-anion exchanger can be disturbed in patients with FS. Defective URAT1 is a predominant cause of the patients with renal hypouricemia who manifest acute renal failure after exercise (34, 35). Hexose transporter gene (*SLC2A9*) is identified as a cause of gout and hyperuricemia (36). This transporter transports both fructose and uric acid. *SLC2A9* produces two isoforms by alternative splicing; the long isoform is expressed in basolateral membrane of proximal tubular cells and the short isoform is expressed in apical membrane of proximal tubular cells. This hexose transporter can be affected in patients with FS. Uric acid is a selective antioxidant, capable especially of reaction with hydroxyradicals and hypochlorous acid, itself being converted to innocuous products such as allantoin, allantoate, glyoxylate, urea, and oxalate (37).

Proteinuria (LMW Proteinuria)

The proximal tubules have a high capacity for uptake of filtered proteins from the glomerulus. The cut off molecular weight for filtration of plasma proteins is assumed to be in the range of 65 KD (kilodaltons) that corresponds to the molecular weight of serum albumin. However, small amount of larger weight proteins including gammaglobulin are filtered from glomerulus in the normal condition. Albumin and LMW proteins (MW<45,000 D) filtered in the glomerulus are considered to be the major source of urinary albumin and LMW proteins. Filtration of albumin and LMW proteins are followed by tubular reabsorption, and thus the resulting albuminuria and LMW proteinuria reflect the combined contribution of these two processes. Dysfunction of both these processes may result in increased urinary excretion of albumin and LMW proteins, and both glomerular injury and tubular impairment have been implicated in the initial events leading to proteinuria. In FS, proteinuria is predominantly caused by the dysfunction of reabsorption in the proximal tubules. Adolescent patients with FS due to Dent disease or Lowe syndrome excrete greatly increased amounts of proteins (1,740 ± 660 mg/day) and peptide (446 ± 145 mg/day). LMW proteins ranging from 2 to 5 KD were present in 12.9 ± 3.9-fold excess in FS compared with normal urine (38). The micropuncture technique in dogs revealed that the filtered load of albumin was 50μg m^{-2} suggesting that a filtered load of albumin is 9 g/day in normal humans (39). However, urinary excretion of protein is less than 0.1–0.15 g/day in the normal condition. Numerous filtered proteins including albumin and LMW proteins from glomerulus are bound to megalin and cubilin in the luminal membrane of proximal tubules. Then, the protein-receptor complex is incorporated into the endosome. The ligand and receptor are disassociated in the endosome; the receptor is recycled back to the luminal membrane and the reabsorbed proteins go into lysosome for further processing. Megalin is a 600 KD glycoprotein and a member of the low-density lipoprotein receptor family. Megalin is expressed in the proximal tubule brush-border and luminal endocytic apparatus. Megalin binds to a number of structurally very different proteins. It contains a large amino-terminal, extracellular domain, a single transmembrane domain and a short carboxy-terminal cytoplasmic tail (*Fig. 42-1*). Cubilin is a multiligand, endocytic receptor. It is a 460 KD protein with little structural homology to known, endocytic receptor (*Fig. 42-1*). Cubilin is expressed in the proximal tubule brush-border and luminal endocytic apparatus. Megalin involved in albumin reabsorption directly as a receptor for albumin, and/or indirectly by affecting the expression and/or endocytic function of cubilin (40, 41). Megalin's expression was decreased in patients with Dent disease. Acidification defect due to endosomal defective ClC-5 in patients with Dent disease disturbs the recycling from intracellular endosome into luminal membrane of the proximal tubule resulting in megalin and cubilin deficiency in the luminal membrane of the proximal tubule.

Etiologies

The causes of FS are divided into three main categories; hereditary, acquired, and exogenous substances (*Table 42-1*). Most of the hereditary FS occurs as one of the manifestations of congenital metabolic disorders or as sporadic or familial disorders. Acquired forms are derived from immunological reactions, nephrotic syndrome or accumulated abnormal proteins. Exogenous substances are composed of drugs, chemical compounds, and heavy metals.

Hereditary Fanconi Syndrome

Dent Disease

Dent disease is an X-linked proximal tubulopathy characterized by LMW protinuria, hypercalciuria, nephrocalcinosis, nephrolithiasis, and eventual end stage renal failure. Hypophosphatemic rickets and metabolic acidosis are sometimes seen (42, 43). Almost all of the patients are males. Adult patients with Dent disease manifest FS. However, children with Dent disease often manifest LMW proteinuria and one or two of the manifestations due to proximal tubular dysfunction and this is called partial FS (44). They usually fall into end stage renal failure by the age of 40s. However, this is highly variable, and one third of patients with Dent disease will not develop end stage renal failure. Patients with Dent disease never manifest extrarenal manifestations, except for rickets, which may itself be a consequence of phosphaturia. School children with Dent disease manifest proteinuria. A lot of school children with Dent disease are detected as proteinuria by school urine mass screening program in Japan, and it was called as idiopathic low molecular weight proteinuria (45, 46). Carrier females are often manifest less severe LMW proteinuria and hypercalciuria, depending on X-chromosome inactivation, but they rarely develop clinically significant problems.

Table 42-1
Causes of Fanconi syndrome

Hereditary
• Dent disease
• Lowe syndrome
• Mitochondriopathies
• Cystinosis
• Galactosemia
• Hereditary fructose intolerance
• Glycogen storage disease type I (von Gierke disease)
• Fanconi-Bickel syndrome
• Tyrosinemia
• Wilson disease
• Idiopathic Fanconi syndrome
Acquired
• Nephrotic syndrome
• Myeloma
• Sjögren syndrome
• Renal transplantation
• Acute tubulointerstitial nephritis with uveitis (TINU) syndrome
• Autoimmune interstitial nephritis and membranous nephropathy
• Anorexia nervosa
• Untreated condition of distal renal tubular acidosis
Exogenous substances
• Drugs
• Chemical compounds
• Heavy metals

Dent disease is associated with inactivating mutations in *CLCN5* gene, which encodes 746 amino acids renal specific chloride channel-5 (ClC-5) (8, 47–49). ClC-5 belongs to the family of voltage-dependent chloride channels, which function as homodimeric proteins. ClC-5 is co-expressed with the vacuolar H^+-ATPase and plays a key role in endosomal acidification that is a crucial function in the receptor-mediated endocytic pathway (50). More than 80 distinct *CLCN5* mutations are reported in patients with Dent disease. They are nonsense, missense, frameshift, splice-site, insertional, and deletional mutations, which result in total or partial loss of function. There are no genotype-phenotype correlations as various mutations are associated with different clinical phenotypes, even within the same family.

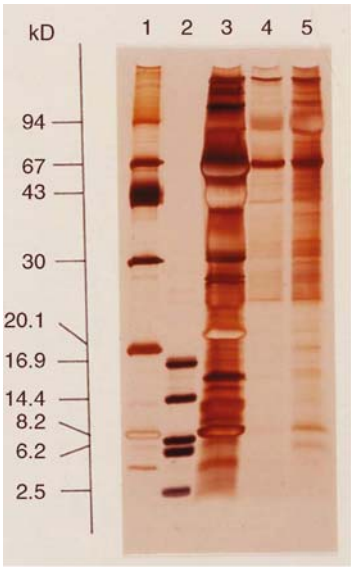
Numerous filtered proteins are bound to megalin and cubilin in the luminal membrane of proximal tubules, and the protein-receptor complex is incorporated into the endosome. The ligand and receptor are disassociated in the endosome; the receptor is recycled back to the luminal membrane and the reabsorbed proteins go into lysosome for further processing. This disassociation is dependent on acidification of the lumen of endosome by increased concentration of H^+ and Cl^- due to the function of H^+-ATPase and ClC-5 chloride channel. An abnormal endocytosis pathway due to ClC-5 dysfunction disturbs the recycling of megalin and cubilin, the back to the luminal membrane, and the expression of megalin and cubilin in the luminal membrane of proximal tubules, leading to LMW proteinuria, hypercalciuria, hyperphosphaturia, and nephrolithiasis. Proper acidification is also important for protein degradation in the endosome. Immunohistochemical analysis of proximal tubule cells in patients with Dent disease revealed an inverted polarity of the H^+-ATPase, with redistribution to basolateral regions, suggesting that the loss of ClC-5 channel alters the function of components that co-distribute and physically interact with it (51).

Total urine protein ranges from 0.5–2.5 g a day, but may reach 4 g or higher in patients with Dent disease (45, 52). More than 60% of the filtered proteins are LMW proteins with molecular weight less than 45 KD (*Fig. 42-3*). Nephrotic syndrome does not occur. LMW proteinuria is the most consistent and one of the earliest presenting abnormalities. Urinary beta 2-microglobulin, a LMW protein (MW = 11.6 KD), is excreted in amounts 100–300 times the upper limit of the normal. Albumin is also excreted in the urine. The pattern of proteins representing the increased excretion of several LMW proteins as well as albumin (MW = 65 KD) is termed as *tubular proteinuria* (52, 53). The terms of LMW proteinuria and tubular proteinuria have usually been used interchangeably (53).

Patients with Dent disease manifest hypercalciuria in the range of 4–10 mg kg^{-1} of body weight a day in children and 4–6 mg kg^{-1} of body weight a day in adults (54). Nephrocalcinosis is also found in children (*Fig. 42-4*). Defective endocytosis of parathyroid hormone (PTH) in patients with Dent disease resulting in its persistence in the lumen of the proximal tubule stimulates 25-hydroxyvitamin D3 1-hydroxylase to produce more 1,25-dihydroxyvitamin D3 resulting in the increased serum levels of this vitamin. 25-hydroxyvitamin D3 is presented to 25-hydroxyvitamin D3 1-hydroxylase in the form of a complex with the vitamin D3-binding protein. As this complex is lost in the urine as a result of defective endocytosis leading to LMW proteinuria, the precursor

Figure 42-3

Electrophoresis of urine from the family members of Dent disease on the polyacrylamide gel and stained by sliver representing LMW proteinuria. Lanes 1 and 2, molecular markers; Lane 3, 12-year-old boy with Dent disease; Lane 4, his mother; Lane 5, his father.



25-hydroxyvitamin D3 could be in short supply. The overall outcome of increased 1, 25-dihydroxyvitamin D3 levels may depend on the delicate balance between these processes. The slightly elevated serum levels of 1, 25-dihydroxyvitamin D3 in patients with Dent disease can lead to increased intestinal Ca^{2+} reabsorption resulting in hypercalciuria (*absorptive hypercalciuria*). In fact, 70% of the patients with *CLCN5* mutations manifest hypercalciuria, even though some of these do exhibit nephrocalcinosis (55). This may be explained by the *CLCN5* knock-out mice model; ClC-5 disruption promotes calcium crystal agglomeration, as well as a redistribution of the crystal-binding molecule annexin A2, in collecting duct epithelial cells (56).

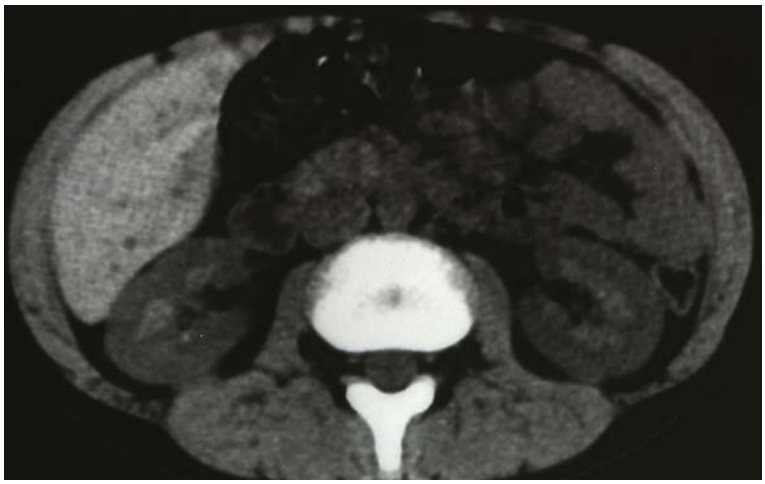
The onset of renal insufficiency and progression to end-stage renal failure are quite variable. Significant decrease of glomerular filtration rate is seen in children with Dent disease, and the patients fall into end-stage renal failure by the end of the age of 40s. Renal biopsy demonstrates normal or focal global glomerulosclerosis with tubular atrophy, tubular dilatation, and interstitial infiltration of monocytes. Medullary nephrocalcinosis is a significant feature in patients with Dent disease. Patients less than 5 years of age manifest medullary nephrocalcinosis. The precise mechanism of progressive renal failure is not known in patients with Dent disease. Nephrocalcinosis can be a candidate to disturb the glomerular filtration rate. High urinary concentrations of potentially bioactive proteins including insulin, insulin like growth factor-1 (IGF-1), and the chemokine monocyte chemoattractant protein-1 (MCP-1) may contribute to interstitial fibrosis that will lead to progressive renal failure in patients with Dent disease (57). Generalized proximal tubule dysfunction is associated with increased cell proliferation, dedifferentiation, and oxidative stress resulting in interstitial fibrosis and eventual renal failure (58). Patients and carrier females often manifest nephrolithiasis, and renal stone is calcium phosphate stone that is also seen in patients with distal renal tubular acidosis.

Dent disease is genetically heterogeneous. Mutations in the *OCRL1* gene are identified in a subset of patients with the Dent disease phenotype (59). Unlike patients with typical Lowe syndrome, typical facial features, mental retardation, metabolic acidosis, and ocular abnormalities are usually absent in patients with Dent disease who have *OCRL1* mutation. The phosphoinositol 4,5-bisphosphate phosphatase (PIP_2 5-phosphatase) activity is markedly reduced in skin fibroblasts cultured from patients with Dent disease due to *OCRL1* mutations, and protein expression, measured by Western blotting, is reduced or absent. PIP_2 5-phosphatase participates the trafficking and recycling of endosome in the proximal tubules. Defective PIP_2 5-phosphatase activity can lead to endosomal dysfunction leading to LMW proteinuria. Unlike the patients with typical Lowe syndrome, none of patients have metabolic acidosis. These observations and findings suggest that *OCRL1* mutations can cause the isolated renal phenotype of Dent disease and affected individuals lack the cataracts, typical facial features, renal tubular acidosis, and neurologic abnormalities that are characteristic to Lowe syndrome. It is difficult to explain that *OCRL1* mutations can cause the isolated renal phenotype of Dent disease. However, it is possible that another phosphatase, *INPP5B*, which shares amino acid homology with *OCRL1*, can compensate phosphatase activity in patients with Dent disease due to *OCRL1* mutations.

There are no specific interventions at present that will change the natural course of renal manifestations and progressive renal failure in patients with Dent disease. Hypercalciuria is corrected by thiazide diuretics therapy in doses similar to effective doses for idiopathic hypercalciuria, presumably by stimulating the reabsorption of calcium in the distal convoluted tubule, where ClC-5 channel is not expressed (60). However, this is not a long-term study which provides the evidence that it is

Figure 42-4

Abdominal CT demonstrating bilateral medullary nephrocalcinosis in a 12-year-old boy with Dent disease.



effective to prevent or delay the progression of end stage renal failure. In animal experiment using *clcn5* (mouse chloride channel 5 gene) knock-out mice, high citrate diets can delay the progression of nephrocalcinosis and end stage renal failure (61, 62). Treatment with an angiotensin-converting enzyme (ACE) inhibitor or angiotensin receptor blocker (ARB) may delay progression of end stage renal failure (63).

Lowe Syndrome

The oculocerebrorenal syndrome of Lowe (OCRL) is a rare X-linked disorder that is characterized by a complex phenotype that involves major abnormalities of the eyes (particularly congenital cataracts), central nervous system abnormality and FS (64). Cataracts are recognized in all the male patients. Lens opacities are present in all carriers of females by slit-lamp examination and this is a reliable screen for carriers (65). Other ocular abnormalities include glaucoma, microphthalmos, and corneal keloid formation. Visual acuity is frequently disturbed. Central nervous system abnormalities include infantile hypotonia (floppy infant), areflexia, and mental retardation. The patients manifest mild ventriculomegaly and cysts in the periventricular regions in the brain. Status epilepticus is sometimes recognized. FS is a major clinical feature and occurs in the 1st year of life, but the severity and age of onset vary. The patients manifest LMW proteinuria, glucosuria, aminoaciduria, hyperphosphaturia, hypercalciuria, hypophosphatemic rickets, hypercholoremic metabolic acidosis, and progressive renal insufficiency (66). Renal insufficiency progresses at variable rate among patients, and it progresses to end-stage renal failure by 30s or 40s.

Like in patients with Dent disease, megalin is nearly absent from the urine in patients with Lowe syndrome suggesting of the decreased expression of megalin in the luminal membrane of the proximal tubule cells. Thus, urinary excretion of retinol-binding protein and the lysosomal enzyme N-acetyl-glucosaminidase are significantly increased in young boys with OCRL (67).

The gene (*OCRL1*) that is responsible for OCRL encodes a 105 KD Golgi protein with phosphoinositol 4,5-bisphosphate phosphatase (PIP_2 5-phosphatase) activity (68). PIP_2 5-phosphatase is mainly a lipid phosphatase that may control cellular levels of a critical metabolite, phosphatidylinositol 4,5-bisphosphate, and is involved in the inositol phosphatase signaling pathway (69). PIP_2 5-phosphatase is present in cultured skin fibroblast and it is not present in peripheral blood cells. PIP_2 5-phosphatase activity is markedly reduced in fibroblasts from patients with Lowe syndrome (70). However, this biochemical test for carrier diagnosis is not reliable; lyonization produces a highly variable pattern of tissue expression in females.

Deficiency of PIP_2 5-phosphatase leads to cellular accumulation of its substrate PIP_2. PIP_2 accumulates in lysosomal membrane (71). PIP_2 is involved in signal transduction, vesicle trafficking and actin polymerization. Absence of PIP_2 5-phosphatase activity leads to a reduction in the number and length of actin stress fibers, a tendency of actin fibers to depolymerize when provoked,

and an abnormal distribution of two actin-binding protein gelsolin and alpha-actinin. This disruption of actin function has significant effects on epithelial function through disrupting cell–cell contacts such as tight junctions or adherent junctions or by altering membrane trafficking such as transport proteins (72). Trans-Golgi dysfunction or altered actin polymerization can explain FS in patients with Lowe syndrome. PIP_2 5-phosphatase is localized to endosome and Golgi membranes along with clathrin, giantin, the mannose 6-phosphate receptor, transferrin, and the early endosomal antigen 1 marker. PIP_2 5-phosphatase interacts with clathrin terminal domain and the clathrin adaptor protein AP-2. This suggests a role for PIP_2 5-phosphatase in endosomal receptor trafficking and sorting (73, 74). OCRL1 is present throughout the early endocytic pathway, including in endocytic clathirin-coated pits, and demonstrate a connection between OCRL1 and adaptor molecules implicated in the endocytic trafficking of receptor in the kidney (75).

OCRL1 has a C-terminal RhoGAP domain. *OCRL1* encodes a PIP_2 5-phosphatase activity that binds to Rac GTPase. Activated Rac GTPase stably associates with the *OCRL1* RhoGAP domain. In this sense, the protein encoded in *OCRL1* can play a bifunctional role. Loss of *OCRL1* RhoGAP domain and the resulting alteration in Rho pathways may contribute to mental retardation in Lowe syndrome, as observed in other forms of X-linked mental retardation (76).

OCRL1 mutations can cause the isolated renal phenotype of Dent disease and affected individuals lack the cataracts, typical facial features, renal tubular acidosis, and neurologic abnormalities that are characteristic to Lowe syndrome. It is difficult to explain that *OCRL1* mutations can cause the isolated renal phenotype of Dent disease. However, another phosphatase, *INPP5B*, which shares amino acid homology with *OCRL1,* can compensate phosphatase activity in patients with Dent disease due to *OCRL* mutations.

More than 70 *OCRL1* mutations have been described in patients with Lowe syndrome; nearly all are clustered in exons 10–23, especially exon 15, and almost none are found in exons 1–9 (70).

Treatment is supportive and includes taking care of ocular manifestations, anticonvulsants, speech therapy, and dental complications. The eye abnormalities usually require therapy early in life. Bicarbonate therapy is usually necessary at a dose of 2–3 mmol kg^{-1} of body weight a day every 6–8 h. Sodium or potassium phosphate can be given in amounts of 1–4 g a day for phosphate depletion and if unsuccessful, vitamin D can be given.

Mitochondriopathies

The mitochondria (mt) have a major role in fatty acid oxidation, tricarboxylic acid cycle, urea cycle, and ATP production through the process of oxidative phosphorylation. Oxidative phosphorylation occurs at the level of the respiratory chain in the inner membrane of the mt (77). The respiratory chain comprises five components (*Fig. 42-5*). Complex I (NADH-coenzyme Q reductase) carries reducing equivalents from NADH to coenzyme Q and consists of different polypeptides, seven of which are encoded by mitochondrial DNA (mtDNA). Complex II (succinate-coenzyme Q reductase) carries reducing equivalents from $FADH_2$ to coenzyme Q and contains five polypeptides that are all encoded only by mtDNA. Complex III (reduced coenzyme Q-cytochrome c reductase) carries reducing equivalents from coenzyme Q to cytochrome c and contains 11 subunits, one of which is encoded by mtDNA. Complex IV (cytochrome c oxidase) transfers reducing equivalents from cytochrome c to oxygen. This complex is composed of cytochromes a and a3, and 13 protein subunits, three of which are encoded by mtDNA. The fifth complex is ATP synthetase.

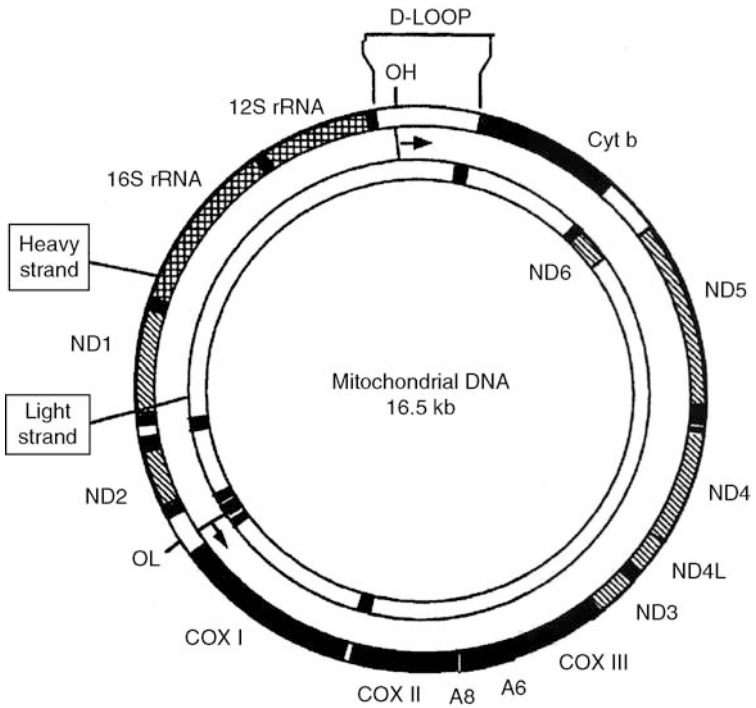
Oxidative phosphorylation consists of oxidative reactions that lead to oxygen consumption and phosphorylation of ADP to ATP. Each mt have its own 2–10 mtDNA. MtDNA genome is a 16.5 kb circular double stranded DNA with an asymmetrical base composition. The heavy strand contains more guanine residues while the light strand contains more cytosine residues. The mtDNA contains 37 genes; 13 encode for polypeptides of the respiratory chain, 2 for the ribosomal RNAs and 22 for transfer RNAs (78). Mitochondrial injury is due to congenital insults or may be the result of secondary events. Genetic defects of one or several polypeptide enzyme complexes of the oxidative phosphorylation system in the mt DNA or nuclear DNA which encodes structural or functional mitochondrial proteins can give rise to mitochondriopathies (mitochondrial cytopathies, mitochondrial diseases) (79).

Mitochondriopathies are multi-systemic disease and may begin at any age. Affected organs are diverse including central nervous system, muscle, liver, heart, kidney, gut, endocrine system, bone marrow, ear, eye, and skin (*Table 42-2*). They display extreme heterogeneity, and make unpredictable the extent and manifestations of disease presentation (80). With the course of the disease, the numbers of organs involved are increased.

The screening for mitochondriopathies includes the determination of plasma lactate, pyruvate, keton bodies and their ratios in fasted and fed patients, polarographic, and spectrometric studies to evaluate the different

Figure 42-5

Map of mitochondria genome. Regions encoding cytochrome b (ctb b), various subunits of NADH-coenzyme Q reductase (ND), cytochrome c oxidase (COX), ATPase, and ribosomal RNAs (rRNA) are indicated (Niaudet P, Rötig A. The kidney in mitochondrial cytopathies. *Kidney Int* 1997;51:1000–1007).



enzymatic complexes of the respiratory chain, muscle histologic studies, and genetic analysis (4).

Renal disease may be the first sign of mitochondriopathies, or it may appear simultaneously with neurological and neuromuscular signs (81). FS is particularly frequent in newborns, infants, and young children (82–84), whereas tubulointerstitial nephropathy is more frequent in children and young adults (85, 86), and can be associated with hereditary focal segmental glomerulosclerosis due to the mitochondrial transfer RNA gene mutation (87–89) and collapsing glomerulopathy due to the mutations in the gene *COQ2* encoding the parahydroxybenzoate-polyprenyl-transferase enzyme of the CoQ10 synthesis pathway (CoQ2 nephropathy) and in the gene *PDSS2* encoding for decaprenyl diphosphate synthase (90, 91).

Most patients with FS due to mitochondriopathies manifest moderate FS including failure to thrive, dehydration, aminoaciduria, glucosuria, proteinuria, LMW proteinuria, phoshaturia, uricosuria, hypercalciuria, and bicarbonaturia. Many patients manifest FS by the age of 2 years. Extra-renal manifestations including neurological symptoms, myopathy, hepatic dysfunction, clinical features of Pearson syndrome, partial adrenal insufficiency, cardiac involvement, diabetes mellitus, deafness, and ophthalmoplegia often manifest in the patients (12, 92, 93). Patients who manifested proximal tubular acidosis with hypercalciuria or Bartter syndrome were described (94, 95). Some patients manifest progressive renal failure (96). Histological analysis reveals tubular dilatations, tubular atrophy and cytoplasmic vacuolization of the tubules. Bizarre giant mitochondria are frequently observed (97).

No satisfactory treatment is presently available to alter the course of mitochondriopathies. The treatment is mainly symptomatic: supplements of sodium bicarbonate, potassium, vitamin D3, phosphate, and water are necessary. Carnitine is given in case of secondary carnitine deficiency. It includes avoidance of drugs that interfere with the respiratory chain such as valproate and barbiturates, or that inhibit mitochondrial protein synthesis such as tetracyclines and chloramphenicol. Dietary recommendations include a high lipid and low carbohydrate diet in patients with complex I deficiency. Hypercaloric diet and parenteral nutrition should be avoided in these patients.

Table 42-2
Clinical symptoms in patients with mitochondriopathies

Affected organs	Symptoms
Central nervous system	Apnea, lethargy, hypotonia, coma, pshycomotor retardation, cerebellar ataxia, stroke-like episodes, myoclonus, seizures, dementia, spasticity, headache, hemiparesis
Muscle	Myopathy, poor head control, limb weakness, myalgia, exercise intolerance
Liver	Hepatomegaly, liver dysfunction
Heart	Cardiomyopathy, arrhythmia
Kidney	Fanconi syndrome, tubulointerstitial nephropathy, nephrotic syndrome (focal segmental glomerulosclerosis, collapsing nephropathy), renal failure
Gut	Vomiting, diarrhea, villous atrophy, cholonic pseudoobstruction, pancreatic dysfunction
Endocrine	Diabetes mellitus, growth hormone deficiency, hypoparathyroidism, hypothyroidism, adrenal insufficiency
Bone marrow	Sideroblastic anemia, neutorpenia, thrombocytopenia
Ear	Hearing loss
Eye	Progressive extrarenal ophthalmoplegia, pigmentary retinal degeneration, ptosis, diplogia, cataract
Skin	Mottled pigmentation, trichothiodystrophy

Cystinosis

Cystinosis is reviewed in detail in Chapter 41 of this textbook, and therefore only a short description is included here. Cystinosis is an autosomal recessive lysosomal storage disorder characterized by an accumulation of cystine, the disulfide of the amino acid cysteine, in the systemic organs, notably kidney, cornea, bone marrow, thyroid, lymph nodes, liver, and spleen (98). Renal manifestations dominate the clinical presentation and course in infantile cystinosis. Cystinosis is the most common familial form of the FS in Western countries. Many patients, particularly in North America, have blonde or reddish-blonde hair. Other organs frequently affected include the cornea and thyroid, causing painful photophobia and hypothyroidism respectively.

Various clinical forms of the disease exist and are based on age at onset and severity of the symptoms (*Table 42-3*). The most severe form, infantile cystinosis, is manifested by failure to thrive, polyuria, polydypsia, dehydration, fluid and electrolyte loss, aminoaciduria, glucosuria, phosphaturia, renal tubular acidosis between 6 and 12 months of age. Some of the patients may develop vitamin D-resistant rickets due to phosphaturia and manifest severe growth failure. Renal function is generally normal at presentation. However, subsequent glomerular impairment leads to focal segmental glomerulosclerosis and eventually to end stage renal failure by 10 years of age without treatment (99). Patients with infantile cystinosis manifest FS, including hyperchloremic metabolic acidosis, aminoaciduria, hypokalemia, hypophosphatemia, glucosuria, and phosphaturia. There have been several patients of nephropathic cystinosis presenting with features of secondary Bartter syndrome (hypokalemia, hyperchloremic metabolic alkalosis, hyperreninemia, and hyperaldosteronism), suggesting abnormalities of Na^+ and Cl^- reabsorption (100, 101). Patients with cystinosis often manifest medullary nephrocalcinosis (102).

Renal histopathologic changes in infantile cystinosis include severe lesions of proximal tubules; typical alterations to the glomerular podocytes, which become multinucleated giant cells; and the presence of cystine crystals, mostly in interstitial cells and podocytes (103). The proximal tubule is the first clinical target of the disease, but cystine crystals are rarely found in the tubular cells of patients with cystinosis. Cystine crystal deposition in the cornea leads to photophobia. Continuous widespread cystine accumulation eventually leads to rickets and retinal, endocrinologic (hypothyroidism and impaired glucose tolerance), hepatic, gastrointestinal, muscular, and neurological abnormalities.

Two less severe and less common form of cystinosis are juvenile (or late-onset) and ocular cystinosis. Patients with juvenile cystinosis manifest glomerular impairment between 12 and 15 years of age but do not suffer from severe tubulopathy or growth failure. Progression to end stage renal failure is slow and occurs at

Table 42-3
Clinical manifestations of infantile cystinosis

At presentation
Common
Failure to thrive
Polyuria and polydipsia
Fanconi syndrome
Vitamin D resistant rickets
Progressive renal failure
Photophobia
Hypothyroidism
Uncommon[a]
Bartter syndrome
Nephrotic syndrome
Diabetes insipidus
Pot-renal transplantation
Dysphagia
Myopathy
Exocrine pancreatic insufficiency
Diabetes mellitus
Central nervous system deterioration
Primary hypogonadism

Adapted from Gahl et al. (114)
[a]may be transient and coexist with common manifestations

variable ages (104). Patients with ocular cystinosis do not involve kidney.

Infantile cystinosis is caused by mutations of the *CNTS* gene encoding cystinosin, a lysosomal transport protein, leading to complete abolition of cystine transport (105). Cystinosin has 367 amino acids and seven transmembrane domains. Cystine transport is dependent on the pH gradient, and the transport of cystine out of the lysosome is driven by the high H^+ content within the lysosomal lumen that is produced by the activity of the H^+-ATPase. A range of mutations in *CNTS* gene has been described, but a single mutation, a 57-kb intragenic deletion, accounts for as many as three quarters of all European cases (105). The adolescent and ocular forms have one severe and one mild *CTNS* mutation, leading to reduced transport activity. The sparing of the kidney in patients with ocular cystinosis reflects tissue-specific expression of splicing factors, or the increased endogenous level of *CTNS* mRNA normally seen in the kidney (106). Individuals who are heterozygous for severe *CTNS* mutations reveal elevated levels of leukocyte cystine but are completely asymptomatic.

The pathophysiology of tubular cystine transport defects in patients with cystinosis is poorly understood, reflecting of an animal model for the disease. Knock-out mice model lacking cystinosin gene do not manifest signs of FS, despite accumulation of lysosomal cystine in the proximal tubules (107). Cystine-loaded proximal tubular cells demonstrate loss of free phosphate and defective ATP production and inhibition of Na^+-dependent transporters (108). ATP depletion can reduce proximal tubular Na^+, K^+-ATPase activity leading to increased Na^+ delivery into the distal tubules and Bartter syndrome (101). A cell culture demonstrated that cells accumulated with intracellular cystine undergo apoptosis at a rate two- to four-fold higher than controls (109). Another works suggests that increased oxidative stress and altered redox status in proximal tubule cells cultured from the urine of patients with cystinosis are associated with proximal tubule dysfunction (110).

The diagnosis of cystinosis is confirmed by demonstrating elevated cystine levels in peripheral leukocytes (97). Corneal crystals detected by slit-lamp examination are diagnostic in childhood cystinosis because these crystals are not seen in patients with other hereditary FS. However, this finding is not sensitive for early diagnosis. The renal pathologic findings in infantile cystinosis consist of a chronic tubulointerstitial nephropathy, with characteristic multinucleated podocytes and intracellular crystalline inclusions in interstitial histiocytes (111). Although numerous multinucleated podocytes are the most characteristic pathologic findings, they are not found in the sclerotic glomeruli and detected only in low frequency (<4%). The cystine crystals are birefringent under polarized light in only alcohol-fixed tissue or in unfixed frozen tissue, because they are water-soluble and not retained in the tissue after routine histologic preparation with aqueous solutions (112).

The management and treatment for infantile cystinosis involve supportive therapy to maintain fluid balance and replace electrolyte losses at initial presentation. Early diagnosis and oral cysteamine, a cystine-depleting agent, can delay the progression of end stage renal failure and other organ involvement. Oral cysteamine therapy given at doses of 60–90 mg kg^{-1} of body weight (or between 1.3 and 1.95 g m^{-2}) a day divided every 6 h generally achieves approximately 90% depletion of cellular cystine, as measured in circulating leukocytes (<1.0 nmol half-cystine/mg protein) (113). The dosage recommended for adults is 500 mg every 6 h, but higher dosages are often required to achieve satisfactory cystine depletion. On the basis of its beneficial effects in maintaining thyroid function and depleting muscle of cystine, oral cysteamine

therapy should continue in patients after renal transplantation to help preserve other organs. Administration of 0.55% cysteamine eye-drops, given 6–12 times a day, can dissolve corneal cystine crystals and lessen visual symptoms (114). Other therapies to supply potassium, alkalinizing agents including citrate or bicarbonate, phosphate, and vitamin D3 are required. When the growth velocity has not improved and the patient remains below the 3rd percentile for height after one year of therapy, growth hormone therapy may be considered.

Galactosemia

Galactosemia is an autosomal recessive disease of galactose metabolism. Nursing infants must move large amounts of galactose through Leloir pathway in order to utilize the carbon skeletons for energy (*Fig. 42-6*). Galactose is the preferred carbon source in mammalian neonates, since it is incorporated into glycogen more efficiently than is glucose (115).

The most frequent form is classic galactosemia that is due to the deficient activity of galactose-1-phosphate uridyl-transferase (GALT) encoded by *GALT1* (116). GALT catalyzes the reaction of galactose-1-phosphate (gal-1-p) plus uridine diphosphate glucose to uridine diphosphate galactose plus glucose-phosphate. Uridine diphophate galactose can be further metabolized to either glucose or CO_2 and H_2O via glycolysis. Milk is a major source of galactose. Accumulated gal-1-p due to defective GALT and exposure to galactose lead to acute deterioration of multiple organ systems, including liver, kidney, ovary, brain, and eye. Affected infant patients manifest vomiting, diarrhea, failure to thrive, developmental delay, liver dysfunction, coagulopathy, renal tubular dysfunction, cerebral edema, vitreous hemorrhage, and *Escherichia coli* sepsis. They sometimes manifest jaundice and unconjugated hyperbilirubinemia and may have severe hemolysis. Liver damage leads to hepatomegaly and cirrhosis that is potentially lethal. Neonatal screening program includes galactosemia, anticipating that early detection and intervention would prevent long-term complications such as mental retardation, premature ovarian failure, and speech delay. Although a galactose-restricted diet prevents the neonatal death, many well-treated patients continue to develop debilitating complications (117, 118). Clinically evident speech delay and cerebellar signs are more frequent than other findings. Premature ovarian failure is nearly universal in females with galactosemia. The predominant manifestation due to kidney damage is FS including hyperaminoaciduria, LMW proteinuria, hyperphosphaturia, and bicarbonaturia (118). Patients placed on a galactose-restricted diet are never truly free of galactose insult, as a significant amount of galactose is found in non-dairy foodstuffs such as vegetables and fruits (119, 120). More importantly, galctose moieties can be produced endogenously from UDP-glucose via the UDP-4-galactose epimerase reaction, and natural turnover of glycoproteins/glycolipid; the rate of endogenous galactose synthesis ranges from 0.53–1.05 mg kg^{-1} of body weight a day (121, 122). Once the lactose is formed intracellularly, it will be converted to gal-1-p by GALT. The less common form of galactosemia is a deficiency of galactose kinase (GALK), which forms gal-1-p from galactose. These patients do not manifest either the acute toxicity syndrome or chronic complications seen in patients with classic galactosemia. They manifest cataracts. Since GALK-deficient patients do not accumulate gal-1-p in their tissues, gal-1-p is considered to play a significant role in the pathogenesis of classic galactosemia (123, 124). GALT deficiency results in accumulation of toxic galactose leading to the unfolded proteins, altered calcium homeostasis and subsequently endoplasmic reticulum (ER) stress (125). ER stress caused by GALT-deficiency might contribute to accelerated apoptosis seen in the granulose cells maturing follicles in galactosemic females, leading to premature ovarian failure (126). Formation of galactitol from galactose by aldose reductase has been proposed as a pathogenetic mechanism and is at least responsible for cataract formation.

The diagnosis is suggested by galactose or galactose 1-phosphate in serum, or in the urine. The diagnosis is confirmed by demonstrating deficient GALT activity in red blood cells, fibroblasts, leukocytes, or hepatocytes.

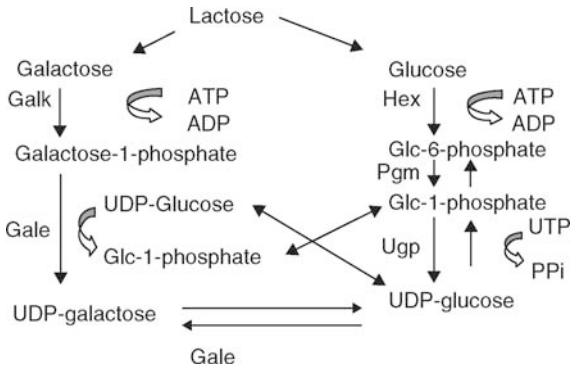
Treatment of this disorder is elimination of galactose from the diet. Acute symptoms and signs resolve within a few days after starting the diet therapy. However, developmental delay, speech disturbance, ovarian dysfunction, and growth retardation are common outcomes in this disorder (127).

Hereditary Fructose Intolerance

Hereditary fructose intolerance (HFI) is an autosomal recessive disorder caused by a deficiency of aldolase B, an enzyme of liver, intestine, and renal cortex catalyzing the metabolism of fructose of exogenous origin (128). Frequency of HFI is estimated at 1 in 20,000 live births. Aldolase B catalyses the specific and reversible cleavage of

Figure 42-6

Composite diagram of the Leloir pathway and uridine diphosphate (UDP)-glucose pyrophosphorylase pathway. *Galk*, galactokinase; *galt*, galactose-1-phosphate uridyltransferase; *Gale*, UDP-galactose 4-epimerase; *Hex*, hexokinase; Pgm, phosphoglucomutase; *Ugp*, UDP-glucose pyrophosphorylase (Leslie ND. Insights into pathogenesis of galatosemia. *Annu Rev Nutr* 2003;23:59–80).



fructose-1,6-bisphophate (FBP) and fructose-1-phosphate (F1P) into dihydroxyacetone phosphate and D-glyceraldehyde-3-phophate, or D-glyceraldehyde, respectively. Aldolase B is equally active with FBP and F1P, whereas aldolase A and aldolase C, the other two vertebrate isozymes, are more active with FBP than with F1P. Aldolase B is encoded in Aldolase B gene *(ALDOB)* mapped to chromosome 9q21.3–q22.2 (129, 130). Missense and nonsense mutations, large and small gene deletions and mutations in the splicing region have been identified in *ALDOB* of HFI patients (131).

Affected individuals manifest symptomatic hypoglycemia, vomiting and life-threatening episodes shortly after the intake of fructose or related sugars including sucrose and sorbitol (132). Prolonged ingestion leads to failure to thrive, hepatomegaly, jaundice, hepatic cirrhosis, and nephrocalcinosis, and may lead to convulsions, coma, and death from severe liver and kidney failure. Symptoms of HFI appear during infancy when infants with HFI are fed a formula or foods including fruits, vegetables, and sweetened cereals that contain sucrose. Patients with HFI may develop a protective aversion to sweets and fruits, which is a reason that diagnosis is frequently missed, and which also explains that reliable prevalence numbers for different populations do not exist.

HFI is associated with proximal tubule dysfunction leading to aminoaciduria, bicarbonaturia, phosphaturia, and lactic acidosis. These manifestations appear rapidly after the ingestion of fructose (133, 134). The development of lactic acidosis adds significantly to the metabolic acidosis (135). Chronic fructose ingestion leads to nephrocalcinosis and impaired distal tubular function. In contrast, resolution of proximal tubule dysfunction can take days or weeks with strict restriction of fructose and sucrose (136).

Aldolase B coexists abundantly in endocytosis zones of the proximal tubule cells with H^+-ATPase (137). Nonfunctional aldolase B impairs the coupling of H^+-ATPase to glycolysis and endosomal acidification that will lead to FS.

Diagnosis includes the metabolic response to an intravenous fructose load or an enzymatic assay of liver or intestinal biopsy samples. However, both of them are bothering and invasive (138). Fructose breath hydrogen test is one of the standard procedures for the diagnosis. However, it can develop life-threatening adverse effects during the test (139). Molecular analysis is available for the diagnosis.

Strict avoidance of foods or drugs containing fructose, sucrose, and sorbitol is the predominant treatment.

Glycogen Storage Disease Type I (von Gierke Disease)

Glycogen storage disease type I (GSD-I) is a group of autosomal recessive disorders with an incidence of 1 in 100,000. There are two major subtypes. Glycogen storage disease type Ia (GSD-Ia, von Gierke disease) is common and is caused by s deficiency in glucose-6-phosphatase-alpha (G6Pase-alpha), a key enzyme in glucose homeostasis that catalyzes the hydrolysis of glucose-6-phosphate (G6P) to glucose and phosphate in the terminal step of gluconeogenesis and glycogenolysis (140). G6Pase-alpha is a hydrophobic endoplasmic reticulum-associated transmembrane protein. Glycogen storage disease type Ib (GSD-Ib) is caused by a deficiency of microsomal glucose-6-phosphatase transporter (G6PT). G6PT translocates G6P from cytoplasm to the lumen of the endoplasmic reticulum. Therefore, G6PT and G6Pase-alpha work in concert to maintain glucose homeostasis. Whereas G6Pase is exclusively expressed in gluconeogenic cells, G6PT is ubiquitously expressed and its deficiency generally causes a more severe phenotype.

Patients with GSD-Ia manifest a phenotype of disturbed glucose homeostasis characterized by fast life-threatening hypoglycemia, hepatomegaly, nephromegaly, hypercholesterolemia, hypertriglyceridemia, hyperuricemia, lactic acidemia, neutrophilia, and growth retardation (141, 142). Infants with GSD-Ia typically present with

seizures and hepatomegaly at 6–8 months of age. Approximately 75% of adolescent and adult patients develop hepatocellular adenoma (HCA), which can lead to considerable morbidity and mortality (143). The incidence of HCA to hepatocellular carcinoma is recently increasing because the patients can live longer than before (144). The presence of GSD-Ia and GSD-Ib are associated with reduced quality of life, independent functioning, and elevated levels of internalizing distress, and parental stress relative to healthy peers.

Renal complications include renal enlargement, gout nephropathy, renal stones, nephrocalcinosis, Fanconi-like syndrome, and chronic renal disease leading to renal insufficiency (145). Hepatomegaly is a common finding in GSD-Ia. Hyperuricemia and uric acid stone in GSD-Ia are explained by a combination of increased synthesis of purine and a competitive inhibition of renal tubular excretion of uric acid (urate) by lactate (146). Proximal tubular dysfunction has been observed in patients with GSD-Ia. Patients manifest proximal renal tubular acidosis due to loss of bicarbonate in the urine, hyperphosphaturia, generalized aminoaciduria and increased excretion of beta 2-microglobulin which are ameliorated by intensive diet therapy (147, 148). This finding suggests that good metabolic control can prevent proximal tubular dysfunction. Chronic renal disease is a long-term complication. Renal biopsies reveal interstitial fibrosis, tubular atrophy, and focal segmental glomerulosclerosis with marked glomerular basement membrane (GBM) thickening and lamellation in patients with GSD-Ia (149–151). Glycogen granules are present in the areas of abnormal GBM. The glycogen content in the mesangium and in the epithelial, mesangial and endothelial cells is increased. Recent treatment has significantly alleviated the metabolic abnormalities and delayed the clinical manifestation of chronic renal disease and renal insufficiency in patients with GSD-Ia. However, glomerular hyperfiltration, hypercalciuria, hypocitraturia that worsens with age, and urinary albumin excretion still occur in metabolically compensated patients with GSD-Ia (152, 153). Although the molecular mechanism responsible for chronic renal disease is still poorly understood, activation of the angiotensin system is suggested to have an important role for the disease progression (154). The expression of TGF-beta 1 in kidney tissue is increased in a patient with GSD-Ia manifesting proteinuria, interstitial fibrosis, and tubular atrophy (155).

The objective of treatment is to maintain normoglycemia to avoid metabolic complications and lactic acidosis. Normoglycemia is accomplished at night with nasogastric feeding of glucose or with orally administered uncooked cornstarch (156). A single dose (1.75–2.5 g kg^{-1} of body weight) of uncooked cornstarch will maintain serum glucose concentration higher than 3.9 mmol L^{-1} for 7 h in most young adults (157, 158).

Liver transplantation is indicated in the patients when medical treatment fails to control the metabolic problems or when HCA or hepatocellular carcinoma develops. Living-donor liver transplantation is a viable option to restore normal metabolic balance and keeping normal renal function (159). Hepatocyte transplantation can be a potential therapeutic intervention to prevent hypoglycemia despite the discontinuation of cornstarch meals (160).

Fanconi-Bickel Syndrome

Fanconi-Bickel syndrome (FBS) is an autosomal recessive disorder characterized by failure to thrive, "doll-like" face, hepatomegaly, nephromegaly, and severe rickets. Patients with FBS manifest glycogen accumulation in hepatocytes and proximal tubular cells, fasting hypoglycemia, galactose intolerance, and FS including glucosuria, aminoaciduria, hyperuricosuria, hyperphosphaturia, proteinuria, and sodium and potassium wastage (161, 162). Some patients manifest cataracts in neonatal period (163). Overall prognosis of FGS is considered as favorable (164). However, some patients manifest neonatal diabetes mellitus and galactosemia and die of hepatic failure during infancy (165).

FBS is caused by the mutations in facilitative glucose transporter gene (*SLC2A2*, also referred to as *GLUT2*) expressed in liver, kidney, intestine, and pancreatic islet cells (166). Over 60 mutations in *SLC2A2* were reported (167). This facilitative glucose transporter is expressed in hepatocytes, pancreatic beta cells, and renal and intestinal epithelial cells and is important for the exchange of glucose between these cell types and the bloodstream (168). Renal histology reveals an increase in mesangial cellularity, glomerulosclerosia, and patchy swelling of epithelial foot process and irregularly thickened lamina rara interna in the glumeruli, and vacuolization of epithelial nuclei in the proximal tubule cells suggesting the presence of glycogen in a 7 year-old patient with FBS (169).

The therapy for FBS is directed at the renal solute losses including sodium bicarbonate and potassium-sodium phosphate; treatment of rickets including active vitamin D3; and frequent feeding including night-time supplementation to prevent ketosis. Uncooked cornstarch has been shown to lessen hypoglycemia and improved growth (170). Galactose–free milk is also used for infant patients (165, 171).

Tyrosinemia I

Hereditary tyrosinemia type I (TI) is an autosomal recessive disorder of an amino acid metabolism. TI is due to the defect in the fumarylacetoacetate hydrolase (FAH) gene (172, 173). FAH is the last enzyme in the tyrosine catabolic pathway.

Patients with TI display a variety of clinical symptoms, such as liver damage from infancy that advances to cirrhosis, reduced coagulation factors, hypoglycemia, high plasma concentrations of methionine, phenylalanine, and aminolevulinic acid, high risk of hepatocellular carcinoma, and tubular and glomerular renal dysfunction (174).

Progressive renal damage begins from early infancy in severe form. Chronic liver damage with a high incidence of hepatoma (hepatocellular carcinoma) is characteristic in milder form (175). Even a patient without clinical manifestations of TI can manifest hepatoma during childhood (176). Accumulated fumarylacetoacetate in the patients with TI is pathogenic for hepatoma. Patients with milder form of TI are at risk for acute exacerbation of liver dysfunction. A common presentation mode is the "acute hepatic crisis" in which ascites, jaundice, and gastrointestinal bleeding are precipitated by an acute event such as an infection. Acute hepatic crises usually resolve spontaneously but on occasion progress to complete liver failure and encephalopathy. Acute, painful peripheral neuropathy may appear and can lead to transient paralysis. Autonomic dysfunction with hypertension and tachycardia can be associated with this acute neuropathy (177). Plasma tyrosine and methionine levels usually are elevated in untreated patients. The presence of succinylacetone in plasma and urine is diagnostic of TI. A rapid ultra performance liquid chromatography tandem mass spectrometric method is used for mass screening of tyrosinemia (178).

FS and developmental hypophosphatemic rickets are features of the kidney involvement. Generalized aminoaciduria, renal tubular acidosis, and mild proteinuria are also often seen, whereas glucosuria is less common because plasma glucose levels are low. Kidney enlargement is common, and nephrocalcinosis can be seen (179). FS leads to carnitine deficiency (180). Glomerulosclerosis and impaired GFR may be seen with time.

Disturbances in tyrosine metabolism lead to increased levels of succinylacetone and succinylacetoacetate. However, the mechanisms causing liver failure, cirrhosis, renal tubular dysfunction, and hepatocarcinoma are still unknown. Apoptosis of hepatocytes and renal tubular epithelial cells are characteristic features of this disease and the apoptotic signal in this disease seems to be initiated by fumarylacetoacetate (181, 182). Accumulated maleylacetoacetate and fumarylacetoacetate in affected tissues can react with free sulfhydryl groups and reduce intracellular levels of glutathione. They may be capable of acting as alkylating agents. Maleylacetoacetate and fumarylacetoacetate are not detectable in plasma or urine but are converted to succinylacetoacetate. Succinylacetone, a metabolite of succinylacetoacetate, is structurally similar to maleic acid, which is known to induce FS and may be the cause of tubular dysfunction of HI. Experimentally, succinylacetone administration to rats leads to FS (183, 184).

Treatment with a low-phenylalanine and low-tyrosine diet dramatically improves the renal tubular dysfunction (185). However, this treatment cannot necessarily improve the hepatic involvement. Moreover, there is a risk of inducing deficiencies of phenylalanine or tyrosine. The formation of pathogenic fumarylacetoacetae is prevented by 2- (2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione (NTBC). NTBC is used for the patients with TI during the first 6 months of life in addition to a diet low in tyrosine and phenylalanine. NTPC clearly improves the vital prognosis and quality of life in the patients (186). However, some patients with NTBC treatment develop hepatoma. A rise of alpha-fetoprotein (AFP), a slow AFP decrease, and never normalizing levels of AFP are important predictors of hepatoma development (187). Liver transplantation has been used for patients with liver failure and to prevent the development of hepatoma (174). Liver transplantation leads to rapid correction of FS (188).

Wilson Disease

Wilson disease (WD, progressive hepatolenticular degeneration) is an autosomal recessive inborn error of copper (Cu) metabolism that affects numerous organ systems (189). Biliary excretion of Cu and incorporation into ceruloplasmin is impaired, leading to liver damage, neuronal degeneration, and impairment of other organs from accumulation of Cu in patients with WD.

The majority of patients with WD presents with either predominantly hepatic or neuropsychiatric symptoms, and with either clinically asymptomatic or symptomatic liver involvement. Approximately 40% of patients presents with liver disease, 40% with extrapyramidal symptoms, and 20% with psychiatric or behavioral abnormalities. Symptoms rarely occur before 6 years of age. Hepatic involvement includes acute hepatitis, fulminant hepatic failure, or progressive chronic liver disease in the form of either chronic

active hepatitis or cirrhosis of the macronodular type. Neuropsychiatric involvements are variable. The most common initial presentation is bulbar symptoms characterized by difficulties with speech and swallowing, and drooling. They frequently manifest dysarthria and coordination defects of voluntary movements accompanied by involuntary movements. One third of the patients with WD manifest psychiatric disturbances. The remaining patients with WD may present with symptoms including hemolytic anemia, bone fracture, arrhythmias, FS, hyperpigmentation, Kayser-Fleisher ring, cataract, and gynecological problems that are attributable to the involvement of the organs.

WD is caused by a mutation in the gene *ATP7B* that encodes a P-type Cu transporting ATPase beta polypeptide enzyme (ATP7B) (190). This ATPase is targeted to the mitochondria, suggesting that its role in Cu dependent processes takes place in this organelle. The disease frequency is estimated to be between 1 in 5,000 and 1 in 30,000, and the carrier frequency is approximately 1 in 90 (191).

Cu is absorbed by the intestinal cells and stored with metallothionein in a non-toxic form. The Cu is later delivered into the circulation by a Cu transporter 15,000 amino acid protein, Cu-transporting P-type ATPase 1 (ATP7A), which is located on the membrane of enterocytes (› *Fig. 42-7*) (192). It is then transported to the liver tagged with albumin, from where it is accepted by hepatocytes. The ATOX1 chaperone protein directs Cu to its binding targets in the hepatocytes. Some of Cu bounds to metallothionein for storage, and the remainders are excreted into ATP7B-regulated biliary canaliculi. ATP7B also mediates the transfer of Cu to apoceruloplasmin to form a six-Cu binding protein, ceruloplasmin (193). Ceruloplasmin is released into the blood, carries 90% of the Cu present in the plasma, and acts as a source for peripheral organs. Mutations of the *ATP7A* gene result in the storage of Cu in enterocytes, preventing entry of Cu into the circulation and thus causing a complete Cu deficiency (Menkes kinky hair disease) (192).

Mutations in *ATP7B* gene lead to a reduction in the conversion of apoceruloplasmin into ceruloplasmin, which is usually present at low levels in the patients of WD. A failure to excrete Cu into the biliary canaliculi leads to toxic effect to hepatocytes. Excess Cu damages mitochondria, which produce oxidative damage to the cells and allows spillage of Cu into the blood, thereby overloading other tissues including the brain, kidney, and red blood cells, initiating toxic effects.

Excessive accumulation of Cu in the kidney leads to renal tubular dysfunction in patients with WD. Patients show most features of FS before the onset of hepatic failure and is characterized by intermittent glucosuria, aminoaciduria, hyperphosphaturia, hyperuricosuria, and proteinuria (194–196). Patients can manifest rickets or osteomalacia. hypercalciuria, urolithiasis, nephrocalcinosis, decreased urine concentrating ability and distal renal tubular acidosis are reported (197). Glomerular function decreases as the disease progresses, but death from extrarenal causes occurs before the onset of renal failure.

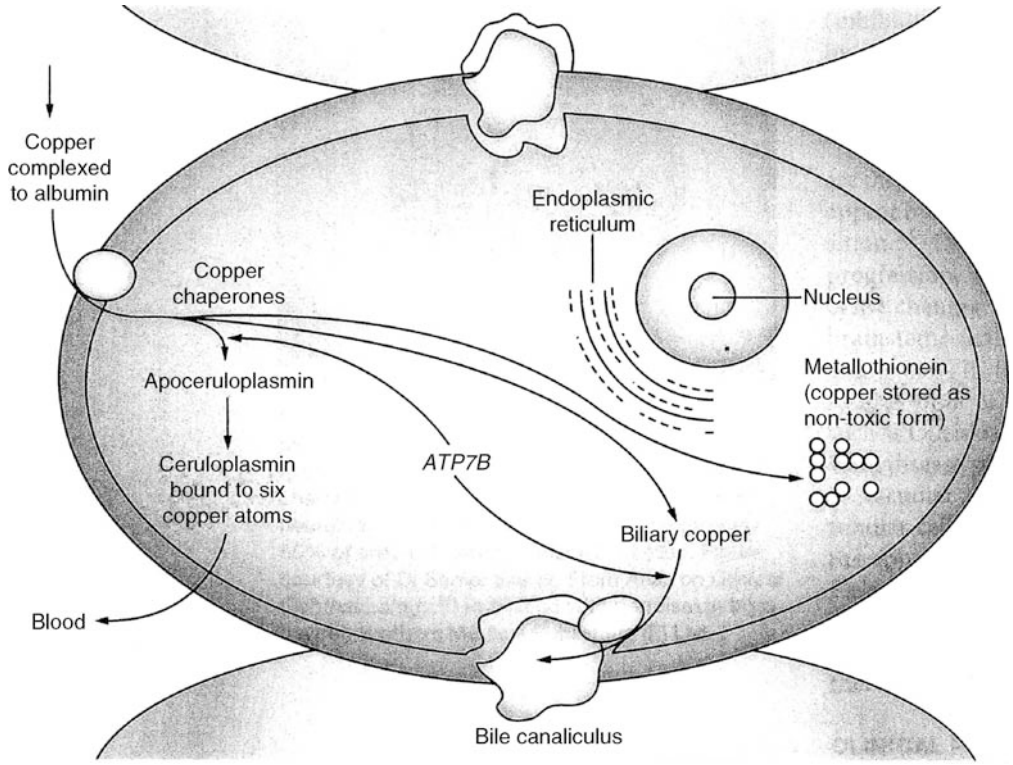
Histological analysis of the patients with WD revealed no alteration on light microscopy or only flattened proximal tubule cells without recognizable brush border (198). Electron microscopy disclosed loss of the brush borders, electron dense deposit in the subapical region of tubular cytoplasm probably representing metalloproteins, and cavitation of the mitochondria with disruption of the normal cristae pattern (199).

All subjects presenting with symptomatic or asymptomatic liver disease with no apparent causes or with extrapyramidal features along with a past or family history of similar hepatic or neurological illnesses in other siblings should be screened for WD (200). Measurement of the serum ceruloplasmin is valuable for diagnosis. Any value below 200 mg L^{-1} is abnormal, and reduced levels are seen in up to 95% of the patients with WD. An estimation of 24 h urinary Cu excretion is another reliable test for the diagnosis of WD. Normal excretion is between 20 and 50 μg a day, and Cu excretion is increased to in excess of 100 μg a day in patients with WD. Serum free Cu is a measure of nonceruloplasmin toxic Cu in the blood, and normal value range from 1.3 to 1.9 μmol L^{-1} (8–12 μg dL^{-1}) in parallel with the increased urinary Cu excretion, because of saturation of the hepatic storage of Cu. A hepatic biopsy and a measurement of its Cu content are helpful for diagnosis. 80% of the patients manifest increased hepatic Cu (>250 μg/g of dry tissue weight). Genetic analysis of *ATP7B* gene is a helpful confirmation of WD. Brain MRI is a very sensitive method for revealing abnormalities in patients with WD. Generalized brain atrophy and hyper-intensity in the basal ganglia, white matter, thalamus, or brainstem are common findings in the patients. Patients exhibit characteristic features on MRI; "face of the giant panda" is seen in the midbrain and "face of the miniature panda" is seen in the tegmentum region of the pons in T2-weighted images (201, 202).

WD has a fatal outcome if not treated appropriately and in a timely manner. The aim of treatment for WD is to remove the toxic deposit of Cu from the body to produce a negative Cu balance, and to prevent its reaccumulation (190, 203). Successful therapy is measured in terms of a restoration of normal levels of free serum Cu and its excretion in the urine. The average daily diet

Figure 42-7

Schematic representation of cooper metabolism within a liver cell. *ATP7b*, causative gene for Wilson disease. (Das SK, Ray K. Wilson's disease: an update. *Nat Clin Pract Neurol* 2006;2:482–493).



contains 2–4 mg of Cu, and 0.8 mg is normally lost into the feces. Patients should avoid Cu-rich foods including chocolate, nuts, shellfish, mushrooms, and liver. D-penicillamine therapy (20 mg kg^{-1} of body weight a day) has been the most commonly used chelating agent that may reverses multiple tissue dysfunction including FS. However, use of D-penicillamine has been questioned, because of reported side effects. The side effects from D-penicillamine can occur both early and late in the treatment period. Early side effects include a hypersensitivity reaction characterized by fever, skin rash, and lymphadenopathy. Delayed side effects including Goodpasture's syndrome, polymyositis, systemic lupus erythematosus, and bone marrow suppression are caused by immunological reactions. Trientine is another effective chelating agent (750–2,000 mg a day for adults). Ammonium tetrathiomolybdate (2–3 mg kg^{-1} of body weight a day in six doses along with meals and in the interval between meals) is a potent agent in removing Cu from the body and may be the drug of choice for patients with neurologic disease to prevent the immediate worsening of symptoms that can occur with D-penicillamine therapy. Zinc acetate or sulfate induces intestinal metallothionein and helps in the prevention of Cu absorption from the gut (3–5 mg kg^{-1} of body weight a day in three divided doses before meals). D-penicillamine should be taken 2 h after meals to avoid any interaction with the zinc. Zinc acetate or zinc sulfate has been used successfully in asymptomatic or presymptomatic affected family with WD, and is equally as effective as D-penicillamine in a group of patients predominantly with neurological disease (204, 205). The use of trientine, tetrathiomolybdate, and zinc has been advocated, although results of long-term trials are awaited. The best therapeutic approach remains controversial and there is no universally accepted regimen. Liver transplantation is effective for the patients with progressive live failure or acute liver failure. Liver transplantation is also indicated for patients with WD in whom medical therapy is ineffective. Symptomatic patients with WD require lifelong treatment, because an interruption to therapy or inadequate treatment can lead to fatalities within 9 months to 3 years (189, 203).

Idiopathic Fanconi Syndrome

Although the significant numbers of genetic causes for the disorders leading to FS are identified, there exist patients with idiopathic FS. Idiopathic FS should be diagnosed when all other known causes have been excludes. Idiopathic FS can be inherited as an autosomal dominant (206–211), autosomal recessive (212, 213), and X-linked form (214). However, most of the familial forms are autosomal dominant inheritance.

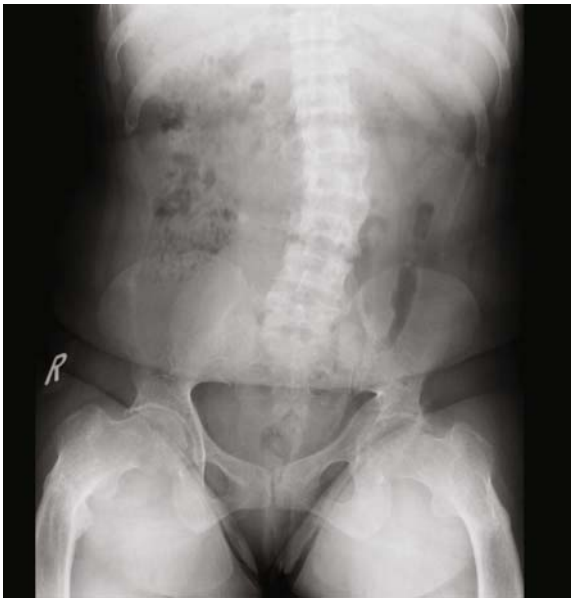
Children with idiopathic FS manifest failure to thrive, frequent bouts of dehydration, and rickets. They often have other features of FS, including polyuria, polydipsia, hypokalemia, hypophosphatemia, proximal renal tubular acidosis, aminoaciduria, glucosuria, and proteinuria. Glomerular filtration rate is usually normal during childhood. However, some develop chronic renal insufficiency or chronic renal failure 10–30 years after the onset of symptoms (208, 209, 213). Nephrocalcinosis and *genu vulgum* (knock knee) are seen in some patients (213). Bone pain, bone fracture, and scoliosis due to osteomalacia can become serious complications in adult patients with idiopathic FS (*Fig. 42-8*).

Renal histology reveals chronic tubulointerstitial fibrosis. The interstitium demonstrates patchy fibrosis associated wit tubular atrophy and focal collections of mononuclear inflammatory cells. Occasional cystically dilated tubules are seen containing eosinophilic proteinaceous material that stains positive for PAS (213).

Treatment for idiopathic FS remains symptomatic. Careful follow-up of these patients is necessary to prevent recurrent bouts of dehydration, electrolyte imbalance, and metabolic bone diseases. Glomerular function and nephrocalcinosis must be checked regularly. Renal transplantation has been done in a few patients who had end-stage renal failure (209).

Figure 42-8

Plain X-ray showing scoliosis and osteopenia in a 35-year-old female with idiopathic Fanconi syndrome.



Acquired Fanconi Syndrome

Nephrotic syndrome is associated with FS (215). The renal pathology is focal segmental glomerulosclerosis. Although the true pathogenesis is not clarified, mitochondriopathies can manifest FS, focal segmental glomerulosclerosis leading to nephrotic syndrome, and both (79–84).

Immunological or hematological disorders are associated with dysproteinuria leading to FS. They are multiple myeloma, Sjögren syndrome, and amyloidosis. Almost all of the patients with theses diseases are adults. In early stages of myeloma, light chain nephrotoxicity often presents with proximal tubular functional abnormalities leading to FS. Proximal tubule dysfunction is the most common mode of renal involvement and it can manifest in a variety of ways. Endocytosis in the proximal tubules is overloaded and cell stress responses that include phosphorylation of MAPKs, prominently, p38 MAPK, and nuclear transcription factors NF-kappa B, AP-1 are activated resulting in production of inflammatory and proinflammatory cytokines, TNF-alpha, interleukin-6, 8 and monocyte chemo-attractant protein-1 (216). These proximal tubule alterations often progress to a severe tubulointerstitial nephritis and end stage renal failure.

Sjögren syndrome is an autoimmune connective tissue disorder that affects exocrine glands. Renal involvement of Sjögren syndrome is mainly manifested as tubular disorders; 70% of the patients manifest distal renal tubular acidosis (217). Urinary concentration defect, proteinuria and LMW proteinuria are often seen in the patients. Only 4% of the patients manifest FS (217). Patients with FS and Sjögren syndrome manifest osteomalacia including bony deformities of rib cage, bilateral humeral shaft fractures, and marked cortical bone thinning (218). Characteristic histological feature of Sjögren syndrome is chronic interstitial nephritis, with diffuse or focal plasmacytoid lymphocytic infiltration. In the late stage of the disease, tubulointerstitial fibrosis is severe. Corticosteroid or/and immunosuppressant therapy can improve the prognosis.

FS has appeared rarely after renal transplantation (219). Acute tubular necrosis, chronic rejection reaction, and nephrotoxic drugs can induce the progression of FS in the patients.

Acute tubulointerstitial nephritis with uveitis (TINU) syndrome is an immunological disease that leads to tubulointerstitital nephritis and anterior uveitis (220). Patients with TINU syndrome manifest asthenia, malaise, weight loss, nocturia, and thirst. Patients also manifest incomplete or complete FS including proteinuria, LMW proteinuria, glucosuria, aminoaciduria, bicarbonaturia, phosphaturia, and uricosuria due to proximal tubule dysfunction and acute renal failure (221). Urine concentration is decreased in the patients. Corticosteroid therapy can improve renal and eye manifestations.

Autoimmune interstitial nephritis and membranous nephropathy is a distinct disorder. The patients manifest failure to thrive, multiple renal tubular disorders including FS and proteinuria (222, 223). Renal biopsy revealed interstitial nephritis with lymphocytic infiltration and fibrosis, and membranous nephropathy. In advanced stage, focal segmental glomerulosclerosis and tubular atrophy develop. Immunofluorescence analysis shows linear staining of IgG along the glomerular capillaries and the tubular basement membrane. These renal lesions result from an autoimmune response to the 58-kD tubular basement membrane autoantibody (224). This disorder is genetically related to HLA B7 serotype (224).

A patient with anorexia nervosa is described to manifest reversible FS like condition including glucosuria, phophaturia, and uricosuria, although the precise pathogenesis is not known (225). These manifestations subside with nutritional recovery.

Untreated patients with distal renal tubular acidosis manifest LMW proteinuria, generalized aminoaciduria, phosphaturia, uricosuria, and hypercalciuria (226, 227). These proximal tubular abnormalities are transient and disappear by the alkali and potassium therapy. Although the precise pathogenic mechanisms underling the development of proximal tubular dysfunction remains unclear, decreased pH in the cytoplasm of the proximal tubule cells resulting from the intracytoplasmic accumulation of H^+ due to luminal membrane H^+-ATPase dysfunction can disturb trafficking of endosome.

Exogenous Factors

Drugs

Numerous drugs and herbs are implicated in the pathogenesis of FS. Drugs and herbs are usually filtered from the glomerulus and reabsorbed in the proximal tubules. They include outdated tetracycline (228), aminoglycosides (229, 230), salicylate (231), valproic acid (232, 233), and Chinese herbs (234, 235). Aminoglycoside antibiotics reduce glucose reabsorption in kidney tissue by reducing mRNA, protein expression, and function of the Na^+-dependent glucose transporter, which is located in the luminal membrane of the proximal tubule (236). Covalent binding of salicylate or its metabolites to mitochondria in proximal tubule cells alters the function of mitochondria (231). Valproic acid produces the defects of mitochondrial respiratory chain and lysosomal enzyme activity in the proximal tubule cells leading to multiple renal transport abnormalities (13, 237). Chinese herbs containing aristolochic acids cause proximal tubular injury, and this is called as aristolochic acid-related nephropathy.

A number of cancer chemotherapy agents are associated with renal glomerular and tubular dysfunctions including FS. The nephrotoxicity of cancer chemotherapy agents is dose dependent and often irreversible. Ifosfamide is an alkylating agent widely used in the treatment of various solid tumors. Chloroacetaldehyde (CAA), one of the main metabolites of ifosfamide, contributes to inhibit endocytosis in the proximal tubule cells (238). CAA decreases total glutathione and ATP levels in the proximal tubule cells. CAA also inhibits endosomal H^+-ATPase activity, which disturbs intracellular vesicle trafficking (239). Patients receiving ifosfamide who have received prior cisplatin are at significantly higher risk of developing FS than are those who have received no prior nephrotoxic therapy (240). When the patients manifest FS, renal sonography reveals hyperechogenicity of the parenchyma with good corticomedullar differentiation (241). Taurine can protect against ifosfamide-induced renal dysfunction without compromising its anti-tumor activity (242). Cisplatin also reduces glucose reabsorption in kidney tissue by reducing mRNA, protein expression, and function of the Na^+-dependent glucose transporter (243). Cisplatin inhibits various types of amino acid transporters in the proximal tubule cells leading to a generalized aminoaciduria (243).

Imanitib mesylate is a specific tyrosine kinase inhibitor that is the first line therapy for patients with chronic myeloid leukemia. This agent induces partial FS including phosphaturia and uricosuria with mild renal failure. Combined blockade of both platelet-derived growth factor receptor and c-Kit receptor tyrosine kinase in proximal tubules causes partial FS (244).

Nucleotide reverse transcriptase inhibitors that are used as anti-human immunodeficiency virus (HIV) agents including adefovir, cidofovir, and tenofovir induce

FS, nephrogenic diabetes insipidus, and acute renal failure (245–248). Adefovir and cidofovir interact with organic anion transporters (OAT); these drugs enter into proximal tubule cells by activated OAT located in the basolateral membrane. However, their efflux into the tubular lumen is decreased by inactivated multidrug-resistance-protein 2 (MRP 2) located in the luminal membrane. Thus, these drugs are accumulated in the proximal tubule cells leading to mitochondrial damage and tubular toxicity. Cytotoxicity of adefovir and cidofovir is proportional to cellular OAT expression (246). Histologic and ultrastructural examination reveals tubular degenerative changes of proximal tubules with swollen and dysmorphic mitochondria. In tubular cells, respiratory chain components encoded by mitochondrial DNA (cytochrome oxidase subunit I) are selectively deficient in renal tubular cells, and mitochondrial DNA is quantitatively reduced (249). In contrast to adefovir and cidofovir, renal toxicity of tenofovir is much less frequent. Tenofovir has little mitochondrial toxicity and it does not interact with OAT (250). Therefore, the precise mechanisms of nephrotoxicity by tenofovir remain unknown.

Chemical Compounds

Paraquat, a non-selective herbicide, and colloidal bismus subcitrate cause FS (251, 252). Large amount of these compounds are usually ingested in a suicide attempt. Patients manifest FS and acute renal failure. Treatment with the chelating agent sodium-2,3-dimercapto-1-propanesulfonate in combination with hemodialysis is highly effective in reducing the serum bismus level. Methyl-3-chromone (diachrome) (253), 6-mercaptopurine (254), and toluene also lead to FS (255).

Heavy Metals

Heavy metals such as lead, cadmium, mercury, chromium, and platinum are a major environmental and occupational hazard. They are very toxic at very low doses. The kidney is the first target organ of heavy metal toxicity. The extent of renal damage by heavy metals depends on the nature, dose, route, and duration of exposure. Both acute and chronic intoxication have been demonstrated to cause nephropathies, with various levels of severity ranging from tubular dysfunctions like acquired FS to severe renal failure (256). Lead poisoning leads to FS, predominantly in children (257). As lead is non-biodegradable with a very long biological half-life, aminoaciduria and glycosuria persist up to 13 years after childhood severe lead poisoning (258).

Cadmium intoxication leads to FS after a long exposure (259). The industrial waste contaminating cadmium in the Jinzu River basin in Toyama prefecture in Japan produced a lot of patients with *Itai-Itai* (*ouch-ouch*) disease that is compatible to FS with severe osteomalacia. Patients complained severe bone pains that are derived from advanced non-traumatic multiple bone fractures. Cadmium produces free radicals that alter mitochondrial activity or induce mitochondrial gene deletion in the proximal tubules (260, 261). Cadmium inhibits H^+-ATPase, which results in a Fanconi-like syndrome (6).

Therapy

Identification of the underling cause for FS is a first step and is critical to direct specific therapy. Avoidance of offending nutrients in galactosemia, HFI, and tyrosinemia and avoidance of Cu-rich foods in WD are therapeutically critical. Specific treatments with Cu-chelating agents including D-penicillamine, trientine, and ammonium tetrathiomolybdate, and zinc are effective for WD. Imuunosuppressive drugs are used for immunologically induced disorders including Sjögren syndrome, TINU syndrome and autoimmune interstitial nephritis and membranous nephropathy. These treatments can completely resolve FS.

When specific therapy does not exist, therapy is directed at the biochemical abnormalities secondary to renal solute and fluid losses and the metabolic bone diseases. Proximal renal tubular acidosis usually requires large amount of alkali (2–15 mEq kg^{-1} of body weight a day) divided into four to six daily doses. High dose of alkali can produce volume expansion, further bicarbonate wasting and potassium loss in the patients with FS. 1–3 mg kg^{-1} of body weight a day of hydrochlorothiazide can reduce the dose of alkali by preventing the volume expansion. Administration of potassium salt of citrate, bicarbonate, or acetate fulfills the dual purpose of treating acidosis and preventing hypokalemia. Sodium wasting and dehydration are treated with combination of sodium bicarbonate, citrate, and chloride, depending on the degree of acidosis. Ensuring adequate fluid and electrolyte intake is essential, especially in the case of infants or gastrointestinal diseases. Early intervention with intravenous replacement therapy is required for the patients with FS who manifest vomiting and diarrhea.

Hypophosphatemia and impaired renal vitamin D3 metabolism in patients with FS lead to rickets and other metabolic bone diseases. 1–3 g of phosphate supplementation

is necessary as neutral phosphate (the mixture of sodium phosphate dibasic 1.94 g and potassium phosphate monobasic 0.34 g contains 0.5 g of phosphate) divided into four to six daily doses. Supplementation of 1,25-dihydroxyvitamin D3 or dihydrotachysterol is effective to treat or prevent rickets and osteomalacia. Vitamin D3 therapy improves the hypophosphatemia and lessens the risk of hyperparathyroidism. Hypercalcemia and hypercalciuria are toxic side effects of vitamin D3 therapy. An adequate amount of physical activity, as well as appropriate diet with calcium, phosphate, and vitamin D3, is necessary to prevent bone deformations, non-traumatic fractures leading to bone pain, deterioration of motor development and disability (262).

Aminoaciduria, glucosuria, proteinuria, LMW proteinuria, and uricosuria usually do not induce clinical symptoms and do not require specific treatments.

Growth failure is a major complication in FS. Despite correction of electrolyte abnormalities, some patients manifest severe growth retardation, especially those with cystinosis and Fanconi-Bickel syndrome. A patient with FS was reported to have growth hormone deficiency (263). Supplemental growth hormone has been used successfully in a few patients with FS.

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43 Primary Hyperoxaluria

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Introduction

Hyperoxaluria may be either a secondary or a primary disease. Two distinct autosomal recessive inherited enzyme defects of glyoxylate metabolism have been related to type 1 and type 2 primary hyperoxalurias (PH), i.e., alanine: glyoxylate aminotransferase (AGT) and glyoxylate reductase/hydroxypyruvate reductase (GRHPR), respectively; in addition non-PH1 non-PH2 patients have been reported. Among all PH patients, type 1 accounts for 77%, type 2 for 9% and non-type 1 non-type 2 for 14% (1).

PH1 is one of the most challenging issues for both adult and pediatric nephrologists worldwide. The diagnostic accuracy has improved during the recent years and can be based on reasonable recommendations. However, due to its rarity and variable phenotype, therapeutic guidelines come, not only from evidence based information, but also expert opinion and experience.

Primary Hyperoxaluria Type 1

Pathophysiology

PH1 (MIM 259900) is an autosomal recessive disorder (~1: 100,000 live births per year in Europe), caused by the functional defect of the liver-specific peroxisomal, pyridoxal phosphate-dependent enzyme AGT (EC 2.6.1.44) leading to oxalate overproduction (*Fig. 43-1*) (2). The disease occurs because AGT activity is insufficient or because AGT is mistargeted to mitochondria which may explain enzymatic heterogeneity. Since calcium oxalate (CaOx) is insoluble in urine, PH1 usually presents with symptoms referable to oxalate deposition in the urinary tract. The median age at initial symptoms is 5–6 years and end-stage renal disease (ESRD) is reached between 25 and 40 years of age in half of patients (2, 3). Indeed it is responsible for less than 0.5% of ESRD in children in Europe *versus* ~10% in Kuwait and ~13% in Tunisia (4, 5), due to genetic make-up and a higher rate of consanguineous marriages. Along with progressive decline of glomerular filtration rate (GFR) due to renal parenchymal involvement, continued overproduction of oxalate by the liver along with reduced oxalate excretion by the kidneys leads to a critical saturation point for plasma oxalate (Pox) so that oxalate deposition occurs in many organs, leading to systemic involvement (named 'oxalosis') and bone is the major compartment of the insoluble oxalate pool. Calcium salts of glycolate are soluble and do not result in any pathology (6).

The infantile form often presents as a life-threatening condition because of rapid progression to ESRD due to both early oxalate load and immature GFR: one-half of affected infants experience ESRD at the time of diagnosis and 80% develop ESRD by the age of 3 years (7, 8).

Recent reviews have focused either on molecular pathophysiology of the disease or on current therapeutic approaches (9, 10). Therefore, clear recommendations may be now made for patients and their families.

Diagnosis

Because of the rarity of the disease and because of an insufficient knowledge of inherited urolithiasis by physicians, there is an average 5-year time interval from initial symptoms to diagnosis of PH1 (2, 3). The combination of both clinical and sonographic signs is a strong argument for PH1, i.e., the association of renal calculi, nephrocalcinosis and renal impairment; in addition family history may bring additional information (1).

PH1, in general, has five presentations: (1) an infantile form with early nephrocalcinosis and kidney failure; (2) recurrent urolithiasis and progressive renal failure leading to a diagnosis of PH1 in childhood or adolescence, (3) a late-onset form with occasional stone passage in adulthood, (4) diagnosis suggested and confirmed only by post-transplantation recurrence and, (5) pre-symptomatic subjects with a family history of PH1 (9).

Crystalluria and infrared spectroscopy are of major interest for identification and quantitative analysis of crystals and stones, showing CaOx monohydrate crystals (type Ic whewellite) with a crystal number $>200/mm^3$ in case of heavy hyperoxaluria (*Fig. 43-2*) (11).

In patients with normal or significant residual GFR, concomitant hyperoxaluria (urine oxalate >1 mmol/1.73 m^2

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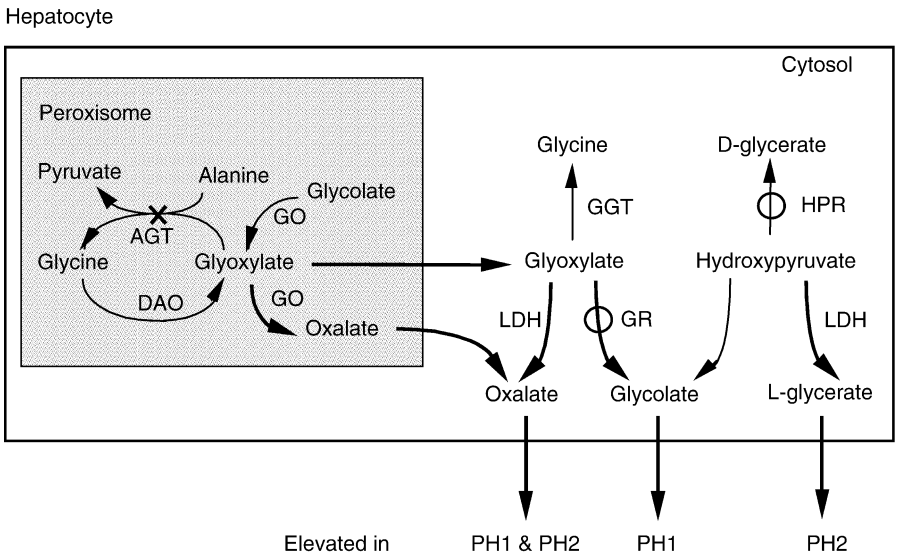
Figure 43-1

Major reactions involved in oxalate, glyoxylate and glycolate metabolism in the human hepatocyte.



AGT : alanine: glyoxylate aminotransferase
DAO : D-amino oxidase
GGT : glutamate: glyoxylate aminotransferase
GO : glycolate oxidase
GR : glyoxylate reductase
HPR : hydroxypyruvate reductase
LDH : lactate dehydrogenase

✕ : metabolic block in PH1
◯ : metabolic block in PH2

Figure 43-2

Infrared spectrophotometry showing monohydrated calcium oxalate.

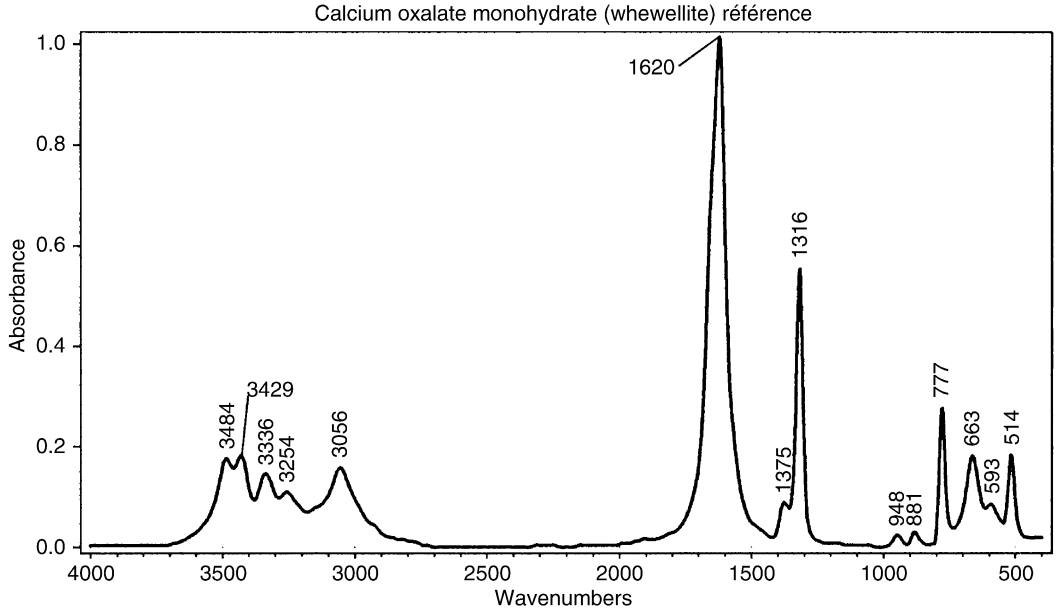


Table 43-1

Approximate cost of specific diagnostic tests and therapeutic procedures in France (in euros, 2007)

Diagnosis	
Crystalluria	27 €
Urine oxalate	135 €
Urine glycolate	135 €
Plasma oxalate	56 €
DNA sequencing	621 €
Prenatal diagnosis (known mutation)	135–270 €
AGT activity on liver tissue	324 €
Conservative treatment	
K/Na citrate	0.05 €/g
Vitamine B6	3 €/day
Transplantation (surgery + first postoperative stay)	
Kidney transplantation	42,685 €
Liver transplantation	86,895 €
Combined liver + kidney transplantation	137,204 €

BSA per day, normal <0.5) and hyperglycoluria (urine glycolate >0.5 mmol/1.73 m^2) are indicative of PH1, but some patients do not present with hyperglycoluria (*Table 43-1*). In ESRD patients, plasma oxalate (±glycolate): creatinine ratio and oxalate (±glycolate) measurement in dialysate might be helpful for screening in children (12). Normal values (mmol/mol) of plasma oxalate: creatinine ratio are <300 for age <2 years, <130 for age 2–5 years, <70 for age 5–15, and <40 for age >16 years [cited in 13]; however such ratios need to be confirmed by complete urine collection (*Table 43-1*). Urinary oxalate excretion is falsely low in patients with deceased GFR because of oxalate retention and systemic deposition as calcium oxalate (13).

In patients with well-defined phenotype, genotyping can be further proposed in order to screen the most common mutations according to local background (*Table 43-1*). Independent of diagnostic value, mutation analysis may provide information on potential pyridoxine responsiveness, on complex enzyme phenotype, and sometimes inform clinical prognosis (14).

In the presence of atypical presentation, a definitive diagnosis requires AGT activity measurement in liver tissue (*Table 43-1*). However, despite controversial information about the relationship between AGT catalytic activity and the severity of the disease (15), liver biopsy is not mandatory if genotype is already known.

Oxalate Burden

When GFR falls to below 30–50 mL/min per 1.73 m^2, continued overproduction of oxalate by the liver along with reduced oxalate excretion by the kidneys leads to a critical saturation point for Pox (Pox >30–50 µmol/L) so that oxalate deposition occurs in many organs. Therefore the presence of any kind of extrarenal involvement is a mark of systemic involvement and therefore may be assessed as a marker of oxalate burden.

Bone is the major compartment of the insoluble oxalate pool and the bone oxalate content is higher (15–910 µmol oxalate/g bony tissue) than among ESRD patients without PH1 (2–9 µmol/g) (16). CaOx crystals first accumulate in the metaphyseal area and form dense suprametaphyseal bands on x-ray. Later on, oxalate osteopathy leads to pain, erythropoetin-resistant anemia, and spontaneous fractures (*Figs. 43-3* and *43-4*).

Along with the skeleton, systemic involvement includes many organs because of progressive vascular lesions: heart, nerves, joints, skin, soft tissues, retina (*Fig. 43-5*) and other visceral lesions. Systemic involvement is responsible for poor quality of life leading to both disability and severe complications. Indeed PH1 is one of the most life-threatening hereditary renal diseases, mainly in developing countries where the mortality rate may reach 100% in the absence of adequate treatment (9).

Figure 43-3
Bone biopsy showing oxalate deposits.

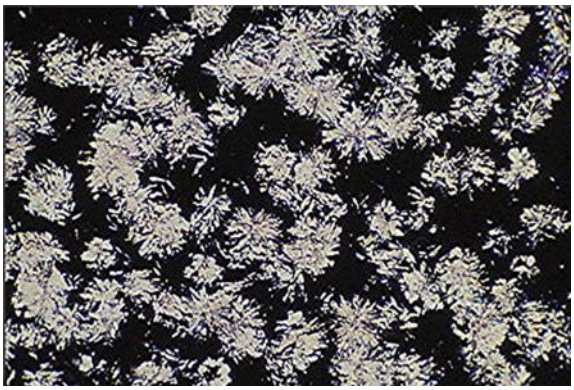


Figure 43-4
Bone biopsy showing oxalate deposits. (See color plate 25)

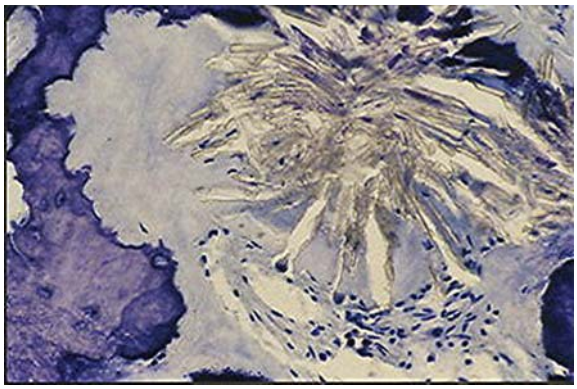
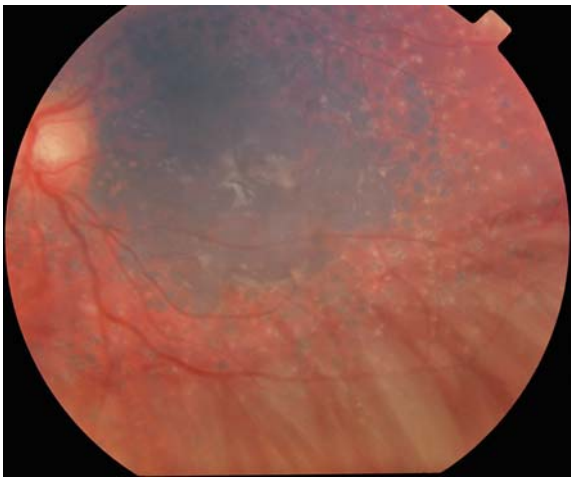


Figure 43-5
Fundus examination showing retinal oxalate deposits. (See color plate 26)



Molecular Approach

Mutation Analysis

Human liver AGT cDNA and genomic DNA have been cloned and sequenced; the normal *AGXT* gene is a single copy gene which is located on chromosome 2q37.3 (11 exons spanning ~10 kb); the protein contains 392 amino acids and has a molecular mass of 43 kDa (14). More than 60 mutations (mainly point missense or nonsense mutations) and polymorphisms have been identified so far and it has been shown that selective exon sequencing can allow a definitive diagnosis in half patients within 2 weeks (6, 17). Some mutations are more frequent (*Table 43-2*) and play a role either in enzyme trafficking or in clinical/biochemical phenotype: Gly82Glu leads to loss of AGT catalytic activity due to inhibition of cofactor (pyridoxal phosphate) binding; Ile244Thr to aggregation and accelerated degradation of AGT; Gly41Arg to AGT intraperoxisomal aggregation as well as accelerated degradation; Gly170Arg to peroxisome-to-mitochondrion trafficking defect, sometimes with significant AGT catalytic activity; Gly170Arg and Phe152Ile to pyridoxine responsiveness; and in some patients homozygous for the 33insC mutation to ESRD during infancy (6, 15, 18–26). Ile244Thr and Gly170Arg mutations are the most common in European and North-American patients (~40% of mutant alleles) and interact with the common Pro11-Leu polymorphism (frequency = 50% in PH1 vs. 15–20% in normal subjects) which plays an important role in phenotype determination (6, 20, 26).

DNA analysis among different ethnic groups has revealed the presence of specific mutations, founder effects and phenotype-genotype correlations among North-African, Spanish, Canary Island, Japanese, Turkish, and Pakistani populations as well as in Arabs in Israel (26–29).

Prenatal Diagnosis

Mutation analysis is based on the sequencing of the 11 exons from the index case and gene segregation is checked in both parents. Prenatal diagnosis can be performed from DNA obtained from crude chorionic villi or amniocytes, on the basis of a restricted analysis of exons including the familial mutation (*Table 43-1*). Such a procedure allows the identification of normal, affected and carrier fetuses.

Table 43-2

Percentage of the most common mutations identified from 200 alleles studied in Lyon, France (9)

Ile244Thr	18.5%	North Africa: 35/37 alleles
Gly170Arg	14.5%	Western Europe: 26/29 alleles
33insC	7.0%	Various countries
Arg122X	5.0%	Middle East: 10/10 alleles
Met195Arg	4.0%	Various countries
Others	49.0%	Various countries

Therapy: General Supportive Principles

Conservative measures should be started as soon as the diagnosis has been made and even suspected. The aims are to increase urinary solubility of CaOx and to decrease oxalate production. The risk of stone formation is increased when urine oxalate exceeds 0.4 mmol/L, especially if urine calcium is plentiful.

Treatment should be initiated with high fluid intake (2–3 L/m^2 per day) aiming at urine dilution by day and night, that may require tube feeding in young children but often fails due to the high non-compliance rate with this difficult management regimen at the time of adolescence. CaOx crystallization inhibitors, such as citrate (potassium or sodium citrate, 100–150 mg/kg per day in 3–4 divided doses, *Table 43-1*) or neutral phosphate (30), are mandatory in order to decrease calcium absorption and therefore calciuria, and to decrease growth and agglomeration of CaOx crystals. Diuretics require careful management: frusemide will increase urine output with the risk of an increased calciuria whereas the diuretic effect of thiazides is less marked but is associated with an appreciable decrease of calcium excretion. Restriction of dietary oxalate intake (spinach, rhubarb, chocolate, tea, etc.) has limited influence on the disease as oxalate of dietary origin contributes very little to hyperoxaluria in PH. Calcium restriction is not recommended, because less calcium would then bind oxalate and form insoluble CaOx complexes in the gut. Ascorbic acid supplementation is not recommended as it is a precursor of oxalate.

The main purpose of therapy is to lower both Pox and plasma CaOx saturation. The effects of conservative measures can be assessed by serial determinations of crystalluria score and CaOx supersaturation software (31).

Pyridoxine is metabolized in the body to pyridoxal phosphate, the main cofactor of AGT. Pyridoxine sensitivity (i.e., >30% reduction of urinary oxalate excretion) is found in 10–30% of PH1 patients, so that it must be tested early at a daily dose of 5–10 mg/kg according to urinary oxalate (*Table 43-1*) (6, 25, 30). Response to pyridoxine may therefore help to preclude or delay the progression to ESRD (31); the patients most likely to respond are those with homozygous Gly170Arg or Phe152Ile mutation, who also experience preserved renal function over time under adequate treatment (25, 26). In case of isolated kidney transplantation in such pyridoxine sensitive patient, pyridoxine should not be discontinued at the time of surgery.

In summary, an aggressive supportive management should be started as soon as the diagnosis of PH1 has been considered that may significantly improve renal survival provided compliance is optimal.

Urologic Therapy

The treatment of stones should avoid open and percutaneous surgery because additional peri-operative renal insults will exacerbate progressive renal injury. The use of extracorporeal shock wave lithotripsy is an available option in selected patients but the presence of nephrocalcinosis may be responsible for parenchymal damage. In patients with repeated renal colic, stone removal can be attained by ureteroscopy and a ureteral JJ stent may be helpful for pain control and protection of renal damage. Management of stones in patients with PH is challenging and important in the long term care of these patients, so that referral to urologists experienced in this field is recommended.

Bilateral nephrectomy may be proposed to at-risk patients while on renal replacement therapy in order to limit the risk of further infection, obstruction and passage of stones.

Failure of Dialysis

Conventional dialysis is unsuitable for patients who have reached ESRD because it cannot overcome the continuous excess production of oxalate in spite of its small molecular mass (COOH-COOH: 1 mmol = 90 mg). Indeed the difference between oxalate generation (4–7 mmol/1.73 m^2 per day) and conventional dialysis removal (1–2 mmol/1.73 m^2 per day) shows that tissue accretion rate is uncontrolled (32). Conventional long-term HD is therefore associated with unacceptable quality of life and is a life threatening option.

In such patients, predialysis Pox usually ranges between 100 and 200 µmol/L (N < 7 µmol/L), which is significantly higher than in non-PH1 HD patients (40–50 µmol/L); calculated CaOx saturation (βCaOx, N ≤ 1 relative unit) is also higher in PH1 (~4.5 relative

units) compared to non-PH1 HD patients (~1.5 relative units) (33). Predialysis Pox is reduced by ~60% following HD (and βCaOx remains more than 1), but returns to 80% of the predialysis levels within 24 h and 95% within 48 h; indeed HD only removes the small fraction of total body oxalate and is followed by a rebound coming from the slow turnover compartment. Therefore daily hemodialysis (HD, >5 h per session) would be the preferred option but it cannot be universally applied and ongoing CaOx tissue deposition continues (34). The challenge is therefore to keep pre-dialysis Pox below 50 μmol/L in order to limit the progression of systemic oxalosis. Peritoneal dialysis (PD) by itself is unable to clear enough oxalate but, in some patients (e.g., children with an aggressive infantile form of oxalosis), combined PD and HD will enhance overall clearance and help in preventing the rebound in Pox after HD (35). On the same pathophysiological bases, HD may be required in selected condition in transplant patients (see below).

In summary, there are limited indications for dialysis in children with PH1: (1) if diagnosis of PH1 has not been established, (2) in small children with infantile oxalosis waiting for organ transplantation, (3) in preparation for kidney transplant, whether before of after liver transplantation, in order to deplete oxalate from the body, (4) following isolated kidney or combined liver-kidney transplantation with any delay in achieving optimal renal function, as a temporary adjunct in the case of high oxalate burden, or with transient loss of transplant function, (5) very exceptionally in older patients if the only alternative is no dialysis, (6) in developing countries, HD (or even less satisfactory, PD) may be indicated as only a preference to absolute withdrawal of all therapy.

Organ Transplantation

Ideally, any kind of transplantation should be a preemptive procedure (*Table 43-1*). Further assessment of oxalate burden needs therefore to be predicted by monitoring sequential GFR, Pox, CaOx saturation and systemic involvement (bone mineral density, bone histology) (9, 36). In addition, recent genotype-phenotype correlations regarding the degree of response to pyridoxine and the presence of Gly170Arg mutation may influence further therapeutic options (25, 26).

Kidney Transplantation

Kidney transplantation allows significant removal of soluble Pox (35). However, because the biochemical defect is in the liver, overproduction of oxalate and subsequent deposition in tissues continues unabated. The high rate of urinary oxalate excretion originates from both ongoing oxalate production from the native liver and oxalate deposits in tissues. Due to oxalate accumulation in the graft, isolated elective kidney transplantation is no longer recommended in ESRD, because frequently recurrence leads to poor graft survival and patient quality of life (37). Again, testing pyridoxine sensitivity is a major issue since it may allow to consider isolated kidney transplantation in selected responsive patients, a condition which is found in patients with the Gly170Arg mutation (25, 26). However most of them should not require renal replacement therapy if diagnosis is made on time and the favorable long term outcome of isolated kidney transplantation together with pyridoxine in these patients remains to be demonstrated. Isolated kidney transplantation may be also regarded as a temporary solution in some developing countries before managing the patient in a specialized center for further combined liver-kidney procedure (*Table 43-1*).

Rationale for Liver Transplantation

Since the liver is the only organ responsible for glyoxylate detoxification by AGT, the excessive production of oxalate will continue as long as the native liver is left in place. Therefore any form of enzyme replacement will succeed only when the deficient host liver has been removed. Liver transplantation is a form of gene therapy as well as enzyme replacement therapy as it will supply the missing enzyme in the correct organ (liver), cell (hepatocyte) and intracellular compartment (peroxisome) (27). The ultimate goal of organ replacement is to change a positive whole-body accretion rate into a negative one by reducing endogenous oxalate synthesis and providing good oxalate clearance *via* either native or transplanted kidney. However the current reason for enzyme replacement (liver transplantation) is only to decrease oxalate, not to cure the disease.

Combined Liver-kidney Transplantation

In Europe, 6–7 combined liver-kidney transplantations per year have been reported, i.e., 61% of all combined liver-kidney transplantations in children (38); the results are encouraging, as patient survival approximates 80% at 5 years and 69% at 10 years (39). Comparable results have

been reported from the United States Renal Data System, with a 76% death-censored graft survival at 8 years post transplantation (37). Such a strategy can be successfully proposed to infants with PH1 (8). In addition, despite the potential risks for the grafted kidney due to oxalate release from the body stores, kidney survival is about 95% three years post-transplantation and the GFR ranges between 40 and 60 mL/min per 1.73 m^2 after 5–10 years.

However the strategy of combined liver-kidney transplantation may be influenced by the stage of the disease (*Table 43-1*). Indeed simultaneous liver and kidney transplantation is logical in patients with a GFR below 40 mL/min per 1.73 m^2 but a metachronous procedure (liver transplantation, then dialysis until sufficient oxalate clearance from the body, followed by kidney transplantation) may be proposed to ESRD patients, mainly infants with a long waiting time (9, 40).

Preemptive Liver Transplantation

Preemptive isolated liver transplantation might be the first-choice treatment in selected patients before advanced chronic renal failure has occurred, i.e., at a GFR between 60 and 40 mL/min/1.73 m^2 (41, 42). Such a strategy has a strong rationale but raises ethical controversies especially when the GFR is superior to 60 mL/min per 1.73m^2. Indeed PH1 is the only peroxisomal disease without psychomotor delay due to cerebral involvement and the conservative management of PH1 patients has significantly improved during the last 10 years; this may influence the role of preemptive liver transplantation in such patients. Several patients have received an isolated liver transplant without uniformly accepted guidelines, since the course of the disease is unpredictable and a sustained improvement in kidney function can follow a transient phase of rapid decrease in native GFR (41, 42).

Post-transplantation Reversal of Renal and Extrarenal Involvement

After combined liver-kidney transplantation, urinary glycolate immediately returns to normal and Pox returns to normal before urine oxalate does. Indeed oxaluria can remain elevated as long as several years due to the slow resolubilization of systemic CaOx deposition. Therefore there is still a risk of recurrent nephrocalcinosis or renal calculi that might jeopardize graft function.

Thus, independent of the transplantation strategy, the kidney must be protected against the damage that can be induced by heavy oxalate load suddenly released from tissues. Forced fluid intake (3–5 L/1.73 m^2 per day) supported by the use of crystallization inhibitors is the most important approach. Pox, crystalluria and CaOx saturation are helpful tools in renal management after combined liver-kidney transplantation (43). The benefit of daily high-efficiency post-transplant HD is still debated and should be limited to patients with significant systemic involvement; it will provide a rapid drop in Pox and therefore reduce the exposure of the transplanted organs to high Pox, but it may also increase the risk of CaOx supersaturation due to reduction in urine volume in the case of inappropriate fluid removal. However it is obvious that post-transplant HD is mandatory in patients with acute tubular necrosis or delayed graft function.

Combined transplantation should be planned when the GFR ranges between 15 and 40 mL/min per 1.73 m^2 because, at this level, oxalate retention increases rapidly (40). In ESRD patients, vigorous HD should be started and urgent liver-kidney transplantation should be performed. Even at these late stages, damaged organs, such as the skeleton or the heart, do benefit from enzyme replacement, which results in an appreciable improvement in quality of life.

Donors for Combined Liver-kidney Transplantation

The transplantation strategy may be based either on immunological bases (i.e., using the same donor for both organs) or on biochemical rationale (i.e., using a two-step procedure according to oxalate body store) (*Table 43-3*) (9). Indeed most publication currently report on the use of cadaver donors but a living related donor may be considered under certain conditions (44).

In summary, there are different approaches to transplantation strategy which may be influenced by the local allocation system. The largest experience has been obtained with a one-step combined liver-kidney transplantation leading to acceptable results. The option of a two-step procedure should be kept in mind according to local experience and when the prospective waiting time is long enough to jeopardize both patient quality of life and survival.

Future Trends

The absence of intestinal oxalate-degrading bacterium *Oxalobacter formigenes* has been found to be associated

Table 43-3

Suggestions for transplantation strategies in pyridoxine resistant PH1 patients according to residual GFR, systemic involvement and local facilities (9)

GFR (mL/min per 1.73 m^2)	Estimated oxalate load	Proposed Tx strategy	Comments
60–40	±	Preemptive CAD L-Tx?	Hazardous (hepatectomy, immunosuppression) Limited experience Ethical issues
<40	+	Preemptive simultaneous (CAD) LK-Tx	Post-Tx HD only if ATN/DFG Exposure to immunosuppression
ESRD	++	Simultaneous Tx procedure	Renal risk Post-Tx HD often required
		2-step Tx procedure: (1) L-Tx, (2) HD proposed to clear, oxalate, (3) K-Tx	DD: needs 2 different donors - loss of immunological benefit LD: immunological benefit – increased risk for the donor

ATN, acute tubular necrosis, *DD* decreased donor, *DFG* delayed graft function, *ESRD* end-stage renal disease, *HD* hemodialysis, *K* kidney, *L* liver, *LD* living donor, *Tx* transplantation

with hyperoxaluria (45), so that a colonization of the gut by direct administration of the bacteria in capsule form may decrease oxalate absorption and might even extract oxalate from the circulation.

The administration of pyridoxamine - which is able to chelate the precursors of oxalate (e.g., glycolate) - to experiment animals has provided encouraging results (46).

At the moment, the only therapeutic option for PH1 patients who are unresponsive to pyridoxine is liver transplantation which is still a palliative measure so that future research would focus on molecular approaches. Different AGT crystal structures have been obtained for polymorphic variants, and aminoacid changes found in these crystals may affect AGT conformation and stability (6). A better understanding of such changes will allow designing pharmacological agents (chemical chaperones) that may stabilize AGT, thus providing a potential treatment for some PH1 patients with missense mutations (such as the most common mutation-polymorphism combinations, i.e., Gly170Arg + Pro11Leu and Ile244Thr + Pro11Leu) without the need for organ transplantation and even gene therapy (6, 10).

However gene therapy has been advocated and recent experience in AGT transfection into hepatocytes has provided encouraging results *in vitro* (47). Many years of research will be required before considering its potential use in humans because of the large number of hepatocytes to be transduced with the normal AGT gene expressed in the liver as a whole (6).

Non-Type 1 Primary Hyperoxaluria

In patients with overt hyperoxaluria, the pattern of urinary metabolites is indicative but no longer diagnosis of PH. In patients with a clinical picture of PH1, 10–30% have normal AGT activity, that may lead to a diagnosis of PH2 or of another disorder causing hyperoxaluria. Enzyme activity measurement in a single needle liver biopsy can confirm or exclude PH1 and PH2.

Primary Hyperoxaluria Type 2 (PH2)

PH2 (MIM 260000) is another rare inherited defect of oxalate metabolism causing raised urine oxalate and L-glycerate (*Fig. 43-1*).

Metabolic Derangement

PH2 is caused by a complete -or nearly complete- absence of the cytosolic/mitochondrial enzyme with glyoxylate/hydroxypyruvate reductase (GR/HPR, EC 1.1.1.26), and D-glycerate dehydrogenase (GD) activities (*Fig. 43-1*) (48–50). Analysis of liver and lymphocyte samples from patients with PH2 showed that GR activity was either very low or undetectable while GD activity was reduced in liver but within the normal range in lymphocytes (51).

Genetics

There is evidence for autosomal recessive transmission and the gene encoding the enzyme glyoxylate reductase/hydroxypyruvate reductase (*GRHPR*) has been located on chromosome 9q11 (52). More than 12 insertions, deletions, or splice site mutations have been identified (6, 50).

Clinical Presentation

PH2 has been documented in less than 40 published patients but there are probably some unreported cases (48, 53). Median age at onset of first symptoms is 1–2 years, and the classical presentation is urolithiasis, including hematuria and obstruction. However stone-forming activity is lower than in PH1 so that nephrocalcinosis and urinary tract infection are less frequent (31). Glomerular filtration rate is usually maintained during childhood and systemic involvement is therefore exceptional (53).

Diagnostic Test

In the presence of hyperoxaluria without hyperglycoluria, a diagnosis of PH2 should be considered, especially when AGT activity is normal. However, hyperoxaluria in PH2 tends to be less pronounced than in PH1. The biochemical hallmark is the increased urinary excretion of L-glycerate (13, 54) but the definitive diagnosis requires measurement of GR activity in a liver biopsy (55) as some PH2 patients have normal L-glycericaciduria (56).

Treatment and Prognosis

The overall long term prognosis is better than for PH1. ESRD occurs in 12% of patients, between 23 and 50 years of age (53). As in PH1, supportive treatment includes high fluid intake, crystallization inhibitors and prevention of complications; there is no rationale to use pyridoxine (6). Kidney transplantation has been performed in some ESRD patients, often leading to recurrence (nephrocalcinosis) including hyperoxaluria and L-glycerate excretion (53, 57, 58). The concept of liver transplantation has therefore been suggested since GR/HPR is present in the liver at higher levels than found in other tissues, but more data are needed because its distribution is the body is more widespread than that of AGT (6). Gene therapy may be expected to work but there is no current available data on this approach. In addition, prospects for using chemical chaperones are rather limited in PH2 since most GR/HPR mutations are not open to stability modification.

Non-type 1 Non-type 2 Primary Hyperoxaluria

Few reports have shown a possible association of PH (1) without AGT nor GR/HPR deficiency, (2) with hyperglycoluria in the absence of AGT deficiency (21). Such unclassified hyperoxalurias suggest the possibility of additional type(s) of PH; hepatic glycolate oxidase (GO) is a candidate enzyme for a third form of inherited hyperoxaluria.

Conclusion

Patients with hyperoxaluria should be referred for diagnosis and management to centers with interest and experience in the conditions and access to the appropriate biochemical and molecular biological facilities. Indeed major advances in biochemistry, enzymology, genetics and management have been achieved during recent years. The understanding of genotype-phenotype relationship and underlying metabolic defects of PH is in progress. The ongoing analysis of transplant strategies from multicenter database will improve individual enzyme replacement and subsequent patient survival and quality of life.

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44 Tubulointerstitial Nephritis

Uri S. Alon

Introduction

Involvement of the tubulointerstitial compartment in renal diseases can be either primary or even more commonly secondary to glomerular, vascular or structural disease (*Table 44-1*). However, even in the latter disorders the magnitude of involvement and damage to the tubulointerstitium can have a significant effect on the outcome of the primary disease (1, 2). This chapter addresses primary tubulointerstitial nephritis (TIN) which is a syndrome with a wide clinical spectrum. TIN is characterized histologically by inflammation and damage of tubulointerstitial structures, with relative sparing of glomerular and vascular elements (3, 4). Acute TIN is typically associated with marked tubulointerstitial inflammation, varying degrees of edema and tubular epithelial cell damage, and mononuclear cell infiltration (5). In contrast, chronic TIN is characterized by tubular epithelial cell damage and atrophy, with tubulointerstitial fibrosis (5). Although there are important clinical and pathophysiologic distinctions between acute and chronic TIN, they are best viewed as manifestations of a continuum of renal injury (6, 7). Acute TIN is often reversible, however, progression to chronic renal disease can occur (8–10).

Structure and Function of the Tubulointerstitial Compartment

The tubulointerstitial compartment comprises about 80% of the renal parenchyma with the majority of its volume contributed by the tubules (11). The interstitium is comprised of matrix and cells. Two main types of cells have been identified: (1) type I interstitial cells are fibroblast-like cells which are capable of producing and degrading extracellular matrix. An inner medullary subgroup of type I cells produce prostaglandins; (2) type II interstitial cells include dendritic antigen-presenting cells located mainly in the cortex, and monocyte-derived macrophages capable of plagocytosis found in all renal zones (12, 13). The matrix is composed of a fibrillar net of basement membrane and interstitial collagens, glycoproteins and proteoglycans as well as interstitial fluid (13). The interstitium provides structural support for the nephrons and capillaries and plays an important role in the transport of solutes. It is also the site where cytokines and hormones such as prostaglandins and erythropoietin are produced. The roles of the tubules in maintaining kidney function and homeostasis of fluid, electrolytes, minerals and acid-base balance are described in Chapters ZZZ (ED-those which refer to tubular structure and Fx). Damage to different segments of tubules will result in metabolic abnormalities related to the specific functions of the affected segments.

Pathophysiology

Tubulointerstitial lesions in all forms of TIN are predominantly characterized by T-cell lymphocytic infiltrates, suggesting immune-mediated mechanisms of renal injury (6, 14). Such mechanisms are likely important in both initiating tubular injury and amplifying damage induced by both immune and nonimmune causes. Studies from experimental models of tubulointerstitial disease indicate that activation of both cell-mediated and humoral immune pathways induce renal injury (15, 16).

Cell-Mediated Immunity

Historically, abnormalities in cell mediated immune responses have been implicated as the major pathophysiologic mechanisms of TIN. Analysis of infiltrating mononuclear cells in human TIN of various etiologies identified the majority as T lymphocytes (17–19). T-cell lymphocytic ($CD4^+$ and $CD8^+$) interstitial infiltrates generally occur in the absence of antibody deposition (5). Immunohistochemical studies conducted on biopsies obtained in drug-induced TIN also indicate the importance of cell-cell interactions in intrarenal inflammation, as there is increased interstitial expression of cellular adhesion molecules. In human forms of acute TIN, increased expression of LFA-1 and VLA-4 cell-surface receptors, as well as their respective ligands, ICAM-1 and VCAM-1, is seen in areas of mononuclear cell infiltration (20). Further support of cell-mediated events in human disease is based on

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Table 44-1
Classification of tubulointerstitial nephritis (TIN)

Primary TIN
1. Medication (antimicrobials, analgesics, lithium, cyclosporine, Chinese herbs)
2. Infection (bacterial pyelonephritis, Hantavirus, Leptospirosis)
3. Immune-mediated (anti-tubular basement membrane disease)
4. Toxins (lead)
5. Hereditary (cystinosis, hyperoxaluria, Wilson disease)
6. Metabolic disorders (hypercalcemia, hyperkalemia, hyperuricosuria)
7. Hematologic disorders (sickle cell disease)
8. Miscellaneous (Balkan nephropathy)
Secondary TIN
1. Glomerular disease
2. Vascular disease
3. Structural disease
a. Cystic diseases
b. Obstructive disease
c. Reflux nephropathy

observations of in vivo and in vitro activation of lymphocytes isolated from affected patients following repeat exposure to specific inciting agents of TIN (21, 22). In view of these observations, the role of cell-mediated events in TIN has been extensively studied in murine models of anti-TBM disease and spontaneous TIN (16, 23, 24). In both of these models, the effector T cell (T_e) lymphocytes that differentiate in diseased animals are $CD8^+$ renal tubular antigen-specific T cells. Nephritogenic $CD8^+$ T_e cells induce renal injury in genetically susceptible animals, and can adoptively transfer renal disease to naive hosts (15, 16, 23, 24).

Cell-mediated responses are initiated by T-cell recognition of relevant antigen presented in the context of appropriate major histocompatibility complex (MHC) molecules. Class I MHC molecules primarily direct $CD8^+$ T cell responses, and class II MHC determinants direct $CD4^+$ T cell responses (25). In vitro studies show that resident renal cells, such as renal tubular epithelial cells, glomerular epithelial cells, and mesangial cells, have the potential to present a variety of antigens (26, 27). Correlation of in vitro antigen-presenting activity to disease activity in vivo, however, has not been established (28). It is noteworthy that renal tubular epithelial cells express class II MHC determinants in inflammatory renal disease and in culture, when stimulated by proinflammatory cytokines such as IFN- and TNF (29, 30). Moreover, class II MHC expression on renal tubular epithelial cells induces in vitro proliferation of antigen-specific T-cell clones and hybridomas and promotes autoimmune renal disease in vivo. These findings suggest that induced class II MHC expression on renal tubular epithelial cells promotes autoimmune injury by facilitating presentation of a self (tubular) antigen by nonlymphoid cells (28). The cause for antigen expression by the tubular cells is unknown. Tubular epithelial cells normally do not express costimulatory molecules such as CD80 and CD86, which likely limits their ability to present antigen under physiologic conditions (31). Genetic susceptibility may play a factor in antigen expression and consequent immune response (16). Other mechanisms, such as drugs or infectious agents, may serve as inciting antigens of cell-mediated responses targeting the kidney (32, 33). In addition, degenerate recognition of peptide antigens by autoreactive T cells in autoimmune disease has been reported (32, 34). The potential relevance of such molecular mimicry in autoimmune T-cell activation to renal immune responses, however, awaits further investigation. There is a large body of research implicating the interaction between effector and suppressor T-cells in the inflammatory process in the renal interstitium. However, a definitive role for such interaction in the pathogenesis of TIN is still unclear (9, 11).

Antibody-Mediated Immunity

Occasionally antibody-mediated TIN can be seen. When antibodies are present by immunofluoresence, they are usually associated with the tubulointerstitial cells, along the basement membrane or as immune complexes. Anti-TBM staining often occurs as part of anti-GBM disease, but can also be a primary phenomenon resembling experimental anti-TBM disease. Anti-TBM antibodies can be seen in drug-induced TIN, and also in renal transplants due to the presence of foreign antigens in the transplanted kidney (35, 36).

Primary immune deposit-mediated TIN is rare. It seems that in such instances the complexes are formed in situ, as circulating complexes would be entrapped in the glomeruli. Indeed, in many of the cases in which immune complexes are detected in the interstitium, they are also observed in the glomeruli. Examples include SLE, IgA nephropathy and membranous nephropathy. The presence of interstitial immune complexes might still require cellular immune response genes for the development of a complete inflammatory reaction. Most studied experimentally is anti-TBM disease. Other animal models

for experimental antibody and immune complex mediated TIN include the Heymann nephritis antigen complex in brush border abnormalities (37) and reaction to Tamm-Horsfall protein (38).

Local and Extrarenal Antigens

Antigens presented to the immune system may be derived from resident cells or arrive at the tubulointerstitial compartment from extrarenal origin. As mentioned previously, resident cells of the tubulointerstitial component and in particular tubular epithelial cells have the potential to present antigens. In addition, local antigens may be related to Tamm-Horsfall protein or, uromodulin, which is a glycoprotein secreted by the cells of the ascending loop of Henle. This glycoprotein can precipitate immune deposits at the base of tubular cells and lymphatic drainage of the ascending limb, and result in binding of neutrophils to the cell-surface (39, 40). In experimental Heymann nephritis, antibodies reacting to the tubular brush border have been detected, at times associated with interstitial infiltration (41, 42). Most extensively studied is the antigen in anti-tubular basement membrane (anti-TBM) disease, named 3M-1 (43, 44). It has been detected both in humans and rodents, showing extensive polymorphic expression (45, 46).

Extrarenal antigens may present to the interstitium as isolated antigens or as part of an immune complex. Antigens arriving at the tissue like those originally derived from drugs like penicillin, cephalosporin and phentoin combine with antibodies and inflammatory cells which initiate interstitial disease. Another mechanism by which external antigens initiate an immune response is by mimicking of epitopes. For instance some antibodies to *Escherichia* coli cross-react with Tamm-Horsfall protein (47). A similar mechanism may be responsible for TIN which follows certain viral illnesses (48, 49). It has also been hypothesized that non-infectious, shared epitopes, may instigate the immune process, (i.e., anti-DNA antibodies) (50, 51).

Circulating immune deposits which settle in the interstitium can cause TIN. This might be the case in SLE, IgA nephropathy and possibly some cases of chronic idiopathic human tubulointerstitial nephritis (52, 53). Experimentally, a model of chronic serum sickness disease in the rabbit results in immune deposits in the interstitium (54).

Cytokines and Amplification of Injury

Events resulting from infiltration of T-cells, and deposition of specific antibodies or immune complexes which augment inflammation and injury are part of the "amplification process." These processes include the release of cytokines and proteases from T cells; the attraction and activation of nonspecific immune effector cells including eosinophyls and macrophages (which themselves release various effector products); and the activation of the complement cascade (19, 55). Interestingly the presence of complement in TIN is infrequent. When present, it is usually associated with deposition of IgG and immune complexes (54–57) or IgE and eosinophils (54, 57, 58). Even more important seems to be the activation of the alternative passway by ammonia, in the absence of antibodies (59). This process may be instrumental in progressive local injury in non-immune mediated TIN. It may also indicate the importance of correction of metabolic acidosis in the prevention of progressive renal damage by diminishing ammonia production. It is possible that both resident epithelial cells and infiltrating cells further amplify damage by expressing chemokines. This sub-class of structurally related cytokines selectively promote the chemotaxis, adhesion and activation of leukocytes (60). Following ureteric obstruction in the mouse, the most potent recruiter of macrophages is monocyte chemoattractent peptide – 1 (MCP-1), which is expressed in tubular epithelium (61). The chemokines induce their effect by binding to specific cell-surface receptors on target cells. Various types of leukocytes are activated by differential chemokines-receptor binding patterns. The pharmocologic blockage of chemokines and their receptors may have a potential in controlling the inflammatory response (62). In humans with ureteropelvic junction obstruction, urinary MCP-1 is increased, and decreases after surgical alleviation of the obstruction (63).

Fibrogenesis and Atrophy

Interstitial fibrosis is the final common injury pathway for a variety of glomerular and tubular disorders, particularly when associated with massive glomerular proteinuria or the presence of inflammatory cells in the tubulointerstitial component (64, 65). It seems that both processes induce local cytokines which transform and activate several types of resident cells in the tubulointerstitial compartment to produce new or modified extracellular matrix (66). Some of the activated cells probably change phenotype (67–69). It is unclear why what is usually a self-limited wound repair process continues unabated. It is possible that failure of homeostatic regulatory mechanisms is responsible for the continuous inflammatory process of chronic TIN.

Tubulointerstitial scars are primarily composed of collagen type I and III, fibronectin and tenascin (70, 71). Early in the development of a scar, the fibrotic tissue may contain monocytes, tubular cells and fibroblasts (65). It appears that local monocytes release cytokines, affecting the activity of their neighboring fibroblasts. The phenotypic expression of the fibroblast and hence the renal glycoprotein synthesized by it may be related to the type of cytokines stimulating the cell (72). Among the strongest morphogenic cytokines driving TIN fibrosis is angiotensin II (73).

The source of the fibroblasts in the tubulointerstitium is yet unknown. There are several lines of evidence to suggest that they may arise from transformation of resident cells like the tubular epithelium through the mechanism of epithelial-mesenchymal transformation (74–76). The transformation is probably activated by immune-mediated mechanisms and various cytokines which result in changes in the basement membrane of the tubular epithelial cell. Once transformed to a mesenchymal cell it acquires motility and becomes responsive to growth factor stimuli (77). Several local chemoattractants stimulate the migration of the newly formed fibroblasts. These chemoattractants are released by macrophages or the fibroblasts themselves (78, 79). The latter, mainly by secreting TGF-β amplify the process (80). Once fibroblasts migrate to their new location they begin to deposit fibronectin matrix which serves as a skeleton for the deposition of other glycoproteins. Concomitantly with the transformation process, the effect of cytokines on tubular cell can result in their atrophy by disturbing their basement membrane synthesis. Additional damage to the tubular cell can be caused by T cell clones with cytotoxic activity resulting in tubular cell destruction and atrophy. Thus tubular atrophy and interstitial fibrosis often coexist.

Acute Tubulointerstitial Nephritis

Epidemiology

Acute TIN accounts for 10–25% of reported cases of acute renal failure (ARF) in adults (3) and up to 7% of children with ARF (81). However, in both children and adults acute TIN may be underreported because many patients with ARF recover spontaneously after removal of the suspected offending agent, and definitive diagnosis based on a renal biopsy is not routinely established (7). Lower reported incidences of acute TIN in children than in adults may reflect more common use of nephrotoxic pharmacologic agents and greater prevalence of preexisting renal abnormalities in older patients.

Clinical, Laboratory and Radiologic Features

The clinical manifestations of TIN are variable and many of them are seen in both acute and chronic TIN (*Table 44-2*). The severity of renal impairment ranges from asymptomatic urinary abnormalities to mild azotemia, and to non-oliguric and oliguric ARF (82). The nonspecific nature of the clinical findings in TIN emphasizes the need for a renal biopsy to make a definitive diagnosis in questionable cases (4, 83). In one series of 13 children with biopsy proven acute TIN, only 6 were suspected of having the diagnosis before the procedure (84). However, in a child suspected to have acute TIN, if improvement in kidney function is noticed after withholding the suspected offending agents and starting treatment, there is no need for a biopsy.

Systemic manifestations of a hypersensitivity reaction, such as fever, rash, and arthralgias, are variable findings. Although these symptoms are more likely to occur in drug-induced acute TIN (81, 85), in a series of nine adults with acute TIN caused by medications, components of this classic triad comprised the presenting symptoms in only about half of the patients (86). In a more recent study on 128 adults with TIN, in which 70% were due to drugs, the triad of rash, fever and eosinophilia was seen in about 10% (87). The rash may be maculopapular, morbilliform, or urticarial, and often it is fleeting (86–90). Arthralgia is seldom a prominent feature (86). Acute TIN associated with infection may present with extrarenal manifestations of disease, such as fever and sore throat (84). Nonspecific constitutional symptoms of fatigue, anorexia, weight loss, nausea and vomiting, and flank pain with macroscopic hematuria may occur (88, 90). Hypertension and edema are seldom noted, except in specific drug-induced lesions, such as the nephrotic syndrome associated with nonsteroidal anti-inflammatory drugs (NSAIDs) (91) or in TIN secondary to glomerular disease (8, 82).

Acute and chronic TIN have quite similar laboratory findings (7). Renal tubular epithelial cell damage is the predominant finding in all forms of TIN, and biochemical abnormalities observed in patients with TIN may reflect injury to specific nephron segments involved in the inflammatory process (*Table 44-3*) (92). In general cortical damage may affect mainly the proximal and distal tubules whereas medullary lesions may affect mainly the loop of Henle and the collecting duct. Proximal tubule injury results in Fanconi syndrome with impaired urinary reabsorption of glucose, bicarbonate, phosphorus, amino acids, and uric acid (93). Damage to the loop of Henle may result in magnesium and sodium losses. Distal tubular lesions result in renal tubular acidosis, impaired

Table 44-2
Clinical presentation of tubulointerstitial nephritis

History
Exposure to toxic substances
Family history of tubulointerstitial nephritis
Nephrotoxic drug use
Past or family history of vesicoureteral reflux
Recurrent urinary tract infections
Uveitis
Symptoms
Abdominal pain
Anorexia[a]
Arthralgias
Diarrhea
Dysuria
Edema
Emesis[a]
Eye tenderness (seen in TINU syndrome)
Fatigue[a]
Fever[a]
Flank or loin pain
Headache
Lymphadenopathy
Malaise
Myalgia
Nocturia
Polydipsia
Polyuria
Rash
Sore throat[a]
Weight loss[a]
Signs
Abdominal pain
Arthritis
Costovertebral tenderness
Edema
Evidence for left ventricular hypertrophy
Fever[a]
Hypertension
Hypertensive retinopathy
Lacrimation (seen in TINU syndrome)
Lethargy
Pallor[a]
Pharyngitis
Poor growth[a]
Rachitic changes[a]
Rash (may be maculopapular, morbilliform, or urticarial)
Volume depletion

[a]Common finding in children

potassium excretion, and sodium wasting (94). Biochemical abnormalities in TIN are an elevated serum creatinine concentration with hyperchloremic metabolic acidosis (3, 84). If proximal tubular involvement is prominent, serum phosphorus, bicarbonate, uric acid, and potassium concentrations may be decreased. Whereas oliguria can occur, many patients have nonoliguric renal failure, and others a urine concentrating defect. The urine microscopic examination may show active or relatively bland changes.

Relevant serologic studies in the investigation of TIN are listed in *Table 44-4*. Normochromic, normocytic anemia is often associated with TIN (81, 92). Hemolytic anemia has also been reported in acute TIN induced by allopurinol, penicillins, and rifampin (4, 92). Leukocytosis may occur, and some but not all patients with drug-induced TIN have peripheral eosinophilia (85, 90, 95). Drug induced liver damage will be reflected in elevated hepatic enzymes (95, 96). Elevated serum IgE titers have been reported in up to 50% of biopsy-proven cases and is suggestive, but not diagnostic, of drug-induced TIN (82, 86). Anti-DNA antibodies, antinuclear antibodies, and complement levels are normal in most forms of TIN, unless associated with a systemic autoimmune disorder (3, 88). Circulating anti-TBM antibodies are detected on rare occasions (89).

Urinary abnormalities vary considerably (*Table 44-4*). Microscopic hematuria is commonly detected and macroscopic hematuria and sterile pyuria also occur (81, 90). Mild to moderate proteinuria (less than 1 g/day) is detected in most patients with TIN (84). Nephrotic range proteinuria is not characteristic of TIN but has been associated with acute TIN induced by certain medications including NSAIDs, lithium, ampicillin, and rifampin (5, 97). Urinary sediment analysis shows granular, hyaline, and white blood cell casts (5). Red blood cell casts are rarely seen in acute TIN (84). Eosinophiluria, defined as greater than 1% of urinary leukocytes stained positively with Hansel's or Wright's stain, is suggestive of acute TIN and has been reported in 50–90% of patients with drug-induced acute TIN (86, 98). However, eosinophiluria can be seen in many inflammatory renal diseases (99). In a study of 51 patients, eosinophiluria had a sensitivity of 40% and specificity of 72%, with a positive

Table 44-3

Patterns of renal tubular dysfunction in tubulointerstitial nephritis

Nephron site	Functional defect	Clinical manifestation
Proximal tubule	Decreased HCO_3, PO_4, amino acids, uric acid, and glucose reabsorption	Fanconi syndrome, hyperchloremic metabolic acidosis, polyuria, hypokalemia
Loop of Henle	Defective NaCl reabsorption, decreased calcium and magnesium reabsorption	Polyuria (polydipsia), salt wasting, calcium, potassium and magnesium losses
Distal tubule	Defective NaCl reabsorption and potassium and hydrogen ion secretion	Hyperkalemia, hyperchloremic metabolic acidosis, sodium and potassium losses
Collecting tubule	Defective water reabsorption	Nephrogenic diabetes insipidus

predictive value for TIN of only 38% (99). In a more recent study the use of Hansel's stain was much superior to Wright's stain in detecting eosinophiluria and differentiating between acute interstitial nephritis and acute tubular necrosis (100).

Renal ultrasound examination usually demonstrates normal or enlarged kidneys, depending on the relative proportions of tubulointerstitial inflammation and edema (3, 81). Gallium citrate scanning may be useful in distinguishing acute TIN from other common causes of ARF (86). However, increased uptake on gallium scan is a relatively nonspecific finding and may be seen in cases of allograft rejection, acute pyelonephritis, and severe minimal change nephrotic syndrome (3). A definitive diagnosis of TIN can be made only by renal biopsy (3, 4).

Pathology

Typical light microscopy findings in acute TIN consist of tubulointerstitial mononuclear cell infiltration and edema, with varying abnormalities in renal tubular epithelial cells (*Fig. 44-1*) (82, 83). In general, vessels and glomerular structures are unaffected (82, 86). Mild mesangial hypercellularity or periglomerular inflammation and fibrosis occasionally are seen (84). Cellular infiltrates, primarily of T-lymphocytes, often are patchy and are variably distributed throughout the kidney (5, 86, 87). Monocytes, macrophages, and plasma cells are also commonly noted in affected areas (84, 88). Eosinophils are most commonly seen with drug-induced acute TIN and can comprise up to 10% of the infiltrate (89). Interstitial granulomas occasionally are seen, particularly in drug-induced or infection-related acute TIN (101, 102). Complement and immunoglobulin deposition are not characteristic of acute TIN (5, 103, 104) but have been noted in children with acute TIN because of systemic lupus erythematosus (SLE), syphilis, hepatitis B, shunt nephritis, and some drug-induced lesions (105).

Tubular epithelial cell damage varies from minimal histologic changes to frank necrosis (85, 103). Most often there is flattening of renal tubular cells with atrophy, degeneration, and loss of the brush border in proximal convoluted tubules (5, 8). Renal tubules are commonly dilated, with splitting or fracturing of the basolateral membrane. The tubule lumens may contain desquamated cells or blood (8, 84). In some instances, infiltrating lymphocytes are observed between tubular epithelial cells (86). Electron microscopy typically reveals striking renal tubular epithelial cell mitochondrial damage, cytoplasmic vacuoles, and significantly dilated rough endoplasmic reticulum (83, 84, 86).

Etiology

The causes of acute TIN can be grouped into four broad categories: medications, infections, immunologic diseases, or idiopathic processes (*Table 44-5*). As described in the pathology section of this chapter, all forms of acute TIN have similar pathologic features. Unique features of distinct forms of acute TIN are described in more detail.

Medications

Medications, rather than infection, are now the leading cause of acute TIN in children (8, 87). An ever increasing list of medications are implicated as causes of acute TIN (*Table 44-6*). However, establishing a clear link between a medication and acute TIN may be difficult, because many case reports of drug-induced acute TIN are in patients who have received several medications simultaneously. In other

Table 44-4

Serum and urine abnormalities often found with tubulointerstitial nephritis

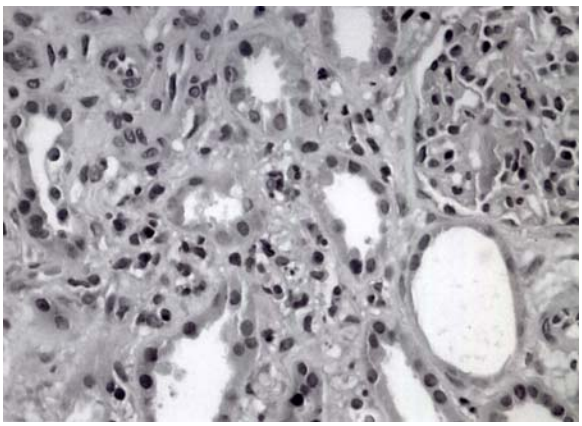
Serum abnormalities
Anemia
Hemolytic
Nonhemolytic[a]
Circulating immune complexes (may be seen in immune-mediated TIN)
Elevated hepatic enzymes (in patients with associated drug-induced liver damage)
Eosinophilia[a]
Hyperchloremic metabolic acidosis (normal anion gap)[a]
Hyperkalemia[a]
Hyponatremia
Hypoaldosteronism
Hypophosphatemia
Hyporeninemia
Hypouricemia
Increased erythrocyte sedimentation rate (ESR)[a]
Increased IgE level
Increased IgG level
Increased BUN[a]
Increased creatinine[a]
Leukocytosis (with or without eosinophilia)
Urine abnormalities
Aminoaciduria
Bacteriuria
Bicarbonaturia
Eosinophiluria (seen usually in drug-induced TIN)
Glucosuria (in absence of hyperglycemia)
Hematuria (usually microscopic)[a]
Hyposthenuria (inability to concentrate urine)
Leukocyturia
Magnesium wasting
Phosphaturia
Proteinuria (usually mild to moderate)[a,b]
Casts
Granular
Hyaline
RBC (seen occasionally in drug-induced TIN)
WBC (seen in infection-mediated acute or chronic TIN)
Salt wasting

[a]Common finding in children

[b]Can be massive with NSAIDs and less commonly with other drugs

Figure 44-1

Acute TIN. Light microscopy of idiopathic TIN in a 14-year-old girl. Cortical renal parenchyma showing a normal glomerulus, extensive interstitial edema, infiltration by lymphocytes and eosinophyls, and cellular epithelial tubular degenerative changes.



instances it might be indeed a combination of medications causing the TIN (106). The major categories of drugs consistently associated with acute TIN in both children and adults include: antibiotics, NSAIDs, anticonvulsants, and diuretics (85, 86, 90). Although symptoms of acute TIN caused by a specific drug can occur within hours or months after starting a medication, they are usually seen 2–3 weeks after starting therapy (4). Antimicrobials and NSAID are the most common drugs causing acute ATN in children (85, 90). The incidence of acute TIN caused by methicillin, which is no longer used, was 16% (90). The incidence of acute TIN with currently available penicillins is significantly less than that previously observed with methicillin. Systemic manifestations of hypersensitivity with fever, rash, and eosinophilia in the setting of ARF can occur in up to 30% of patients with penicillin-induced acute TIN (4). By contrast, these hypersensitivity manifestations are not typically associated with NSAID-induced acute TIN (91). NSAID-induced TIN may be associated with minimal change nephrotic syndrome (97). Although NSAID-induced acute TIN in children occurs less often than in adults, the increasing availability of over-the-counter NSAIDs preparations for children may result in an increase in cases in that age group. Proton-pump inhibitors have recently become another common cause of acute TIN (107).

A category growing in significance is renal damage, including TIN, secondary to dietary supplements (108).

Table 44-5
Etiologic classification of acute tubulointerstitial nephritis

Immune-mediated[a]
Drug hypersensitivity (see *Table 44-6*)
Lactam antibiotics[a]
Other antibiotics
Diuretics
Nonsteroidal anti-inflammatory drugs
Other drugs
Immunologic diseases
Usually associated with glomerulonephritis
IgA nephropathy
Membranous glomerulonephritis
Syphilis
Systemic lupus erythematosus[a]
Usually *not* associated with glomerulonephritis
Allograft rejection
TINU syndrome
Infection-mediated[a] (see *Table 44-7*)
Direct infection of renal parenchyma (infectious agents identified in the interstitium)
Reactive (sterile) interstitial nephritis (infectious agents *not* identified in the interstitium)
Idiopathic

[a]Common cause in children

Drug-induced acute TIN is an idiosyncratic reaction, and as such, it is difficult to predict which patients will be affected. No specific risk factors have been consistently identified. Acute TIN has been reported with various routes of administration including oral, intravenous, intramuscular, and rectal (85, 90, 103, 109). Duration and the dosage of therapy also do not correlate well with the development of disease, although several reports suggest that acute TIN is more common with high-dose therapy (7, 90).

The association of drug-induced acute TIN with fever, rash, and eosinophilia suggests an underlying allergic reaction to administered drugs. Reports of accelerated, recurrent TIN on drug rechallenge also implicate drug hypersensitivity (109, 110). Administration of structurally similar medications may induce cross-reactivity as evidenced by recurrent acute TIN after cephalosporin administration in individuals with previous penicillin-induced TIN (90).

Drug induced acute interstitial nephritis was found to be a cause for graft dysfunction in kidney transplant recipients. Early diagnosis and treatment prevented permanent damage (111).

Infections

In the preantibiotic era, infection, especially Streptococcal, was the predominant cause of acute TIN in children. Councilman's initial postmortem findings of TIN involved 42 children who succumbed to scarlet fever (112). With the development of effective antimicrobial therapy, the incidence of streptococcus-associated acute TIN has decreased. Other infectious organisms have become important causes of acute TIN. These include unusual bacteria such as *Rickettsia* species, *Yersinia*, and mycoplasma. Viruses, such as adenovirus and human immunodeficiency virus (HIV), and parasites are also causes of acute TIN (*Table 44-7*) (5, 113–115). Naturally HIV infection/AIDS may serve as background for other infectious or drug induced TIN (116). Infections may induce acute TIN by two distinct processes. Organisms may directly invade the renal parenchyma, producing local renal infection and inflammation. This form of acute TIN may respond to treatment of the underlying infection. Alternatively, organisms may induce "reactive" intrarenal inflammation without evidence of renal infection. The latter mechanism is implicated by the observation that group A streptococcal infections can cause acute TIN in children, even with appropriate antibiotic therapy (84). Mechanisms for the renal inflammatory reaction in the absence of renal infection are not clearly elucidated but are presumed to be immunologically mediated (84).

Immunologic Diseases

Immunologic diseases elicit acute TIN in two distinct settings (*Table 44-5*). TIN occurs with either a primary glomerular lesion or, less commonly, as an isolated primary TIN. SLE is the most important cause of acute TIN seen in association with glomerulonephritis in children. Tubulointerstitial immune deposits may be present in up to 60% of SLE patients who are biopsied and correlate with both severity of interstitial inflammation and degree of functional renal impairment (117, 118). As is the case with other glomerular diseases, severe tubulointerstitial involvement with fibrosis is a poor prognostic indicator for renal function (105). Acute TIN occasionally is seen in children with membranous nephropathy, postinfectious glomerulonephritis, and shunt nephritis (105). IgA nephropathy was found to be associated with significant TIN in 37% of 51 patients (119). At follow-up, patients with renal tubular deposits had significantly worse renal function than those with isolated glomerular findings (119). Immunologic diseases that present with isolated tubulointerstitial involvement are tubulointerstitial nephritis with uveitis (TINU) syndrome, allograft rejection,

Table 44-6

Drugs associated with acute tubulointerstitial nephritis

Anticonvulsants
Carbamazepine[a]
Lamotrigine
Phenobarbital[a]
Phenytoin[a]
Sodium valproate[a]
Anti-inflammatory drugs and analgesics
Benoxaprofen
Diclofenac
Diflunisal
Fenoprofen
Floctafenine
Glafenin[a]
Ibuprofen
Indomethacin
Ketoprofen
Mefenamic acid
Naproxen[a]
Niflumic acid[a]
Phenazone
Phenylbutazone
Piroxicam
Rofecoxib
Sulfasalazine
Sulfinpyrazone
Sulindac
Suprofen
Tolmetin[a]
Zomepirac
Lactam antibiotics
Cephalosporins
Cefaclor
Cefotaxime
Cefoxitin
Ceftriaxone
Cephalexin
Cephaloridine
Cephalothin
Cephradine
Penicillins and derivatives
Amoxicillin
Ampicillin[a]
Carbenicillin
Cloxacillin[a]
Flucloxacillin
Methicillin[a]
Mezlocillin
Nafcillin[a]
Oxacillin
Penicillin G[a]
Other antibiotics
p-Acyclovir
Azithromycin
Aztreonam
Chloramphenicol
Ciprofloxacin
Clarithromycin
Clotrimazole
Erythromycin[a]
Gentamicin
Indinavir
Isoniazid
Lincomycin
Loracarbef[a]
Nitrofurantoin
Norfloxacin
p-Aminosalicylic acid
Piromidic acid
Polymixin sulfate
Rifampin
Spiramycin
Sulfonamides[a]
Sulfadiazine
Trimethaprim-sulfamethoxazole[a]
Tetracyclines
Minocycline[a]
Vancomycin
Diuretics
Chlorthalidone
Ethacrynic acid
Furosemide
Thiazides
Ticrynafen
Tienilic acid
Triamterene
Other drugs
Aldomet
Allopurinol
Amlodipine

Table 44-6 (Continued)

Amphetamine
Anti-CD4 antibodies
Aspirin
Azathioprine
Bevacizumab
Captopril
Chlorprothixene
Cimetidine
Clofibrate
Clozapine
Coumadin
Crack cocaine
Creatine
Cyclosporin[a]
Cytosine-Arabinoside
Diazepam
Doxepin
Etanercept
Ethambutol
Haloperidol
Heroin
Herbal medicines
Imipramine
Interleukin 2
Mesalazine[a]
Omeprazole
Pamidronate
Pantoprazole
Phenazone
Phenindione[a]
Phenylpropanolamine[a]
Propranolol
Propylthiouracil
Quinine
Radiographic contrast agents
Ranitidine
Recombinant interferon
Streptokinase

[a]Cases reported in children

and rarely, SLE (120–122). Allograft rejection is discussed in detail in Chapter ZZZ.

TINU syndrome was first described in 1975 in a report of two adolescent girls that developed ARF with eosinophilic TIN associated with anterior uveitis and bone marrow granulomas (123). Subsequent reports have highlighted the significant adolescent female predominance (120). The pathogenesis of this syndrome remains unclear and preliminary evidence suggests roles for both humoral and cell-mediated immune mechanisms. Circulating or deposited immune complexes were detected in up to 60% of patients (124, 125). A significant number of patients, however, have no evidence of tubulointerstitial immune deposits on renal biopsy. Analysis of the lymphocytic infiltrates on biopsies of patients with TINU demonstrated a predominance of activated memory T helper cells, thus providing further evidence for cell-mediated events (120). A genetic predisposition is suggested by the occurrence of TINU in identical twins within the same year (126). In other cases, prior infection, use of certain drugs or the presence of an autoimmune disorder have been implicated (127, 128). Among others TINU has been described in association with granulomatous hepatitis (129), hyperthyroidism (130) and Epstein-Barr virus infection (131). Patients usually have anorexia, fever, weight loss, abdominal pain and polyuria. Eye tenderness may not be evident at presentation, as uveitis could occur at any time with respect to the onset of renal disease (132). Laboratory abnormalities include an elevated erythrocyte sedimentation rate, increased serum IgG levels, azotemia, and nonhemolytic anemia. Urinary abnormalities include proteinuria, glucosuria, and sterile pyuria. In most cases the interstitial nephritis resolves completely, either spontaneously or after steroid therapy. In a study on 21 children with acute TIN, TINU patients required a longer period of time for the renal function to recover in comparison with other etiologies (133). The uveitis often requires multiple steroid courses and other immunosuppressives like methotrexate, azathioprine and cyclosporin A, and may relapse (105, 134).

Idiopathic

Idiopathic cases of acute TIN are uncommon in childhood. In a series of 12 children with biopsy-proven acute TIN, only 1 had idiopathic disease (8). These cases are diagnosed by exclusion of the previously described entities and usually are not associated with hypersensitivity symptoms of fever, rash, or eosinophilia (3).

Treatment and Prognosis

The initial treatment of acute TIN is primarily supportive, with dialysis therapy as indicated (3, 4). It is important to

Table 44-7

Classification of tubulointerstitial nephritis associated with infection

Direct infection of renal parenchyma (infectious agents identified in the interstitium)
Bacteria
Leptospira spp.[a]
Mycobacteria
Various species commonly associated with pyelonephritis[a]
Viruses
Adenovirus[a]
Cytomegalovirus[a]
Hantaviruses[a]
Polyoma virus (BK type)
Fungi
Histoplasma
Various species commonly associated with pyelonephritis
Rickettsia
Rickettsia diaporica (Q fever)
Rickettsia rickettsii
Reactive (sterile) interstitial nephritis (infectious agents *not* identified in the interstitium)
Bacteria
Brucella spp.
Corynebacterium diphtheriae
Francisella tularensis[b]
Group A -hemolytic streptococcus[a]
Legionella pneumophila
Mycoplasma hominis
Salmonella typhi
Streptococcus pneumoniae[a]
Treponema pallidum[b]
Yersinia pseudotuberculosis[a]
Viruses
Epstein-Barr virus[a]
Hepatitis B virus[b]
Human immunodeficiency virus (HIV)[a]
Mumps[a]
Rubella virus[b] (togavirus)
Rubeola virus (paramyxovirus)
Parasites
Ascaris[b]
Leishmania donovani
Toxoplasma gondii[a]
Other
Kawasaki disease

[a]Cases reported in children
[b]Incompletely documented or isolated cases

immediately discontinue all possible offending medications which in many patients might be multiple. When replacing the suspected medications, it is important to select medications that are not potentially cross-reactive (e.g., a cephalosporin to replace a -lactam penicillin) or other potentially nephrotoxic agents (90). In infection-related acute TIN, specific treatment of the underlying infection is indicated (84).

The use of corticosteroid therapy for acute TIN remains controversial (7, 135). Anecdotal case reports and uncontrolled trials with small numbers of patients suggest a therapeutic benefit (9, 81, 84). However, prospective controlled studies of corticosteroids or other cytotoxic agents in acute TIN are lacking, and it is possible that many reported patients would have recovered merely from the withdrawal of the inciting agent. Various steroid treatment regimens for acute TIN have been reported, primarily in the adult literature (86, 136). Some children have been treated with daily prednisone at a dosage of 2 mg/kg per day, which is tapered rapidly over 2–4 weeks (84). Pulse methylprednisolone followed by high-dose daily or alternate-day prednisone has also been used in adults (136). It was found recently to be significantly more effective if introduced early in the course of the disease (137). In idiopathic cases and those in which removal of the offending agent do not result in improvement we use a similar protocol of pulse methylprednisolone followed by daily oral prednisone of 2 mg/kg (maximum dose 80 mg) for 4 weeks, then changed to alternate day treatment and tapered over a period of several months depending on the response.

In patients who are steroid-dependent or steroid-resistant, several case reports and small series reported successful use of cyclosporine and mycophenilate (138, 139). It seems that especially the latter may have a room in treating patients who cannot tolerate steroids or are steroid-dependent or steroid-resistant (139). Whether the treatment with mycophenilate can be further potentiated by co-administration of low dose steroids requires further investigation. It seems likely that in coming years, new family of medications targeted at specific sites of the immunologic and injury mechanisms, currently under development, will be ready for clinical application

(140, 141). Their use will be complemented by the identifications of new markers better delineating the nature of kidney injury and response to treatment (142).

Prognosis for recovery of renal function in children with acute TIN is excellent (8, 81, 84, 90). Most affected patients recover renal function completely within weeks to months of onset of ARF. The mean renal recovery time of 13 children with acute TIN from various causes was 69.5 ±34.7 days (84). All patients had normal serum creatinine and urinalysis at follow-up examination 11/2 to 10 years after presentation. Of note, the clinical or histologic severity of disease at presentation did not correlate with rate of recovery of renal function. Another study of 7 children with acute TIN, 42% of whom required dialysis, reported a renal recovery rate of 86% (81). In two studies of adults with acute TIN, poor prognostic factors included the severity of the interstitial inflammation on renal biopsy and the duration of acute renal failure (143, 144).

Chronic Interstitial Nephritis

Epidemiology

Chronic TIN in children occurs primarily in the setting of obstructive uropathy, vesicoureteral reflux, and inherited conditions (3, 9). Obstructive uropathy together with reflux nephropathy account for almost 30% of all children who develop chronic renal insufficiency (145, 146). Primary chronic TIN from other causes accounts for only 2–4% of children with chronic renal failure, and chronic TIN plays a role in some patients with transplant nephropathy (146). In a few families chronic TIN has been described in association with cholestatic liver disease (95, 96) and in others with mitochondrial abnormalities (147, 148).

Clinical, Laboratory and Radiologic Features

Chronic TIN tends to progress more slowly than other forms of chronic renal diseases, and therefore, affected individuals often have no clinical evidence of disease until late in the course of renal insufficiency (3). Many patients have nonspecific constitutional symptoms characteristic of chronic renal failure, with weight loss, growth retardation, fatigue, anorexia, vomiting, and occasionally polyuria and polydipsia (149). Hypertension may also occur (150). Patients with the syndrome of chronic TIN associated with cholestatic liver disease may also have symptoms of hepatic dysfunction, such as pruritis and scleral icterus (151). Naturally in children with chronic TIN a major manifestation may include growth failure.

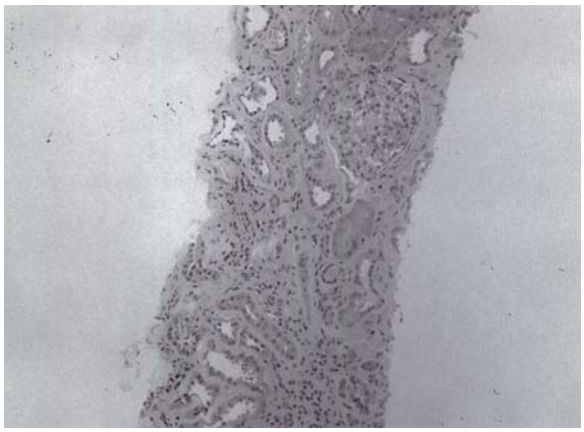
The clinical, laboratory and radiologic findings in chronic TIN are in essence similar to those observed in acute TIN. However, as in other cases of chronic kidney failure, the deterioration in kidney function is insidious and the medical history might extend back to a longer period. In contrast to some patients with acute TIN, patients with chronic TIN do not have oliguria; on the contrary some may be polyuric. In advanced cases, renal ultrasonography shows small and hyperchogenic kidneys. Skeletal radiographs may show renal osteodystrophy and in the case of Fraconi syndrome rickets and osteomalacia.

Pathology

Histologic findings in chronic TIN are characterized by tubulointerstitial fibrosis with a lymphocytic infiltrate, as well as tubular atrophy and thickening of the tubular basement membrane (3, 152). When renal damage is associated with high intratubular pressure, as occurs with vesicoureteral reflux and urinary tract obstruction, dilated renal tubules are particularly evident (83). In primary chronic TIN, glomeruli are often normal until late in the course of disease, when periglomerular fibrosis and global sclerosis are seen (*Fig. 44-2*) (151, 152).

Figure 44-2

Chronic TIN. Light microscopy of idiopathic chronic TIN in a 12-year-old boy. Renal cortex showing moderate interstitial fibrosis, tubular atrophy and tortuosity. The interstitium is infiltrated by scattered mononuclear cells. A preserved glomerulus with periglomerular fibrosis is seen.



Etiology

The causes of chronic TIN are summarized in Table 44-8. Of note, acute TIN from any cause can progress to chronic TIN if the disease process is not abated by removal of the inciting agent or steroid therapy. Secondary chronic TIN associated with primary glomerular or vascular disorders, especially focal segmental glomerulosclerosis and hemolytic uremic syndrome, is a common histologic finding in patients with progressive renal disease (153).

Chronic TIN in children most commonly is due to obstructive uropathy or high grade vesicoureteral reflux, especially when associated with urinary tract infections (146). Urinary tract obstruction of only a few weeks' duration may result in irreversible renal damage (7). Isolated vesicoureteral reflux, without obstruction, might also be associated with chronic TIN in children. Interstitial damage and progressive fibrosis in these disorders may result from renal immune responses that amplify tubulointerstitial injury initially induced by high urinary tract pressures at time starting in utero. Persistent and progressive renal inflammation and damage can occur, however, despite relief of the obstruction or surgical correction of the vesicoureteral reflux, mostly seen nowadays in severe prenatal obstructive and reflux nephropathy (147, 154, 155).

Treatment and Prognosis

There is currently no known effective therapy for chronic TIN. Naturally, when an offending agent is detected it should be removed. However, even in such cases the damage may be irreversible and its progression self perpetuating. Some experimental data indicate the potential for pharmacotherapy in stopping or slowing the inflammatory, apoptotic and fibrotic mechanisms by blocking their stimulating signals and activity. For instance, in the mouse model of unilateral obstruction, injury to the kidneys starts with increased angiotensin II production, which activates TGF-β in a cascade leading to tubulointerstitial inflammation and fibrosis (61). The use of ACE inhibitors in this model diminished transformation of renal cells to interstitial myofibroblasts and decreased migration of inflammatory cells into the interstitium. It is possible that the addition of aldosterone receptor blocker to the ACE inhibitor augments its beneficiary effect (156).

In a study in the mouse model of unilateral ureteral obstruction, injection of exogenous hepatocyte growth factor blocked myofibroblast activation and dramatically prevented interstitial fibrosis (157). The study suggested

Table 44-8

Etiologic classification of chronic interstitial nephritis

Drug-related
Acetaminophen
Antiretroviral
Aspirin
cis-Platinum
Cyclosporine[a]
Lithium
L-Lysine
Methoxyfluorane
Nitrosoureas (streptozotocin, CCNU, BCNU)
Phenacetin
Phenylbutazone
Propylthiouracil
Heavy metal-related
Arsenic
Bismuth
Cadmium
Copper
Gold
Iron
Lead
Mercury
Uranium
Hereditary
Acute intermittent porphyria
Alport's syndrome
Alstrom syndrome
Cystinosis
Lesch-Nyhan syndrome
Medullary cystic disease – Juvenile nephronophthisis complex
Medullary sponge kidney
Oxalosis
Methylmalonic acidemia
Fabry disease
Polycystic kidney disease
Sickle cell disease
Wilson disease
Idiopathic
Infection-mediated (see Table 44-7)
Direct infection of renal parenchyma (chronic or recurrent acute pyelonephritis)
Reactive (sterile) interstitial nephritis

Table 44-8 (Continued)

Immune-mediated
Diseases usually associated with glomerulonephritis
Systemic lupus erythematosus[a]
Antiglomerular basement membrane disease
IgG4-related autoimmune disease
Mixed cryoglobulinemia
Polyarteritis nodosa
Wegener granulomatosis
Diseases *not* usually associated with glomerulonephritis
Allograft rejection
Chronic active hepatitis
Familial immune complex interstitial nephritis
Sjogren syndrome
Tubulointerstitial nephritis and uveitis syndrome (TINU syndrome)
Radiation-induced (induces cytotoxic reactive oxygen molecules)
Metabolic
Hypercalcemia
Hypercalciuria
Hyperphosphatemia
Hyperuricemia
Hyperoxaluria
Hyperparathyroidism
Hypokalemia (potassium-losing nephropathy)
Miscellaneous
Anorexia nervosa
Balkan nephropathy
Hemorrhagic fever
Syndrome of tubulointerstitial nephritis and chronic cholestatic liver disease
Hypoxic disorders
Sarcoidosis
Neoplastic
Leukemia
Lymphoma
Multiple myeloma
Urologic[a]
Urinary tract obstruction
Calculi
Congenital
Posterior urethral valves[a]
Prune belly syndrome[a]
Ureteropelvic junction obstruction[a]
Surgery
Tumor
Vesicoureteral reflux[a]

[a]Common cause in children

that blockage of the transition of tubular epithelial cells to myofibroblasts may provide a novel therapeutic approach in halting fibrosis. In another study on the same animal model administration of bone morphometric protein-7 (BMP-7) prevented tubulointerstitial fibrosis and inhibited tubular atrophy by prevention of apoptosis. The exact mechanism of action by which BMP-7 provides renoprotective effect is yet unknown (61). Several studies have demonstrated the potential of 3-hydroxy-3-methylglutaryl CoA (HMGCoA) reductase inhibitors, like simvastatin and fluvastatin, in ameliorating interstitial fibrosis in animal models (158, 159). Other studies examined the effect of blocking TGF-β and its mediators in tissue fibrosis, like connective tissue growth factor (160). A different direction of investigation is focused on the potential protective effect of nitric oxide in chronic TIN (63, 161, 162).

At the moment, patients with chronic TIN are treated with supportive therapy. Whether the use of ACE inhibitors or HMGCoA reductase inhibitors in human chronic TIN is effective as is in some glomerular diseases associated with proteinuria has yet to be proven.

The prognosis for normalization of renal function in chronic TIN is less favorable than in acute disease. In specific forms of chronic TIN, such as juvenile nephronophthisis, progression to end-stage renal disease is almost inevitable (149, 150). By contrast, patients with chronic TIN in the setting of obstructive uropathy have a more variable clinical course (147, 154).

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Systemic Disease

45 Vasculitis

Seza Ozen

Vasculitis is the inflammation in a blood vessel wall. This inflammation leads to narrowing or aneurysm of the vessel resulting in protean manifestations in the tissue that it nourishes. Kidney is one of the target organs frequently affected in vasculitis. The vasculitis syndromes have certain predilections for the size of the vessel and the organ they affect. A number of attempts have been made to classify the vasculitis syndromes based on these features. The nomenclature criteria suggested by the Chapel Hill Consensus Conference has been the most widely accepted (1). However, these criteria are mainly definition criteria. The American College of Rheumatology has suggested classification criteria for each vasculitis (2–4). However, these are strictly based on adult experience. Thus, in 2006 a group of pediatric rheumatologists and nephrologists published the working classification for childhood vasculitides (5) (*Table 45-1*) and classification criteria for the common childhood vasculitides (5). One should remember that all the mentioned criteria are classification criteria and the diagnosis of these diseases depend on the judicious assessment of the patient.

The most common vasculitides of children are Henoch Schonlein purpura (HSP) and Kawasaki Disease (KD), depending on the geographic area. HSP is reviewed in a separate chapter.

Kawasaki Disease (KD)

Definition and classification: KD is a vasculitis that affects the skin and mucosa as well. KD is characterized by a special preference for the coronary vessels. According to the Chapel Hill nomenclature criteria, KD is defined as arteritis involving large, medium-sized and small arteries and associated with mucocutaneous lymph node syndrome (1). KD is classified according to the presence of fever of at least 5 days duration plus four of the following five features (5–7):

Changes in peripheral extremities (edema and/or erythema of the hands and feet and subsequent periungual desquamation) or desquamation of the perineal area (*Fig. 45-1*).
Polymorphous exanthema mainly truncal,
Bilateral nonpurulent conjunctival injection,
Changes of lips and oral cavity: injection of oral and pharyngeal mucosa and
Cervical lymphadenopathy, usually unilateral.

As is the case in many vasculitides, KD is an abnormal immune response to environmental or infectious trigger (s), in a certain individual who is predisposed by virtue of their genetic constitution. Superantigens have been frequently implicated as the causative agents. This has been supported by the skewing of the T cell receptor Vβ repertoire (8). The fact that KD mainly affects the young children may be because of timing of the exposure of the environmental agent in a naïve immune pathway.

Clinical features: The clinical features of the disease are those included in the diagnostic criteria (7). Renal involvement is not common. However, pyuria and urethritis are frequent (8). Since this is a vasculitis affecting medium size arteries, it is perhaps not surprising that renal artery has been involved in a significant portion of historic biopsies (9). With improving imaging techniques, there have been reports of renal artery aneurysms in patients with KD albeit rare. Thus angiographic evaluation of other mid size arteries should be done as deemed necessary. Renal parenchymal involvement and acute renal failure have been reported only in rare case reports; one with a biopsy showing acute interstitial nephritis (10).

In the laboratory work-up, a complete blood count is mandatory. Thrombocyte counts are elevated at the second week of the disease; however, in the early phase of the disease they may be abnormally low (8). Acute phase reactants are high. Every child suspected of having KD should have an echocardiography for the search of coronary artery involvement.

Treatment: The treatment of KD consists of intravenous immunoglobulin (IVIG) administered at a dose of 2 g/kg along with salicylates at a dose of 60–80 mg/kg/d (7, 8). Steroids may be indicated in patients resistant to IVIG.

KD is the top cause of acquired heart disease in the western world (7). Thus, early recognition of the disease and administration of IVIG in the first 10 days of initiation is crucial. Aspirin should be given for 6 weeks

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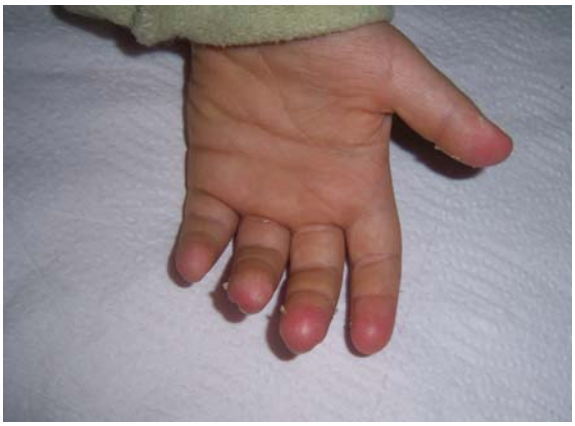
Table 45-1

New classification of childhood vasculitides (with permission, (5)

I. Predominantly large vessel vasculitis
Takayasu arteritis
II. Predominantly medium-sized vessel vasculitis
Childhood Polyarteritis Nodosa
Cutaneous Polyarteritis
Kawasaki Disease
III. Predominantly small vessels vasculitis
A. Granulomatous
Wegener granulomatosis
Churg-Strauss syndrome
B. Nongranulomatous
Microscopic polyangitis
HSP
Isolated cutaneous leukocytoclastic vasculitis
Hypocomplementic urticarial vasculitis
IV. Other vasculitides
Behçet disease
Vasculitis secondary to infection (including Hep B-associated PAN), malignancies, and drugs, including hypersensitivity vasculitis
Vasculitis associated with connective tissue diseases
Isolated vasculitis of the CNS
Cogan syndrome
Unclassified

Figure 45-1

Peeling in the hands of a patient with Kawasaki Disease. (See color plate 27)



or until the ESR and thrombocyte counts are normal and lifelong for those who have any cardiac involvement.

Childhood Polyarteritis (PAN)

The Chapel Hill Nomenclature criteria (CHCC) defines polyarteritis in two separate groups (1): Classic PAN is defined by the CHCC as the necrotizing inflammation of medium-sized or small arteries without glomerulonephritis or vasculitis in arterioles capillaries or venules. The same group has defined microscopic polyarteritis or polyangiitis (MPA) as necrotizing vasculitis, with few or no immune deposits, affecting small vessels (i.e., capillaries, venules or arterioles). Necrotizing arteritis involving small and medium sized arteries may be present. Necrotizing glomerulonephritis is very common and pulmonary capillaritis often occurs (1).

The consensus conference held with pediatric rheumatologists and nephrologists have revised the ACR and other existing pediatric criteria of polyarteritis nodosa (PAN) (*Table 45-2*) (5). Childhood PAN differs from microscopic polyangiitis through the absence of necrotizing glomerulonephritis and ANCA. In childhood PAN, kidney involvement occurs through necrotizing arteritis of the renal vascular bed whereas, as mentioned above, the presence of necrotizing glomerulonephritis classifies the child as MPA (11). In a large adult series, Agard et al. reported that the initial manifestations of both classic PAN and MPA were very similar except for gastrointestinal involvement and peripheral neuropathy being more frequent in PAN (12).

Childhood PAN also differs from adult PAN with the possible involvement of small size arteries as well (13). Furthermore the HBsAg associated PAN is very rare in children.

The new suggested criteria of childhood PAN include the demonstration of necrotizing arteritis in small-medium size arteries or aneurysms in mid-size arteries by imaging techniques (5).

Pathogenesis: As in KD, infections are implicated as a triggering agent in a predisposed host. In fact, a number of reports point to the role of streptococci in childhood PAN (13). In animal models, polyarteritis-like vascular lesions have been induced by infectious agents (14). On the other hand, other forms of PAN have more specific pathogenesis: HBs Ag-related classic PAN is an immune complex disease. MPA is a disease related to MPO-ANCA where the role of ANCA is vascular and capillary injury is similar to that suggested for WG (see below).

Table 45-2

Classification criteria for childhood PAN (reference 5)

A systemic illness characterized by a biopsy showing small and mid-size artery necrotizing vasculitis OR *Angiographic abnormalities* (aneurysms or occlusions)*
In the presence of at least two out of the following seven criteria
1. Skin involvement (livedo reticularis, tender subcutaneous nodules, other vasculitic lesions)
2. Myalgia or muscle tenderness
3. Systemic hypertension, relative to childhood normative data
4. Mononeuropathy or polyneuropathy
5. Abnormal urine analysis and/or impaired renal function
6. Testicular pain or tenderness
7. Signs or symptoms suggesting vasculitis of any other major organ system (gastrointestinal, cardiac, pulmonary, or central nervous system)

Clinical features: PAN often presents with general findings and wide range of systemic symptoms. In an effort to define the characteristics of childhood PAN throughout the world, we have recruited 110 patients from 21 pediatric centers from over the world (13). The mean age of the patients was 9.05 ± 3.57 years with a range of 1 to 16 years. There were an equal number of girls and boys (13). These features were similar to those reported in small series and were different from the adult data. The average ages of patients were 9.3 and 7.5 years in two series both including infants (15,16). Children almost always have general symptoms such as malaise, fever and/or weight loss. A number of patients do not have typical presentations of microscopic or classic PAN, but have inflammation in varying sized arteries of skin, musculoskeletal, and other organ systems (15,17).

Kidney involvement presents with systemic vasculitis along with an ischemic kidney involvement ranging from hypertension to tubulointerstitial nephritis or glomerular features. In previous small series before the Chapel Hill criteria were introduced, renal involvement was present in 65–80% (18,19). In the aforementioned multinational study, renal involvement was present in a significant proportion of the patients. The participating pediatricians classified 57.2% of their patients to have a systemic PAN disease, 30% as cutaneous PAN and 8.1% as microscopic PAN or polyangiitis (13). The clinical features of the childhood PAN are clearly reflected in this large series of childhood patients. Vessel size was not segregated in the classification of these patients. The disease often has an insidious onset with constitutional symptoms, typical skin lesions, abdominal pain and musculoskeletal complaints (13).

Renal involvement in childhood PAN may present as hypertension reflecting the necrotizing vasculitis of the renal artery. In fact, in the series of 110 children with PAN, 43% of the patients with systemic form of the disease had hypertension. Eighteen had abnormalities in the urinalysis whereas seven (11.1%) had impaired renal function during the course of their diseases. As to other organ systems involved, one-third had CNS involvement. Pulmonary and cardiac disease was reported in 11% and 14%, respectively (13).

Diagnosis was reached by angiographies in 52.5% of the patients, whereas it was through biopsies from various sites of involvement in the rest (13). The biopsies demonstrated the typical pauci-immune necrotizing arteritis.

In the last decade, a number of childhood series have been reported (15). Five patients with PAN and six patients with MPA have been reported from India (20). General symptoms, musculoskeletal complaints, peripheral neuropathy and skin lesions dominated the clinical picture. All the patients with PAN were HBsAg and ANCA negative and had normal urinalysis findings. In contrast, all patients with MPA demonstrated an active urine sediment and 83.3% were pANCA positive (20). Among the series of 7 patients reported by Peco-Antic et al., 29% presented with acute renal failure and three had deterioration of renal function (21).

Complete blood count may reveal anemia, leukocytosis and thrombocytosis. The work-up should include urinalysis, monitoring renal function, acute phase reactants to reflect disease activity and study of ANCA. It should be remembered that ANCA is often negative in childhood PAN although there may be nonspecific indirect immunofluorescence staining (13). Acute phase reactants are often elevated. Imaging for mid-size arteries is required for the patient for a search of aneurysm in a patient who presents with hypertension or ischemic abdominal pain. In a childhood series, children with medium or large vessel aneurysms or those with renal perfusion defects on angiography had a higher likelihood of having renal impairment (5, 16). In a review of the angiograms in PAN patients, Brogan et al. have concluded that the most reliable non- aneurysmal findings were of perfusion defects, presence of collateral arteries, lack of crossing of peripheral renal arteries, and delayed emptying of small arteries (16). In patients without hypertension, renal involvement is unlikely and mesenteric or hepatic arteritis may be the cause of abdominal pain and ischemia.

Treatment: Childhood PAN is a severe vasculitis that necessitates efficient treatment. Corticosteroids are the basis of treatment. Treatment of the systemic disease is similar to that of ANCA-related vasculitides. The pediatric literature reveals that additional immunosuppressive treatment is often used for these children. However, we need long-term and multicenter studies to define the need of additional immunosuppressive treatment and the length of steroid therapy in patients with limited disease.

Microscopic Polyangiitis

If the child with vasculitis presents with a rapidly progressive glomerulonephritis, then one needs to consider necrotizing glomerulonephritis (*capillaritis*) which is associated with ANCA (1) (see above). During the aforementioned conference of pediatricians, the presence of antineutrophil cytoplasmic antibody (ANCA) directed against the MPO antigen shown by ELISA and/or strong perinuclear staining pattern by indirect immunoflourescence (IIF) has been added to the previous definition of CHCC (5).

Pathogenesis: Both in vitro and in vivo experimental data support the pathogenic role of ANCA in MPA (22). In fact, a recent mice model has introduced direct role of the injury by anti-MPO: anti-MPO IgG led to focal necrotizing and crescentic GN with a paucity of glomerular immune deposition which mimics the human disease (23). On the other hand, we still need further data on the factors that are effective in the production of ANCA at the first place and the genetic factors involved.

Clinical and laboratory features: The presence of histopathologically proven necrotizing glomerulonephritis along with a positive MPO-ANCA titer in the absence of granulomatous lesions and upper respiratory tract involvement leads to a diagnosis of MPA (5). In the largest adult series of 85 MPA patients, 78.8% had renal symptoms and 47 had renal insufficiency (24). Childhood cases have been reported as small series. Hattori et al. (25) have reviewed 34 ANCA-seropositive Japanese pediatric patients with biopsy-proven pauci-immune necrotizing crescentic glomerulonephritis and 21 were classified as microscopic polyangiitis. The authors concluded that the disease was similar to that of adults except for female predominance (25). Patients who subsequently developed end-stage renal disease (n = 9) had significantly higher average peak serum creatinine levels and more chronic pathologic lesions at diagnosis compared with patients with favorable renal outcome (25).

In a pediatric series reported by Peco-Antic et al., 57% of patients had pulmonary-renal syndrome and two had acute renal failure progressing to ESRD (21). Another one developed chronic renal failure (42.8%). In another series of 6 pediatric patients from India, 50% presented with pulmonary renal syndrome (20). We reported a series of 25 patients with PAN, 10 of whom were classified as small vessel necrotizing arteritis (microscopic PAN) (15). Six developed renal failure and three of them had pulmonary-renal syndrome. All had high MPO-ANCA levels. Six had renal biopsies showing pauci-immune, necrotizing crescentic glomerulonephritis. Four of them (40%) progressed to chronic renal insufficiency (15). All the 19 MPA patients and 1 WG from China presented with hematuria and proteinuria and 80% had acute renal failure. Eighty five percent had various multiorgan involvement (26).

Laboratory investigations: An immuno-fluorescence testing for ANCA often reveals a p-ANCA pattern. Further ELISA testing should also be ordered which typically will show a high titer of MPO-ANCA (5, 22, 24). Urinalysis will reflect the glomerular disease with proteinuria, hematuria and an active urine sediment with casts. Renal function tests were impaired in 30–60% of the pediatric patients reported in the pediatric series (15, 20, 21). A chest X-ray is required in all patients. In patients with X-ray abnormalities, more sophisticated techniques such as CT are needed to define the extent of the lung disease.

Kidney biopsy is indicated in children with kidney involvement. The renal biopsy will typically reveal a pauci-immune necrotizing glomerulonephritis (*Fig. 45-2*). Immunofluorescence shows no specific staining and few if any electron dense deposits are present on electron microscopy (17, 24).

Differential diagnosis includes other ANCA-associated vasculitides such as Wegener Granulomatosis and Churg Strauss syndrome, lupus nephritis, severe cases of Henoch Schonlein nephritis, and coagulation disorders.

Treatment and Prognosis: The prognosis of ANCA-related vasculitides in children, MPA and WG, have substantially improved with the new treatment modalities in adult patients (27).

Treatment can be divided into two parts as induction and maintenance:

For induction treatment, corticosteroids and cyclophosphamide are widely accepted. Daily oral corticosteroids (1–2 mg/kg/d) and cyclophosphamide (2 mg/kg/d p.o. or monthly IV pulses 0.75gr/sqm) may be initiated as induction therapy. Whether cyclophosphamide is to be given via an oral or IV route is still a matter of debate. Although we wait for the results of the CYCLOPS trial, there is probably no significant difference between the two modalities. In patients with severe disease, intravenous methylprednisolone for 3 days is also suggested.

Figure 45-2
Glomerulus with segmental necrotizing lesion, inflammatory cell infiltrate and epithelial cresent (HE, original magnification: ×400). (See color plate 28)

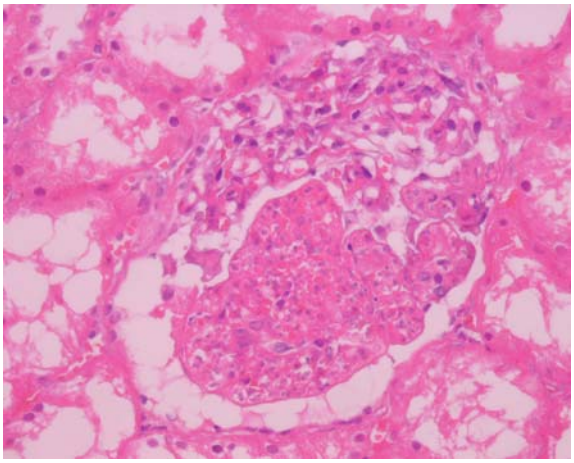
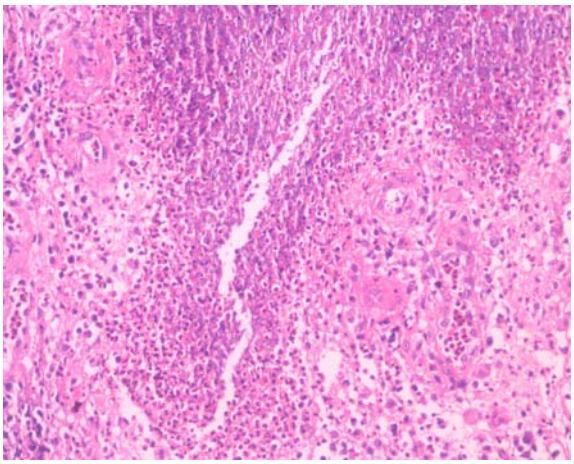


Figure 45-3
Maxillar sinus biopsy revealing a vessel wall showing evidence of vascular necrosis with transmural vasculitis and granulomatous inflammation with multinuclear giant cells around the vessel (HE, original magnification: ×200). (With the courtesy of Dr D Orhan, Hacettepe University). (See color plate 29)



For maintenance treatment, the steroid dose is tapered following clinical and laboratory response but not before 4 weeks to 1 mg/kg/d and the dose is decreased by 10 mg every 2 weeks to reach a minimum dose of 10 mg/d and subsequently every other day. This dose is continued for a year after clinical remission. For maintenance regimen, total cyclophosphamide dose has always been a concern for pediatricians. The CYCAZAREM study has shown that the replacement of cyclophosphamide with azathioprine at 3 months is also effective for disease control (27). Methotrexate has also been shown to be an alternative for maintenance treatment (28). A number of adult series have suggested the continuation of oral cyclophosphamide after remission.

Plasmapheresis, in addition to immunosuppressive treatment, may be used for life-threatening disease. The MEPEX study has shown that plasmapheresis is advantageous over pulse steroids in patients with renal failure (29).

The prognosis in children seems to be better than that reported in adults. In the childhood multinational series of 110 patients, there was only one (1.1%) death and two (2.2%) end-stage renal disease (ESRD) (13). In the adult series, the morbidity and mortality rate are significant.

Wegener Granulomatosis

WG is defined as a granulomatous inflammation involving the respiratory tract and necrotizing vasculitis affecting small to medium-sized vessels. Necrotizing glomerulonephritis is common (1). WG is a vasculitides with major kidney involvement like microscopic polyangiitis. According to the criteria revised for childhood, three of the following six should be present to classify a child as having WG (5):

granulomatous inflammation on biopsy (*Fig. 45-3*),
abnormal urinalysis,
nasal-sinus inflammation,
subglottic, tracheal or endobronchial stenosis,
an abnormal chest x-ray or CT and
PR3 ANCA or C-ANCA staining.

Pathogenesis: The etiology of WG remains unknown; however, the endothelium itself is the target for initial injury. Recent studies suggest a direct involvement of ANCA in the pathogenesis of the disease and that inflammatory cells primed with ANCA are cytotoxic to the endothelial cell (22, 30). It has been shown that In vitro ANCA induces endothelial injury through the activation of neutrophils and the reactive oxygen molecules, proteases and cytokines that they release. In WG, ANCA is "active" against the proteinase 3 (PR3) on neutrophils. In vitro ANCA can further activate neutrophils to release reactive oxygen species, which in conjunction with neutrophils can damage and start the inflammation of endothelial cells (30). However, interestingly the mice model defined with MPO-ANCA was not been able to be repeated in PR3-deficient mice (22).

There are a number of hypothesis about why ANCA is produced in the first place (31). There have been a number of genetic associations with the disease including HLA antigens, a region on chromosome 6p21.3 (31). The polymorphisms of the natural inhibitor of PR3, alpha-1-antitrypsin was also studied as a susceptibility factor for the disease: heterozygosity for alpha1-antitrypsin deficiency has been associated with an increased risk and morbidity of WG (32). As to environmental factors, respiratory infections especially staphylococcal infections may have a triggering role during onset and relapses (32). The reader is referred to an excellent review on the existing hypotheses on the etiology of ANCA-associated vasculitis (31).

Clinical and laboratory Features: Data from small pediatric series reflect that 90% of children with Wegener granulomatosis present with symptoms related to the upper and lower respiratory tract vasculitis (33–35). This consists of sinusitis, epistaxis and nasal inflammation. Lower-airway inflammation will manifest as cough, dyspnea and hemoptysis and 30% will have abnormalities in the chest X-ray (33–35). In a recent series, upper airway involvement occurred in 21 patients at presentation and 24 during follow-up (35). Twenty patients had initial pulmonary involvement, most commonly nodules (44%) and pulmonary hemorrhage (44%). Four patients (16%) had venous thrombotic events. (35).

Kidney involvement occurs in 10 to 100% of affected children (33–35). In patients with kidney involvement, the findings range from proteinuria and/or hematuria to impairment of renal function. According to a recent series of 25 children, glomerulonephritis was present in 22 patients at presentation; Only one of 11 patients who presented with or developed renal impairment had normalization of serum creatinine (35). Necrotizing glomerulonephritis is one of the most serious manifestations in WG: a significant proportion of patients develop renal insufficiency (32).

Other clinical findings may include blurred vision, eye pain, conjunctivitis, episcleritis, persistent otitis media (33–35). CNS involvement may occur and present as cranial nerve palsies, seizures or neuropathies (32). Skin lesions include purpuric maculas, nodules, ulcerations and gangrene. Musculoskeletal complaints may be present. Cardiac involvement is very rare (32). Gastrointestinal involvement may occasionally be present in the form of nonspecific pain, nausea and vomiting (32, 33).

ANCA are present (1, 5). A cytoplasmic pattern on immunofluorescent staining of ANCA (c-ANCA) is present in 70–90% of patients with active WG (32, 35). ELISA tests detecting PR3-ANCA (Proteinase 3) will confirm this specificity in a high proportion of the patients.

White blood count and acute phase reactants are elevated (32). RF may be positive in low titers. Urinalysis is essential and will show hematuria, proteinuria casts when there is kidney involvement. Chest X-ray and pulmonary function tests are indicated for the assessment of lung disease (5, 35). The chest X-ray may show nodular infiltrates and nodules. Further assessment with a chest CT may be required.

Biopsies of airways show the characteristic granulomatous inflammation with necrotizing vasculitis and patchy necrosis (*Fig. 45-3*). Kidney biopsy shows pauci-immune focal necrotizing crescentic glomerulonephritis (1, 32–35). In about half of the cases, renal vasculitis can also be seen. Unlike the biopsy of lung or sinuses, the renal biopsy rarely shows a granuloma.

Differential diagnosis is similar to MPA. In addition, other granulomatous diseases such as sarcoidosis, lymphomatoid granulomatosis and tuberculosis are also included in the differential diagnosis of a child with WG.

Treatment and Outcome: Treatment of WG and microscopic polyangiitis is similar (36). In fact, most of the adult studies published by the EUVAS (European Vasculitis Study group) include MPA and WG patients together (27, 28).

Etanercept which is an TNF receptor blocker, has not been shown to be effective in WG in a prospective and randomized trial (37). Rituximab, an anti-CD20 monoclonal antibody, has been shown to be effective in small series (36).

Relapses are frequent as in the adults. Stegmayr et al. have found that relapses occurred after a median of 28 months (4–120) (34).

In WG, treatment with trimethoprim sulfamethoxazole is justified by the reports showing its prevention of relapses (32, 36). The overall data support the strong prognostic impact of renal function at diagnosis and of renal relapses during follow-up.

Churg Strauss Syndrome (CSS)

CSS is defined by the CHCC as a systemic necrotizing vasculitis with hypereosinophilia and extravascular granulomas along with asthma (1). The classification criteria have been developed by the ACR (38). CSS is very rare in childhood; the literature consists of case reports only.

The disease presents with long history of asthma. As indicated in the ACR criteria, the children are expected

to have features of eosinophilia, a history of allergy, peripheral nervous system involvement, or sinus involvement. Skin involvement and vasculitis is similar to that of PAN. However, this pauci-immune small vessel vasculitis is distinguished form WG and MPA by the presence of granulomatous inflammation and asthma along with hypereosinophilia. Among adult patients, about 20% develop renal involvement in the form of glomerulonephritis and renal insufficiency (39).

The laboratory work-up is similar to MPA or WG. This is one of the ANCA-associated vasculitides. Typically, a p-ANCA pattern is expected. Specific laboratory tests are required for the ACR criteria and differential diagnosis.

Treatment is similar to that of other ANCA-associated vasculitides (28).

Takayasu Arteritis (TA)

Takayasu arteritis is defined as a granulomatous inflammation of the aorta and its main branches (1, 2). Although this has not been confirmed, children seem to have a higher incidence of renal artery stenosis. This led to the inclusion of hypertension as a criterion to the new childhood criteria. According to the revised classification of TA for childhood, *angiographic abnormalities* (conventional, CT or MR) of aorta or its main branches is a mandatory criterion and at least one of the following four should be present (5):

1. Decreased peripheral artery pulse(s) and/or claudication of extremities
2. Bruits over aorta and/or its major branches
3. Blood pressure difference >10 mmHg
4. Hypertension (related to childhood normative data)

The exact etiology and pathogenesis are unknown. Inflammation of the vasa vasorum and myointimal proliferation of the involved large arteries is a marked feature of the disease (40).

Clinical and laboratory features: General symptoms may occur. End-organ ischemic features ensue according to the involved vessel site. Relevant symptoms may be claudication, abdominal pain, headache, syncope, stroke, and renal hypertension due to renal artery involvement (2). Hypertension is the most common renal manifestation. In a report of 10/25 kidney specimens for autopsies, 10 (40%) were classified as diffuse mesangial proliferative glomerulonephritis and 4 (16%) as other associated glomerulopathies (41).

In adults, the disease is divided into prepulseless phase and a pulseless phase. The prepulseless disease is not always evident in children. Aorta and the large arteries on both sides of the diaphragm may be involved. Symptoms depend on the anatomical location of the artery and on the type of lesion (stenotic, occlusive or aneurysmal).

Laboratory findings frequently include elevated ESR and CRP (42). Autoantibodies to nuclear antigens and ANCA are negative. The diagnosis depends on imaging techniques that demonstrate stenosis, and/or aneurysmal changes and/or aortitis in the aorta and its main branches. An MR-angiography or CT- angiography may provide the evidence of TA. In fact these latter technologies also provide information for the thickening of the vessel wall which reflects the inflammation. Recent reports highlight the value of PET-scan in the diagnosis of this disease. However, PET-scan is expensive and should be reserved for rare instances where differential diagnosis of fibromuscular dysplasia pose a problem.

Treatment and prognosis: Corticosteroids constitute the main treatment as other vasculitides. Concomitant use of methotrexate provides a higher remission rate and lower dose of maintenance steroid (40, 42). Cyclophosphamide may be required for patients with severe or resistant disease (42). Anti-TNF treatment also appears to be promising in selected cases (40). For patients who require revascularization intervention, both surgical and endovascular procedures can be performed, with low morbidity and mortality (40). The prognosis has improved with lower relapse rates. However, the evaluation of disease activity is a major problem in the management and follow-up. Any sign of vascular ischemia, a rising ESR, and new angiographic abnormalities should suggest a relapse of the disease and patients should be managed accordingly.

Behcet Disease

Behcet disease is classified according to the criteria used for adults where the presence of oral ulcers is a mandatory criterion. Two other criteria are needed: genital ulcers, skin findings, eye involvement and a positive pathergy test (43).

Behcet disease is rare in childhood (42). However, it has certain characteristics that would be of interest. Behcet disease is the only vasculitis that affects vessels of all sizes and both arteries and veins – except SLE. Behcet disease is a vasculitis that affects the mucosal membranes and the skin just as KD. The epidemiology suggests a certain spread through the ancient "silk road." BD has wide range of renal involvement (44). The common types of glomerular lesions among the reported cases

are crescentic GN, proliferative GN, and immunoglobulin A (IgA) nephritis (44). Aneurysms may be located throughout the renal artery, from the orifice of the main artery to intrarenal microaneurysms.

Treatment depends on the organ system involved and frequently requires immunosuppressive treatment.

In conclusion, kidney is an organ that is frequently involved in vasculitides. It has a major impact on the morbidity of the disease. Careful assessment and effective management of these patients are essential.

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46 Henoch-Schoenlein Purpura

Rosanna Coppo · Alessandro Amore

History and Diagnostic Criteria

Heberden was the first to report, the beginning of 1800, the observation of one child presenting with petechial hemorrhages on lower limbs, joint, and abdominal pain, bloody stools and gross hematuria (1). The syndrome was named after the description by Schoenlein of the clinical entity characterized by purpura and joint pain (2) and by Henoch of the frequent association of gastrointestinal symptoms and kidney involvement (3). Henoch-Schoenlein purpura (HSP) is a small vessel vasculitis with multiorgan involvement including skin, gastro-intestinal tract, joints, and kidneys with variable clinical expression (4, 5). The past ambiguous definitions of hypersensitivity angiitis, anaphylactoid purpura or streptococcal rheumatic peliosis indicate the frequent clinical relationship between HSP and either allergy or infections.

The 1994 Consensus Conference on Nomenclature of Systemic defined HSP as a small vessels vasculitis (involving capillaries, arterioles, venules) with immunoglobulin (Ig) A (IgA)-dominant immune deposits, typically involving skin, gut, and glomeruli and associated with arthralgias or arthritis (6). The European League against Rheumatism/Pediatric Rheumatology European Society (EULAR/PReS) endorsed consensus criteria for the classification of childhood vasculitis proposes as classification criteria for HSP the presence of palpable purpura (mandatory criterion) and at least one of the following features: diffuse abdominal pain; any biopsy showing predominant IgA deposits; arthritis or arthralgia; renal involvement (any hematuria and/or proteinuria) (7).

The differential diagnosis, which is sometimes difficult in adults, is thought to be easy in children as many pediatricians consider that purpura without thrombocytopenia is sufficient to allow the diagnosis of HSP. However, skin biopsy confirms the suspect in only half of children (8), showing granulocytes in the walls of small arterioles or venules (leukocytoclastic vasculitis) and IgA vascular deposits.

Pathogenesis

The finding of IgA deposited in the vessel wall, as well as in the glomerular mesangial area, allows a clear-cut distinction of HSP and other systemic vasculitides or collagen diseases with similar multiorgan involvement.

IgA is found in serum and mostly in external secretions, playing a major role in mucosal immunity. Two distinct subclasses of IgA exist, IgA1 and IgA2, differing with the insertion of 19 aminoacids, peculiar to IgA1 and deleted in IgA2 subclass in the hinge region connecting the CH1 and CH2 domains, at the junction between the Fab and the Fc portions of the IgA1 molecule (9). Either subclass is synthesized by plasma cells as a 155 kD protein consisting of two α heavy chains and two κ or λ light chains, or as dimers or polymers of the basic 4-chain Ig structure, with molecular weights multiple of 155 kD. Dimers are joined by a J chain and can be transported from the basolateral to the luminal surface of secretory epithelium via a specialized glycoprotein receptor, the secretory component. In humans, serum IgA is predominantly monomeric, of the IgA1 subclass, and is derived from plasmocytes within the bone marrow and spleen. Mucosal-derived plasmocytes produce predominantly dimeric IgA containing J-chain (10) of both IgA1 and IgA2 subclasses. Glomerular deposits in HSP as well an in primary IgAN are made of polymeric IgA1, leaving open the possibility of either bone-marrow or mucosal origin, by a somehow disturbed synthetic pathway (11).

HSP, as well as primary IgAN, can be triggered by mucosal infections. Since IgA is the prominent Ig in mucosal secretions acting as a defense against viral and bacterial agents, several authors have tried to identify the eliciting antigen(s). HSP could be secondary to an immune response to antigen/s because eluted mesangial IgA cross-react with the mesangial area of other biopsy samples from different HPS patients (12). The concept of HSP as an antigen-dependent process was further emphasized by the experimental observation that most closely reproduces HSP: systemic vasculitis and nephritis can be

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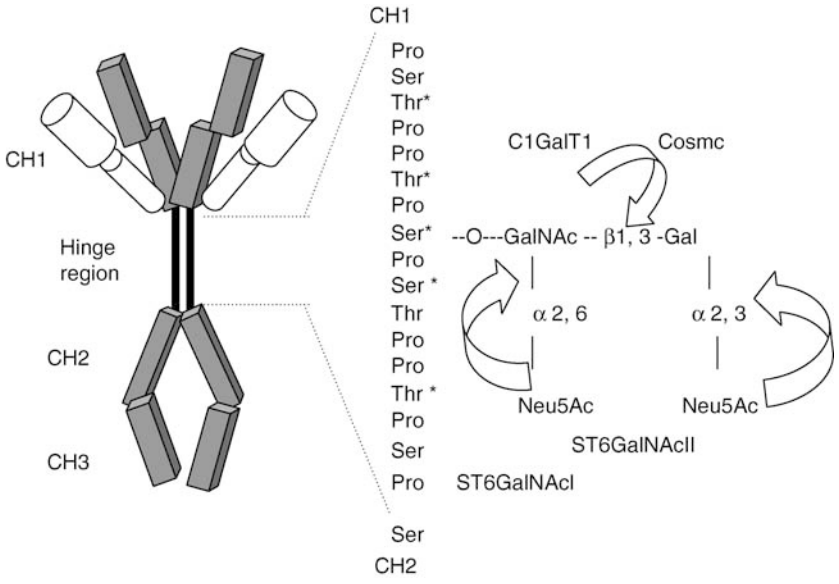
induced by injecting animals with a complement-activating carbohydrate antigen (13, 14). Food antigens or infectious organisms have been suspected (15–17), but the possibility of a single eliciting antigen remains unproved (18, 19), and environmental antigens rather play the role of triggering factors.

The accumulation of IgA immune complexes (IgAICs) within glomeruli has been considered the major pathogenic mechanism for HSP nephritis as for idiopathic IgAN. High levels of IgAICs have been detected during clinically active phases of purpuric rashes or HSP nephritis (20–23). However, IgAICs are not detected in all patients with renal involvement, suggesting the involvement of other pathogenic mechanisms. In HSP patients and particularly in those with nephritis, circulating IgA molecules react with α-galactosyl residues (23), fibronectin (24),gluten-derived molecules (19), mesangial matrix glycoproteins (25), or endothelial cells (26). Hence, it was postulated that these interactions were not true antigen-antibody reactions but rather related to the affinity of circulating IgA to various molecules, mostly glycoproteins.

Attention has been focused on the carbohydrate moieties of IgA, particularly IgA1, which is the predominant subclass deposited in both primary IgAN and HSP nephritis. IgA1 is highly glycosylated (9) (*Fig. 46-1*). In addition to the *N*-linked oligosaccharides typically present in the carboxyl terminal portion of all classes of Ig heavy chain, IgA1 contains 5 short *O*-linked oligosaccharide chains composed of *N*-acetylgalactosamine (GalNAc), galactose (Gal) and sialic acid (Neu5Ac). These oligosaccharides are coupled to serine and/or threonine residues which lie in the aminoacid insertion typical of IgA1 in the hinge region (9). The core 1 structure, Gal β1–3 bound to 3GalNAcα1-R, is synthesized from GalNAcα1-R by the action of core 1 β1,3-galactosyltransferase (core 1β3-Gal-T) and its chaperone, the core 1 β3-Gal-T (Cosmc). Sialyltransferases ST6GalNAcI and ST6GalNAcII bind the Neu5Ac residues to GalNAc in α2–6 and to Gal in α 2–3 respectively (9). The gene encoding the core galactosyltransferase is C1GALT1 is mapped on chromosome 7p14-p13, and the gene encoding for the specific chaperone Cosmc, C1GALT1C1, is mapped on chromosome Xq2 (27). Aberrant IgA1 glycosylation with defective sialylation and galactosylation and increased exposure of GalNAc residues has been detected in patients with HSP as well as in primary IgAN (28–31). A defective activity of core β3-Gal-T in B-cells has been postulated as for primary IgAN (32), but results are still inconclusive. A down-regulation of Cosmc expression in B lymphocyte of patients with IgAN has been recently

Figure 46-1

Human IgA1 contains five short *O*-linked oligosaccharide chains composed of *N*-Acetylgalactosamine (Gal NAc), Galactose (Gal) and Neuraminic Acid (Neu5Ac). These oligosaccharides are coupled to serine (Ser) and/or threonine (Thr) residues which lie in the aminoacid insertion typical of IgA1 in the hinge region connecting the CH1 and CH2 domains. The core 1 structure, Gal β1–3 bound to GalNAc, is synthesized by the β1,3-galactosyltransferase (C1GalT1) and its specific molecular chaperone (Cosmc). Sialyltransferases ST6GalNAcI and ST6GalNAcII bind the Neu5Ac residues to the core chain.



reported (33), however variants of C1GALT1 gene, rather than C1GALT1C1 have been found to be associated with the genetic susceptibility to IgAN (34). An imbalance in lymphocyte function, with a prevalence of T-helper 2 over T-helper 1 T cell subsets, can lead to altered IgA glycosylation in mice (35).

In vitro, desialylated or degalactosylated IgA molecules show a high tendency for self-aggregation, resulting in the formation of macromolecules with a molecular weight similar to IgAICs (36). Moreover, aberrantly glycosylated IgA1 trigger the synthesis of autoreactive IgA with formation of mixed IgA1/IgG ICs (37). Hence aberrantly glycosylated IgA1 can circulate in monomeric form or participate in the formation of self-aggregates or true IgA1ICs. Whether present in IgA1ICs or in self-aggregates, such aberrantly glycosylated IgA1 likely escapes clearance by hepatic receptors for asialoglycoproteins (38) because of the lack of Gal and possibly because of the size of the aggregate, which excludes them from the space of Disse. Abnormally glycosylated IgA1 may deposit in the glomerular mesangium more readily than normal IgA1 by virtue of enhanced inter-carbohydrate (lectin-like) reactivity with the fibronectin, laminin, and collagen within the mesangial matrix (25).

An enhanced interaction of aberrantly glycosylated IgA1with the transferrin receptor (TfR) and Fcα receptors on mesangial cells results in cellular activation and phlogistic mediator synthesis (39). We demonstrated an increased expression of integrin adhesion molecules (40), and of the inducible form of nitric oxide synthase (41) in mesangial cells after incubation with desialylated/degalactosylated IgA or IgA1 isolated from patients with IgAN. The resultant increase in the production of intraglomerular nitric oxide may lead to peroxidative damage, apoptosis, and sclerosis. This effect can be further enhanced by the concomitant depressed expression of vascular endothelial growth factor induced by aberrantly glycosylated IgA1 on mesangial cells, leading to an impaired repair process (42). Aggregates of IgA also stimulate the synthesis of a variety of cytokines (e.g., interleukin-6, platelet-derived growth factor, interleukin-1, tumor necrosis factor α, transforming factor β), vasoactive factors (e.g., prostaglandins, thromboxane, leukotrienes, endothelin, platelet-aggregating factor, nitric oxide) or chemokines (monocyte chemotactic protein-1, interleukin-8) by mesangial cells.

Complement activation may have a role in the pathogenesis of HSP nephritis. It is of interest that aberrantly glycosylated IgA can activate complement more efficiently than normal IgA (43, 44). In children with active HSP, CH50, and properdin levels are often reduced with increase in levels of C3d (21, 45), even though serum levels of C3 and C54 are within the normal range. This suggest a complement activation, possibly via the alternative pathway, balanced by enhanced factor synthesis, thus masking the consumption.

Antineutrophil cytoplasmic antibodies (ANCA) of the IgA isotype (IgA-ANCA) have been found in adults with HSP, but other reports failed to confirm these findings (46, 47). We demonstrated that IgA molecules from children and adults with HSP nephritis show an increased binding to sonicated neutrophil extracts and to purified myeloperoxidase but not to serine protease-3 (29). Of interest, this reactivity was never observed in sera of patients with primary IgAN, even though they have aberrantly glycosylated IgA1. This binding is affected by electrical charge and carbohydrate interactions, suggesting a lectin-like binding of aberrantly glycosylated IgA1 to ANCA antigens, rather than a true antigen-antibody reaction. Myeloperoxidase may be released in excess during phlogistic processes, leading to circulating IgA1-ANCA complexes, which, in the presence of increased levels of eosinophilic cationic proteins (48) as well as other phlogistic mediators, may favor vascular deposition of IgA1. Plasma IgE levels are increased in HSP and are significantly higher than in IgAN (49), which might be consequent to a prevalence of T-helper 2 lymphocytes. The vascular affinity of circulating IgA-containing immune material leads to purpura and systemic vasculitis.

Signs of increased oxidative stress can be detected in children with HSP, likely a result of a systemic immune system activation (50). Increased number of B cells synthesizing IgA and abnormalities of T-suppressor activity have been observed during the acute phase (51). In patients with HSP nephritis a reduction in Fcγ, C3b, and Fn receptor function of mononuclear phagocytes has been reported (52). These abnormalities were transient, probably secondary to saturation of receptors more than a primary event.

Several reports indicate that HLA Class II gene polymorphisms can to be a risk factor for HSP. A positive association with DRB1*01 and DRB1*11 (64% vs. 48% in the controls) as well as a negative association with HLA-DRB1*07 was first reported by our group in an Italian cohort (53) and confirmed in another study (54). An increase in DQA1*0301 (55) was found in Japanese children. An increased frequency of homozygous C4A or C4B null phenotype was observed in whites (56) and in Japanese patients (55). No association of angiotensin-converting enzyme genes polymorphism and manifestations or progression of HSP nephritis was found (57). The risk of developing severe gastrointestinal complications is

negatively associated with polymorphism of the intercellular adhesion molecule 1 (ICAM-1) and it has been suggested that it might also reduce the risk of renal involvement (58).

Clinical Data

Epidemiology

HSP is the most frequent vasculitis in childhood. Its annual incidence is approximately 14 cases of 100,000 children (59). In a recent Dutch epidemiology study the incidence was 6.1 cases of 10,000 children (8) whereas the incidence in United Kingdom was 22.1 in another study (60). HSP is frequent in the first decade of life; however it rarely affects children younger than 2 years of age. The median age at onset is 4–5 years (61). The sex ratio shows a male preponderance (male to female ratio, 1.4–1.7:1). Renal involvement is most frequent between 6 and 10 years of age (62–64). The geographic distribution of HSP is similar to that of IgAN. HSP is common in Europe [particularly in France, Italy, Spain, UK, and Finland] and Asia (e.g., Japan, Singapore, and China), whereas it is less common in North America and Africa (65, 66). The disease may be favored by racial factors, as HSP rarely affects blacks and American Indians (67).

Factors Triggering Henoch-Schonlein Purpura

The disease may be triggered by an infectious disease (62). *Streptococcus β, Yersina, Mycoplasma, Toxoplasma, varicella*, measles, rubella, adenovirus, HIV, and several other agents have been recorded among the triggering factors but without direct evidence of causality (66). There is a peak incidence of HSP in winter in North Europe and in June in Italy (68). Some epidemic clusters and familial cases of HSP have been reported (69).

The role of allergic reactions has been questioned, as well as the role of vaccinations (e.g., against smallpox or influenza), drugs (e.g., including ciprofloxacin, vancomycin, minocycline, carbamazepine), or other allergens (66).

Systemic Manifestations

HSP syndrome is characterized by a multiorgan involvement (61, 64, 67).

Skin

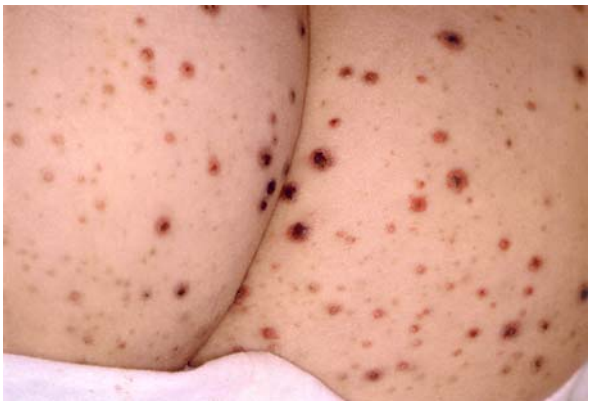
The characteristic skin lesions consist of slightly raised "palpable" purpuric macules that do not disappear on pressure with normal platelet count. They mostly begin with erythematous macules, some of which evolve into slightly raised urticarial papules, which soon become purpuric and eventually take a fawn color as they fade. Individual petechiae often become confluent in large patches. The purpuric rash often has a symmetrical distribution over the extensor surfaces of the lower limbs and forearms and the buttock sides (*Fig. 46-2*). The purpura is mostly present in the ankle area and in the milder cases it can be present only there. It also affects pressure areas (e.g., belt and pants) and occasionally involves earlobes, nose, and genitalia. Sometimes the rash is accompanied by fever and general malaise, with a clinical picture of infectious purpura or allergic reaction.

At microscopic examination of skin biopsy, leukocytoclastic vasculitis of dermal vessels is observed with IgA deposits in the vascular wall.

Purpura is concomitant with renal involvement in two-thirds of the cases. In 25% of children purpura precedes and in 10% follows the urinary abnormalities by 3–12 months (64). The purpura lasts for a few days but often relapses. Subsequent flare-ups of purpura occur 25% of children with severe renal disease (70, 71). Relapses of purpura may be accompanied by macroscopic hematuria and a transient increase in proteinuria, although the extent and duration of purpuric rash are not correlated with the severity of the renal lesions.

Figure 46-2

Purpuric rash of Henoch-Schoenlein purpura over the sides of the buttocks.



Gastrointestinal Tract

Gastrointestinal symptoms are reported in 50–70% of patients (4), more frequently in children than in adults (64). The most typical manifestation is diffuse abdominal pain, increasing after meals, referred to as *bowel angina*. Vomiting, hematemesis, and hematochezia or melena is frequent (5). Major abdominal complications, like intussusception, intestinal infarction, and bowel perforation are rare events. The pain is often severe and mimics an acute surgical emergency. There is a range of endoscopic findings including gastritis, duodenitis, ulceration, and purpura, mostly involving the second portion of the duodenum. Intestinal biopsies show IgA deposition and leukocytoclastic vasculitis in the submucosal vessels. Steroid therapy may be useful in relieving bowel-wall edema and pain, however most children improve spontaneously.

Joints

Transient arthralgia is reported in 50–70% of cases (4). They mostly involves lower limb articulations, ankles, and knees, with a picture of oligoarticular synovitis, A periarticular edema may be present. The articular involvement does not lead to joint erosions, deformities or functional limitations.

Kidney

The selection of HSP patients strongly affects the data concerning the prevalence of renal involvement (4, 59–65). Variations in the prevalence of renal involvement among different series may also depend on the methods of detection of nephritis and it may increase if serial urinalyses are performed. In unselected cohorts of children, the prevalence of the renal involvement during the course of HSP is approximately 33%, ranging form 20–55% (63). When serial urinalyses are performed for one year, the percentage of renal involvement among children with HSP increases progressively, (71). The urinary abnormalities detected in unselected cohorts of children with HSP include hematuria and various degrees of proteinuria, up to nephrotic-ranges (*Fig. 46-3*).

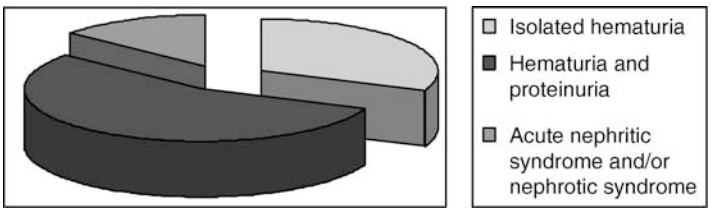
In cohorts of children with severe renal disease, as in the cohort of the Italian register of renal biopsies in children (72), the prevalence of glomerulonephritis related to HSP is 11.6% of all renal diseases. These figures are similar to French series, where HSP was detected in 10 to 15% of cases (73).

The pre selection of children investigated also affects the clinical picture. General pediatricians often report a systemic disease with modest and transient urinary abnormalities (73), whereas in pediatric nephrology departments the renal disease is more severe (74, 75).

The manifestations of glomerulonephritis secondary to HSP include isolated microscopic or macroscopic hematuria, mild or heavy proteinuria with or without nephrotic syndrome, renal failure, and hypertension (63). The more frequent clinical presentation of HSP nephritis in non selected pediatric series is an isolated microscopic hematuria (73, 76). In 80% of cases, it is detected within 4 weeks after the onset of the disease. It is often transient, detectable only by routine urinalysis during the acute phase, followed by a complete recovery. In some patients, proteinuria of variable amounts is present. Nephrotic syndrome is more common in referral centers but is rare in unselected series. Renal function is normal in most children but may be impaired in children with severe nephritic syndrome It is most often a moderate renal failure, which rarely requires dialysis. Hypertension is rare at onset and has been reported in children with minimal urinary abnormalities.

Figure 46-3

Clinical presentation of children with nephritis of Henoch-Schoenlein purpura. Cumulative data from 666 children (76, 77, 87, 88, 92, 93).



Other Non Renal Manifestations

HSP can be complicated by a cerebral vasculitic process, resulting in convulsions, encephalopathy, chorea or blindness (74).

Other rare manifestations include hemorrhage in the calf or subcutaneously, pulmonary-renal syndrome, cardiopulmonary syndrome, pancreatitis (77), adrenal bleeding or testicular involvement mimicking torsion (66).

The vasculitic process can affect the ureter (78). In these cases it is generally associated with loin pain. The ureteral lesion of necrotizing vasculitis may evolve into sclerotic lesions and progress to stenosis, requiring correction (78, 79). An early diagnosis by repeated ultrasound can limit can the consequences of this complication (80).

Laboratory Data

In HSP, as in the idiopathic IgAN, serum IgA levels are increased, in up to 70% of patients, but the increase is often limited to the acute initial phase, returning to normal levels when the children enter remission. The subclass mostly increased is polymeric IgA1 (66). IgA1 with aberrant glycosylation and increased exposure of GalNAc residues can be detected particularly during acute phases as well as IgA1ICs (28–31) and IgA/fibronectin aggregates (24). IgA antibodies to endogenous or exogenous antigens have been reported, including IgA rheumatoid factor (81). High levels of IgA reacting with sonicated neutrophil extracts (IgA-ANCA) and with purified cytoplasmatic antigens (myeloperoxidase) have also been detected in children with HSP nephritis (29, 46).

Platelet count is in the normal range and the activity of the clotting factors is normal. Conversely abnormalities and low levels of fibrin-stabilizing factor (factor XIII) have been reported (82) as well as increased von Willebrand factor plasma levels (83), in acute phases of HSP, indicating an endothelial damage that favors fibrin deposition and crescent formation.

Recent investigations are focused on a renal damage pattern found in urine of patients with IgA-associated glomerulonephritis by capillary electrophoresis (84) as well as increased podocyturia (85) but these new biomarkers need further validation.

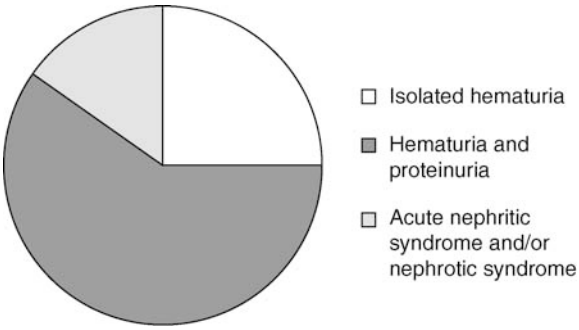
Pathology

Light Microscopy Features

HSP nephritis is characterized by mesangial damage with different degrees of hypercellularity (*Fig. 46-4*), ranging from isolated mesangial proliferation to focal and segmental proliferation and severe crescentic glomerulonephritis.

Figure 46-4

Renal biopsy specimen from a patient with nephritis of Schoenlein-Henoch purpura showing expansion of the mesangial matrix and increase in mesangial cellularity (PAS × 250).



Light microscopic examination shows a wide range in terms of the type and severity of glomerular involvement from one patient to another and also within the same biopsy, from glomerulus to glomerulus (86–88). Classifications of HSP nephritis are mostly based on the severity of the proliferative lesions (4, 67, 87). Adhesions of the tuft to Bowman's capsule can be found in coincidence with segmental proliferative lesions, often with splitting and duplication of glomerular basement membranes. In rare cases the severe mesangial proliferation is associated with mesangial interposition in which cells and matrix migrate into the capillary walls, between the basement membrane and endothelial cytoplasm, mimicking a membranoproliferative glomerulonephritis. Polymorphonuclear cells may infiltrate the glomerular tufts, sometimes as severely as in acute post-streptococcal glomerulonephritis. Segmental necrosis of the glomerulus is often present at onset. It is not rare to detect intracapillary glomerular thrombi.

It is common to find crescent formation varying in size from a limited segment to circumferential crescents. At onset, crescents are cellular, then evolve into fibrous crescents, generating segmental scars or global sclerosis. The most common histological feature is a predominance of small crescents. In children with severe renal symptoms, extra-capillary proliferation is detected in more than half of the cases (65, 89). In these cases, crescents often involve less than 50% of glomeruli. Some biopsies show periglomerular inflammatory infiltrates, mostly associated with crescents (15% of the children) (71).

Histologic classes of HSP nephritis can be distinguished according to the presence or absence and the extension of extra capillary proliferation, and degree of the endocapillary lesions (*Table 46-1*) (67). The six classes include minimal or moderate glomerular lesions and absence of crescents (Class I or II), various extent of extra-capillary cellular proliferation (in less than 50% of glomeruli, in 50–75% or in more than 75%, Class III, IV, and V respectively). Class VI includes pseudo-membranoproliferative glomerulonephritis.

The distribution of histologic changes in 270 children (65, 89, 90), showed in half of the cases endocapillary focal or diffuse proliferation with crescents involving less than 50% of glomeruli. Severe extracapillary proliferation involving more than 50% of glomeruli was limited to approximately 25% of the cases (*Fig. 46-5*).

Hyalin changes or accumulation of fibrinoid material, or necrosis with inflammatory infiltration and clear findings of vasculitis are present in approximately 10% of children. Blood vessels may show medial hypertrophy and intimal fibroelastosis. Necrosis of the capillary tuft has been reported in 8% of children, coincident with extra-capillary proliferation. The renal biopsies form children with HSP nephritis frequently show degenerative tubular alterations with flattening, vacuolization, desquamation, and focal loss of the brush-border microvilli, in the cortex (67). Tubular cylinders and blood casts are detected in 30% of patients.

Immunohistochemistry

Mesangial IgA granular deposits are the hallmark of the disease which, in contrast with the frequent focal and segmental proliferative changes, is always diffuse as in primary IgAN (*Fig. 46-6*). IgA1 is the dominant subclass with equal distribution of light chains. The J chain is detectable, indicating that IgA in deposits is dimeric, but the secretory tract is absent. Extensive sub-endothelial deposits are associated with the most severe histologic forms with endocapillary proliferation or crescents.

C3 is codeposited in 75–85% of the cases similarly to idiopathic IgAN. The membrane attack complex C5–C9 and the alternative complement pathway components are always detected. Early classical pathway complement components C1q and C4 are present only rarely and stain with low intensity (67). IgG and IgM codeposits are present in the 40% of the cases. Fibrin or fibrinogen deposits are found in 60–70% of patients, both in mesangial and in parietal areas, often coincident with mesangial proliferation, suggesting a role of the clotting cascade activation. Glomerular fibrin-related deposits are much more frequently present in HSP nephritis than in IgAN (65) and are often related to active phases with extracapillary proliferation. Deposits of IgA and C3 can be found in arterioles or cortical peritubular capillaries in children with severe glomerular changes.

Electron Microscopy

Electron-dense deposits are detectable in a setting of mesangial matrix expansion and variable degree of cellular hyperplasia (67). The deposits, initially paramesangial and small, become larger and of non uniform

Table 46-1

Classification of Henoch Schoenlein nephritis lesions according to Emancipator (67)

Class I:	Minimal glomerular lesions and absence of crescents
Class II:	No crescents IIa: Pure mesangial proliferation IIb: Focal-segmental endo-capillary proliferation IIc: Diffuse endo-capillary proliferation
Class III:	Presence of extra-capillary cellular proliferation in less than 50% of glomeruli IIIa: In association with focal and segmental endo-capillary proliferation IIIb: With diffuse endo-capillary proliferation
Class IV:	Florid extra-capillary proliferation in 50–75% of glomeruli IVa: In association with focal and segmental endo-capillary proliferation IVb: With diffuse endo-capillary proliferation
Class V:	Extra-capillary proliferation in more than 75% of glomeruli Va: In association with focal and segmental endo-capillary proliferation Vb: With diffuse endo-capillary proliferation
Class VI:	Pseudo-membranoproliferative glomerulonephritis

Figure 46-5

Distribution of histological grades in Henoch-Schoenlein purpura nephritis. Cumulative data from 270 renal biopsies (65, 89, 90).

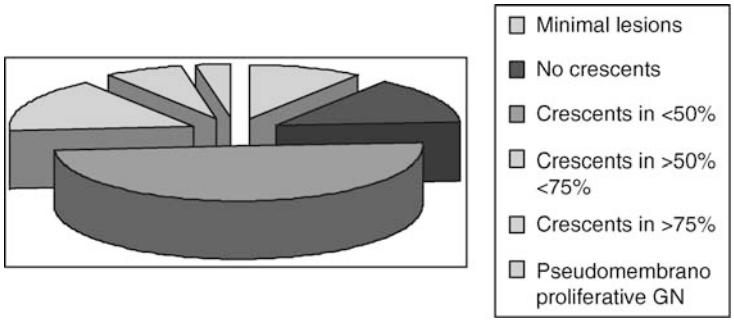
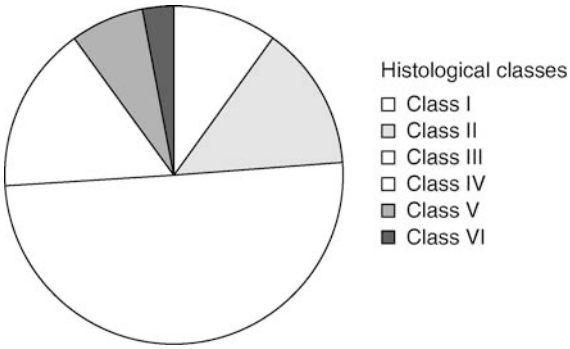


Figure 46-6

Immunopathology of Henoch-Schoenlein nephritis: Mesangial deposits of IgA (anti IgA × 250).



density, in strict connection with the mesangial matrix. Most deposits have a mesangial localization with parietal extension (in 60% of the cases), are purely mesangial only in 30% of cases. The parietal, paramesangial electron-dense material is generally subendothelial, more rarely subepithelial. Sometimes, electron-dense deposits, "hump" like with "garland" shape or fluffy aspects, are detectable. In other cases, the deposits show with a "woolly" aspect in the external rara lamina, placed in the periphery of the capillary loops and delimited by a thick layer of new basement membrane. As a possible consequence of the membrane reactivity to immune deposits, the presence of parietal deposits modifies the capillary basal membrane profile because of the widening of the rara internal and external lamina, with neo-formed layers.

Follow-Up Renal Biopsies

Studies of serial biopsies in children with HSP showed a good correlation between the histologic changes, clinical data – particularly at follow-up – and outcome (91). When patients undergo clinical resolution, mesangial proliferation disappears, and small crescents regress while others evolve into segmental synechiae. IgA deposits substantially decrease and sometimes completely disappear. Conversely, when repeat biopsies are performed in patients with persistently active or progressing nephritis, the proliferation continues to be severe, and evolution into fibrotic lesions is detected. The importance of careful clinical monitoring during the follow-up of children with HSP nephritis must be stressed also on the basis of the good correlation between clinical and histological data reported by this study.

Non Renal Lesions

The typical lesion of HSP, either in skin, gut or in kidney vessels is leukocytoclastic vasculitis with fragmentation of leukocyte nuclei in and around arterioles, capillaries, and venules, surrounded by infiltrating neutrophils and monocyte cells in the presence of nuclear residues (nuclear dust) in the wall of arterioles. Fibrinoid deposits and arteriolar and venular necrosis can be found. Deposits of IgA and C3 are present in the dermal capillaries in purpuric lesions and uninvolved skin and are considered a valid diagnostic criterion, with 100% specificity in combination with leukocytoclastic vasculitis. IgG and IgM can be codeposited in approximately 20% of the cases, whereas C1q and C4 are absent. Similar deposits have been reported in superficial derma capillaries of IgAN patients. In dermatitis herpetiformis, IgA deposits are found as well, but they are located on the top of the derma papilla. In systemic lupus erythematosous, the dermal-epidermal junction is mostly positive for IgG, C1q, and C4. The diagnosis may be difficult in the rare cases in which skin

eruption entirely consists of urticaria lesions without purpura. Hypersentitivity vasculitis is sometimes overlapping, but the dermal IgA vascular deposits are specific for HSP lesions.

Correlations between Clinical and Pathologic Data

In the Italian HSP series of 74 children (64, 70), patients with minimal proteinuria had higher prevalence of histologic lesions without crescents (classes I and II) (92%). In cases with significant proteinuria, more severe renal lesions were frequently found. Extracapillary proliferation was often found in children with nephrotic proteinuria (33% of class III and IV). Gross hematuria at presentation was associated with crescent formations in the 22% of the children. The clinical feature mostly predictive for severe histologic lesions was the onset with renal function impairment, as 62% of children with severe acute nephritic syndrome and severe kidney function impairment, had crescents, and other authors reported similar findings (86).

Clinical Course and Prognosis

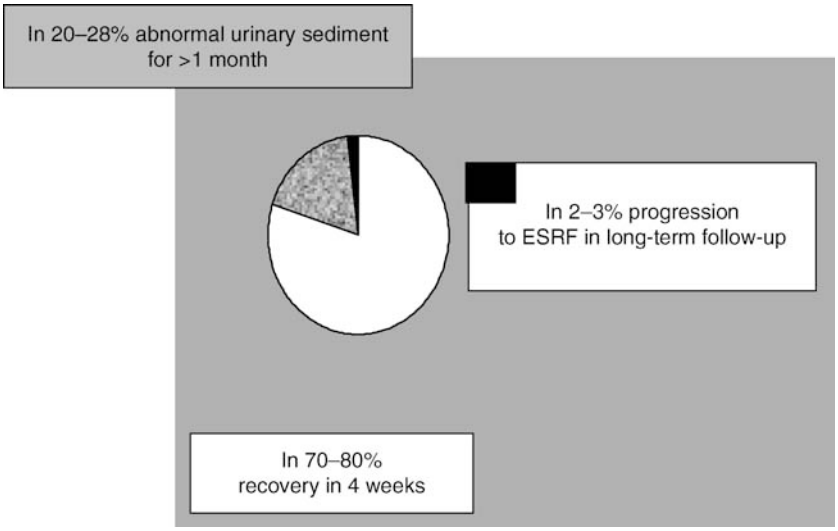
HSP nephritis accounts for 5.1% of children with end-stage renal failure (ESRF) in Europe (4, 68).

The clinical course and long-term outcome vary according to the cohorts examined, particularly when unselected cohorts are compared with children followed by pediatric nephrology units. In unselected series, HSP is a mild disease, with renal involvement in a minority of cases, mostly presenting with isolated hematuria or minimal proteinuria and with long-term concerning no more than 1% of patients (91). Reports of series of children admitted into general pediatric hospitals indicate a prevalence of urinary sediment abnormalities in 20–54% of the cases, which disappear within four weeks in 70–80% of the cases, or last for more than one month in 20–28% of the cases (81, 87, 88, 92, 93) (*Fig. 46-7*). With a follow-up of more than 10 years, a progression to ESRF is observed in approximately 2–3% of the children with initial signs of renal involvement (94).

The remission rates reported by tertiary reference centers are below 50%, with poor outcome in 10–25% of children (75, 95). In cohorts of children with a severe disease, long-term prognosis is poorer, as 15–30% of them progress to renal failure with wide variability depending on the duration of follow-up (63). In a long-term follow-up study, a late progression was observed after 25 years in 25% of children, not only in cases with persistently active renal disease, but also in others having experienced clinical improvement after the acute phase (75). These authors reported persistent urinary abnormalities in 15% of children with a nephrotic onset or persistent heavy proteinuria, 40% of those

Figure 46-7

In children admitted into general pediatric hospitals, the urinary sediment abnormalities disappear within four weeks in 70–80% of the cases, last for more than one month in 20–28% of the cases. Progression over long-term follow-up is observed in 2–3% of the children (81, 87, 88, 92–94).



with a nephritic presentation and 50% of those with mixed nephritic-nephrotic syndrome at onset. Some of these patients ended in ESRF. It is clear that renal function may deteriorate during the follow-up even in patients who apparently completely recovered two years after onset (75), perhaps due to sequences of glomerular hypertension. Even in some cases who had a second renal biopsy years after the initial vasculitis episode and no urinary abnormalities, IgA mesangial deposits were detected, indicating a protracted silent renal disease (96).

In the Italian multicenter study (70, 89) after 1–20 (mean 6.7) years, one-third of the 83 children who had had a renal biopsy were in remission (*Fig. 46-7*). In 40% of the cases only minimal or moderate proteinuria was left. Chronic renal failure or doubling of baseline creatinine occurred in 14.5% of children and 7.2% reached ESRF needing dialysis after 4.5–12.0 years (89). When comparing children and adults with a HSP nephritis severe enough to warrant renal biopsy, the outcomes are similar over the first years (70). However, over longer follow-up the final outcome results worse in adults, as renal survival at 10 years was 90% in children and 75% in adults (relative risk 3.5) when considering dialysis as the end-point, and 86% and 67% respectively (relative risk 14.9) when considering doubling of baseline creatinine as the end-point (89). Physiological long-term function decline in old subject should also be considered.

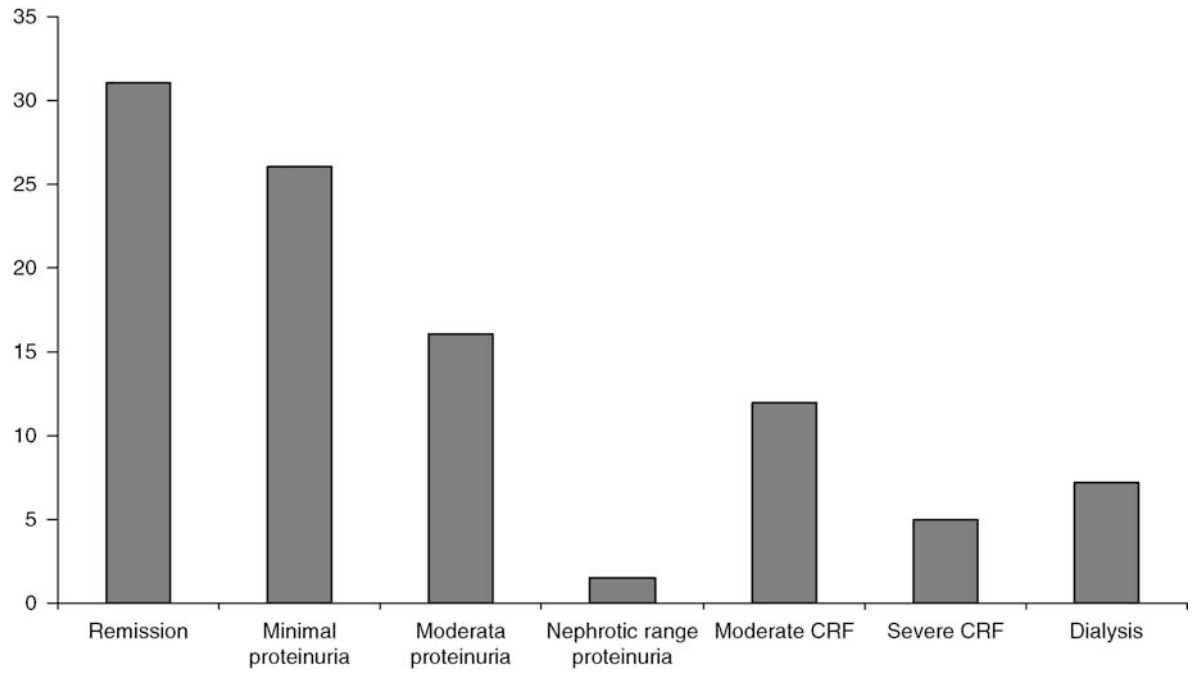
Children at Risk for Progression

It is of paramount importance to identify children with potentially progressive renal disease. As reported above, the clinical presentation is, in general terms, indicative of the long-term outcome (76). In some reports, nephrotic syndrome or renal insufficiency at onset were risk factors (44%) for renal failure after two decades of follow-up (75) (*Fig. 46-8*).

Conversely, in the Italian cohort (70, 89), no significant prognostic factor was found at presentation, including renal function impairment at onset or hypertension. Mild proteinuria or, at the opposite, nephrotic-range levels, were respectively associated with high frequency of remission or functional deterioration. However, children with mild proteinuria also frequently displayed severe histologic lesions, with extracapillary proliferation and nephrotic and nonnephrotic children had similar outcomes. The variable found at multivariate analysis to be

Figure 46-8

Long-term renal outcome of patients with Henoch-Schoenlein nephritis. Mean 5-year follow-up of 57 children with severe Henoch-Schoenlein nephritis, warranting renal biopsy (From the Italian Group of Renal Immunopathology (70)). Moderate CFR: chronic renal failure with <90 to >60% glomerular filtration rate. Severe CRF: chronic renal failure with <60 to >30% glomerular filtration rate. (Adapted from reference (70).



strongly predictive for both progression to doubling of creatinine and dialysis, was mean proteinuria values during follow-up (89).

The percentage of glomeruli involved by extracapillary proliferation, the extent of Bowman's space occupied by individual crescents, the fresh/fibrous crescents ratio are important risk factors (74). There is a close relationship between the extent of crescents and long-term sequela in patients with Class IV and V, with extensive glomerular involvement by crescents in more than 50% and more than 75% of glomeruli, respectively. In a Japanese series, 33% of Class IV and 83% of Class V, ended in ESRF (97). The predictive value of mild extracapillary proliferation is low when crescents involved less than 50% of glomeruli, because many of these cases recover, and also some children without crescents experience an unfavorable outcome (98). According to multivariate analysis, the presence of crescents on renal biopsy (classes III–V) was not an independent risk factor for progression to doubling of serum creatinine or to dialysis, when compared to the survival of patients without crescents at renal biopsy (Class I and II) (89).

Renal Transplantation in Children with HSP Nephritis

Recurrence of IgA mesangial deposits is frequent after renal transplantation, even though the recurrence of clinical symptoms of HSP is unusual. In adults, the actuarial risk for histologic or immunofluorescent recurrence is 35% at 5 years, with graft loss in 11% (99, 100). In most patients, no clinical manifestations or minimal hematuria accompany histologic recurrence in grafted kidneys (101). Recurrence is not prevented by triple therapy, including cyclosporine and is was more frequent in rapidly progressive cases. Delaying transplantation of one year after the last systemic signs was not effective in decreasing the rate of recurrence. The recurrence rate appears to be increased in good HLA match (99) and recipients of living-related grafts, suggesting a role for genetic factors. This is controversial, however, and the issue of whether using living donors for renal transplantation in HSP is still debated (102, 103).

Therapy

Mild cases do not require treatment, provided urine monitoring is ensured to detect modifications in the clinical follow-up, mostly during the first 2–3 months after the purpuric rash. The abdominal pain is generally controlled by small doses of steroids which favor a rapid resolution. Patients with recurring necrotizing purpura or severe abdominal pain have been successfully treated by intravenous Ig infusion (104).

Because in most cases the purpuric rash precedes by days or weeks the detection of urinary abnormalities, attempts have been made to prevent the development of the nephropathy by giving prednisone (1–2.5 mg/Kg/day for 1–3 weeks). A beneficial effect was not observed in retrospective studies, which compared treated versus untreated patients. However, the treatment was often reserved to the more active cases, thus introducing a selection bias which may have influenced the outcome (105–107). Conversely, a prospective trial (albeit not strictly randomized) suggested a protective effect of two weeks small doses of prednisone (108). The prevention consisted in avoiding the appearance of microscopic hematuria, which is not associated with a high risk for progression to renal failure. A recent systematic review of reported outcomes of children with HSP who were treated at diagnosis with corticosteroids compared with those with supportive care only, concluded that corticosteroid, given early in the course of illness seem to produce consistent benefits for major HSP outcomes, including the resolution of abdominal pain, the need of surgical intervention and the odds of developing persistent renal disease (109).

Once the nephritis is developed, it is possible either to treat or to wait for a spontaneous remission. Based on observations dating decades ago, oral corticosteroids were believed to be ineffective for treating HSP nephritis. Indeed, retrospective analyses failed to demonstrate any benefit of prednisone in children with HSP nephritis (110). However, again a selection bias favoring treatment of more severe cases was likely, because the severity of the disease was a major determinant in the decision to treat. More recently, a one year treatment with oral prednisone and azathioprine in patients with moderately severe renal involvement gave positive results in comparison with historical untreated cases (95, 111). However, as the historical group included particularly severe cases, no recommendation for treatment of moderately severe HSP nephritis is presently possible (112). One small series of children suggested that the addition of azathioprine to steroids may ameliorate histopathological features and the clinical course of severe HSP nephritis (113).

Tonsillectomy has been proposed for HSP nephritis, as for primary IgAN, with the aim of limiting the mucosal immune response. The overall analysis does not support

the recommendation for this intervention to treat HSP nephritis (114).

Nephrotic range proteinuria and crescents in >50% of glomeruli were mostly associated with poor outcome. There is a need for an efficient treatment for children with severe endocapillary and extracapillary proliferation and clinical presentation of nephrotic or nephritic syndrome with impaired renal function. In severe forms of HSP nephritis, long-term immunosuppressive therapy, based on daily steroids and cyclophosphamide for 8–12 weeks followed by azathioprine and a reducing regimen of alternate-day steroids for 8–12 months, did not avoid progression to ESRF in 15% of the cases and persistent renal abnormalities on long-term follow-up (115). An aggressive therapeutic regimen resulted in a favorable clinical outcome in 11/12 children with 60–90% glomeruli with crescent formations. This regimen consisted in a triple therapy of 3-methylprednisolone pulses followed by a 6-month course of prednisone, dipyridamole, and cyclophosphamide for 3 months (116). More than 60% of these patients experienced a complete remission. Similar positive results have been reported using high dose steroids in association with oral cyclophosphamide (117).

A larger French series confirmed that methylprednisone pulses, followed by a 3-month course of oral prednisone and, in some patients, with a 2-month course of oral cyclophosphamide, resulted in clinical recovery in 71% of children with nephrotic syndrome or crescentic involvement in greater than 50% of the glomeruli, versus 40% in a historical group of untreated children (118). Similarly, in children with greater than 50% of glomeruli involved with crescents, good results were reported with oral prednisone for 4 months in association with 2 months of cyclophosphamide and 4 months of heparin or warfarin (119). A combination of prednisone, azathioprine or cyclophosphamide also gave positive results (120), as well as methylprednisolone and urokinase pulse therapy combined with cyclophosphamide in severe histologic forms (121).

Our group (122), and more recently others (123–125), successfully treated children with rapidly progressive HSP nephritis with plasma exchanges, corticosteroids, and cytotoxic drugs. In our series, even though some cases experienced a complete remission, most had subsequent renal relapses and, in spite of a new cycle of plasma exchanges, renal function did not improve, and patients progressed to ESRF. The progression was delayed by 1–4 years. In the Japanese series, 60% of the treated patients had a complete remission of renal disease after 10 years (123). A German series showed that aggressive treatment, including PE may delay the rate of progression in the majority of patients with severe crescentic HSP nephritis, but the treatment was unable to definitely prevent the progression to ESFR (126). Of interest, the report of a difficult case of recurrent HSP nephritis in renal allograft, successfully treated with PE (127).

The analysis of uncontrolled studies with a combination of corticosteroids (either intravenous pulses or oral), immunosuppressive drugs (cyclophosphamide or azathioprine), sometimes in association with anticoagulation (warfarin or dipyridamole) in children with nephrotic-range proteinuria, or major extracapillary proliferation on biopsy, allows us to conclude that aggressive therapeutic regimens are effective in decreasing the rapidity of progression to chronic renal failure. However, these treatments should be started early during the course of the disease before the crescents become fibrotic. At this late stage, the treatments are ineffective.

Cyclosporine A in association with steroids in cases with nephrotic-range proteinuria is effective in reducing proteinuria, and improving the activity index at control renal biopsy, however the risk of nephrotoxicity should be considered (128).

In patients with heavy proteinuria, favorable results with decrease in proteinuria and improvement of histological index of renal activity have been reported using intravenous Ig infusions (129, 130). However, a rebound was noticed shortly after the therapeutic cycles, which somehow banished the positive results obtained (129). On the contrary, Ig infusion may be useful in cases with severe recurrent purpuric rash and mild urinary symptoms. Nephrotoxicity can be a serious rare complication of i. v. Ig therapy, mostly associated with sucrose-containing preparations in volume-depleted subjects (131). New treatments, like leukocytapheresis have been successfully tried for the treatment of refractory HSP resistant to both prednisolone and i. v. Ig therapy (132).

Relationship between Henoch-Schoenlein Purpura and primary Immunoglobulin A Nephropathy

The main reason for which a relationship between HSP and IgA nephropathy has been suggested is that these two conditions are indistinguishable by renal histopathology. Furthermore, other features are in favor of an unifying concept. Occasional patients with IgA nephropathy have extrarenal symptoms. A few patients may present with IgA nephropathy and develop a purpuric rash months or years later. Some patients with HSP may

develop episodes of macroscopic hematuria suggestive of IgA nephropathy, in the following years. The occurrence of both diseases in the same family is not uncommon (133). In a French nation survey on 40 families with two or more members affected by primary IgAN, five had members presenting with complete HSP syndrome, confirming a possible genetic link between the two diseases (134). Most patients with HSP nephritis who undergo renal transplantation have a recurrence of IgA deposits in the graft in the absence of extrarenal symptoms, as do patients with IgA nephropaty. Last, a male preponderance is seen in both diseases, and the high prevalence of both conditions is observed in the same geographic areas (e.g., Europe, Asia).

HSP and primary IgAN share similar disturbances in the IgA system. Both diseases present with high levels of serum IgA, as well as high levels of circulating IgA-ICs, IgA1-IC, and IgA-fibronectin aggregates, as discussed above, even though the levels in acute phases of HSP are generally higher than in primary IgAN (20, 22). Both diseases have aberrantly glycosylated IgA1 in circulation (28–31). The present hypothesis of the factors which condition the development of HSP nephritis rather than primary IgAN, stresses the role of complement and coagulation pathways activation, with involvement of eosinophilic reactivity possibly due to different IgA1 glycosylation properties. However, this remains an unproved hypothesis.

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47 Systemic Lupus Erythematosus

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Introduction

Systemic lupus erythematosus (SLE) is a chronic inflammatory disease characterized by highly diverse clinical manifestations and the presence in the serum of a variety of autoantibodies reacting with different cell components. The skin, joints, lungs, heart, kidneys, nervous system, and other organs are involved. The role of autoantibodies in the pathogenesis of the disease is subject to debate. Certain autoantibodies, such as those reacting with cell surface determinants or circulating proteins, are directly responsible for specific clinical manifestations of the disease. The clinical course of the disease is characterized by flares, periods of chronic disease and periods of remissions.

The American Association of Rheumatology (AAR) developed diagnostic criteria of SLE (1, 2) to established with certainty the diagnosis of SLE and distinguishing it from other rheumatic diseases (*Table 47-1*). When four of these criteria are present, the sensitivity for the diagnosis of SLE is 95% and the specificity is 96%. Some patients with the disease may not satisfy initially the criteria but may develop other manifestations later and then have the full criteria for the diagnosis of SLE. For example, some patients may have isolated nephritis, such as membranous nephropathy, without serological evidence of SLE at first.

The prevalence of SLE is approximately 40 in 100,000 in Europe and North America (3). SLE is three times more frequent in Afro-Americans than in Caucasians but is exceptional in blacks from Africa (4, 5). In a survey of pediatric lupus in the United Kingdom, the relative risk was 6.7 in Asian populations and 6.1 in black populations compared with the white population (6). In more than 80% of cases, SLE affects females after puberty. In children, the prevalence is 1 in 100,000 with girls more frequently affected than boys. The female:male ratio increases from 2:1 in prebubertal children to 4.5:1 in adolescents and 8:1 in adults (7). In children, most cases of lupus occur after age five, with a peak incidence in late childhood and adolescence (8). Twenty percent of SLE cases begin in childhood.

The presentation of the disease in children varies both in terms of the gravity of symptoms and the diversity of clinical manifestations. However, the disease is often more acute and severe, affecting multiple organs in children compared to adults (9–12). In addition to organ damage, SLE has a major detrimental impact on growth and psychological development. It is important to promote a multidisciplinary approach to SLE to assure better individual treatment of patients.

Pathogenesis

The etiology of the SLE remains unknown despite the progress that has been made during the recent years in understanding the pathogenic mechanisms of the disease. SLE is characterized by the development of autoantibodies directed against a variety of self components and, contrary to other autoimmune diseases, there are no stigma of organ-specific autoimmunity.

Genetic factors – The evidence for a genetic susceptibility to SLE in humans is based on familial aggregation. The prevalence of SLE is estimated to be 2.6–3.5% in first-degree relatives of SLE probands compared with 0.3–0.4% in relatives of match controls. Moreover, an increase in concordance rate is observed in monozygotic twins (24–56%) compared to dizygotic twins (2–5%). The risk of developing the disease in siblings of SLE patients is 10–40 times higher than that in the general population. An analysis of 125 lupus multiplex families including at least two cases recruited through a French national survey demonstrated in most cases a non-Mendelian inheritance suggesting a complex multifactorial inheritance pattern with several genes interacting together in a single individual (13). Recent technological advances have allowed rapid analysis of the whole genome in patients with complex disease and matched controls. Several genomewide association studies in large international cohorts of patients have identified genetic variants in susceptibility genes that confer a significant risk of SLE. This approach gives some new insights in the comprehension of the disease mechanisms and open avenues for therapeutic intervention. In these lines, it has been demonstrated that the genes encoding the interferon regulatory factor

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5 (IRF5) (14), the protein tyrosine phosphatase type 22 (PTPN22) (15) as well as the B-cell-specific tyrosine kinase (BLK) (16) are implicated in the pathogenesis as some variants in these genes are significantly associated with the disease.

As lupus is a multifactorial disease with a multigenic inheritance, one can assume that many more polymorphisms with subtle functional impact have a role in inducing the full-blown disease in conjunction with environmental factors.

Studies in various mouse models suggest that lupus may be mediated by a multitude of genetic abnormalities that impact on specific checkpoints, starting with a loss of immunologic tolerance to nuclear antigens, leading to a pathogenic autoimmunity and organ targeting. While the genes involved in these perturbations are still unknown, recent findings have provided insights into specific clue elements implicated in these pathways. Several studies suggest that increased liberation or disturbed clearance of nuclear DNA–protein complexes after cell death may initiate the disease. Dnase1 deficient mice generated by gene targeting exhibit a phenotype very similar to classical human SLE (17). Moreover, mutations responsible for a loss of function of this enzyme have been found in patients with SLE (17). *Table 47-2* shows the different genes for which variants have been shown to be associated with SLE in humans and in mice (17–28).Variation in gene copy number is increasingly recognized as a source of genomic variability. Loss of the rat-specific *Fcγr3* related gene is responsible for macrophage overactivity and glomerulonephritis in Wistar Kyoto rats. (29). In human, a strong association between *FCγR3B* copy number and risk of systemic lupus erythematosus, microscopic polyangeitis and Wegener's granumalomatosis in two independent cohorts from the UK and France has been demonstrated (30).

Table 47-1

The 1982 revised ARA criteria for classification of systemic lupus erythematosus

Malar rash
Discoid rash
Photosensitivity
Oral ulcers
Arthritis
Serositis (pleuritis or pericarditis)
Proteinuria >0.5 g/day or red casts
Psychosis or seizures
Hemolytic anemia or leucopenia (<4,000/mm^3) or lymphopenia (<1,500/mm^3) or thombopenia (<100,000/mm^3)
Antinuclear antibodies
Anti-dsDNA antibodies or anti-Sm antibodies or false positive TPI/VDRL or positive LE cell test

Table 47-2

Mouse and human genes that cause lupus*

Categories	Mouse with null mutation	Patients with SLE	References
Regulatory machinery			
Activation threshold of lymphocytes			
PD-1	Autoimmune cardiomyopathy	A polymorphism in intron 1 is more frequent in patients than in control (12 vs. 5%)	16, 18
Lyn	Autoreactive antibodies and severe glomerulonephritis	Lyn in significantly decreased in B-cells at the mRNA and protein levels	19, 20
Deletion of autoreactive T-cells			
P21	Anti-dsDNA, glomerulonephritis	Reduced expression of p21 in lymphocytes	21, 22
Scavenger machinery			
C1q	High autoantibodies titers, 25% had glomerulonephritis with great numbers glomerular apoptotic bodies	C1q deficiency is strongly associated with SLE	23, 24
DNAse1	Antinuclear autoimmunity and glomerulonephritis	Heterozygous nonsense mutation and decreased Dnase1 activity in serum of patients	17, 25
Serum amyloid P (SAP)	Antinuclear autoimmunity and severe glomerulonephritis	Low levels of SAP-DNA complexes in serum of patients	26, 27

*This list is not exhaustive

Environmental and infectious factors – Based on twin concordance studies, additional factors appear to be necessary to induce the disease, indicating a multifactorial origin for SLE. Environmental, hormonal, toxic and infectious factors have been suspected. The fact that seroconversion against EBV was observed in 99% of children with SLE as compared to only 70% of their controls is consistent with but does not in itself establishes EBV as a causative factor in SLE (31). Antibodies to 60 Kda RO (a ribonucleoprotein complex) are the earliest autoantibodies detected in the preclinical period as individual progress towards clinical disease (32). The initial autoantigenic 60 Kda RO crossreacts with a peptide from the latent viral protein Epstein-Barr virus nuclear antigen-1 (EBNA-1). Moreover, mice immunized with either the first epitope of 60 KdA or cross-reactive EBNA-1 epitope acquire clinical symptoms of lupus including renal dysfunction (33). These data suggest that autoimmunity may arise through molecular mimicry between EBNA- and some lupus autoantigens.

Photosensitivity is a common feature of SLE since specific lupus lesions occur on sun-exposed areas and the disease is aggravated by sun exposure in 40–70% of patients. The current theory is that UV light induces apoptosis of keratinocytes, which develop small surface blebs containing lupus autoantigens such as the Ro particle (34). Similarly drugs such as chlorpromazine (35) or environmental toxics such as silica dust have been shown capable of inducing apoptosis.

Hormonal factors – The female predominance in SLE is particularly strong during childbearing ages, suggesting that hormonal differences may contribute to the increased risk of the disease (36, 37). Reduced androgen levels, increased estradiol and prolactin levels have been reported in SLE patients. Moreover, studies in mouse models have shown disease exacerbation by estrogen and prolactin and improvement by androgens. However, a population-based study of 240 female SLE patients found little evidence that estrogen- or prolactin-related exposures are associated with an increased risk of lupus (38).

Role of T and B lymphocytes – In SLE, autoantibodies, immune complexes and autoreactive T cells may cause tissue damage. While an absence of autoreactive T-cells deletion in central lymphoid organs in SLE is still controversial, a loss of self-tolerance in the periphery appears to be critical in the pathogenesis of SLE (39). In normal mature B cells the immunoglobulin FcγRIIB receptor opposes signals for proliferation that are generated when B cell receptor binds antigen. FcγRIIB deficiency coupled with failure to eliminate immature self-reactive B cells in the lupus-prone mice B6.Sle1z, allows self-reactive B cells to escape clonal anergy that normally control the production of autoantibodies (40). The partial restoration of inhibitory Fc receptor (FcγRIIB) levels on B cells is sufficient to restore tolerance and prevent autoimmunity (41).

Regulatory T-cells (Tregs) are a specialized subpopulation of T lymphocytes that act to inhibit the autoreactive lymphocytes. Tregs have been shown to have a reduced ability to suppress the proliferation of helper T-cells in patients with active lupus compared to patients with inactive lupus (42). Self-antigen can trigger autoimmunity by several ways. The level of autoantigens may be increased by an impaired clearance of immune complexes or of apoptotic cells. Defects in the complement system or lymphocyte Fc receptor alterations may enhance the level of available autoantigen (43–45). Perturbations of the cytokine network could activate antigen-presenting cells (APC) and thereby reduce the signaling threshold of self-reactive peripheral T or B cells to a given concentration of autoantigen (46). Alterations in these different regulatory pathways have been described in lupus-prone natural and knockout mouse models (*Table 42-2*). Moreover, polymorphisms or mutations of several genes implicated in these processes have been reported in lupus patients.

The production of autoantibodies against components of the cell nucleus is a hallmark of SLE, the most prominent being those against double-stranded DNA (dsDNA) and histones. There is growing evidence that the nucleosome, the fundamental unit of chromatin composed of histones and dsDNA is a major autoantigen that drives a T-cell-dependent autoimmune response. Its implication in the pathogenesis of SLE seems to be closely related to an abnormal regulation of apoptosis and an impaired clearance of apoptotic products. High levels of these products and particularly of nucleosomes have been observed in lupus patients in comparison to controls (47).

Dendritic cells are key regulators of the immune system. These professional antigen-presenting cells induce the activation of naïve T-cells and stimulate growth and proliferation of B-cells. It has been shown that SLE patient's serum induces monocytes to differentiate into dendritic cells able to capture dying allogeneic cells and to present their antigens to autologous CD4+ T cells leading to their proliferation. Furthermore this capacity to induce dendritic cell differentiation is correlated with disease activity (48) and is abolished by blocking interferon-γ suggesting that this cytokine is the major mediator of monocyte to dentritic cell differentiation. The dendritic cells might capture apoptotic cells and nucleosomes, present in SLE patient's blood. Subsequent presentation of autoantigens to CD4+ T cells could then initiate the expansion of autoreactive T cells, followed by differentiation of autoantibody-producing B cells.

Complement deficiency – Activation of the complement system is implicated in the defense against microbes and in the adaptive immune response and inflammation. The complement system plays a paradoxical role in the development and expression of SLE. While the activation of complement is involved in tissue damage as illustrated by several studies in mice, inherited deficiencies of components of the classical pathway are strongly associated with the development of SLE. Lupus develops in many patients with complete deficiencies in C1q (90%) and C4 (75%) suggesting that these molecules have a protective role against the development of the disease (49). The protective role of C1q has been confirmed by the observation of severe lupus nephritis in C1q deficient mice. The complement system is involved in the clearance of apoptotic cells notably through the binding of the C1q and mannan-binding lectins molecules to the apoptotic cells and the subsequent complement activation (50).

Clinical Manifestations

SLE is often acute in onset and manifests with general symptoms of fever and fatigue associated with cutaneous and articular symptoms (11, 51). Such a cluster of symptoms is suggestive of SLE. In a French multicenter study, it was found that the most common initial manifestations were hematologic (72%), cutaneous (70%), musculoskeletal (64%), renal (50%), and fever (58%). Thirty-two percent of children had atypical symptoms, mainly including abdominal involvement in 26 patients (52). In some cases, SLE begins with an isolated symptom such as a hemolytic anemia, a nephritic syndrome with hematuria, chorea, or pericarditis. The performance of systematic serologic tests permits the linkage of these manifestations to SLE (53).

General symptoms, particularly weight loss, anorexia, and asthenia, are virtually constant in SLE. Episodic fever is frequent during acute phase of the disease or during infection. Pulmonary or urinary tract infections are frequent during the course of SLE. Opportunistic infections may be life threatening. A number of factors may favor a relapse of the disease, including exposure to ultraviolet light (54), infection (55), stress (56), surgery or pregnancy.

Joints and muscles – Arthralgia is the most common manifestation, observed in 80% of cases during the evolution of the disease (57). Both large and small articulations, especially the hands, are affected, causing moderate pain and articular swelling. The damage of periarticular tissues is often more pronounced than synovial damage, particularly in the form of tendinitis. Arthritis is very rarely deforming and synovial fluid contains relatively few cells, essentially mononuclear. Myalgia is less frequent. Avascular osteonecrosis which often affects femoral heads may occur in the absence of treatment with corticosteroids. This complication can be asymptomatic at first or accompanied by pain. In early stages, X-ray results are often normal while MRI reveals necrotic lesions.

Skin – The most classic skin lesion is the "butterfly" rash over the cheeks and nose, present in a third of cases. It often appears after sun exposure (58). Maculopapulus eruptions on the upper portion of the trunk as well as cutaneous zones exposed to light are more common. Papular and squamous lesions on the trunk, face, and palms of the hands can be observed in less severe, strictly cutaneous forms of the disease, often linked to the presence of anti-Ro antibodies. Raynaud's phenomena of the extremities causes painful white marble-like lesions, leg ulcers, and peri-ungual or finger-pad erythema. Urticarian eruptions and pigmentation anomalies are sometimes observed. Cutaneous photosensitivity is noted, especially in white patients. Mucosal lesions, particularly in the form of oral ulcerations, are common. Alopecia is observed in some children, especially those with active forms of the disease.

Lungs – Pulmonary involvement occurs in more than 50% of children (59). Pleuritis, observed in 40% of children, is often asymptomatic. It may be revealed by thoracic pain. Pleural liquid, more or less abundant, is an exudate, containing an excessive number of neutrophils and lymphocytes. The levels of C3 and C4 are diminished while the level of antinuclear antibodies is elevated. Anomalies in diaphragm function have been reported, responsible for dyspnea. Pulmonary damage may manifest as an acute pneumonitis with thoracic pain, dyspnoea, and fever (60). Poorly defined opacities can be observed on X-ray. Histologically, lymphoplasmocytic infiltrates may be present. Chronic fibrosing alveolitis have been described. Pulmonary arterial hypertension is a rare but severe problem, which may be secondary to repeated pulmonary embolism (61). Pulmonary hemorrhage during acute phase of the disease or during infections may be life threatening.

Heart – Pericarditis is frequent and is often diagnosed on echocardiography. Myocarditis with congestive heart failure is rare. Endocarditis of Liebman-Sachs which often affects the mitral valve and is associated with antiphospholipid (APL) antibodies is rare (62, 63).

Nervous system – Neuropsychiatric symptoms are present in 30–45% of children with SLE (64–66). These symptoms can either be a direct result of SLE or secondary to arterial hypertension, renal insufficiency, infectious

complication, hemostasis abnormalities, or various treatments, particularly corticosteroids (67). Mood disorders or behavior disturbances are often difficult to interpret in this chronic disease. Headache is the most frequent neurological manifestation of SLE and is often accompanied by localized or generalized seizures (68). Symptoms suggestive of a cerebral tumor have been described. Chorea is frequent in children, sometimes in association with APL antibodies. Cranial nerve palsies have been described, particularly ptoses and hemiparesis. Psychiatric symptoms consist of difficulties with attention or memory, disorientation, behavioral problems, depressive syndrome, or psychotic states with hallucinations and delirium (69–71). These symptoms can occur in an acute, transitory manner, during a flare of the disease, or can be more prolonged. They are often linked to ischemic cerebral vascular lesions that can be visualized by magnetic resonance imaging (72). Damage to the large arteries is rare. The role of antineuronal antibodies that are found in half of patients in the pathophysiology of the lesions is uncertain. Thrombotic phenomena secondary to the APL antibodies may play a role in the pathogenesis of neurological manifestations (73, 74). Behavioral problems, with a decrease in academic achievements, are often noted in children with SLE. These problems could be linked to the disease itself, to the treatment with corticosteroids, or the psychological response to a serious chronic illness. If these problems are secondary to SLE, the symptoms can respond favorably to an increase in corticotsteroid doses.

Eye – Both the retina and the cornea can be affected. Keratoconjunctivitis sicca is the most common ocular manifestation. The retinal lesions consist of white, cottony exudative lesions of the optic nerve, papillary edema, or thrombosis of the central retinal artery, causing blindness (75).

Hematopoietic system – Hematological manifestations are common in SLE (76). Inflammatory cervical or diffuse adenopathies occur in children with SLE. Anemia, observed in more than half of cases, is usually normochromic and normocytic and its degree reflects the activity of the disease. Hemolytic anemia is present in less than 10% of cases, accompanied by reticulocytosis, a positive Coombs test, an increase in free bilirubin levels and a decrease in haptoglobin levels. Aplastic anemia has been reported and responds to treatment with corticosteroids. Leukopenia, noted in more than 50% of cases, is in part responsible for an increased sensitivity to infections. Neutropenia may be linked to medullary suppression, hypersplenism, or antineutrophil antibodies. Lymphopenia affects both T and B lymphocytes. Thrombopenia, noted in 10–25% of cases, is accompanied by an increase in megacarycotyes in the marrow, indicating peripheral destruction of platelets. The acute, severe forms of thrombocytopenia are often observed during an acute phase of the disease and are responsive to treatment with corticosteroids, while more chronic forms are less symptomatic and less responsive.

Antiphospholipid antibodies – APL antibodies may be directed against epitopes on oxidized phospholipids complexed with a glycoprotein, β2-glycoprotein I, or against the glycoprotein itself. The principal APL antibodies include anticardiolipin antibodies, β2-glycoprotein-I antibodies and the lupus anticoagulant (77). These antibodies are responsible for thrombosis in vivo while they prolong phospholipid-dependent coagulation tests in vitro (78). Patients with SLE have a high incidence of antiphospholipid antibodies with a consequent high risk of thrombosis (79).

A patient with the APL syndrome must meet at least one of the two clinical criteria (e.g., vascular thrombosis or complications of pregnancy) and at least one of two laboratory criteria (e.g., anticardiolipin antibodies or lupus anticoagulant antibodies). Patients with APL antibodies develop thrombotic arterial or venous accidents, thrombocytopenia, and the experience of repeated abortions. Primary APL syndrome occurs in patients without clinical evidence of another autoimmune disease, whereas secondary APL syndrome occurs in association with autoimmune or other diseases (80). The prevalence of APL antibodies is 1–5% among apparently healthy subjects in the young and increases with age. Among patients with SLE, the prevalence of APL is much higher, ranging from 12 to 30% for anticardiolipin antibodies and 15 to 34% for lupus anticoagulant antibodies (81).The APL syndrome may develop in 50–70% of patients with SLE and APL antibodies within 20 years of the initial diagnosis.

Patients with primary and secondary APL syndrome have similar clinical consequences of APL antibodies (82). The clinical hallmark of the APL syndrome is the presence of vascular thrombosis. Venous thromboses are more frequent than arterial thrombosis. Involvement of the small vessels, particularly in the kidneys can mimics the hemolytic-uremic syndrome and the thrombotic thrombocytopenic purpura. The APL syndrome may also occur as a more chronic process, resulting in progressive loss of organ function (83).

In a cross-sectional cohort study in 59 consecutive SLE patients, thirteen thrombotic events occurred in 10 of the 59 patients (17%). There was a direct relationship between the presence of a lupus anticoagulant and a thrombotic event (84). Similar findings were reported in a retrospective study in 36 children with lupus nephritis.

No difference in the incidence of APL positivity was detected between the eight children who experienced thrombotic complications and those who did not (85).

Altered fibrinolysis secondary to an increase of the inhibitor of the plasminogen activator and a decrease of protein S have also been described in patients with SLE. Other more rare circulating anticoagulants have been observed. They are true in vivo anticoagulants, including antifactor VIII, antifactor XI, or antifactor XII.

Endocrine glands – An autoimmune thyroiditis, with antithyroglogulin and antimicrosome antibodies may be responsible for symptoms of hypothyroidism and less often symptoms of hyperthyroidism. A study found a high frequency of antithyroid antibodies in children with SLE (86). Diabetes mellitus is often secondary to high doses of corticosteroids but it may be secondary to SLE with the presence of antiinsulin receptor antibodies. Hormonal contraception may promote lupus activity and thromboses, particularly in patients with antiphospholipid antibodies. Therefore, it is important that appropriate contraception is adapted when necessary and patients must be informed of the risks of future pregnancy, which would need specialized monitoring (87).

Gastrointestinal manifestations – Gastrointestinal manifestations are often secondary to the side effects of treatments. Gastritis and peptic ulcers may be secondary to the use of nonsteroidal antiinflammatory agents or corticosteroids. Oral ulcers are frequent during the course of SLE and patients may complain from dysphagia. Esophageal reflux or ulceration may occur. Abdominal pain is a frequent complain in patients with SLE. It may be related to serositis, peritonitis, intestinal vasculitis, intra-abdominal thrombosis, small intestinal bacterial overgrowth or pancreatitis (88). Vascular lesions can cause intestinal ischemia and/or perforations. Clinical manifestations linked to these gastrointestinal lesions can be masked by corticosteroids. Pancreatitis may be directly related to SLE or secondary to corticosteroid therapy.

Hepatomegalia is frequent but rarely accompanied by significant liver dysfunction. The presence of liver abnormalities with antinuclear antibodies is more likely to be due to chronic active hepatitis, also called "lupoid hepatitis," than to SLE.

Renal Disease in SLE

Pathogenesis of Lupus Nephritis

Genetic or acquired susceptibility factors are probably associated with the onset of renal disease. Numerous mouse models have improved our understanding of the pathogenesis of lupus nephritis.

Nucleosome play a central role in the pathogenesis of SLE. There is growing evidence that increased apoptosis and impaired clearance of apoptotic cells facilitate the emergence of anti-DNA antibodies and immune complexes. Nucleosomes are also important for triggering tissue lesions, including glomerular lesions. They are at least two mechanisms that explain how autoantibodies mediate tissue damage: (a) deposition of preformed immune complexes in the kidney or *in situ* formation of these complexes via the interaction between nucleosome already deposited in the glomerulus and anti-dsDNA antibodies, (b) cross-reactivity of antibodies with glomerular basement membrane components. The recent demonstration by means of electron microscopy in mice and humans that anti-dsDNA antibodies colocalize with extracellular chromatin in glomerular-membrane associated electron-dense structures supports the first hypothesis (89, 90). α-actinin has been identified as a cross-reactive target bound by nephritogenic anti-dsDNA in a lupus mouse model (91). α-actinin is an actin-bundling protein expressed in podocytes, monocytes, capillaries and larger vessels. Mutations of the gene encoding the isoform α-actinin-4 have been identified in familial focal and segmental glomerulosclerosis. α-actinin may also be a target for anti-dsDNA in human.

Inherited deficiencies of the early components of the complement classical pathway are the strongest susceptibility genes for the development of SLE in humans. C1q deficient mice have been generated by homologous recombination (19). The presence of multiple apoptotic bodies in the altered glomeruli suggests a critical role for C1q in the clearance of apoptotic cells. This finding is consistent with the report that C1q binds to apoptotic keratinocyte (92). Apoptotic cells are thought to be a major source of the autoantigens of the SLE, and impairment of their removal by complement may explain the link between hereditary complement deficiency and the development of SLE.

Another study on lupus-prone New Zealand black and New Zealand white mouse has shown that the γ chain of the T-lymphocyte receptor is mandatory to initiate the inflammatory cascade in the kidney. Indeed, when the NZB/NZW mice were backcrossed with FCγR deficient mice, glomeruli were not affected despite the deposition of immune complexes and C3 (93). In humans, decreased binding affinity of the FcγRIIIA-158F allele may result in ineffective clearance of antigen–antibody complexes, resulting in an increased susceptibility to immune-complex-mediated nephritis (94). Of interest,

another polymorphism in the gene coding for the FcγRIIIA receptor represents a significant risk factor for SLE but has no clear effect on susceptibility for lupus nephritis (95). A study of inflammatory markers after remission in NZB/NZW mice indicates that activated macrophages type II have a central role in the genesis of lupus nephritis (96).

Clinical Findings

Clinical symptoms of renal involvement are noted in 40–80% of patients, appearing most often during the first years (7). Renal involvement is variable, with some patients showing minimal urinary anomalies and other having nephritic syndrome with rapidly progressive renal failure. Proteinuria is the most frequent symptom. Proteinuria is frequently abundant accompanied by a nephrotic syndrome responsible for edema. Microscopic hematuria is frequently associated to proteinuria but is rarely seen in isolation. The urinary sediment contains granular and red cells casts. Patients with severe nephritis are often hypertensive and have a reduced renal function. Up to 50% of children with lupus nephritis have a decreased glomerular filtration rate. Signs of renal tubular dysfunction may be observed and are related to the presence of immune deposits along the tubular basement membranes and to interstitial cellular infiltrates. Increased excretion of ß2 microglobulin is frequently found. Renal tubular acidosis with hyperkalemia has been reported.

Histologic Classification

Most authors believe that the presence of urinary abnormalities or renal function impairment is an indication for renal biopsy, especially to guide the initial treatment (97). There is no close correlation between symptoms and the class of glomerular lesions (focal or diffuse proliferative nephritis with varying degrees of severity or membranous nephritis). The type of histological lesions is most important to decide the best therapy. A renal biopsy may also be indicated in a patient with glomerular disease without extra-renal manifestations of SLE. It is more difficult to decide whether to perform a renal biopsy in patients with no clinical evidence of renal disease. Although the clinical signs of renal damage are present in only 40–75% of patients, renal biopsy studies show nearly universal histologic changes. The majority of clinically asymptomatic patients show moderate histological damage most often mesangial deposits and mild mesangial proliferation but some may have more severe histological lesions such as focal and segmental glomerulonephritis or very rarely diffuse proliferative glomerulonephritis (silent lupus nephritis).

Histological anomalies are present in 90% of patients and immune deposits are almost always found on immunofluorescence examination or by electron microscopy. There is a wide diversity of glomerular damage in SLE. Moreover, tubulointerstitial and vascular lesions can be observed in addition to glomerular lesions. The first World Health Organization (WHO) classification proposed by Pirani and Pollack in 1974 was based primarily on the histological changes observed by light microscopy. It was modified in 1982 by the ISKDC (International Study of Kidney Disease in Children). A revised classification based on glomerular lesions has recently been proposed with the objectives to standardize definitions, emphasize clinically relevant lesions and facilitate reproducible reporting (98–100) (*Table 47-3*). The main

Table 47-3

International society of nephrology/renal pathology society (ISN/RPS) classification of lupus nephritis

Class I – Minimal mesangial lupus nephritis
Normal by light microscopy but mesangial deposits by immunofluorescence
Class II – Mesangial proliferative lupus nephritis
Mesangial hypercellularity and mesangial deposits by immunofluorecence
Class III – Focal lupus nephritis
Class III (A): with active lesions
Class III (B): with active and chronic, sclerosing lesions
Class III (C): with chronic, sclerosing lesions
Class IV – Diffuse lupus nephritis
Class IV-S (A): diffuse segmental proliferative lupus nephritis with active lesions
Class IV-G (A): diffuse global proliferative lupus nephritis with active lesions
Class IV-S (A/C): diffuse segmental proliferative and sclerosing lupus nephritis
Class IV-S (C): diffuse segmental sclerosing lupus nephritis
Class IV-G (C): diffuse global sclerosing lupus nephritis
Class V – Membranous lupus nephritis
Global or segmental subepithelial immune deposits
Class V lupus nephritis In combination with class III or class IV
Class V with sclerosing lesions
Class VI – Advanced sclerosing lupus nephritis
>90% sclerosed glomeruli without active lesions

changes concern the elimination of the subcategories membranous lupus nephritis which are close to class III or class IV lupus nephritis and the introduction of a subclassification of class IV lupus nephritis with segmental (IV-s) or global (IV-g) distribution of the lesions. An adequate renal specimen, containing at least ten glomeruli, must be obtained for light microscopy analysis. Immunofluorecence examination is also mandatory. Electron microscopy may aid the classification but is not necessary for diagnosing lupus nephritis. Furthermore, renal biopsies have permitted clinicopathologic correlations with the natural outcome of the disease. Of importance, the histologic categories guide therapeutic decisions (101, 102).

Class I is defined as minimal mesangial lupus nephritis with normal glomeruli by light microscopy but mesangial immune deposits by immunofluorescence (*Fig. 47-1*).

Class II is defined as mesangial proliferative lupus nephritis with mesangial hypercellularity and mesangial immune deposits. Immunofluorescence and electron microscopy examination show mesangial deposits whereas the glomerular walls are normal. Urine examination can be normal or reveal microscopic hematuria and moderate proteinuria. The prognosis is excellent except in cases of transformation to a more severe form (103).

Class III is defined as focal lupus nephritis involving less than 50% of glomeruli. The affected glomeruli show segmental lesions of endocapillary proliferation or inactive segmental scars. Necrotic lesions and cellular crescents may be present. Immunofluorescence examination reveals subendothelial deposits along the glomerular capillary walls with focal or diffuse mesangial immune deposits. Tubulointerstitial or vascular lesions are often present in addition to glomerular lesions. When less than 20% of glomeruli are affected by small segmental lesions, patients usually have mild renal symptoms with low-grade proteinuria without nephrotic syndrome and normal GFR. The long-term prognosis is favorable with probably less than 5% risk of progression to end stage renal failure after 5 years (104). Conversely, when cellular proliferation, necrosis and large subendothelial deposits involve more than 40% of the glomeruli, the clinical symptoms are more severe with active urine sediment, nephrotic syndrome, hypertension and moderate renal insufficiency in some patients. The prognosis is the same as for diffuse lupus nephritis (101, 104).

Class IV is defined by diffuse lupus nephritis involving more than 50% of glomeruli, The lesions may be segmental, affecting less than 50% of the glomerular tuft, or global, affecting more than 50% of the glomerular tuft. Therefore, this class may be subdivided in diffuse segmental lupus nephritis (IV-s) and diffuse global lupus nephritis (IV-g). In diffuse segmental lupus nephritis more than 50% of the glomeruli show segmental lesions of endocapillary proliferation with or without necrosis and with or without segmental scars. In diffuse global lupus nephritis more than 50% of the glomeruli show global and diffuse endocapillary proliferative lesions with often extracapillary proliferation.

In addition, active lesions are often seen such as necrotic lesions, karyorrhexys, hematoxylic bodies, leukocytic infiltrates, wire-loops and hyaline thrombi. The proportion of glomeruli with epithelial crescents is an important pronostic factor. Tubulointerstitial lesions with mononuclear cell infiltrates are common. Immunofluorescence and electron microscopy show diffuse mesangial and more or less extensive subendothelial immune deposits. Deposits are also found frequently along the tubular basement membranes and the capillary walls. The clinical symptoms are often severe: hematuria with casts, nephrotic syndrome, hypertension and moderate or severe renal insufficiency. Patients with class IV nephritis are at high risk of progression to end stage renal disease if adequate therapy is not undertaken.

Class V is defined as membranous lupus nephritis, characterized by a thickening of the glomeruli capillary walls and the presence of global or segmental continuous subepithelial immune deposits separated by "spikes," In pure class V, there is little or no cellular proliferation. Compared to the idiopathic form of membranous nephropathy, mesangial deposits are almost constant. In some patients, mesangial hypercellularity and subendothelial deposits are present, in which case class V is associated with lesions of class II, class III or class IV lupus nephritis. Membranous nephritis may precede other manifestations of lupus. Moderate proteinuria is accompanied in 50% of patients by hematuria. Nephrotic syndrome often develops. Moderate renal failure and hypertension are observed in 25% of patients.

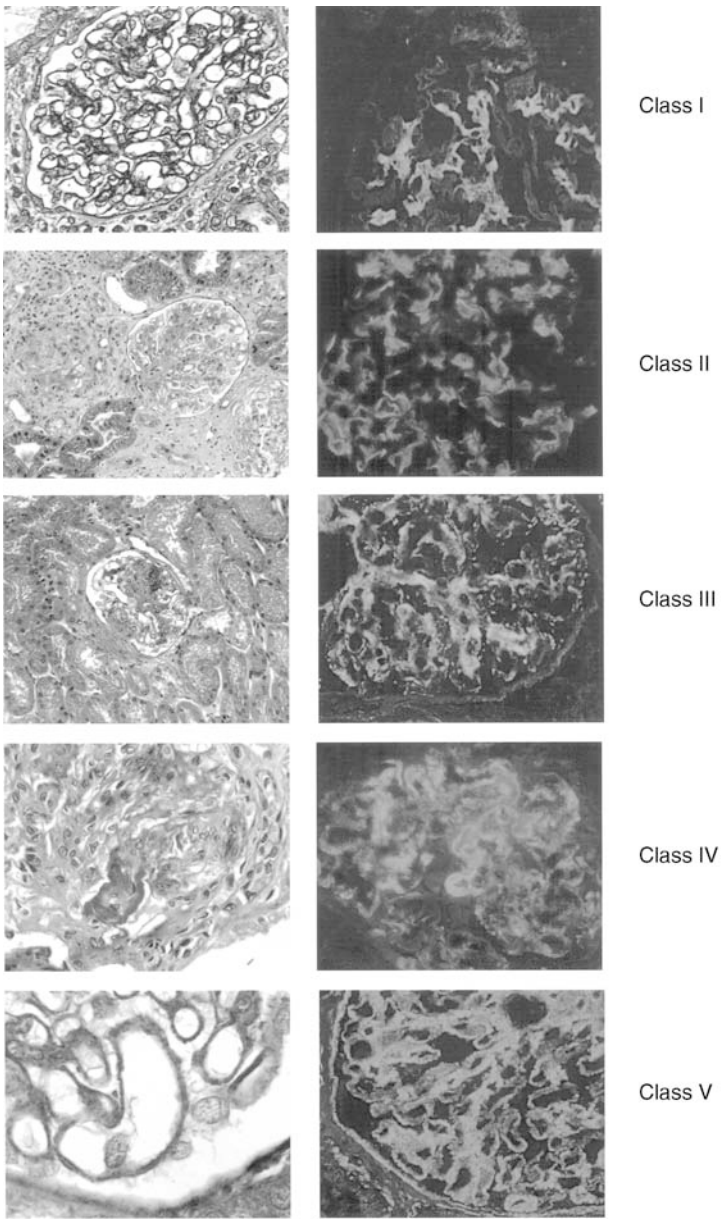
Class VI corresponds to an advanced stage of lupus nephritis where most glomeruli are sclerotic but without evidence of active glomerular lesions.

In addition to glomerular lesions, tubulointertitial and vascular changes are often observed including inflammatory vasculitis, vascular thrombosis, interstitial inflammation, interstitial fibrosis and tubular changes.

By immunofluorescence, the composition of deposits is remarkable no matter where they are located. In these deposits, IgG is dominant, mostly IgG1 and IgG3. IgA and IgM are also present, as well as early complement components, C4 and C1q along with C3. Such positivity for all three Ig classes and C3, C4 and C1q is called *full house* and is highly suggestive of lupus nephritis. Fibrin deposits are also found, particularly in class IV biopsies.

Figure 47-1

Class I: Normal glomerulus by light microscopy (right-hand side) but mesangial immune deposits by immunofluorescence study (left-hand side). Class II: Purely mesangial hypercellularity and mesangial matrix expansion in glomerulus by light microscopy with mesangial immune deposits by immunofluorescence study. Class III: Segmental endo- and extracapillary proliferation involving $<$ 50% of all glomeruli by light microscopy with mesangial and some subendothelial immune deposits by immunofluorescence study. Class IV: Global endo- and extracapillary proliferation and subendothelial deposits by light microscopy and numerous immune deposits by immunofluorescence study. Class V: Membranous glomerulonephritis with subepithelial deposits by light microscopy, clearly defined by immunofluorescence study. (Courtesy of Dr Laure-Hélène Noël) (See color plate 30)



Activity and Chronicity Indexes

Pirani et al. and Morel-Maroger et al. introduced the concept of histological signs of activity and chronicity (105). Later, Austin et al. introduced the activity and the chronicity indices, which may help to define to prognosis (106). Active histological lesions include cellular crescents, endocapillary proliferation, fibrinoid necrosis, karyorrhexis, thrombi, wire-loops with subendothelial immune deposits, glomerular leucocyte infiltration and interstitial mononuclear cell infiltration (*Table 47-4*). These active lesions are each graded 0–3 (with both necrosis and cellular crescents graded 0–6) to give an activity index graded 0–24. Active lesions are potentially reversible with the treatment. Glomerular capillary wall necrosis is associated with poor outcome when these lesions affect a high percentage of glomeruli (107). Extramembranous deposits are not considered as active lesions. Conversely, irreversible lesions, including glomerular sclerosis, fibrous crescents, tubular fibrosis and interstitial fibrosis, permit the definition of chronicity index. These lesions do not respond to treatment. If the activity and the chronicity indices are important to decide the best therapy for individual patients, their prognostic significance is still debated. The NIH group found that an activity index >12/24 or an elevated chronicity index were indicative of a poor renal prognosis (106). A chronicity index less than 2 was associated with a 10-year renal survival rate of 100% whereas when the chronicity index was comprised between 2 and 4 the renal survival rate was only 70%. The renal survival rate at 10 years was only 35% in patients with a chronicity index greater than 4. However, a study including unselected patients with lupus nephritis found that activity and chronicity indices did not accurately predict renal outcome (108).

Table 47-4
Active and chronic histological renal lesions

Active lesions
Glomerular
Endocapillary hypercellularity
Fibrinoid necrosis
Karyorrhexis
Hyalin thrombi
Wire loops (subendothelial deposits)
Hematoxylic bodies
Cellular crescents
Leukocyte infiltrates
Tubulo-interstitial
Mononuclear cell infiltrates
Tubular necrosis
Chronic lesions
Glomerular
Glomerular sclerosis (segmental or global)
Fibrous crescents
Fibrous adhesions
Extramembranous deposits
Tubulo-interstitial
Interstitial fibrosis
Tubular atrophy

Repeat Renal Biopsies

Repeat biopsies have shown that transformations from one class to another are possible, either via aggravation or amelioration of the histological lesions. A class II nephritis can transform into class III nephritis or class IV nephritis. These, in turn, are susceptible to improvement under treatment. In contrast, transformation is rare in cases of class V nephritis. Hill et al. reported that a repeat renal biopsy at 6 months carries pronostic significance (109). Repeat renal biopsies allow for the evaluation of the efficacy of treatment and are useful in considering therapeutic changes, particularly in cases of worsening clinical symptoms (proteinuria, renal insufficiency). In some cases, clinical aggravation may be secondary to sclerotic lesions, for which treatment is ineffective. Sclerotic lesions are especially seen in patients who have had insufficient treatment for an active nephropathy. In other cases, deterioration may be related to active histological lesions, which require a more aggressive treatment. Also, deterioration of renal function may be secondary to nephrotoxic treatments such as non steroidal antiinflammatory drugs, cyclosporine or antibiotics rather than SLE itself. Therefore, a repeat renal biopsy may be indicated in case of persistent nephrotic syndrome or persistent active urine sediment despite adequate therapy, recurrent proteinuria and/or hematuria after remission or an increase in serum creatinine without clear explanation.

Prognostic Factors in Patients with Severe Lupus Nephritis

Several studies have analyzed the factors that are associated with a higher risk of progression. Extensive crescent

formation and necrotizing glomerular lesions are associated with an adverse outcome (110–115). In addition to these histological signs of severity, other factors may be of prognostic significance, including age, gender, race, hypertension, initial serum creatinine concentration, the delay between onset of renal disease and treatment, the occurrence of exacerbations of the nephropathy, the response to therapy after the first year (106, 110, 116–120).

An analysis of these prognostic factors is important in order to propose the most effective and safest therapy for individual patients. Several studies have shown that lupus nephritis has a worse prognosis in men compared to women. Renal survival in black Americans with diffuse lupus nephritis is poorer as compared to white patients (121). Renal survival rate in black patients was 58% at 5 years compared to 95% of white patients who retained renal function following pulse cyclophosphamide therapy. Several possible reasons for the significance of race in the poorer prognosis in lupus nephritis have been proposed and include socioeconomic factors, different HLA phenotypes and inherited susceptibility for progression to renal failure.

In a study of 86 patients with severe class III or class IV lupus nephritis who were treated with prednisone and oral cyclophosphamide, an elevation of serum creatinine was the only initial feature predictive of progression to renal failure (122). Of interest, the same authors found that the subsequent clinical course in response to therapy supplemented prognosis based on initial serum creatinine. The risk of progression to renal failure was higher in those patients who did not show resolution of renal abnormalities within 48 weeks of follow-up. Patients with normal initial serum creatinine and patients with resolution of initial serum creatinine elevations had a similar and much lower risk of progression to renal failure. Other authors have also reported the poor prognostic significance of elevated serum creatinine at presentation (106, 110, 116, 123, 124). However, these findings were not confirmed in several studies with longer periods of follow-up (101, 115, 120, 125).

In a cohort of 87 patients, the duration of renal disease prior to renal biopsy and initiation of therapy was a statistically significant predictor for renal failure or death due to renal disease (126). The most likely explanation for these findings is that early treatment of renal disease is associated with better outcome. There was a significant increase in serum creatinine, in urinary protein excretion and in activity and chronicity indexes on renal biopsy in those patients in whom the treatment was delayed. Lim et al. found that chronicity index, male sex and initial renal insufficiency were prognostic factors for class IV lupus nephritis (127). Similarly, a retrospective study of 43 patients with severe lupus nephritis who were treated with IV cyclophosphamide found that increased serum creatinine and a high degree of interstitial fibrosis on renal biopsy before treatment were associated with a worse renal prognosis (118).

Moroni et al. studied the prognostic significance of renal flares defined as a rapid increase of serum creatinine and/or in proteinuria in a retrospective study of 70 patients with lupus nephritis (120). Patients with renal flares had a higher probability of having a persistent doubling of serum creatinine compared to those who had no renal flares. The relative risk of having a persistent doubling of serum creatinine was 27 times higher in patients with rapid increases in serum creatinine. This outcome was more frequent in patients who did not respond rapidly to therapy. These data support the need for aggressive treatment of lupus nephritis exacerbations. In a study of 189 patients, Mok et al. found that the occurrence of nephritic flares was the only predictor of creatinine doubling (128). Moroni et al. found that the risk of relapse was related to the duration of initial treatment and the duration of remission before withdrawal of therapy (129). The mean duration of treatment in the group of patients who did not relapse was 57 vs. 30 months in patients who relapsed after cessation of therapy. In a retrospective study of 35 patients with lupus nephritis who were followed for up to 10 years, Barber et al. found that female gender, older age, higher nonrenal SLEDAI scores at the time of diagnosis were predictive of sustained remission (130).

A retrospective study of 93 African Americans, 100 Hispanics, and 20 Caucasians identified predictors of outcome. At baseline, mean blood pressure and serum creatinine were higher in African Americans compared to Hispanics and Caucasians. Class IV lupus nephritis was seen more often in African Americans and Hispanics compared to Caucasians. Renal deterioration or death occurred more frequently in African Americans and Hispanics than in Caucasians indicating that these populations have a more aggressive disease and a worse outcome (131). Similarly, Korbet et al. in a study of 86 patients with severe lupus nephritis reported a more aggressive renal disease resulting in worse outcomes in black patients compared with white patients (132).

Several reports have claimed a poorer prognosis of lupus nephritis in children as compared to adults. However, in a series of 80 patients with onset of disease under the age of 20 years, Cameron et al. reported a renal survival rate of 85% at 5 years and 82% at 10 years (133). Platt et al., in a series of 70 patients, found rates of 85% and 81% (8). Several reports have identified the risk factors for renal failure in children with lupus nephritis.

In a retrospective study of 167 children, persistent hypertension, anemia, increased serum creatinine concentration at the time of biopsy, low titer of CH50 were associated with a higher risk of renal failure (134). In this study, the overall renal and patient 5-year survival rates were 87.7 and 91.1% respectively. McCurdy et al. reported on a group of 71 children of whom 22% progressed to end stage renal failure (124). They also found that persistent hypertension, anemia, abnormalities of the urineanalysis and elevated serum creatinine levels were significantly associated with progression to renal failure. Class IV nephritis, active and chronic lesions were also associated with progression to renal failure. Emre et al. found that the prognosis of lupus nephritis in children is primarily dependent on the histopathological lesions. Moreover, the severity of the clinical renal disease at admission and the presence of persistent hypertension are the main poor prognostic factors (135). Baqi et al. analyzed the risk factors for renal failure in 56 children with lupus nephritis (117). Univariate analysis revealed that with an elevated serum creatinine level, a decreased C3 complement level, hypertension and class IV lupus nephritis were associated with the rate of progression to renal failure. Multivariate analysis showed that progression to renal failure was independently associated with class IV lupus nephritis, hypertension at presentation and low C3 complement level in association with elevated serum creatinine levels. These three reports as well as other studies in adults found that hypertension was a significant risk factor for renal failure. Therefore, children with hypertension should be treated appropriately with antihypertensive drugs, as control of blood pressure may be as important as antiinflammatory treatment.

Prognostic Factors in Patients with Membranous Lupus Nephritis

Patients with class V lupus nephritis, without mesangial hypercellularity have a good prognosis with a 5-year renal survival close to 85% (136–138). Conversely, Sloan et al. found that when membranous nephropathy was associated with segmental endocapillary proliferation and/or necrosis in less than 50% of glomeruli, the 5-year renal survival was 72% and only 49% when more than 50% of glomeruli were affected (137). Therefore, associated glomerular inflammation and/or necrosis are important prognostic factors in membranous lupus nephritis. Elevation of initial serum creatinine or worsening renal function and severe nephrotic syndrome are also risk factors for poor renal outcome.

Laboratory Investigations

SLE is characterized by a large variety of autoantibodies of which certain, such as anti-DNA antibodies, are found almost exclusively in patients suffering from this disease (139). These autoantibodies, associated with the corresponding antigen, form immune complexes which activate the classic complement pathway. Immune complexes can be detected in the circulation or are deposited in different tissues where they are detectable by immunofluorescence techniques.

The detection of antinuclear antibodies (ANA) by indirect immunofluorescence is a simple and sensitive test in which the serum is incubated with tissue section of a suspension of cells such as polynuclear cells. ANA are present in significant titer in 95% of patients and the diagnosis of SLE is unlikely in their absence (140). The pattern of fluorescence gives an indication on the specificity of the antibodies. A homogenous or diffuse aspect of fluorescence is related to the presence of anti desoxyribonucleoprotein antibodies and is strongly associated with SLE. The same antibodies are responsible for the formation of Hargraves cells (LE cells). Following the reaction of the antibodies with the nucleus of leukocytes, the chromatin pattern is altered and the DNA forms a large mass with the antibodies and the complement that is then ingested by a phagocyte. A peripheral aspect of the fluorescence points to the presence of antinative DNA antibodies, characteristic of active SLE. A speckled aspect of the fluorescence is related to the presence of antibodies reacting with various nuclear antigens, such as anti-Sm or anti-RNP antibodies. Such a pattern of fluorescence may be observed in SLE or mixed connective tissue disease but is more frequently associated with scleroderma. The nucleolar pattern is observed in only 25% of patients with SLE.

The presence of ANA is not unique to SLE as one finds them in other rheumatologic disorders such as chronic juvenile arthritis, scleroderma or mixed connective tissue disease, autoimmune hepatitis, thyroiditis, or after taking certain medications. ANA titers should be determined, as if low levels are found in around 2% of the general population, high levels are highly suggestive of SLE. A small proportion of patients with SLE does not have ANA and over half of them have anti-SSA antibodies. Anti-SSA antibodies are not detected by indirect immunofluorescence tests, which explains negative results. Clinically, these patients have photosensitivity, arthralgia, and serous effusions.

Antinative, double stranded, DNA antibodies are relatively specific for SLE (97%) and therefore very useful for diagnosis (141–143). They can be detected in the serum by measuring their capacity to bind radiolabeled native

DNA (Farr test) or by an immunoenzymatic technique. Anti-DNA antibodies can also be detected by indirect immunofluorescence on the microorganism Crithidia Luciliae, which have a kinetoplast in their cytoplasm containing native DNA. The Farr assay detects high affinity antibodies whereas the immunoenzymatic technique detects both high and low affinity antibodies. The Farr assay correlates with disease activity especially with proliferative nephritis (144). Moreover, the Farr assay gives estimation on the level of antibodies, which is used to monitor disease activity. Normal or stable levels are positively correlated to a stability of the disease, while an increase often precedes the reappearance or aggravation of clinical manifestations of the disease. The ELISA is of interest for screening diagnosis. The antibody titer correlates well with disease activity but moderately well with the activity of nephritis.

The Smith (Sm) antigen is a nuclear nonhistone protein (145). Anti-Sm antibodies are specific to SLE but are only found in 30% of cases and are often associated to a less severe form of the disease (146). Antiribonucleoprotein antibodies, present in 35% of SLE cases, are not specific for SLE. These antibodies bind to proteins containing U1-RNA (147). Antierythrocyte antibodies, detected by the Coombs test can be responsible for severe hemolytic anemia. Antiplatelet antibodies are often present, sometimes causing thrombopenia. Antilymphocyte antibodies, directed against T lymphocytes, may play a role in the pathogenesis of the disease. Siegert et al. found a correlation between the levels of anti-C1q antibodies and the occurrence of nephritis, the titers of anti-DNA antibodies and hypocomplementemia (148). The increase of anti-C1q titers was correlated with the development of class IV lupus nephritis. Thus, the serial measurements of these antibodies may be useful to predict relapses of nephritis. Because C1q is found in glomerular immune deposits, C1q may act as a planted antigen for anti-C1q antibodies, thus increasing the inflammatory reaction (149).

APL antibodies, including anticardiolipin and the lupus anticoagulant, may be found in SLE and are responsible for an increased risk of thrombotic complications.

Many of these autoantibodies can be responsible for the formation of immune complexes, either in the serum or localized in tissues. Circulating immune complexes can be detected by several tests but these tests are not useful in the diagnosis or the monitoring of SLE as many other conditions can be accompanied by circulating immune complexes. Immune complexes deposited in the tissues are detected by direct immunofluorescence techniques. Deposits are very frequently found in the kidneys. Skin biopsy may show immunoglobulin, principally IgG, and complement fraction deposits at the dermo-epidermic junction. These fine-grained deposits principally contain IgG. This is the lupus band test that is highly suggestive of SLE (150).

Hypocomplementemia is observed in 75% of cases at presentation, particularly in patients with nephritis. Decreased levels of CH50, C1q and C4 are related to the activation of the classical pathway of the complement system. A permanent low C4 level may also be secondary null C4 alleles. A deficiency of other complement factors may be observed. Levels of C4, C1q and C3 are used to monitor disease activity in conjunction with anti-DNA antibodies. In most patients with nephritis, flares are preceded by a decrease of these complement components. Concentrations of properdin and factor B are also depressed due to the activation of the alternative pathway.

Regular testing of biological parameters (blood cell count, erythrocyte sedimentation rate, serum albumin, proteinuria, urinary sediment, GFR, anti-DNA antibodies, CH50, C4 et C3) every 3–6 months is important during the evolution of the disease. This testing allows for the detection of hematological or renal involvement as well as a serological evidence of disease activity prior to the appearance of clinical manifestations (151).

Drug-Induced Lupus

Several drugs, particularly those which are metabolized by acetylation in the liver, such as procainamide or hydralazine, can induce a lupus like syndrome, (152, 153). The syndrome is more likely to develop in slow acetylers, which have a genetically determined deficit in hepatic *N*-acetyltransferase (154).

One characteristics of drug-induced lupus is the presence of antihistone antibodies, which are found in 95% of patients. Other autoantibodies, such as anti-DNA antibodies are rarely found in those with drug-induced lupus. These autoantibodies are formed against a complex between DNA and a histone dimer of H2A and H2B. Of interest, although the drugs responsible for drug-induced lupus are quite heterogeneous, all of these antibodies are directed against the same epitope (155). With hydralazine, the complex is composed of H1 and H3-H4 and DNA.

Procainamide, hydralazine and penicillamine, isoniazid, interferon-alpha, methyldopa, chlorpromazine, diltiazem are all likely causes of lupus. Anticonvulsants (e.g., phenytoin, mephenytoin, trimethadione, ethosuximide), quinidine, antithyroid drugs, antimicrobial agents (sulfonamides, rifampin) may possibly induce lupus.

Clinical manifestations associated with drug-induced lupus include fever, cutaneous rash, arthralgia and serositis, while renal, hematologic or neurological symptoms are rare. Anti-DNA antibodies are often absent, and the level of complement fractions normal.

The differentiation between SLE and lupus syndrome secondary to a medication is not always clear. The principal criterion is the spontaneous recovery from the syndrome within 6 months after withdrawal of the suspected medication.

Neonatal Lupus

Neonatal lupus occurs in 1–2% of children born to mothers with lupus and is due to passively transferred autoantibodies. The main complication is a complete and irreversible atrioventricular block secondary to anti-SSA (Ro) and anti-SSB antibodies, which interferes directly with the development of the conduction system (156). Some infants require insertion of a pacemaker. The incidence of congenital heart block in children from mothers with anti-SSA (Ro) antibodies ranges from 0.2 to 7.5% (157).

Other newborns, particularly girls, present with erythematous rash that often develops after exposure to ultraviolet light. The rash regress totally after 6 months. Other manifestations may include anemia, moderate leukopenia and thrombocytopenia, or hepatomegalia with cytolysis (158).

Treatment of Lupus Nephritis

Therapeutic options for patients with lupus nephritis vary depending on the histological lesions observed on renal biopsy.

Therapy of Patients with Mild Renal Lesions

Patients with class II lupus nephritis do not need specific therapy, as there is little probability of progression (103). Nevertheless, careful follow-up of the patient is necessary, as transformation to a more severe renal disease is possible.

Therapy of Patients with Class III Lupus Nephritis

Patients with class II lupus nephritis and less than 20% of the glomeruli affected by small segmental lesions have a favorable long-term outcome. In this setting, there is no indication for specific therapy, which may however be required for extra-renal symptoms. Conversely, when cellular proliferation and necrosis involve more than 40% of the glomeruli, the course of the disease is close to that of class IV lupus nephritis and the same aggressive therapy is needed (101, 108).

Therapy of Patients with Class IV Lupus Nephritis

The therapeutic regimen of class IV lupus nephritis have changed over the years (159). Although there are a large number of reports on this topic, there are only few randomized studies, which deal with limited numbers of patients, and more importantly many of them do not indicate the long-term outcome after 10 years of follow-up. The optimal therapy for patients with class IV lupus nephritis remains a challenge but it has been shown that aggressive immunosuppressive therapies have improved the prognosis. Most nephrologists agree that initial therapy should be intensive in order to achieve complete remission which is associated with a better long term outcome. Indeed, in a study involving 86 patients with severe lupus nephritis, patients who achieved remission had a 94% renal survival rate at 5 and 10 years compared to 46 and 31% renal survival rate at 5 and 10 years in patients who did not achieve complete remission (160). The aim of the treatment is also to prevent renal flares and avoid deterioration of renal function. Indeed, renal flares are associated with a worse prognosis (128, 161). Finally the optimal therapy should be associated with limited side effects.

Induction Therapy

Pulse Methylprednisolone

Patients with class IV lupus nephritis are treated with prednisone 1–2 mg/kg/day for several months. However, high doses of oral prednisone alone not only give poor results on the long term but also are often associated with serious side effects. Many authors have proposed to initiate therapy with three intravenous methylprednisolone pulses, which have potent and rapid antiinflammatory and immunosuppressive effects (162–164). Following methylprednisolone pulses, extra-renal symptoms disappear rapidly and serum creatinine returns more rapidly to normal. The NIH protocole gives monthly methylprednisolone pulses in addition to IV cyclophosphamide.

Following methylprednisolone pulses, oral prednisone is started but the optimal initial dose is not well defined. Similarly, there is no consensus on how the dosage should be decreased.

Cyclophosphamide: High-dose vs. Low-dose

Induction therapy with cytotoxic drugs and corticosteroids was shown to be superior to corticosteroids alone in preventing the progression to renal failure with a follow-up of 5 years (165, 166). The "standard" therapeutic regimen combines cyclophosphamide and corticosteroids. The NIH trials showed that this association was the best option to preserve long-term renal function. The first trial showed that patients receiving IV or oral cyclophophamide with glucocorticoids had a better renal survival than those receiving glucocorticoids alone (165, 167). In the second NIH trial, a long course IV cyclophospphamide over 30 months was compared to a short course over 6 months and to IV methylprednisolone pulses The probability of doubling serum creatinine after 5 years of follow-up was higher in patients treated with methylprednisolone pulses compared to those receiving IV cyclophosphamide (48 vs. 25%). The probability of nephritis flares was significantly higher in patients receiving cyclophosphamide pulses for 6 months compared to those receiving the long-course cyclophosphamide regimen (55 vs. 10% after 5 years) (168). In the third NIH trial, patients receiving both IV cyclophosphamide and IV methylprednisolone had a higher rate of remission compared to those receiving IV methylprednisolone alone and a reduced risk of long-term deterioration of renal function (169). However, these therapeutic regimen did not decrease overall mortality (170) and were associated with significant side effects, including a high rate of amenorrhea, infections and possibly neoplasias (171).

The Lupus Nephritis Collaborative Study treated patients with severe lupus nephritis with oral prednisone at a starting dose of 60–80 mg/day and oral cyclophosphamide for only 8 weeks, a shorter and therefore less toxic course of cyclophosphamide than the NIH protocoles (122). Exacerbations were treated using increased doses of corticosteroids. Only 16% of the 31 patients who had normal serum creatinine concentration at the start of the treatment had a rise of serum creatinine at latest follow-up and only two of them (6.5%) progressed to renal failure. Conversely, 16 of the 55 patients (29%) with elevated initial serum creatinine levels progressed to renal failure during the follow-up period. These patients may have benefited from a more aggressive regimen. Patients with severe lupus nephritis and normal initial serum creatinine may thus achieve excellent outcome without cyclophosphamide pulses, although the duration of follow-up in this study was rather short.

The possibility to control the disease with lower doses of IV cyclophosphamide was also investigated in Europe considering that lupus nephritis might be less severe in Caucasians and that lower doses may result in fewer side-effects. The Euro-Lupus nephritis trial included 90 adult patients with diffuse proliferative lupus nephritis and compared the efficacy and the toxicity of a high-dose IV cyclophosphamide regimen (6 monthly pulses and two quarterly pulses) and a low-dose IV cyclophosphamide regimen (6 fortnightly pulses at a fixed dose of 500 mg) (172). In both arms, azathioprine was given as maintenance therapy for at least 30 months. The rate of renal remission was not statistically different in both groups but adverse side-effects were less frequent in the low-dose group. With a mean follow-up of 97 months, the cumulative probability of doubling serum creatinine or reaching end stage renal failure was not different in both groups (173). The best predictor of good long-term renal outcome was an initial favorable response (174).

In children with active lupus nephritis, the data on the efficacy of cyclophosphamide pulses remain scant (175). Lehman et al. treated 16 children with cyclophosphamide monthly for 6 months and then every 3 months (176). They reported significant improvement at 1 year in urine protein excretion, hemoglogin levels, C3 and C4 and creatinine clearance despite a significant reduction in prednisone dosage. Baqi et al. compared the results of two treatment modalities in children with class II and class IV lupus nephritis: high-dose pulse methylprednisolone for 10 days, followed by oral prednisone (20 patients) or IV cyclophosphamide given monthly for 6 months and every 3 months for a period of 3 years with oral prednisone (30 patients) (117). The authors did not find any difference in outcome between the two treatment modalities.

Azathioprine

The Dutch Working group on SLE compared IV cyclophosphamide monthly for 6 months and every 3 months for a total of 7 doses and azathioprine for 2 years with methylprednisolone pulses (three series of 3 pulses within 6 weeks). After 2 years, all patients received azathioprine with prednisone. Although there were no differences between the two groups in the rate of complete and partial

remissions during the first 2 years, a higher proportion of patients in the azathioprine group experienced relapses with a mean follow-up of 5.7 years (177).

Mycophenolate Mofetil

Following the observations of the improvement of nephritis in murine lupus with the use of mycophenolate mofetil (MMF) (178, 179), several uncontrolled studies in adults and in children confirmed the potential benefits of this drug in lupus nephritis (180–183). The first controlled trial involving 42 patients with class IV lupus nephritis compared prednisolone and MMF given for 12 months with prednisolone and oral cyclophosphamide given for 6 months, followed by prednisolone and azathioprine for 6 months. After 1 year, both groups showed similar rates of complete or partial remissions, relapses and treatment discontinuations (184). Adverse effects of treatment were more frequent in the group of patients receiving cyclophosphamide. In a trial involving 44 patients with class IV lupus nephritis, Ong et al. reported a remission rate of 26% in the MMF group compared to 12% in the IV cyclophosphamide group but the difference was not significant due to the small number of patients (185). Ginzler et al. compared MMF and IV cyclophosphamide given as induction therapy for 6 months in 140 patients (186). Complete remissions were significantly more frequent in the MMF group (23%) compared to the IV cyclophosphamide group (6%) ($p = 0.005$). Severe infections and death in two patients only occurred in the cyclophosphamide group. A metanalysis performed in 2007 evaluated the outcome of 268 patients and concluded that MMF compared with cyclophosphamide reduces the risk for failure to induce remission during induction therapy and may reduce the risk for death or end-stage renal disease (187). The authors conclude that MMF may be considered as a first-line induction therapy for the treatment of lupus nephritis in patients without severe renal dysfunction. It is not known whether MMF would be as efficient in patients with more severe renal dysfunction.

Plasma exchanges have been proposed with the aim of removing immune complexes that may be involved in the pathogenesis of lupus nephritis. A randomized controlled trial compared a standard therapy with prednisone and oral cyclophosphamide to the same treatment with plasma exchanges, three times a week for 4 weeks in 86 patients with severe lupus nephritis (188). The mean follow-up was 136 weeks. There were no differences between both groups in term of patient survival, renal survival, clinical activity of the disease and complications. However, it does not mean that plasma exchanges are not of help in individual patients (189, 190).

Maintenance Therapy

The maintenance therapy is aimed to maintain remission, prevent relapses and reduce the risk of progression to renal failure. The optimal drug and the optimal duration for maintenance therapy are still debated.

Contreras et al. performed a prospective controlled trial in 59 patients with class IV lupus nephritis comparing three maintenance regimen (oral MMF, oral azathioprine and quarterly pulse cyclophosphamide) following the same induction therapy in all patients (4–7 monthly cyclophosphamide pulses plus prednisone) (191, 192). With a mean follow-up of 30 months of maintenance therapy, there were five deaths (four in the IV cyclophosphamide group and one in the MMF group) and five patients progressed to end stage renal failure (three in the IV cyclophosphamide group and one in each of the two other groups). Event-free survival (based on a composite end point of death and renal failure) was therefore significantly better in the MMF and the azathioprine groups compared to the IV cyclophosphamide group. Moreover, the relapse rate was lower in the MMF group compared to the IV cyclophosphamide group.

Another study from Italy compared cyclosporine and azathioprine in 69 patients who had first received methylprednisolone pulses and oral prednisone plus oral cyclophosphamide for 3 months. At 4 year follow-up, there were no difference in the change in creatinine clearance, in the reduction in proteinuria or the occurrence of flares between the two groups.

The results of two ongoing controlled studies, Maintain and ALMS, should answer wether MMF is superior to azathioprine for maintenance therapy.

The optimal duration of maintenance therapy is not known and there is no study comparing different durations of treatment. Some authors maintain the treatment for 18–24 months while others propose a longer duration up to 5–8 years (177, 193).

Treatment of Relapses

Relapse of the disease may be defined by a worsening of renal symptoms once patients have achieved remission. Some patients may present with active urinary sediment often accompanied by increasing serum creatinine and proteinuria (194). The presence of red cell and/or white

cell casts is a strong predictor of a renal relapse. An elevation in the titer of anti-DNA antibodies and a decrease in the complement levels (C4 and C3) are often associated with renal "flare." These serological parameters should be checked regularly, as their changes precede clinical relapse (144, 195). These patients should be closely monitored but should not be treated solely for changes in serologic activity. In cases with worsening of renal symptoms, repeat renal biopsy may also be of help before deciding a change in the therapy. Patients who show active lesions on repeat renal biopsy may need aggressive treatment. Other patients with increased serum creatinine may have extensive tubulointerstitial lesions without active glomerular lesions, which represent a nonimmunologic progression of the renal disease and do not respond to therapy. Other patients treated initially for diffuse proliferative glomerulonephritis and who responded well to therapy may have an increased proteinuria several months later. A repeat renal biopsy is useful in this situation as it may show active lesions or pure membranous nephropathy which require different therapeutic decisions. Therefore, a repeat renal biopsy is often needed in patients with renal relapses in order to decide the best therapy and avoid unnecessary aggressive treatments. Some authors treat renal exacerbations with methylprednisolone pulses followed by oral prednisone with tapering doses (196).

Treatment of Patients with Membranous Nephropathy

Patients with pure membranous nephropathy, mild proteinuria and normal renal function have a good prognosis and there is general agreement that no immunosuppressive treatment is needed (136, 137). The treatment of patients with nephrotic syndrome is controversial. These patients are at risk of thrombotic complications. A NIH trial including 41 patients led to the conclusion that patients treated with either cyclophosphamide or cyclosporine had higher remission rates (46 vs. 13%) and lower rates of persistent nephrotic syndrome (19 vs. 60%) compared with patients treated with prednisone alone. There was a trend to more relapses with cyclosporine compared to cyclophosphamide (197).

Moroni et al. performed a trial where patients were randomly assigned to receive either a 6 month regimen consisting of corticosteroids (3 methylprednisolone pulses followed by oral prednisone 0.5 mg/kg/day during months 1, 3 and 5) and chlorambucil (0.2 mg/kg/day during months 2, 4 and 6) or a symptomatic treatment (198). The probability of surviving without developing renal failure at 10 years was 92% in the treated group vs. 60% in the control group. Complete or partial remissions were significantly more frequent in the treated group. Radhakrishnan et al. treated 10 nephrotic patients with cyclosporine for periods up to 43 months (199). Proteinuria decreased to less than 1 g/day in 6 patients and between 1 and 2 g/day in two. Repeat renal biopsies in five patients showed a decrease in the histological activity index but a rise in the chronicity index. Serum creatinine levels were not significantly increased at the end of the study. In addition to immunosuppressive treatment, aggressive renoprotective therapies are recommended with ACE inhibitors and/or angiotensin II receptor blockers which often are accompanied by a reduction in proteinuria (200).

Patients with a worsening of renal function should have a repeat renal biopsy, as membranous nephropathy may be associated with proliferative glomerulonephritis. In this setting, patients should be treated with the same regimen as that used for diffuse proliferative glomerulonephritis

Other Therapeutic Approaches

Rituximab

Rituximab, a chimeric mouse/human monoclonal antibody which recognizes B lymphocytes, is being increasingly used in autoimmune diseases. Rituximab induces a prolonged depletion of peripheral blood CD20 positive cells but has no effect on plasma cells. In SLE there are arguments for the role of B lymphocytes in the pathogenesis of the disease. Indeed, B lymphocytes not only produce autoantibodies but may also act as antigen-presenting cells and recruit clones of autoreactive T lymphocytes. Interestingly, Sfikakis et al. studied prospectively peripheral blood lymphocytes markers in ten patients with severe lupus nephritis who received rituximab. The expression of the costimulatory molecule CD-40 ligand on CD4+ T lymphocytes was significantly decreased as well as the expression of several markers of T lymphocyte activation (201). These changes were correlated with the clinical efficacy of rituximab. Vigna-Perez et al. found a significant increase of regulatory T cells (TREG) and an increase in the apoptotic T cells in patients with lupus nephritis following rituximab therapy. There are several reports suggesting that rituximab may have a beneficial role in patients with severe active lupus nephritis resistant to standard treatments. Overall, the rate of clinical remission is about 80% (202–207).

However, as most patients receive rituximab in combination with other immunosuppressants, including in several studies IV cyclophosphamide, it is difficult, in the absence of controlled studies to ascertain the benefit of rituximab. In a study of seven patients with proliferative lupus nephritis, Gunnarsson et al. observed an improvement of histopathologic class of nephritis on repeat renal biopsies in the majority of patients with a decrease in the activity index and a decrease in the number of T and B lymphoctes in the interstitium in 50% of the patients (208). Willems et al. reported a series of 11 children including eight with renal involvement (209). A complete renal remission was observed in two children, a partial remission in four while rituximab had no effect in two patients. Serious adverse events occurred in five of the 11 children including septicemia in two and severe hematologic toxicity in four. Thrombocytopenia and severe neutropenia occurred 1–3 days after rituximab infusion in two patients and resolved within 10 days.

Intravenous immunoglobulins have been reported to be effective in individual patients, including patients with proliferative lupus nephritis (210, 211). A recent meta-analysis found a response rate ranging from 33 to 100% and controlled trials are warranted (212).

Several biological agents have recently been studied in SLE. LJP-394, which consists of four-linked oligonucleotides, binds to anti-DNA producing B lymphocytes. Although LJP-394 has been shown to decrease anti-DNA titers (213), the clinical benefit has not been proven (214).

Anti-CD40 ligand monoclonal antibodies improve renal disease in murine lupus (215). However, studies in human were abandoned because of thrombotic complications (216).

CTLA4Ig, abatacept, is a fusion protein between CTLA4 (the human cytotoxic T-lymphocyte associated antigen) and the constant region of IgG1 heavy chain. CTLA4Ig binds to CD80 and CD86 on antigen-presenting cells, blocking the engagement of CD28 on T cells and preventing T-cell activation (217). CTLA4Ig treatment prevents onset of SLE in several murine models (218) and reduces anti-dsDNA antibodies, increases C3 concentrations, and decreases hematuria in patients with proliferative lupus nephritis (219).

Prognosis and Complications

Until 30 years ago, the prognosis of patients with lupus nephritis was very poor. Better management of lupus has improved the prognosis with a 10-year survival rate exceeding 80% (220). The disease is often marked by flares and periods of remission. The prognosis of SLE must also take into consideration iatrogenic complications since they are often linked with flares of the disease, particularly infections, vascular complications, osteonecrosis, or thrombosis.

Infectious complications are a significant cause of death during the course of SLE (220–222). These are favored by leukopenia and the reduction of the phagocytic functions, functional asplenia, hypocomplementemia, and, more important, by treatment with corticosteroids and immunosuppressive agents (223–225). Some authors have recommended systematic preventive antibiotherapy and antipneumococcal vaccination. Sepsis may be difficult to distinguish from a relapse of lupus. Herpes zoster is very common among the viral complications (8, 226). Early treatment with acyclovir avoids dissemination.

Accelerated atherosclerosis is now recognized as a frequent cause of morbidity and mortality in young adults with lupus and the process may start during childhood (226–231). Myocardial infarction has been reported in very young patients (232). The pathogenesis is probably multifactorial and includes inflammatory vascular disease, APL antibodies, hypertension, lipid abnormalities associated with the nephrotic syndrome or renal insufficiency, and corticosteroids (233–238). The prevalence of coronary-artery calcification is elevated and the age at onset is reduced compared to control subjects (239).

Thrombosis is another common complication of SLE (240). The risk factors include antiphospholipid antibodies, decreased levels of plasminogen activator, decreased levels of protein S and, in some patients, the additional risks related to the nephrotic syndrome (84, 241–246). Arterial thromboses are observed in lupus patients with APL antibodies and often involve cerebral arteries (247). Venous thrombosis are more common (248). Pulmonary embolism is frequent and may be complicated by pulmonary hypertension.

End stage renal failure occurs in 10–20% of patients with severe renal disease after a mean period of 5 years (7, 249, 250). While lupus nephritis progresses to renal insufficiency, the activity of SLE often diminishes in terms of other clinical manifestations and biological symptoms (251). Dialysis can be started and these patients do as well as nonlupus patients with end stage renal disease. Some patients, however, have a rapid course to renal failure, maintaining clinical and serological signs of activity. Therapeutic decisions are difficult as aggressive treatments may allow a recovery of renal function and discontinuation of dialysis. On the other hand, these patients are at higher risk of iatrogenic complications, particularly infection, which may be life-threatening (252, 253). Clinical and biological symptoms of the disease most often

improve in patients on chronic dialysis, thus allowing stopping of corticosteroids and immunosuppressive therapy. However, clinical manifestations can persist or even appear at this stage.

Renal transplantation is the treatment of choice for those who progress to renal failure. The outcome after renal transplantation in these patients is similar to that of patients with other diseases in adults (254–258) as well as in children (259). The risk for thrombotic complications is greater in patients with SLE, particularly those with APL-antibodies (260). However, it is advisable to wait before proposing transplantation until the clinical and serological activity of lupus has decreased. Moreover, a period without corticosteroids and immunosuppressive agents may be beneficial to the child. After renal transplantation, the activity of the disease declines and recurrence on the graft is unusual. The very low rate of recurrence of the disease after transplantation is a strong argument against a direct role of circulating immune complexes in the pathogenesis of the nephropathy. An analysis of patient and graft outcomes in patients with lupus nephritis was conducted by UNOS. The study concludes that patients with lupus have outcomes comparable to nonlupus transplant recipients (258).

Ischemic thrombosis of the bone is a rare complication of lupus in children. Several factors may be involved including the presence of APL antibodies and corticosteroid therapy. It may affect any bone but the femoral head and the femoral condyle are more commonly involved (261). Bone mineral density measurements may show osteoporosis and a study found an inverse correlation between bone mineral density and the cumulative dosage of corticosteroid but no significant correlation between bone mineral density and duration or activity of the disease (262).

Growth retardation is a major concern in children treated for several years with corticosteroids, particularly those who need to be maintain on daily steroid therapy. Other complications of corticosteroids include cataracts, gastrointestinal hemorrhage, and diabetes mellitus. A cushingoid aspect is frequent and often badly tolerated by adolescent girls. It may explain poor compliance leading to severe relapses.

Side effects of cytotoxic drugs are common. The incidence of hemorrhagic cystitis following IV cyclophosphamide is very low provided adequate hydration using the IV route is given (263). To minimize the risk of hemorrhagic cystitis, mesna, which binds to cyclophosphamide metabolites in the urine, may be helpful. Nausea and vomiting may be in part prevented by the concomitant use of antiemetic agents such as ondansetron (Zofren). Cyclophosphamide pulses often results in neutropenia with a serious risk of infection, which may be life-threatening (225). Children and their families should be aware of the risk of transient alopecia. Ovarian toxicity is another serious complication (264, 265). The risk of amenorrhea depends on the age of the patient at start of the treatment and the total number of pulses (266–268). When treatment is given for 6 months, the risk of amenorrhea is very low if the patient is less than 25 years of age, whereas 25% of patients older than 30 years of age will develop this complication. When the total number of pulses exceed 15, the probability to develop amenorrhea is 17% for patients less than 25 years and nearly 100% for those older than 30 years. Gonadotrophin-releasing hormone antagonist may preserve ovarian function (269–272). There are no published data on long term gonadal toxicity of cyclophosphamide pulses given to prepubertal girls. The gonadal toxicity of pulse cyclophosphamide in men has not been studied. However, studies in children with idiopathic nephrotic syndrome suggest that this toxicity may occur if the cumulative dosage is higher than 200 mg/kg. Severe infections have been reported in children with lupus nephritis after IV cyclophosphamide (273). It should be noted that no disseminated malignancies have been reported in patients treated with cyclophosphamide pulses although longer follow-up periods are needed before any definite conclusions may be drawn.

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48 Hemolytic Uremic Syndrome

S. Johnson · C. Mark Taylor

Introduction

The origin of the diagnostic term Hemolytic Uremic Syndrome (HUS) is found in the seminal report of five children by Gasser et al. (1). The children all had microangiopathic hemolytic anemia died of renal failure and were discovered to have acute cortical necrosis at post-mortem. From this emerged the triad of clinical features, *microangiopathic hemolytic anemia*, *thrombocytopenia*, and *renal impairment* that are used to diagnose the syndrome.

These clinical features are not exclusive to HUS and further conventions have evolved to focus more clearly on the syndrome. For example, patients with pre-existing sepsis and multi-organ failure who develop these clinical features are usually excluded from a diagnosis of HUS. Typically in these cases there is disseminated intravascular coagulation with consumption of circulating coagulation factors. By comparison, patients with HUS have activated coagulation and preservation of circulating coagulation factors even though platelets are consumed. The assumption is that in HUS, intravascular thrombosis is focal and not generalized. There are two exceptions to this convention. Septicemia with *Streptococcus pneumoniae* or *Shigella dysentery type 1* can lead to HUS in which there is accelerated coagulation, at least initially (discussed below). Patients with malignant hypertension can also develop the same triad of clinical features but would not be described as HUS. Given that some forms of HUS are associated with severe hypertension it is sometimes difficult, at least initially, to make the diagnostic distinction.

A generation before Gasser, Moscovitz described a teenage girl with fluctuating neurological disease, fever, mild renal impairment and microangiopathic hemolytic anemia, the condition now known as thrombotic thrombocytopenic purpura (TTP) (2). TTP overlaps HUS if the diagnostic criteria are restricted to the *clinical presentation alone*. Rarely, patients with TTP may have prominent renal impairment and therefore resemble HUS. More often, patients with HUS have neurological involvement and risk being inappropriately described as TTP.

Diagnostic precision can be improved by moving towards an etiological based classification. One such attempt by the European Paediatric Research Group for HUS is used in this chapter (3). Following a contemporary review of the literature subgroups of HUS and TTP were assigned to two diagnostic levels. In the first there was reasonable evidence of causation, and in the second there were clinical associations but evidence of causation was weak or absent. It seems increasingly likely that TTP will become clearly separated from HUS on the grounds that the etiology and pathogenesis are specifically related to deficiency or inactivation of the protease that cleaves von Willebrand factor. Because of this assumption only a brief review of TTP is included in this chapter.

For historical reasons some of the terminology surrounding HUS has become confusing. *Table 48-1* addresses the terminology.

Making the Diagnosis of HUS

Microangiopathic hemolytic anemia (MAHA) is a Coombs test negative hemolytic anemia in which fragmented red cells (schistocytes) appear in the peripheral blood, usually accompanied by thrombocytopenia (*Fig. 48-1*). The plasma concentration of lactate dehydrogenase is elevated and haptoglobin is reduced. In clinical practice the latter is a more sensitive test of intravascular hemolysis and is often the last parameter to normalize when MAHA resolves. Although a consistent feature of HUS, the severity of MAHA does not correlate with clinical outcome. Severe oligoanuria and acute cortical necrosis may occur with relatively mild anemia. It seems likely that MAHA is partly the result of mechanical damage to red cells as they pass through the renal microcirculation in which there is thrombotic microangiopathy. Red cell production is increased and reticulocytosis, macrocytosis and polychromasia are seen.

Thrombocytopenia is common to all types of HUS but is variable, transient, and can therefore be missed. Thrombocytopenia is due to platelet consumption. While some of this consumption occurs in the thrombotic microangiopathic lesion itself, labeling studies have shown that the majority of platelets are removed in the reticuloendothelial system. Despite thrombocytopenia, clinically significant hemorrhage is uncommon and this may be due to

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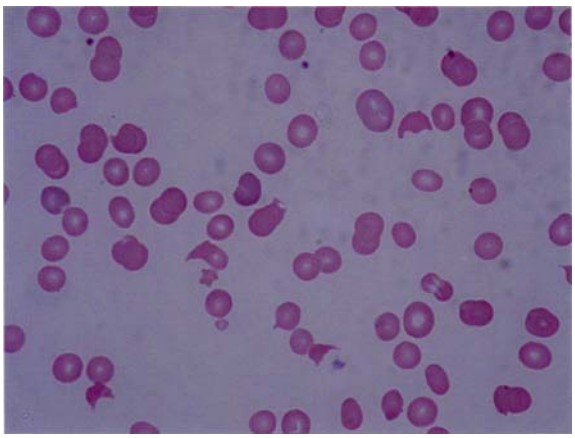
Table 48-1

Terminology used to describe HUS

Term	Comment
D + HUS	Refers to patients presenting with a prodrome of diarrhea, usually less than 2 weeks before disclosing HUS
Typical HUS	Refers to children with D + HUS. Used retrospectively it implies a single, self limited clinical event
Atypical HUS	Literally applies to patients with any pattern of clinical presentation other than D + HUS. There is drift of usage to imply those forms of HUS associated with complement dysregulation
Recurrent or relapsing HUS	Further episode of thrombocytopenia and/or MAHA after normalization of hematological parameters in a former episode. Implies that further renal injury is occurring or imminent. Also strongly suggests that the patient has an ongoing risk factor e.g., genetic, autoimmune
Thrombotic microangiopathy, glomerular thrombotic microangiopathy	These are pathological terms describing the characteristic findings in HUS, and should not be used to describe a clinical condition e.g., microangiopathic hemolytic anemia
Familial HUS	Needs to distinguish between *synchronous* HUS in family members, for example in an outbreak of enterohemorrhagic E. coli infection, and *asynchronous* disease that implies an inherited risk factor. Without this distinction the term lacks meaning

Figure 48-1

Photograph of a blood film characteristic of Microangiophathic hemolytic anemia. Fragmented erythrocytes (schistocytes) are seen.



activation of the coagulation system (4, 5). Typically, plasma concentrations of fibrinogen and factor V are normal and prothrombin and partial thromboplastin times are normal or short. Thrombin-antithrombin III complexes, prothrombin fragments, tissue plasminogen activator, plasminogen activator inhibitor-1 and D-dimers are increased reflecting increases in both thrombus formation and lysis. Overall the fibrinolytic activity of plasma is impaired.

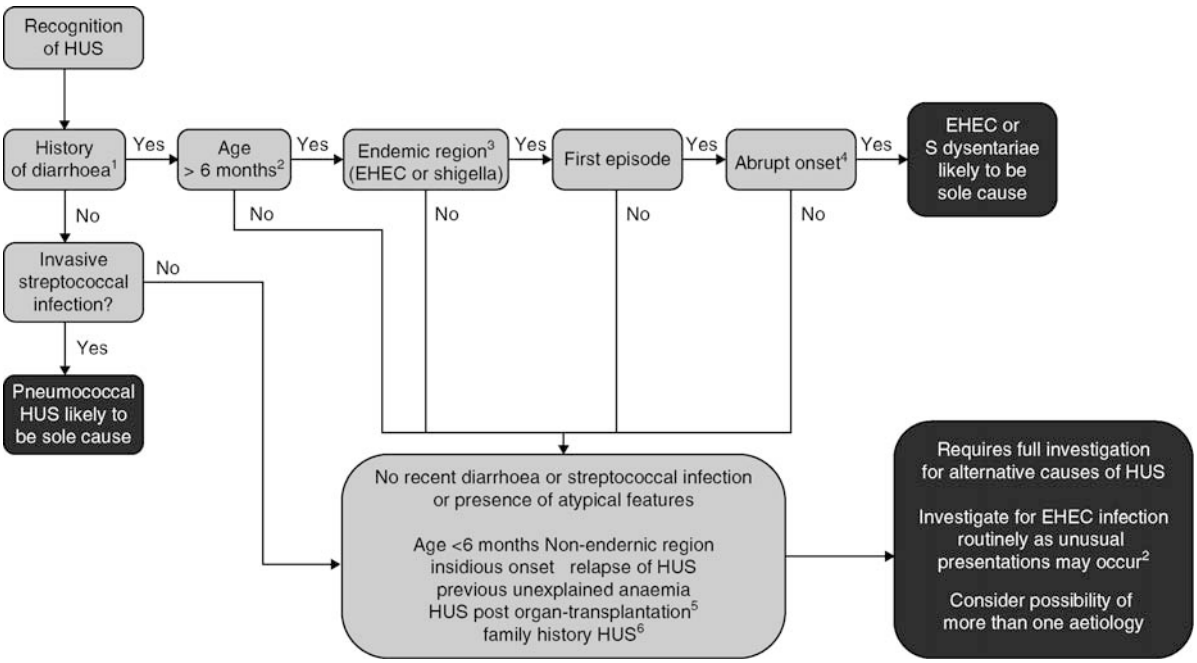
Renal failure occurs abruptly in post-infectious forms of HUS and is typically oligoanuric. Renal failure with high urine output is recognized but rare. In other forms of the syndrome that do not follow acute infections, the onset of renal impairment can be either abrupt or insidious. Proteinuria is a consistent finding in all types of HUS. Hypertension at onset is usually severe and difficult to control in patients with HUS associated with complement dysregulation. By contrast those with infection-induced HUS usually have normal blood pressure or only mild hypertension at onset, but commonly develop transient hypertension during the recovery of oliguric renal failure.

Classification of HUS

The clinical presentation provides a useful indication of the likely sub-group diagnosis of HUS. A diagnostic flow diagram for this is shown in *Fig. 48-2*, and a summary of sub-groups appears in *Table 48-2*. By far the commonest subgroup (>90% of childhood HUS) is induced by shiga toxin producing bacteria, usually enterohemorrhagic

Figure 48-2

Diagnostic flow diagram for HUS based upon clinical presentation. (1) Bloody or non-bloody diarrhea within 2 weeks prior to onset. (2) Infants less than 6 months of age are less likely to have EHEC infection, although this varies according to the local epidemiology of EHEC infection. (3) Refer to up to date reference on prevalence. (4) Insidious onset with fluctuating clinical signs and laboratory parameters increase the likelihood of a non-infective cause. (5) HUS can follow transplantation of several organs including kidney. The role of drugs, especially calcineurin inhibitors, has been suspected but not proven. (6) In an outbreak of EHEC infection several family members may develop HUS and do not require investigation beyond confirmation of the EHEC infection. Families with asynchronous HUS are likely to have inherited risk factors and require full investigation. (7) EHEC infection can cause HUS without diarrhea. Use locally developed public health/microbiological services to identify EHEC.



Escherichia coli (EHEC). *Shigella dysenteriae type 1* can induce the syndrome in endemic regions, and rarely other organisms such as *Citrobacter freundii* are responsible (6). These patients typically have a prodrome of diarrhea, often bloody diarrhea, a few days before the recognition of HUS (D + HUS) and are usually over 6 months of age.

Another important subgroup that is readily identified on clinical grounds follows invasive *Streptococcus pneumoniae* infection (7, 8). These infants tend to be younger than those with D + HUS, and the syndrome is very rare after the age of 4 years. They present with pneumonia, empyema, meningitis and less often, isolated septicemia.

Beyond the infection-induced forms, all other etiologies are rare and require specialist laboratory support to reach a diagnosis. An inherited predisposition to HUS is suggested by very early onset (< 6 months of age), lack of a diarrheal prodrome, insidious onset with lethargy, pallor, feeding difficulties, severe hypertension or a family history of HUS. Within this group patients may have mutations in complement regulators, and less often inherited deficiency of von Willebrand protease or an inborn error of cobalamin metabolism. There remains a small group of patients in whom the cause of HUS is not known in spite of extensive investigation, and no clinical association such as a suspected drug effect, cancer or bone marrow transplant that might hint at a causative process. In practice this is less than 5% of all childhood HUS. The majority of these have a single episode, and as long they make a good recovery of renal function, the prognosis appears favorable. However, amongst undiagnosed patients is a small group with relapsing or familial disease in whom novel risk factors await discovery.

Table 48-2

Subgroups of HUS based upon etiology

Diagnostic subgroup	Comment
HUS associated with shiga toxin (stx)-producing infections; enterohemorrhagic	D + HUS closely maps to this group where entero-hemorrhagic Escherichia coli infection is the source of toxin. Shigella dysenteriae type 1 is a well recognized and broadly similar form in parts of sub-Saharan Africa and Indian subcontinent. Occasionally other bacterial species such as *Citrobacter* produce stx and induce HUS. Stx is implicated in pathogenesis. This subgroup covers > 90% of HUS in children
Escherichia coli	
Shigella dysenteriae type 1	
Other stx producing organisms	
HUS associated with invasive *Streptococcus pneumoniae* infection	Rare but distinctive form of HUS usually with evidence of desialyation of host cell membranes and T-activation of erythrocytes induced by neuraminidase, a product of *pneumococcus*
HUS associated with complement dysregulation	May be genetic, sometimes familial, with mutations affecting the alternative pathway of complement (Factor H, Factor I, Membrane co-factor protein CD46, Factor B, C3). May also be acquired by autoantibody to Factor H
Inherited or acquired severe deficiency of von Willebrand cleaving protease	Inherited or acquired severe deficiency of vonWillebrand cleaving protease, ADAMTS13. Prominent early renal impairment is rare. These patients are better described as TTP rather than HUS
HUS associated with cobalamin deficiency	Usually due to homozygous mutation in methylenetetrahydrofolate reductase gene; cobalamin C deficiency
HUS associated with quinine	Adult patients only described so far, children rarely exposed to quinine

Pathological Findings in HUS

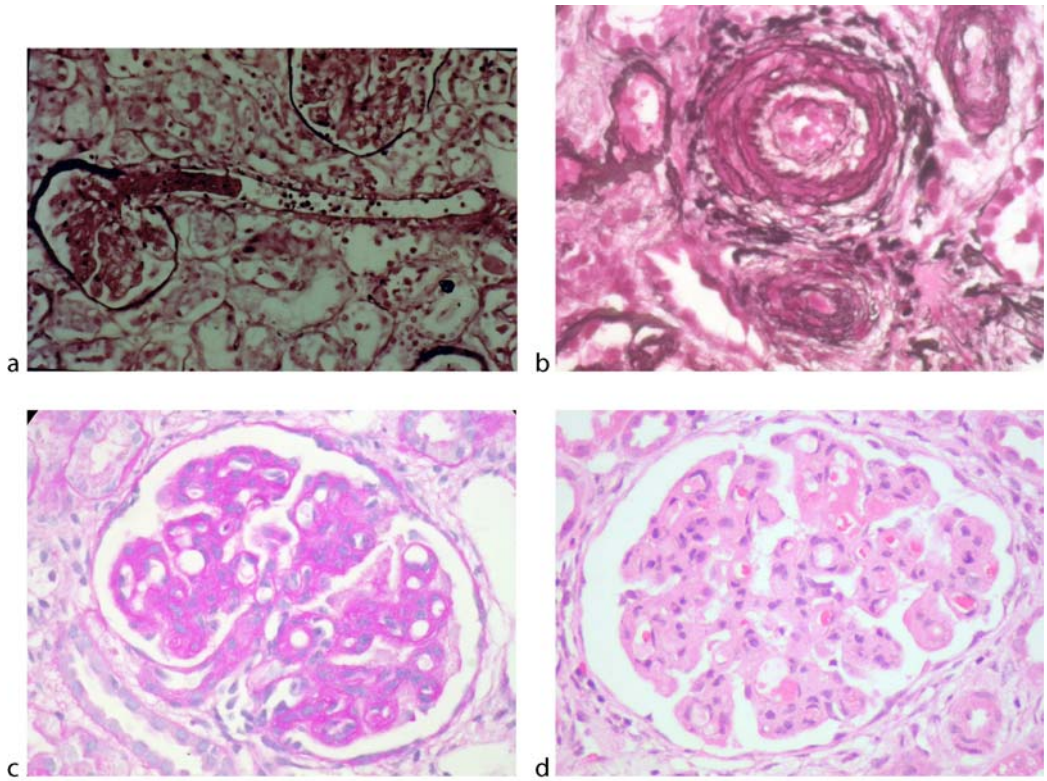
The term *thrombotic microangiopathy* (TMA) was coined by Symmers to describe hyaline thrombi that occurred in a distinctive arteriolo-capillary distribution in the absence of primary vascular inflammation (9). He found it in many organs of patients that would fit the current clinical description of TTP. Subsequently, Habib identified microvascular thrombi in the glomeruli of children with HUS, and with permission from Symmers proposed the term glomerular TMA (10). Broadly three types of TMA are seen. In the most common *glomerular type*, glomerular capillaries are distended with platelet-fibrin thrombi. Endothelial cells are swollen and may be detached from the underlying basement membrane, and the capillary wall appears thickened (*Fig. 48-3a*). *Acute cortical necrosis* may occur, in which case it is claimed that a careful search will reveal vascular thrombotic lesions. *Arterial TMA* is a form in which the afferent arteriole or the interlobular arteries are involved with endothelial swelling and replication, narrowing of the lumen, and varying amounts of intraluminal thrombus in differing stages of organization (*Fig. 48-3b*). Downstream, the glomeruli are shrunken and ischemic with wrinkled basement membranes and loss of resident cells.

The three histological groups offer some prognostic value (10). Cortical necrosis is irreversible and, if extensive, correlates with a failure to recover renal function. Pure glomerular TMA is characteristic of HUS induced by EHEC infection, and is to some extent reversible. Arterial forms are more often seen in adults, in relapsing disease, and associated with both severe hypertension and poor prognosis.

While there are good data to link EHEC-induced HUS to glomerular TMA, much of it obtained at post mortem, the correlation between other etiological subgroups of HUS and pathological findings is far less clear. Biopsies are not undertaken in HUS during the period of thrombocytopenia because of the risk of hemorrhage, and so they usually represent a later stage in the process. In patients with HUS secondary to complement dysregulation, the light microscopy findings include lobulated glomeruli and thickened capillary walls (*Fig. 48-3c*). In these biopsies, thrombus may be scanty or absent. In some this resembles mesangiocapillary glomerulonephritis. The assumption is that more thrombosis would have been seen if tissue had been obtained during the period of microangiopathic hemolytic anemia and thrombocytopenia (*Fig. 48-3d*). Complement C3 can be identified in the capillary walls in some cases but it is usually scanty. Overlap between complement dysregulation associated HUS and primary glomerulonephritis with isolated C3 deposition is recognized (11).

Figure 48-3

(a) Glomerular thrombotic microangiopathy: post mortem specimen from child with EHEC-induced HUS. (b) Arteriole showing endothelial swelling and replication in a patient with HUS secondary to a complement factor H mutation. (c) Lobulated glomerulus with thickened capillary walls. (d) Active thrombotic microangiopathy in a patient with factor H mutation showing capillary thrombi. (See color plate 31)



Other pathological changes such as mesangiolysis occur, but the cause is unclear and it remains unknown if this feature associates with specific subgroups of HUS. It is rare in early EHEC infection, perhaps because mesangial cells in culture are less sensitive to shiga toxin than other resident glomerular cells. Mesangiolysis is reported in the forms of glomerular TMA that complicate treatment with vascular endothelial growth factor, radiation and chemotherapy (10).

For most patients with HUS, TMA is an acute event, although in some it can be recurrent or chronic. Unless there is a rapid resolution of glomerular thrombosis, the lesion progresses to global glomerular sclerosis, downstream ischemia, interstitial fibrosis and tubular atrophy. Following an episode of TMA with moderate or severe nephron loss, surviving glomeruli and nephrons undergo compensatory hypertrophy, as with any other nephron destructive process. Later, hypertrophied glomeruli may show signs of focal segmental sclerosis and hyalinosis as a secondary process, typically associated with albuminuria (12).

TMA outside the renal microcirculation is recognized. In EHEC-induced HUS microthrombi at post mortem have been seen in gut, brain, heart and pancreas (13). In TTP platelet rich microthrombi can be widespread. By contrast extra-renal TMA is infrequently reported in HUS associated with complement disorders, although cerebral and retinal vascular damage has been reported (14–16).

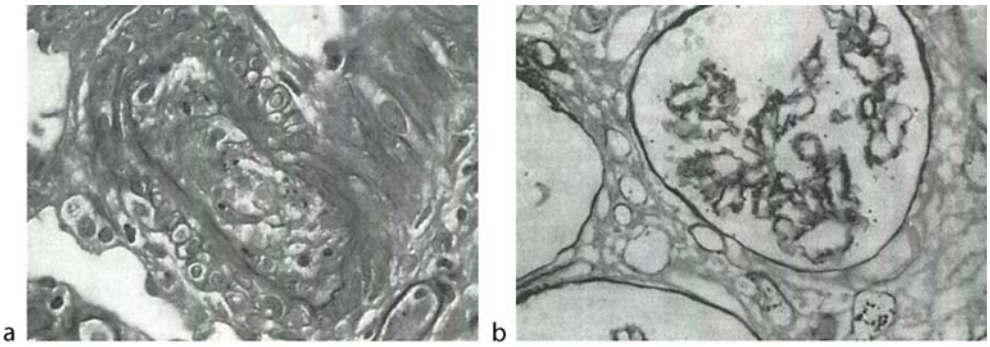
HUS Caused by Enterohemorrhagic *Escherichia coli*

Clinical Features

Prior to the identification of EHEC as a human pathogen, it was recognized that children who had diarrhea shortly before the diagnosis of HUS had a better outcome than

Figure 48-4

Extensive cerebral necrosis demonstrated by magnetic resonance imaging. Reproduced courtesy of Chantal Loirat.



those without diarrhea (17). The term D + HUS was subsequently adopted to describe the former group. Following the seminal paper by Karmali et al. (18) it was quickly established that D + HUS was attributable to infection with EHEC (19–24). EHEC infection is not always confirmed in patients with D + HUS, since its excretion can be transient. However, the clinical features are similar whether or not the organism is identified. This suggests that in practice nearly all D + HUS in temperate regions is attributable to these pathogens. In Europe, the Americas and Australia more than 90% of all childhood HUS is D + HUS (25, 26).

The peak age of presentation is between 1 and 5 years of age, with a small number of older children and adolescents affected. It is rare in adults and in the first 6 months of life. Diarrhea and abdominal cramps are abrupt in onset, and fresh blood is visible in the stools in about two thirds of cases. Fever is not a prominent feature. The infective colitis causes hemorrhage and infarction through all layers of the intestine. Toxic dilatation of the colon, perforation and massive hemorrhage are surgical emergencies. Delay in dealing with this risks cardiovascular collapse that is difficult to reverse. In the child who presents in a collapsed state these complications should be carefully considered.

Pallor and oliguria herald the onset of HUS and usually appear on about the fourth day of diarrhea with a range of 1–10 days. Purpura and bruising are usually mild or absent. Mild jaundice occurs in about a third of cases and there is often mild and transient elevation of liver enzymes, and less often hepatomegaly. Some patients are dehydrated and hypovolemic at the time of presentation, while others who have maintained fluid intake in the presence of oligoanuria may have hypervolemia. Hypertension at onset is rather unusual, but often appears transiently later in the course of the acute renal failure.

Extra-renal manifestations are an important cause of added morbidity and are the main cause of death (20). Central nervous system (CNS) disturbances are common and usually present early in the course of the illness. They include irritability, confusion, hallucination and seizures. Diffuse massive cerebral edema can induce hind brain compression leading to a decerebrate state and brainstem death. Less common is focal brain infarction leading to stroke (*Fig. 48-4*). A rare but well recognized complication is pancreatic involvement that can lead to overt diabetes mellitus (27). It usually appears a week or more after the onset of HUS. Cardiomyopathy and myocarditis are described and may occur several weeks after onset and may not be secondary to hypertension or vascular volume overload and may be secondary (28).

Microbiology of EHEC

Escherichia coli that are capable of inducing bloody diarrhea in humans, with or without HUS, are referred to as EHEC. These organisms have various virulence factors but the principal one is the ability to excrete a species of shiga toxin (stx) to which humans have receptors. The terms stx and verocytotoxin are equivalent, although the former is preferred as it indicates the relationship with the family of toxins that resemble the exotoxin of *Shigella dysentery* type 1. The latter is a historic term referring to the extreme sensitivity of vero cells to stx in culture.

Shiga toxins are species restricted. Not all stx are toxic to humans, thus not all shiga toxin-producing *E. coli* (STEC) are necessarily EHEC, but all EHEC are STEC (29). EHEC have not acquired enteroinvasive properties and patients almost never develop septicemia. However, HUS has been reported secondary to urinary infection with EHEC. These cases can occur without typical

gastrointestinal symptoms and septicemia in this circumstance is described (30). It is important to culture the urine from patients with HUS, and if *E. coli* are found, to investigate for toxigenic properties.

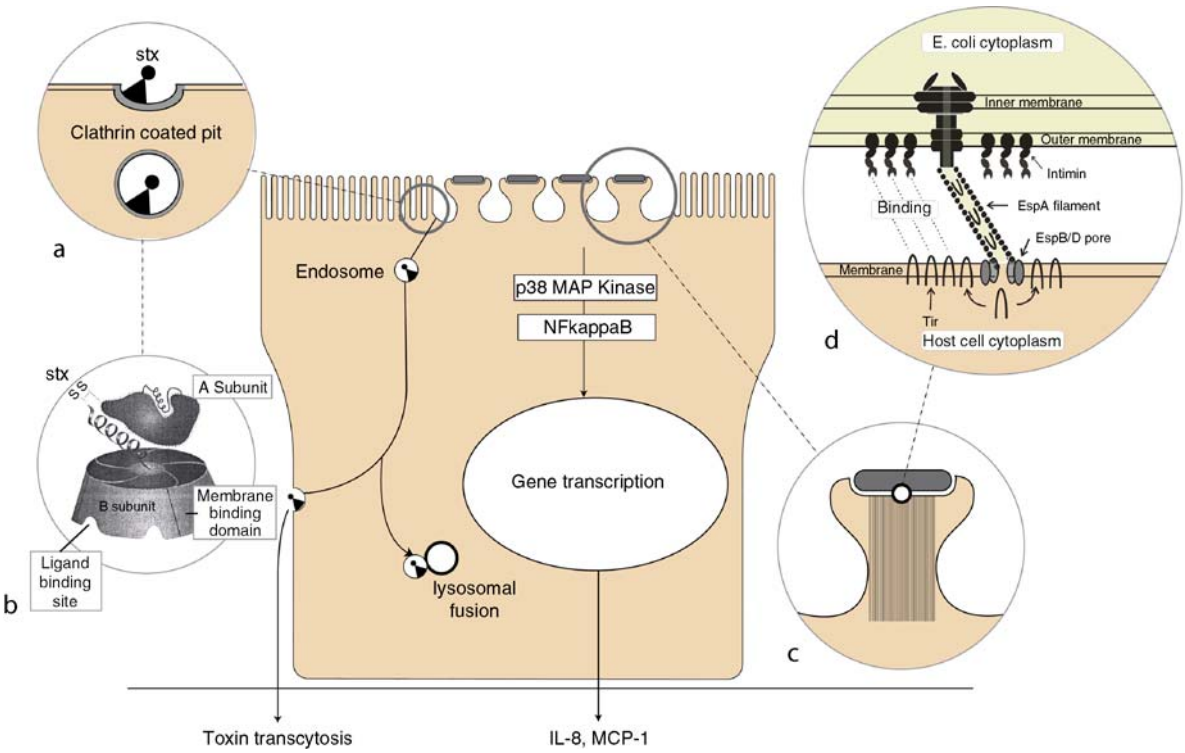
EHEC usually possess additional virulence factors such as the ability to attach to the luminal surface of host enterocytes and to cause effacement of the microvilli (Fig. 48-5) (31). This property is characteristic of enteropathogenic *E. coli* (EPEC) and explains their ability to cause watery diarrhea through loss of absorptive surface. The attaching and effacing lesion is a two-step process. During the first step the *E. coli* expresses the adhesin intimin on its surface. In the second step E. coli injects the intimin receptor into the host cell through a microtubular structure known as a type 3 secretion system. The intimin receptor in the host becomes oriented in the host cell membrane permitting the *E. coli* to adhere

Many of the bacterial proteins essential to type 3 secretion are known, and the genes that encode them occur in the *locus for enterocyte effacement* (LEE) (31). These include *eaeA* the gene for intimin and *tir* the gene for the translocated intimin receptor. EHEC seem to preferentially locate to the epithelium associated with Payer's patches, the clinical significance of which is unknown (31). Attachment induces various signaling events in the enterocyte, resulting in cytoskeletal rearrangements whereby the microvilli are lost and replaced by a pedestal on which the *E. coli* become firmly bound. It seems likely that "attachment and effacement" is an added virulence factor for EHEC and allows delivery of stx in immediate proximity to host enterocytes. Transcytosis of toxin across the intestinal epithelium has been demonstrated in vitro (32). However, attachment is not an absolute requirement for EHEC, and organisms that lack this ability occasionally cause HUS.

Many EHEC also secrete calcium-dependent alpha-hemolysin or possess the gene encoding it, *ehxA* (33, 34). Alpha-hemolysin is a pore-forming toxin that induces

Figure 48-5

Effect of enterohemorrhagic *Escherichia coli* on intestinal epithelial cells. (a) Shiga toxin released on the luminal side of the cell is taken up into an endosome. From here it is either transported and secreted from the basal surface, or degraded by a lysosome. (b) Cartoon of the structure of shiga toxin (from Donnelly JJ, Rappuoli R. *Nat Med* 200;6:257–258 with permission). (c) Attaching and effacing lesion with rearrangement of actin cytoskeleton. (d) Type 3 secretion system structure and protein components (courtesy of Dr S Knutton).



lysis of non-human red cells and is toxic to human brain microvascular cells in vitro (35). The association suggests that this is a virulence factor in human infection but it is not essential and its exact pathological role in hemorrhagic colitis and HUS is not known.

The *E. coli* serotypes that are associated with HUS vary in different parts of the world. In North America and North West Europe the dominant serotype is O157:H7, but other serotypes occur either sporadically or as causes of outbreaks of enterocolitis and HUS (19, 24, 36–38). In Southern Europe a high proportion of HUS is associated with O26 (24). In Australia, human infection with O157 is rare even though Australian cattle are known to harbor it, and O111 is the dominant causative strain (26). Several O-serotypes of EHEC are also well known as the same O-serotypes of EPEC, for example O26, O55, O111, further illustrating the importance of the combined virulence factor of attachment and effacement. Novel serotypes are continuously reported and sometimes clearly traced back to animal or environmental sources (39).

Toxicology

Shiga toxins (stx) have a common structure of a single A-subunit linked to five B-subunits (40). Whereas the gene for Shiga toxin itself is encoded in the chromosome of *Shigella dysenteriae-type 1*, genes for stx-1 and stx-2 are encoded in STEC by temperate bacteriophages which can integrate with the host genome. Exposure of STEC to certain antibiotics appears to increase toxin release in vitro (41). There has been concern that antibiotic use in the diarrheal phase of the illness might promote HUS by amplifying toxin release, but a meta-analysis, admittedly under-powered, did not confirm this (42). Stx bacteriophages, released during lysis, are available for uptake by other bacterial species including commensal organisms, not always *E. coli.* This may confer new pathogenetic properties and is thought to be the mechanism by which organisms not normally associated with toxigenic properties have caused HUS (e.g., *Citrobacter freudii* (6)). STEC may have more than one bacteriophage and therefore produce more than one toxin, e.g., stx-1 and stx-2.

Stx-1 is identical to Shiga toxin, the product of *Shigella dysenteriae* type 1. Stx-2 is approximately 60% homologous to stx-1 with different subtypes denoted by a suffix.

Within an EHEC serotype, e.g., O157, different stx phage types have geographical and temporal associations. EHEC responsible for HUS express stx-2 more often than stx-1 (4, 18, 19, 23, 43). Stx-2c is positively associated with HUS disease but Stx-2d, Stx-2e and Stx-2f are not. Recently a variant of Stx-2d, Stx-$2d_{(activatable)}$ has been associated with HUS (44). This toxin is modified by enzymes in intestinal mucus to increase its virulence. Moreover EHEC producing Stx-$2d_{(activatable)}$ do not seem to require the added virulence properties of attachment and effacement. Stx-2e is responsible for "edema disease" of pigs and recognizes Gb4 expressed in that species. Stx-2f has been associated with avians.

It seems likely that *E. coli* can lose stx bacteriophages as well as acquire them since O157 strains that do not possess stx genes are found in patients with D + HUS (45).

STEC Zoonosis

STEC that are clearly pathogenic in man may colonize animals particularly ruminants, without causing disease. In cows, the carriage of *E. coli* O157 varies according to age and feeding practices. The organisms locate to mucosal lymphoid tissue in the rectum (46). While contamination of meat and milk products has been a major concern for food hygiene, driven by well-publicized outbreaks involving undercooked ground beef, this is probably not the most common route for human exposure. STEC shed on to pastureland survive over a wide range of temperature and pH, and resist composting. Because they persist in soil they can readily contaminate surface water, well water, and water for bathing as well as fruits, salads and vegetables. However, in small children an important risk appears to be direct contact with animals for example from visits to farms (47). In industrialized countries, infection is more prevalent in summer than winter reflecting the seasonal exposure to countryside, animals and fresh uncooked foods.

Humans acquire antibodies to stx-2 during childhood and teenage years so that about half of adults have antibodies detectable by western blotting. Stx antibodies wane in old age. Family studies have found antibodies in close contacts of children with hemorrhagic colitis and HUS. Some of these will have had minor gastrointestinal symptoms and others may be asymptomatic which suggests that sub-clinical infection is common. A high proportion of abattoir workers have been shown to excrete STEC, although not necessarily EHEC (48). This raises the possibility that contact with STEC that lack the necessary additional virulence factors needed for a human pathogen might be immunogenic. Experimentally, anti-stx antibodies are protective in several models of EHEC infection and it is a reasonable hypothesis that adults are to some extent protected by acquiring anti-stx2, and that the high

incidence of HUS in pre-school children reflects immunological naivety.

Epidemiology

The greatest incidence of EHEC-induced HUS in Europe and North America is in children aged 1–5 years, whereas in Argentina the age of onset is lower, between 6 months and 4 years. The onset of HUS before 6 months of age raises concern that EHEC is unlikely to be the only cause. The incidence also differs between regions, generally being higher in cooler, temperate regions. For example the incidence in Scotland (3.4 per 10^5 children < 5 years of age) is greater than in England (1.54 per 10^5 children < 5 years (25). The incidence in England and France is similar, and greater than Italy (24). While the diagnosis of EHEC infection has increased over time, perhaps reflecting better laboratory techniques, the number of cases of children presenting with HUS has remained stable over the last 20 years in economically developed states, and the mortality has reduced (49). However, fluctuations in incidence may occur with local epidemics sometimes linked to a common source of infection. In such outbreaks it has been estimated that one in ten exposed to the infection develop symptoms of colicky abdominal pain and diarrhea, and 15% of children with diarrhea or bloody diarrhea will get HUS. Outbreaks may be biphasic, a second wave occurring 2 weeks later from person to person transmission. The time from exposure to onset of diarrhea is usually less than a week, mostly 3–4 days, and the mean interval between onset of diarrhea and disclosure of HUS is 4 days, range 1–10 days.

Laboratory Investigation

In regions where O157 is the predominant EHEC, it has been common practice to culture stools on sorbitol MacConkey agar enriched with tellurite to promote the growth of O157. Because this serotype is usually unable to ferment sorbitol, colonies can be inspected, picked and then tested specifically for O157 with agglutination or enzyme linked immunoassay. This approach will miss other serotypes of EHEC and any O157 that is capable of fermenting sorbitol. It will also identify stx-negative strains of O157.

Given that stx production is an essential feature of EHEC and central to the epidemiology, it is logical to investigate directly for this property rather than rely on identifying O-serotypes that may or may not be toxin producers (50). Historically, toxin identification was laborious and expensive. Stools were filtered to obtain free toxin that was tested on cell cultures, confirmation being sought by neutralizing the toxin with specific antibodies. Sensitive immunoassays to detect toxin in bacterial cultures or even in stool samples are becoming available (51). These can give quick results and are likely to become first line investigations. Polymerase chain reaction and DNA hybridization can also be used to amplify and detect stx genes. Neither of these methods identifies the organism, but colonies with these properties can be further identified and serotyped.

Genetic profiling of EHEC is valuable in research and in identifying emerging pathogenic strains, particularly in outbreaks. Lytic phage typing can also be useful to identify different strains of O157.

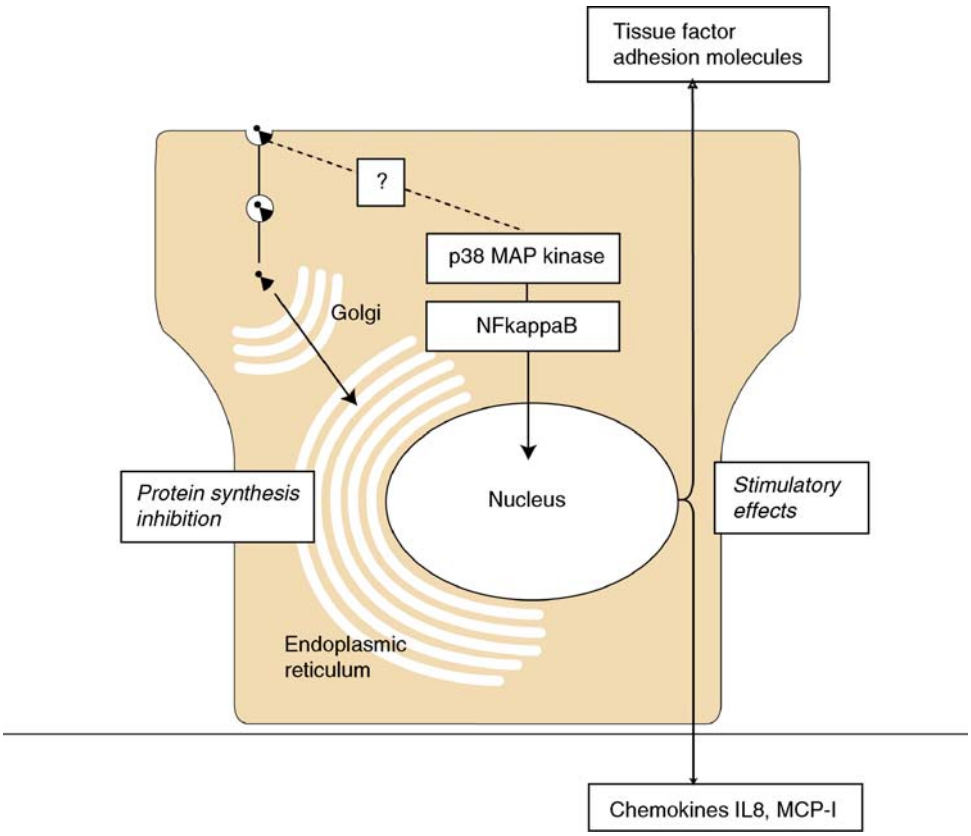
Pathogenesis

There is good evidence that stx can be transcytosed across the intestinal epithelium to reach the submucosa and the microvascular thrombosis and hemorrhage of the intestinal lesion is almost certainly a direct result of the toxin (*Fig. 48-5*). It is less clear how the glomerular and other distant vascular sites are affected. Toxin is very difficult to identify in blood, probably because the amount required to induce disease is small, and because the affinity with cellular glycolipid receptors would result in rapid clearance. A suggestion that stx was transported on neutrophils has been rescinded (52).

At target sites, the B subunit recognizes and binds to a eukaryotic cell glycolipid that is expressed differently in different species. In humans this is globotriaosylceremide, Gb3, and it is expressed on renal tubular cells and vascular cells in kidney, brain and intestine, in Paneth cells in the intestine but not on intestinal epithelium (53). Human glomerular endothelium has a high expression of Gb3 that is further upregulated by the inflammatory cytokine TNF-alpha (54, 55). Toxicity is dependent on recognition, binding and internalization of the toxin followed by cleavage and cytoplasmic release of the A-subunit (*Fig. 48-6*). The A-subunit is an N-glycosidase that cleaves ribosomal RNA, effectively blocking transcription. Stx cytotoxicity in vitro differs between cells types (56) and between stages in the cell cycle. It may result in cell death, but sub-lethal effects are also likely to be relevant to the pathogenesis. Experimentally, human vascular endothelium exposed to sub-lethal concentrations of stx exhibits upregulation of adhesion molecules, a change in phenotype from anticoagulant to procoagulant with the expression of tissue

Figure 48-6

Action of shiga toxin on target cells such as glomerular endothelium. High levels of toxin exposure induce protein synthesis inhibition, the toxin being transported in a retrograde fashion from the Golgi apparatus to the endoplasmic reticulum where it cleaves ribosomal RNA. Sublethal exposure induces cell signaling via p38 MAP kinase and nuclear factor kappa-B.



factor, and release of chemokines such as monocyte chemoattractant protein-1 (5). Whether endothelial cell death occurs in vivo is not known. Histology shows glomerular endothelial cell swelling and detachment from basement membrane, but not extensive endothelial loss. However, exposed basement membrane from cell retraction or detachment would in theory present a prothrombotic surface to platelets and support the coagulation cascade.

There is strong evidence that increased procoagulant and thrombolytic processes precede the impairment of kidney function in EHEC-induced HUS (57). This is indicated by a rise in pro-thrombin fragments, D-dimers, tissue plasminogen activator and its inhibitor before oliguria or a change in plasma creatinine. This fits well with the pathology of intraglomerular thrombosis seen post mortem in early deaths from HUS. Cortical necrosis is probably caused by severe ischemia secondary to glomerular thrombosis, however proximal tubular epithelial cells are sensitive to stx in vitro and direct toxic injury has also been proposed.

There is also evidence that acute inflammation plays a role in the development of HUS. Patients with HUS demonstrate a rise in C reactive protein, neutrophilia and an increase in circulating proinflammatory cytokines such as interleukin-8 and granulocyte colony-stimulating factor (5). The urinary excretion of MCP-1 and Interleukin-8 is also increased (58). Neutrophilia at onset (neutrophils > 20,000/mm^3) is a biomarker for an adverse outcome (5).

Treatment and Outcome

At present there is no treatment that directly reduces stx production, absorption or its cytopathic consequences.

Antibiotic therapy of EHEC infection is unnecessary as the intestinal infection is self limiting and non-invasive, and there remains a concern that antibiotics might encourage bacterial release of stx and increase the clinical risk. An attempt to sequester stx in the intestinal tract was unsuccessful even with a binding agent (Synsorb) that was capable of avid toxin uptake in vitro (59). Plasma exchange therapy has not shown benefit (60). Passive immunization with engineered monoclonal antibody to stx can reduce toxicity in animal studies but has not reached the stage of human clinical trials (61).

The mainstay of treatment is supportive, with particular attention to hydration and electrolyte balance. Children are often saline depleted during the initial period of diarrhea. Correction of the volume deficit with hyponatremic fluid is contraindicated. Even using oral rehydration, a dilute replacement fluid risks rapid onset hyponatremia, cerebral edema and seizures. Oral fluids often accentuate the gastro-colic reflex and worsen abdominal pain, a frequent symptom in the early course of the disease. Vomiting also makes oral rehydration unreliable. Volume is better replaced intravenously using isotonic saline. There is some evidence that vigorous initial rehydration with isotonic saline can reduce the risk of later oliguria, and even defer or prevent renal failure (62). Once oliguric renal failure has occurred, salt and water intake has to be restricted to prevent vascular volume overload. Acidosis is common and should be corrected with bicarbonate. Plasma potassium should be closely monitored as it can rise steeply in the presence of red cell fragmentation or with blood transfusion.

There is no advantage in early dialysis, and dialysis should only be instituted once it is clearly needed to correct biochemical abnormalities that carry short term clinical risks. Peritoneal dialysis can be undertaken as long as the colitis does not pose a surgical risk (see below). Hemodialysis is usually satisfactorily, but vascular access can be challenging in infants who are very anemic and thrombocytopenic. Approximately 80% of patients require transfusion of packed red cells to stabilize the anemia (25). The transfusion should be given slowly with careful monitoring of intravascular volume status. A target hemoglobin of 8–9 g/dL is sufficient. Where possible platelet transfusion should be avoided in HUS, as this has been associated with a deterioration when administered during other microangiopathic processes, perhaps acting as a substrate for further microthrombus formation (63). Use of platelets should be restricted to clinically significant hemorrhage or for invasive procedures.

Occasionally the colitis itself is life threatening. Children who present with abdominal distension may develop toxic megacolon, obstruction, bowel perforation and shock. Close surgical review is mandatory and an emergency resection may be life saving.

In most cases the blood pressure is in high normal range for most of the clinical course and it is common to see a transient hypertension for a few days about the time of recovery of oliguria. However there are exceptions, and safe limits for blood pressure need to be set. In cases where there are neurological complications, full intensive care facilities are needed including CNS imaging, control of seizures, and pre-emptive treatment of any respiratory dysfunction in addition to the fluid, electrolyte and blood pressure corrections outlined above. Endocrine pancreatic failure and cardiomyopathy can occur, sometimes relatively late when other aspects such the hematological component appear to be resolving. Monitoring for hyperglycemia and clinical observation to detect cardiac decompensation is required.

The 1 year survival for D + HUS has steadily improved since the 1980s, and is now above 98% (7). Early deaths are usually due to CNS complications, less often to abdominal catastrophes related to colitis. It is very unusual today for patients to progress immediately to end stage renal failure; almost all regain some independent function. However, between 2 and 5 years from onset about a quarter of survivors will have renal impairment or proteinuria (64). Hypertension may occur but usually in association with the former. The risk of developing a second episode of HUS is remote; certainly less than 1 in 500.

Late outcome after D + HUS is unknown. There are sufficient unpublished cases of patients showing worsening proteinuria and renal impairment after an apparently full early recovery to suggest that all survivors need long term follow up. It is also prudent to consider additional life-time renal risks that may be compounded by the nephron loss induced by HUS. Survivors should be advised against smoking and obesity. Women with proteinuria or hypertension need individual advice about the risks of pregnancy.

HUS and *Shigella dysenteriae* type 1

HUS is a well-recognized complication of *Shigella dysenteriae* type 1 infection (65). Many of the features of the syndrome resemble EHEC-induced HUS. The age range is wider, the median age of presentation being around 3 years, and the median time from the onset of diarrhea to the presentation of HUS is 7 days compared to 4 for most EHEC infections.

Shigella dysenteriae can be enteroinvasive while EHEC typically are not. Therefore, unlike EHEC infection, early and appropriate antibiotic treatment is indicated and appears to reduce the incidence of HUS (66). Shiga toxin is implicated in the pathogenesis. In some laboratory models of HUS a combination of a ribotoxin and lipopolysaccharide is more likely to induce glomerular thrombosis than toxin alone (67). Children with *Shigella dysenteriae*-induced HUS are exposed to bacterial lipopolysaccharide because of the enteroinvasive nature of the organism and this added stimulus is likely to be pathogenic (68). The neutrophilia at onset is typically greater than with EHEC but has a similar prediction for the development and severity of HUS (69, 70). In some patients disseminated intravascular coagulation leads to consumption of coagulation factors, a very rare event in EHEC-induced HUS. Cases of disseminated intravascular coagulation with consumption of coagulation factors, such as that seen in sepsis and multiorgan failure, are usually excluded from the definition of HUS, however *Shigella dysenteriae* and invasive *Steptococcus pneumoniae* infections that cause HUS are exceptions to that rule.

There is the general impression that HUS complicating *Shigella dysenteriae* is more severe, but the condition mostly occurs in economically disadvantaged regions where children may have co-morbidities and poor access to health care. Catastrophic dehydration, hyponatremia and central nervous system complications may in part reflect this. In epidemics in sub-Saharan Africa, mortality rates of 17 and 43% are described (65) whereas in an outbreak in France all five affected children recovered with normal renal function (71).

HUS in Pneumococcal Sepsis

A distinctive form of HUS occurs as a complication of infection with *Streptococcus pneumoniae* (8). This was first described by Klein et al. (8), who reported two infants who died from pneumococcal pneumonia with clinical features of HUS. Post mortem revealed renal cortical necrosis and glomerular arteriolar and capillary thromboses. Since then over 150 cases have been published (39, 72, 73). This form of HUS appears to be increasing in incidence, in parallel with an increase in empyema. This is a paradox since the overall incidence of invasive pneumococcal infection in infants is falling, partly because of immunization against prevalent strains of pneumococcus. Epidemiological studies in the UK identified no cases of pneumococcal HUS between 1985 and 1988, but 43 cases between 2000 and 2005 (39, 74, 75).

Patients are usually infants or children below 3 years of age. Pneumococcal infection typically precedes the onset of HUS by a few days. The majority of cases follow pneumococcal pneumonia, and two thirds of these have loculated infection in the form of empyema (64). HUS has also been reported following pneumococcal meningitis with or without subdural collections and less often pneumococcal septicemia without a discernable focus (76). Profound anemia, thrombocytopenia and oliguric renal failure occur abruptly with a rapid deterioration in the child's clinical condition.

Pathogenesis

Pneumococci all have the ability to secrete one or more species of neuraminidase, an enzyme capable of stripping sialic acid (N-acetyl-neuraminic acid, NANA) from host cell membranes. Neuraminidase activity has been identified in the serum of children with pneumococcal HUS (77). Cleavage of NANA by neuraminidase from the normally disialylated tetrasaccharides of the red cell membrane occurs in vivo. This exposes a crypt-antigen known as the Thomsen-Friedenreich (T)-antigen which has a distinctive terminal beta-linked galactosyl residue (Gal-β (1–3)-GalNAc) (78, 79). T-antigen exposure can be readily confirmed on a patient's erythrocytes using the lectin *arachis hypogea* that identifies the exposed T-antigen. T-antigen exposure has also been shown on platelets and endothelium in this form of HUS (7, 72, 80).

A frequent laboratory finding is that the patient's red cells are agglutinated in vitro by ABO compatible serum, a phenomenon known as red cell polyagglutination (81). Polyagglutination is explained by a naturally occurring anti-T IgM class antibody that is found in most individuals by 3 months of age, adult concentrations being reached by 2 years (39, 82–84). Although often referred to as "naturally occurring," these antibodies are probably generated following exposure to ubiquitous intestinal flora that possess antigens similar to T.

It was postulated that these pre-formed IgM antibodies bind to T and initiate a cascade of events leading to TMA (84, 85). However, the importance of anti-T in HUS is questionable. Hemolysis in the setting of T activation may have alternative causes, such as direct hemolytic action of bacterial enzymes and toxins, altered interaction of complement components with desialyated red cells (78, 85) or shortened survival of red cells with reduced membrane sialic acid content (86, 87). HUS with T-antigen detection has been described in the absence of detectable anti-T antibodies (88–90). In vivo studies show that treatment

of red cells with neuraminidase decreases red-cell survival independent of anti-T titer (7, 72). IgM anti-T antibodies are cold reactive and do not cause red-cell agglutination or complement activation at 37°C in vitro (8). Clinical observations do not consistently support a relationship between transfusion and hemolysis in patients with T-activated red cells. There are reports describing cases of T activation in which administration of plasma did not lead to hemolysis (7,91).

Treatment and Outcome

Thorough medical and surgical treatment of the invasive pneumococcal infection is essential. Intensive care is often needed because of the multi-organ involvement in these cases. Beyond that the management of this condition is only supportive. Because of the uncertainty over the role of anti-T in the pathogenesis of this form of HUS, many still advocate the strict avoidance of plasma containing products (11, 92–104) and advise that for transfusion, red cells and platelets are washed to avoid plasma administration. Some authors advocate screening donors for anti-T and use of low titer products only (although this is not well defined). However there are no documented reports of clinical deterioration following use of plasma products in this condition. In contrast, a number of cases have received plasma products without adverse effects (105).

The mortality associated with this form of HUS is at least five times higher than patients with EHEC-induced HUS, 11–25% in recent reports. Death is usually related to the complications of sepsis, pneumonia or central nervous system infection, but failure to recover renal function contributes to the late mortality. The morbidity exceeds that of D + HUS with the duration of oligoanuria, thrombocytopenia and hospital stay being twice as long, and there is a greater requirement for red cell and platelet transfusions (106). About a quarter of survivors have renal impairment, but those who fully recover renal function appear to do well, although long term follows up data are lacking. No cases of relapse of this form of HUS have been reported.

HUS and Disorders of Complement Regulation

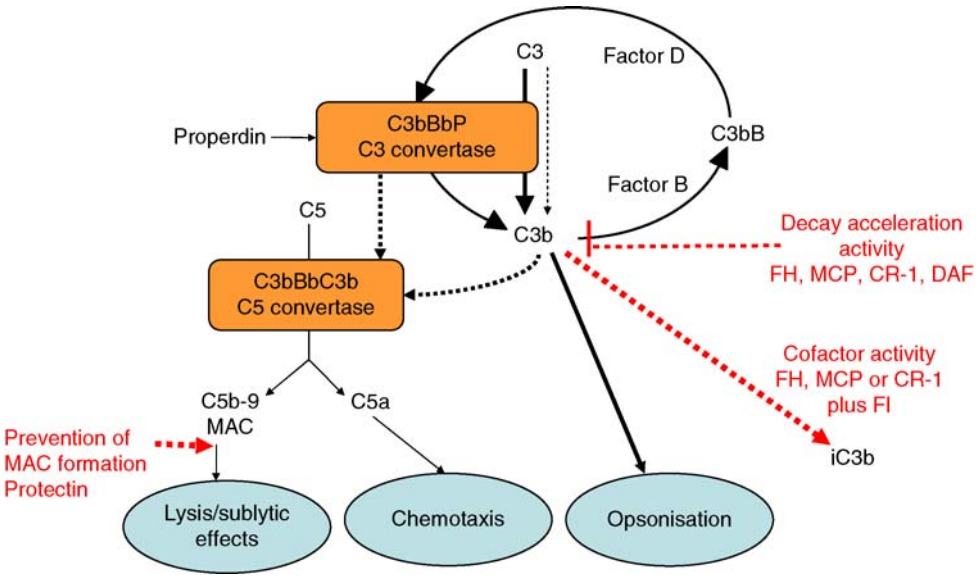
The seminal report of Warwicker et al. (107), in which HUS was associated with a complement factor H(FH) gene mutation, heralded the discovery of a number of different gene mutations in patients with HUS, all within genes encoding proteins of the alternative complement pathway (108). Various patterns of inheritance have been described, and genotype-phenotype correlations are starting to emerge. The likelihood of an underlying complement disorder is high if HUS present before 6 months of age, or if there is a family history of HUS occurring asynchronously, or unexplained past episodes of anemia, or HUS presents without an obvious infective cause, or if there is a relapse. Some patients with HUS secondary to complement dysregulation will have a low plasma concentration of C3, but these are a minority. While a low C3 increases the probability of a complement disorder, a normal C3 does not exclude it. The same is true for plasma FH concentration.

To understand the pathogenesis, a concept of the alternative complement pathway (ACP) is necessary (*Fig. 48-7*). The ACP provides rapid antibody-independent defense against micro-organisms. It is spontaneously activated by the slow hydrolysis of plasma C3 to a form of C3b that is able to combine with factor B (FB) to form C3bBb, the alternative pathway C3 convertase (109). This convertase cleaves further C3 into C3b, and also forms a C5 convertase, triggering the formation of C5b6789, the membrane attack complex (MAC). The pathway is strongly activated by microbial fragments (110) which become rapidly coated with C3b. C3b is an opsonin that assists effective phagocytosis and clearance of microbial components. The MAC is a pore-like structure that inserts into cell membranes resulting in cell lysis of invading pathogens. Host cells exposed to sub-lytic doses of the MAC undergo cell activation (111–113).

Since the ACP is spontaneously activated and positively amplified, its regulation is essential for host protection (*Fig. 48-7*). Host cells are protected by an array of membrane-bound complement regulatory proteins which interact with C3b to prevent the convertase from forming or promote its rapid dissociation ("decay acceleration" activity) (114). These include membrane co-factor protein (MCP, CD46), decay-accelerating factor (DAF, CD55) and complement receptor 1 (CR-1, CD35) (11, 115). The plasma protein FH performs this function in the fluid phase, and in addition binds to cell membranes to act as a membrane bound regulator (113). Additional regulation is provided by the inactivation of C3b by the plasma serine protease factor I (FI) in conjunction with co-factor activity from other regulators, including MCP, FH and CR-1 (113, 116, 117). Further defense against the effects of complement activation is provided by the cell membrane regulator protectin (CD59) (118). This regulator restricts the polymerization of C9 molecules essential for MAC formation.

Figure 48-7

Activation, amplification and regulation of the alternative complement pathway. The pathway is continuously activated through the spontaneous hydrolysis of C3 (black dashed line) to a form of C3b that combines with factor B to form a C3 convertase. This generates further C3b from C3 and a stable C3 convertase (C3bBb) is formed, which is further stabilized by the binding of properdin, amplifying the production of C3b. C3b is an opsonin, and triggers the formation of the terminal products of complement activation, C5a and C5b-9, the membrane attack complex (MAC). Host cell protection from complement activation is provided by complement regulatory proteins including membrane co-factor protein (MCP), decay-accelerating factor (DAF), complement receptor 1 (CR-1) and factor H (FH). Activation steps are shown in black and regulatory steps are shown in red.



Mutations in FH, MCP, FI, C3 and FB are all implicated in the pathogenesis of HUS (118).

Pathogenesis

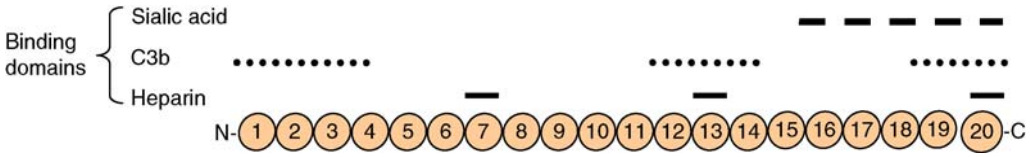
The mechanisms by which complement gene mutations lead to atypical HUS have been partly elucidated, and are summarized below.

Factor H (FH)

FH is a glycoprotein, produced by the liver. The gene (CFH) is located on chromosome 1q32, in the "Regulators of Complement Activation" (RCA) cluster. It acts as a co-factor for the proteolytic cleavage of C3b to inactivated C3b (iC3b) by FI (35) and promotes the decay of formed C3 convertases by competing with FB for binding to C3b (119–121). It is the main mechanism by which the ACP discriminates between foreign and host surfaces (121–123). Unlike many pathogens, host surfaces are rich in polyanions such as sialic acid and glycosaminoglycans. FH has high affinity for these anionic sites (124). FH consists of 20 globular domains known as short consensus repeats (SCRs) (*Fig. 48-8*) (125–129). The complement regulatory domains are found within the N-terminus (SCR 1–4) (130). The C-terminus (SCR 16–20) contains binding sites for surface-bound C3b, sialic acid and glycosaminoglycans and is important for attaching FH to host cell surfaces (125, 130). Over 60 different FH mutations have so far been described in HUS (131). Mutations are usually heterozygous and mostly occur in SCR 16–20. Most patients have normal plasma FH and C3 concentrations. Several important functions of mutant FH proteins have been studied. Mutations in SCR 20 generally lead to reduced binding of FH to heparin, to surface bound C3b and to endothelial cells (132). Mutant proteins display normal complement regulatory activity in the fluid phase (132), but reduced cell surface complement regulation (133). Taken together, this evidence suggests that mutant FH proteins are capable of controlling fluid

Figure 48-8

Schematic representation of complement factor H (FH). FH consists of 20 globular domains known as short consensus repeats (SCRs). The numbering of SCRs is indicated from 1 to 20. Domains known to interact with specific ligands are indicated. The N-terminus (SCR 1–4) exhibits complement regulatory activity whilst the C-terminus contains binding sites for polyanions and surface bound C3b, and thus mediates binding of FH onto cell surfaces. Adapted from (94).



phase complement activation, but their ability to bind to host cell surfaces and protect from complement mediated damage is impaired.

Auto-antibodies to FH have been reported in patients with HUS (108, 134). They appear to mimic the effect of C-terminal FH mutations, as they inhibit the regulatory function of FH at cell surfaces by blocking its C-terminal recognition region (92). In an intriguing development, the presence of FH autoantibodies appears to correlate with a genetic absence of the FH related proteins, FHR-1 and FHR-3 (11, 93, 95, 98).

Membrane Co-factor Protein (MCP, CD46)

MCP is a transmembrane protein expressed on almost every human cell except erythrocytes (95, 98). It is a co-factor for FI in the cleavage of C3b and C4b and also has decay acceleration activity (98). The gene encoding MCP is located in the RCA cluster. Mutations in MCP, usually heterozygous (113), have been detected in 8–14% of patients with familial or relapsing HUS (135). Most patients with MCP mutations show a 50% reduction in surface expression of MCP on peripheral blood mononuclear cells (PBMCs) (11, 136, 137). Other mutations produce a protein with normal surface expression but reduced binding to C3b (115). MCP mutations in HUS therefore lead to impaired MCP function, and presumably reduced cell surface protection from complement mediated damage.

Factor I

FI is a serine protease that cleaves the alpha chains of C3b and C4b, inactivating them and preventing convertase formation (11). FH and MCP are co-factors for its activity. The gene is located outside the RCA cluster on chromosome 4q25 (92, 99, 138, 139). FI mutations have been described in 4–10% of cases of familial or relapsing HUS and these mutations are heterozygous, and associated with either normal or reduced FI concentration, and a variable reduction of complement C3. Certain mutant proteins demonstrate reduced inactivation of C3b and C4b (95, 98).

Factor B

A recent publication reported mutations in the gene encoding FB and persistent activation of the ACP in two families with atypical HUS (24). Functional analyses demonstrated that the two mutations caused gain-of-function that resulted in enhanced formation of the C3bBb convertase or increased resistance to inactivation by complement regulators.

C3

C3 mutations have been found in a small number of patients in whom other complement mutations have not been identified (24). The majority were heterozygous missense mutations, and most gave rise to a gain of function of the ACP. Two mutations caused haplotype insufficiency of C3 and their role is presently unclear.

Together these findings indicate that increased ACP activation is central in the pathogenesis of this form of HUS (see *Table 48-3*).

Clinical Features

Patients with these forms of HUS more often have an insidious onset unlike the abrupt presentation of the post infective forms. Both initial onset and relapses may be precipitated by relatively minor illness such as upper respiratory tract infections. Consistent findings at onset

Table 48-3

Incidence of gene mutations in cohorts of familial or relapsing HUS patients

Gene	Cohort	Incidence of mutations	Reference
Factor H	Newcastle, UK	5 of 50 cases (10%)	Richards (99)
	Spain	4 of 13 cases (30%)	Perez-Caballero (98)
	Italy	4 of 4 pedigrees[a]	Caprioli (93)
	German speaking countries	17 of 111 cases (15%)	Neumann (95)
	France	10/46 children (22%)	Sellier-Leclerc (11)
MCP	Newcastle, UK	3 of 30 families (10%)	Richards (11)
	Spain	6 of 41 cases (14%)	Esparza-Gordillo (11)
	Italy	2 of 25 cases (8%)	Noris (93)
	France	10 of 77 cases (13%)	Fremeaux-Bacchi (115)
Factor I	France	8 of 77 cases (10%)	Fremeaux-Bacchi (11)
	Newcastle, UK	3 of 75 cases (4%)	Fremeaux-Bacchi
	Spain	2 of 41 cases (5%)	Esparza-Gordillo
Factor B	Spain	Not yet determined	Goicoechea de Jorge
C3	Newcastle, UK	Not yet determined	Fremeaux-Bacchi

[a]Cohort of atypical HUS with low C3

are hypertension which is often severe and can lead to heart failure and encephalopathy, and heavy proteinuria and hematuria in those who retain urine production. Extra renal manifestations may occur but are less frequent than in EHEC-induced HUS. Some, such as retinal thrombotic microangiopathy, seem to be reported particularly with FH mutations. This presents with pain in the eye and visual loss. Hematological relapse is common. This has been arbitrarily defined as a return of MAHA after a period of 2 weeks with normal platelet count. Patients presenting in early infancy have a high risk of progressing to end-stage in the first episode. Once end-stage renal failure has occurred the MAHA and thrombocytopenia tend to resolve.

Seventy percent of patients with FH mutations have their onset before 12 years of age, and historically 70% progress to irreversible renal failure or death (93). Those with FI mutations have a similarly adverse outcome. Patients with MCP mutations fare slightly better but also have a relapsing course with gradual progression to end-stage renal failure in up to half of patients (11). Those with autoantibody-induced FH deficiency tend to present in the later half of childhood and their outcome is unclear as yet.

The penetrance of disease in pedigrees with complement mutations varies with different mutations and pattern of inheritance. Overall approximately 50% of those carrying a heterozygous FH disease-associated mutation risk developing HUS themselves (140). Other genetic factors such as polymorphisms in FH and the MCP genes influence disease expression (141–148). This has led to the conclusion that the co-inheritance of different susceptibility alleles influences complement regulation, predisposes to HUS and explains the incomplete penetrance of HUS in mutation carriers (141, 142).

Therapy of HUS Associated with Disorders of Complement Regulation

Prior to the recognition of complement gene mutations in atypical HUS, anecdotal reports suggested benefit from plasma exchange or infusion (92, 99). More recently, the effects of plasma infusion have been described in children with homozygous or compound heterozygous deficiency of FH (92, 99). All improved, but one child became refractory to this regime (149, 150). Repeated plasma infusion is limited in oliguric patients by the risk of volume overload.

Plasma therapy has also been described in patients with heterozygous FH mutations with variable results (49). Patients with isolated MCP dysfunction do not appear to benefit from plasma therapy (99), probably because MCP is a membrane bound complement regulator not a circulating one, and there is a high spontaneous remission rate. Little is known about the role of plasma

therapy for other more recently identified forms of complement dysregulation.

The complex laboratory investigations needed to confirm the etiology of HUS take time, and therefore initial treatment is empirical. The European Paediatric Study Group for HUS argues for urgent plasma exchange, replacing with fresh frozen plasma or a standardized whole plasma product as a first line treatment. This is recommended on the basis that it would replace mutant complement proteins responsible for the disease and remove autoantibodies to FH. They justify the urgency and invasive nature of this approach on the fact that glomerular thrombotic microangiopathy is a destructive process and a first episode of HUS may lead rapidly to end-stage failure (92, 99).

Since complement dysregulation appears to be at the heart of the pathogenesis, synthetic complement inhibitors may become a therapeutic option, and a number of compounds are becoming available for clinical trials (151). Correcting the abnormal circulating complement regulator by liver transplantation is considered below.

Transplantation in HUS

Where EHEC infection is the cause of end-stage renal failure, renal transplantation is not complicated by an increased risk of graft loss through thrombosis or HUS recurrence (3). The only caveat is that very rarely EHEC can induce HUS in a patient with an added risk factor such as a disorder of complement regulation, and this may not be known at the time of their original presentation. In this unusual circumstance the possibility of recurrence cannot be excluded (92).

Prior to the recognition of complement mutations, up to 60% of patients with so-called atypical HUS progressed to ESRF, and about 50% of those who were transplanted lost their grafts to disease recurrence or thrombosis (11, 92, 99, 136). The application of complement genomics has now shown that for patients with FH mutations the rate of graft loss is 80% (92, 152, 153), with a broadly similar picture emerging for those with FI mutations (154). Worse, in families where living-related kidney donation was undertaken, HUS occurred in the donor who was carrying the disease associated FH mutation (155). By contrast, patients with MCP mutations rarely develop disease recurrence after renal transplantation, probably because of normal surface expression of MCP on the donor endothelium (92, 156, 157). In one case of recurrence in a MCP patient, the graft exhibited endothelial microchimerism, with recipient endothelial cells detected in the graft (158). Calcineurin inhibitor-free immunosuppression does not reduce the risk of recurrence despite previous concerns that they may contribute to post-transplant TMA (159).

Based upon the rational that FH is synthesized by the liver, isolated liver or combined liver-kidney transplantation has been attempted as a treatment for HUS associated with FH mutation (159). The former can be considered for patients who are dependent on frequent plasma exchange to maintain a remission, especially if they experience breakthrough relapses or worsening proteinuria or renal function. In such cases the procedure would need to be undertaken before extensive and irrevocable renal damage has occurred. For those already in chronic renal failure the decision to perform a combined liver and kidney graft trades the early high risks of the procedure against the late but accumulative risks of chronic dialysis. Although initial cases were unsuccessful, subsequent reports are more optimistic, with disease-free survival reported in 3/7 patients (160). Given this complexity, transplant management for HUS patients should be undertaken only in the full knowledge of the genotype. Liver or liver and kidney transplantation should only be performed in experienced centers, and with a clear strategy to minimize the risks of uncontrolled complement activation during surgery and increased peri-operative thrombosis.

HUS and Deficiency of von Willebrand Protease (ADAMTS13): Thrombotic Thrombocytopenic Purpura

HUS has a clinical overlap with TTP. The clinical "pentad" for the diagnosis of TTP comprises thrombocytopenia, microangiopathic hemolytic anemia, neurological signs, renal dysfunction and fever (62, 92), although the insidious onset and fluctuation of clinical findings may mean that not all of the pentad appears at once, and this can pose a diagnostic difficulty. The distinctive pathogenesis of TTP as outlined below permits the clinical definition of TTP to be aligned to severe deficiency of the von Willebrand protease, ADAMTS13, and patients with this etiology should be described as TTP rather than HUS (161).

Pathogenesis of TTP

The overwhelming majority of contemporary cases of TTP are associated with genetic or acquired abnormalities

von Willebrand Factor cleaving protease (also known as ADAMTS13, A Dysintegrin And Metalloprotease with Thrombospondin-like motifs type 13) (162). Von Willebrand factor (vWF) is synthesized by endothelial cells, stored in the Weibel-Palade bodies and secreted in multimeric form where it remains associated with the endothelial cell surface to provide binding sites for platelets, and promotes platelet aggregation (49). Injury to the endothelium, or stimulation by vasopressin increases vWF release. Physiologically the vWF multimers are cleaved after secretion to reduce the ability of platelets to aggregate.

Patients with relapsing TTP were initially shown to have ultra-large vWF multimers in their circulation, and deficiency of ADAMTS13 activity was confirmed later (163). Rarely, the ADAMTS13 deficiency is caused by genetic mutation that code for loss of function, and this presents as congenital TTP (Upshaw-Schulman syndrome) (164). More commonly, the deficiency is caused by an acquired inhibitor, usually an IgG auto-antibody (163) sometimes in association with the use of certain anti-platelet drugs such as *clopidogrel* and *ticlopidine*. For deficiency of the protease to be a primary cause of microvascular thrombosis the enzyme activity needs to be very low, typically less that 5% of normal. Moderate reductions in activity, down to 30% of normal, are found in several disease and physiological states and are not independent causes of TTP or HUS. The platelet rich microvascular thrombi of TTP are to be found at the arteriolar-capillary junction, a position of high sheer stress, in many tissues including brain, heart, spleen, kidneys, pancreas, adrenals, lungs and eyes.

In all cases of atypical HUS ADAMTS13 activity should be determined, and if it is low plasma mixing studies are indicated to determine the presence of an inhibitor. Veyradier et al. studied ADAMTS13 activity in a cohort of children with HUS (cases with and without diarrheal prodrome) (165). During the acute phase, only a single patient (1/41 cases) with post-diarrheal HUS exhibited a very low level (<5%) of ADAMTS13 activity, but this recovered to normal after 3 months and the significance of the finding is unclear. However, in the group without prodromal diarrhea, 6/23 cases had severely decreased ADAMTS13 activity (<5%) which remained undetectable during remission. Review of clinical data revealed that disease started at birth in all six children, with hemolysis and jaundice requiring exchange transfusion. This is typical of congenital ADAMTS13 deficiency. All had significant renal involvement, and half had neurological episodes.

Clinical Management

Historically the mortality of TTP was around 95% of cases. This fell to 20% with the institution of plasma therapy (97). In cases of congenital ADAMTS13 deficiency, regular plasma infusion, and more recently cryosupernatant, is very effective in preventing relapses (96). However in TTP generally, plasmapheresis is more effective than plasma infusion alone, presumably because it removes the inhibitor present in the majority of cases (92).

HUS and Cobalamin Metabolism

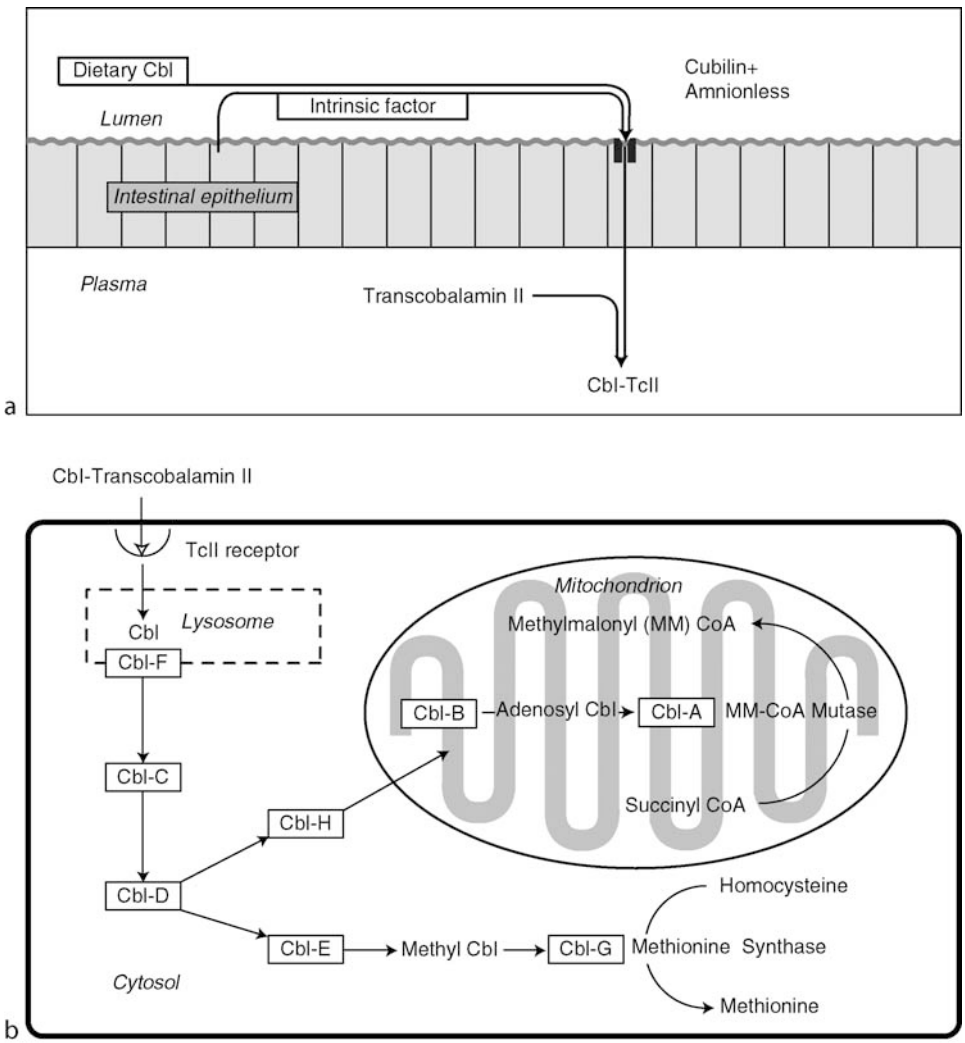
A rare form of HUS complicates an inherited defect of *intracellular* cobalamin (Cbl) metabolism. Defects in absorption and plasma transport have not been associated with HUS per se although anemia, thrombocytopenia and renal impairment can occur.

Cyanocobalamin, vitamin B12, is obtained entirely from the diet. Its absorption and plasma transport is outlined in *Fig. 48-9a*. All cells have a requirement for Cbl, and it is essential for two pathways. As methyl-cobalamin it is a cofactor for the methionine synthase that converts homocysteine to methionine in the cytosol: this methylation step requires 5-methyl tetrahydrofolate. As adenyl-cobalamin it is a cofactor for methylmalonyl-CoA mutase that converts L-methylmalonyl-CoA to succinyl-CoA within mitochondria. An outline of the intracellular pathway and the complementation steps is given in *Fig. 48-9b*. Some idea of the level at which Cbl metabolism is deranged can be gauged by whether there is an excess of methylmalonic acid or homocysteine in plasma or urine. Combined hyper-excretion of both products suggests an abnormality in the proximal part of the pathway, usually deficiency of Cbl-C deficiency, the commonest inherited defect of cobalamin metabolism, and rarely Cbl-D or Cbl-F (166). Patients who manifest HUS almost always have Cbl-C deficiency.

Defects in the intracellular metabolism of cobalamin were initially investigated by complementation studies. Cobalamin-C deficiency is attributable to homozygous or compound heterozygous mutations in the gene, methylmalonic aciduria and homocystinuria (*MMACHC*) on chromosome 1p34 (167). The condition is inherited as an autosomal recessive disorder. Over 40 mutations have been reported, but one mutation, 271dupA, accounts for 40% of cases mostly of European origin (168). It is predicted that this and other mutations code for loss of normal protein function, although the precise function

Figure 48-9

Metabolism of cobalamin. Gastric intrinsic factor is a prerequisite for vitamin B12 absorption in the terminal ileum. The B12-intrinsic factor complex enters a specific endocytotic pathway that depends on a receptor composed of cubilin and amnionless. Inherited deficiency of B12 absorption can be due to mutations in cubilin, amnionless or gastric intrinsic factor, and constitute Imerslund-Glasbeck syndrome. Patients with Imerslund-Glasbeck syndrome caused by a cubilin mutation have proteinuria because cubilin is a multi-ligand receptor that is essential in scavenging filtered proteins in the proximal tubule of the kidney. In the enterocyte, cobalamin is transferred onto the carrier protein transcobalamin-II (TCII) a 43 kDa globulin that is important for cellular uptake of the vitamin, and exported into the plasma. Recirculation of cobalamin occurs in that the Cbl-TCII complex is filtered in the kidney, avidly recovered in the proximal tubule and returned to the circulation attached to re-synthesized TCII. Cells take up the Cbl-TCII complex via a TCII receptor. The intracellular pathway of cobalamine is shown. Figure courtesy of Joanna Clothier.



of the *MMACHC* product in the metabolism of cobalamin has not yet been resolved.

Not all patients with Cbl-C deficiency develop HUS. How this metabolic defect promotes HUS is also unknown. Hyperhomocysteinemia is injurious to endothelium and shifts its normal anti-thrombotic property towards a procoagulant one. For example, patients with hyperhomocysteinemia secondary to cystathione synthase

deficiency exhibit intravascular thrombosis in arteries and veins but not TMA or HUS (169). Methylmalonicacidemia induces interstitial renal disease probably because of a disturbance in the production on intracellular energy, but not vascular disease.

Interestingly two siblings with thrombotic microangiopathy, renal failure and neurological disease were reported to have Cbl deficiency, a homozygous mutation in methylenetetrahydrofolate reductase (MTHFR), and a heterozygous mutation in complement factor H (170). While there is no support for MTHFR polymorphisms modifying the clinical presentation of patients with *MMACHC* mutations (168), the role of compounding risk factors such as a complement disorder should be considered in those who develop HUS.

The clinical presentation of patients with Cbl-C deficiency differs depending on the age of onset. The more severe is early presentation in the neonatal period and up to about 6 months. Multi-system involvement is typical with failure to thrive, vomiting, hypotonia poor feeding, hydrocephalus, seizures, pancytopenia, hemolysis, hypogammaglobulinemia, acidosis, gastric hemorrhage, hypoproteinemia and edema, hypertension and renal failure. The mortality is high. A later presentation is increasingly recognized and includes neurological deficit, retinopathy, developmental delay and lung disease. HUS can occur in either early or late onset cases. Glomerular thrombosis and endothelial swelling and detachment typical of TMA is seen. In post mortem cases thrombi can be found in other sites such as pulmonary arteries or brain. Hepatic steatosis and a distinctive atrophic gastritis have also been reported (169).

Prompt diagnosis is essential as delay contributes to adverse outcome. The finding of excess homocystine and methylmalonate in urine is a first step towards the diagnosis. Confirmation of the Cbl-C defect is made either on complementation studies on fibroblasts, or by genetic investigation for mutations in *MMACHC*. Parenteral administration of high doses of hydroxycobalamin (1–5 mg daily) has been shown to partly override the transport defects and control HUS. It does not consistently reverse or prevent the late acquisition of neurological deficits (171). Some authors have added betaine, folic acid and carnitine supplementation.

HUS and Quinine

HUS induced by quinine or quinidine dependant autoantibodies has been reported in adult patients, usually elderly women (172–174). Exposure to quinine may be slight, for example from quinine used for its bitter flavor in tonic water, although more often it occurs after taking quinine in tablet form, often used to prevent muscular cramp. The clinical presentation usually consists of nausea, vomiting, diarrhea, "chills," myalgia, abdominal pain, dyspnea and oliguric renal failure. Bruising and petechia are common. These symptoms occur as little as 12 h from the ingestion of quinine in a sensitized person (173). Neurological signs and symptoms are common and include confusional states (174). Schistocytes appear in the blood film with varying degrees of anemia. Thrombocytopenia is profound and there is evidence of increased fibrin degradation products. Prothrombin and partial thromboplastin times are normal, and there is no consumption of coagulation factors. Lactate dehydrogenase is increased and haptoglobin reduced. Glomerular thrombosis typical of TMA has been reported. So far there is no information about the role if any of ADAMTS13.

Initially it was thought that the renal impairment that accompanied the syndrome was relatively mild, with some individuals escaping renal involvement although having neurological signs. However more recent experience from the Oklahoma TTP-HUS registry (174) indicates that 4 of 17 patients died and 7 survivors had chronic renal failure. Plasma exchange has been undertaken and makes logical sense as it would seem likely to remove antibody.

HUS and Other Disease Associations

Glomerular endothelium is dependent on vascular endothelial growth factor (VEGF) produced by the opposing podocytes to maintain its fenestrated phenotype and the integrity of the capillary structure (175). Anti-VEGF monoclonal antibodies *Bevacizumab* and *Sunitinib* used to prevent metastatic vascular development in cancers has been associated with proteinuria, sometimes heavy enough to induce nephrotic syndrome, with or without hypertension in about a quarter of patients. In a few cases it has been associated with thrombocytopenia and renal failure. The full picture of MAHA appears to be inconsistent and intravascular hemolysis is mild, limiting qualification for the clinical diagnosis of HUS. Nevertheless, renal histology has revealed convincing glomerular thrombotic microangiopathy (176, 177). Further proof of the role for VEGF was provided by a murine model in which the podocyte-specific production of VEGF was conditionally knocked down. Affected animals developed proteinuria and hypertension and over half had red cell schistocytes in the blood film. Whether or not reduction

in locally produced VEGF has a role in other forms of HUS has yet to be investigated.

Cancer, particularly *disseminated adenocarcinoma* has been associated with HUS, usually in adults with primary gastric, colonic or prostatic disease, but rarely in children. Many but not all these patients were recipients of chemotherapeutic agents including *Mitomycin-C*, either alone or in combination with *5-fluorouracil and Doxorubicin* (178). Also implicated are *Bleomycin, Epirubicin, Cisplatin and Gemcitabine. Ionizing radiation* has been associated with HUS, again in the clinical setting of cancer therapy. The mode of action of the chemotherapeutic agents is not known. Experimentally, *Mitomycin* was shown to induce TMA in a rat model (179) and *ionizing radiation* has been shown to injure and detach vascular endothelial cells.

Transplantation of solid organs and bone marrow may be complicated by HUS. Calcineurin inhibitors *Cyclosporine A* and *Tacrolimus* have been implicated. They mediate vasoconstriction through endothelin-1 but this has not been shown to participate in the pathogenesis. The incidence of HUS and TTP in allogenic bone marrow transplantation varies between series. Coexisting infection with Aspergillus species, cytomegalovirus, adenovirus, human herpes virus 6, and parvovirus B19 may have contributed. The reviewers found evidence that von Willebrand protease activity was not severely reduced in these patients and no benefit could be found from plasma therapy. The mortality was high. While some cases has clear evidence of glomerular thrombotic microangiopathy, this was absent in some cases that had been described as HUS or TTP. Extra-renal TMA was not found (180).

HUS or TTP has been described in association with *pregnancy* (180) the *HELLP syndrome* (hemolytic anemia, elevated liver enzymes and low platelets) and with the use of *oral contraceptives*. Teenage girls presenting with HUS should be asked directly about their use of oral contraception and the possibility of pregnancy. In these associations the mechanism of microangiopathy is often unknown. However, some have severely reduced ADAMTS13 activity or mutations in complement regulators, and investigation for this possibility is needed.

HUS or TTP can complicate either *Systemic Lupus Erythematosus* (181, 182), usually in the presence of lupus anti-coagulant or anti-cardiolipin antibodies, or the *anti-phospholipid syndrome*. In a couple of cases of the latter, profound reduction was found in ADAMTS13 activity due to an autoantibody (183), again indicating the need for full investigation of the cause. There is some evidence that patients with anti phospholipids antibodies and HUS or TTP benefit more from plasma exchange than immunosuppression with steroids alone (184).

Human immunodeficiency virus infection-associated nephropathy (HIVAN) occurs late in the course of HIV infection and is often compounded by other chronic infections and immune abnormalities, and occasionally HUS (185). HIV in a primate model induces vascular endothelial damage that might suggest a direct mechanism.

Acknowledgments

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49 Sickle Cell Nephropathy

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Introduction

The major clinical consequences of sickle cell disease (SCD) are vascular obstruction by sickled cells and anemia because of red blood cell (RBC) destruction. The sickling process may cause hematuria, renal papillary necrosis (RPN) and a urinary concentrating defect. There also is a chronic sickle cell glomerulopathy, which is less directly related to sickling, as well as unusual susceptibility to infections and to a specific form of malignancy.

Sickle hemoglobin (HbS) differs from normal (HbA) by the substitution of valine for glutamine in the 6-position of the β-globin chain (1). Under low oxygen tension in concentrated solution, HbS aggregates and forms intracellular fibers. These fibers prevent RBCs from deforming normally, so they do not pass easily through the microcirculation and thus cause occlusion. Their increased density, related to the Gardos channels, increase the tendency to sickle (2). Repeated cycles make some cells irreversibly sickled, and even in well-oxygenated blood they cause abnormal viscosity. In addition, these abnormal cells are easily lysed, causing anemia. The different measures of renal involvement and time line in SCD are illustrated in *Fig. 49-1* (adapted from (3)).

Hematuria and Renal Papillary Necrosis (RPN)

Clinical

Gross hematuria may be the most dramatic clinical event in SCD (4). It is often painless and usually unilateral, more commonly on the left side (5) explained by increased venous pressure due to the greater length of the left renal vein. Hematuria can occur at any age. It is more often reported with sickle trait (HbAS), probably because of a higher genetic frequency than HbSS (6). RPN is usually discovered in patients with painless gross hematuria (7). However, hematuria is not invariably present, and RPN can occur even in young children (5). When sought systematically, 40% of patients in one Nigerian series had RPN (8). In another series, there was no difference in the incidence of RPN between symptomatic (65%) and asymptomatic (62%) patients (9). The frequency of RPN on urography therefore suggests that the process develops subclinically without gross hematuria.

Pathogenesis

The pathology associated with isolated hematuria is rarely examined. In the past, kidneys removed from patients with uncontrolled bleeding in SCD showed relatively insignificant changes, primarily medullary congestion (6). The hematuria probably results from the sequence of renal medullary sickling, vascular obstruction, and RBC extravasation. This is precipitated by those factors present in the renal medulla that lead to sickling: The PaO_2 (35–40 mm Hg) is below the threshold (45 mm Hg) for sickling (10); the high osmolality of the medulla draws water from the RBC, leaving the HbS concentrated; and promotes the formation of hemoglobin polymers. The acidic environment of the renal medulla further increases the likelihood of sickling. This mechanism of microvascular occlusion explains many of the complications of SCD, but is overly simplistic. The distorted cell is also partly explained by dehydration of the cell, by enhanced KCl co-transport, and Gardos channel hyperactivity (2,11), in part induced by cell swelling and acidification. Further, K+ and water efflux are enhanced by transiently increased SS cell cytosolic Ca, induced by the membrane distortion.

Pathology

The pathology of RPN in SCD is a focal process, with some collecting ducts surviving within a diffuse area of fibrosis. Within the medullary fibrosis the vasa rectae are destroyed, following initial dilation and engorgement (12). The dependence of the papilla on that circulation results from repeated small focal infarctions of the papilla (13). This differs from the RPN found in analgesic abuse, in which the vasa rectae typically are spared, and most

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Figure 49-1

Time and event line for the development of sickle cell nephropathy. Average ages of onset are paired with clinical or laboratory markers underlying the processes, endogenous physiologic modifiers and therapeutic possibilities. COX, cyclooxygenase; Cys-C, cystatin-C;GFR, glomerular filtration rate. (Adapted from (3).)

Average age (y)	Clinical observation Harms ↓ Helps ↑	Process Harms ↓ Helps ↑	Modifier Aggravates ↓ Improves ↑	Therapeutic effort Inhibits ↓ Improves ↑
1	Anemia ↓	Sickling ↓	High HbF ↑	Hypertransfusion ↑
	Pain ↓			Hydroxyurea ↑
				Gardos block ↑ Zn ↑
			Chg β-globin ↑	β-globin gene Rx ↑
			Incr. HbA ↑	Bone marrow transplant ↑
5	GFR ↑ SCr. ↓	Medullary congestion ↓	Incr. adhesion ↓	
	Hematuria ↓		High oxygen ↓	
		Hyperperfusion ↑	High COX-1 ↑	ACEI ↑
		Hypertrophy-EGF ↑	Hypoxia ↓	Somatostatin analogue ↑
		Papillary necrosis ↓	High NO ↑	
			High caspase-3 ↑	
			High HSP70 ↑	
			Incr. COX-2 ↑	
10			High CRP ↓	
			High HO-1 ↑	
		Hyperfiltration ↑	Incr. AII ↓	ACE ARB ↑
			High PGI_2 ↑	PGI_2 analogue ↑
			High HSP70 ↑	
15	High microalbuminuria ↔	Hyperperfusion ↓		ACEI ↑
20	High NAG ↑	Tubular function ↓		ACEI ↑
20	High β_2-M ↑	Tubular function ↓		
30	High proteinuria ↑	Glomerular Htx ↓		ACEI ↑
30	NI GFR	FSGS ↓		
35	Low GFR ↓			
35	Incr. Cys-C ↓			
40	Low Cr Cl ↓			
50	High Cr ↓			

lesions occur in peritubular capillaries (13). These same factors are present in sickle trait, although with a smaller proportion of sickling cells. Because calyces are affected separately and sequentially in SCD, acute obstruction, with sloughing of papillae, is uncommon (9,14).

Diagnosis

Continued gross hematuria likely represents a form of renal "sickle crisis" in a known HbSS or HbAS patient. Other treatable causes for hematuria, including the renal medullary carcinoma in patients with sickle hemoglobin, must be excluded (15). Severe pain makes the diagnosis of renal sickle crisis less likely, whereas moderate discomfort often lateralizes the bleeding. Renal and bladder ultrasound can rule out bleeding from a stone or tumor and an diagnose renal papillary necrosis (see below). The increased echodensity of medullary pyramids on ultrasound is typical of SCD, and in the absence of hypercalciuria, medullary echodensity in a patient with hematuria should suggest a sickle hemoglobinopathy (16). Walker (17) reviewed ultrasound reflectivity in young (age 10–20 year) SCD patients, and found diffuse echogenicity in 9%, and medullary echo-density in 3%. Surprisingly this was greater in the milder genotypes, 37% in SC and 79% of Sβ+ Thalassemia patients. This finding is unexplained, but is unlikely to represent RPN. This was found in 20% of patients with sickling processes and interpreted to suggest subclinical nephrocalcinosis or iron deposition.

The diagnosis of RPN in SCD was traditionally made by urography. In McCall's series 39% of 189 patients had calyceal clubbing, 23% with definite RPN (18). Cortical scarring as found in pyelonephritis does not accompany

the calyceal clubbing of SCD (19). Other urographic findings of RPN in SCD are distinctive. A "medullary" form in which there is an irregular medullary cavity, often with sinus tracts, is common (9). The base of the calyx and its normal outline are preserved (19). Sonography can sometimes identify the early medullary form of papillary necrosis. A later finding is calcification of the medullary pyramids in a "garland" pattern surrounding the pelvis. This pattern of "shadowing" echo density may be distinctive. The progression to the "papillary" form results in clubbing and caliectasis (*Figs. 49-2* and *49-3*). This is more common in analgesic nephropathy, in which an area of sequestration is often found, resulting from infarction of a large area of the papilla (20). A prospective survey of symptomatic SCD patients (9) found that 11 of 18 SA patients had a form of RPN, but 8 of these 11 had evidence of infection. Nine of 11 symptomatic SS patients had RPN, of which 5 had evidence of infection. Asymptomatic patients included 16 of 22 SS patients with RPN, 1 of 3 SA patients, 3 of 4 SC patients, and 5 of 8 S-Thal patients. It is not necessary to perform contrast urography to visualize the renal architecture in SCD.

Treatment

In view of the benign pathology in SCD hematuria, conservative management is appropriate (6). Bed rest is often recommended to avoid dislodging hemostatic clots. It is advisable to maintain high rates of urine flow by both hypotonic fluid intake (4 L/1.73 m^2 surface area per day) and diuretics (a thiazide or a loop diuretic such as furosemide), which should help clear clots from the bladder (5). In addition, a diuresis reduces medullary osmolarity and may therefore help alleviate sickling in the vasa rectae. Some caution is necessary in the administration of sodium-containing fluids because of potential sodium retention (see below).

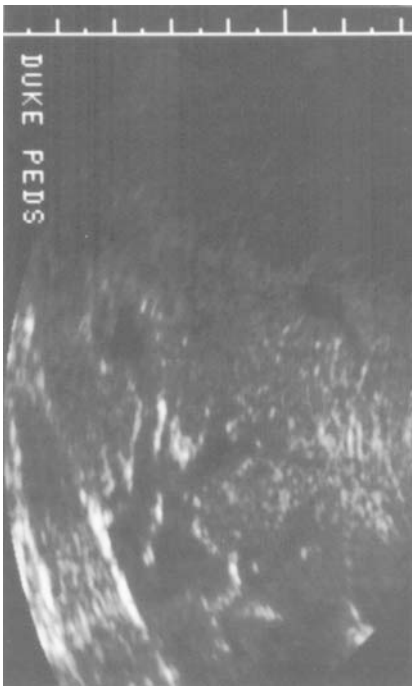
The combination of vasopressin and administration of hypotonic fluid to reduce plasma osmolality has been suggested. Hyponatremia induces water uptake by RBCs in vitro, thereby reducing the effective HbS concentration and making them less likely to sickle (6). This treatment is neither safe nor proven effective (6). Saline volume expansion would be especially ineffective, and coupled with hypertransfusion would predispose to congestive

Figure 49-2

Tomographic pyelography of an 18-year-old patient with abdominal pain and hematuria. Papillary necrosis is evident from blunted medullary cavities, especially the upper pole. The bases of the calyces are preserved. The middle pole calyx has a possible sinus tract.

Figure 49-3

Ultrasonographic visualization of the same kidney as in Figure 49-2. The middle pole exhibits deep extensions into the papilla, likely sinus tracts, typical of the "papillary" form of RPN.



heart failure. It would also be surprising if vasopressin were to increase the patient's urine concentration sufficiently to inhibit water diuresis (see below).

Because sickling is increased in an acid environment, alkalinization of the urine by 8–12 g $NaHCO_3$ (per 1.73 m^2) per day may reduce sickling in the urine environment, but this may not be relevant for medullary sickling (5). Alkalinizing the patient to increase the O_2 affinity of Hb is theoretically valid but not of proven value in practice (6). Transfusions may be necessary for blood loss and could be helpful by increasing the proportion of normal HbAA cells, reducing sickling cells.

Epsilon-aminocaproic acid (EACA) inhibits fibrinolysis, allowing clots to mediate hemostasis. The effective dosage in an adult is 8 g/day. In one series, 4 of 12 cases required EACA after failure of treatment with fluid administration and alkalinization (5). Unless other measures have failed, the risk of thrombosis should impose some caution in using EACA. Lower dosages may be adequate to arrest hematuria, starting with 1 g (per 1.73 m^2) orally three times daily and increasing the dose until hemostasis occurs (6). Nephrectomy is rarely required for uncontrolled bleeding. Arteriographic localization and local embolization of the involved renal segment may avoid nephrectomy.

RPN can be prevented experimentally by either diabetes insipidus or water diuresis, thereby eliminating the medullary concentration gradient (21). Thus the fluid administration prescribed for gross hematuria is also appropriate to prevent RPN. Angiotensin-converting enzyme (ACE) inhibition can experimentally induce a 50% increase in papillary blood flow (21). This may help prevent RPN, but it could aggravate hematuria acutely by increasing blood flow to a bleeding area.

Tubular Dysfunction

The relevant medullary pathology in SCD is found in the region of the collecting ducts, the inner medulla, and the papilla. This results in dysfunction of the collecting duct and juxtamedullary nephrons (21). Sickling and vascular congestion of the medulla, seen in gross hematuria (6), are probably responsible for reversible concentrating defects. The basis of irreversible medullary dysfunction in RPN is likely the medullary fibrosis and destruction of vasa rectae. Juxtamedullary nephrons and collecting ducts are destroyed, as is found in the model of RPN induced by bromoethylene-HBr in rats (22).

Clinicopathologic Features

Urinary Concentration

The most common tubular abnormality in SCD is a urinary concentrating defect. Typically, HbSS patients achieve a urine concentration of 414 mOsm/kg after 8–10 h of thirst, compared with 911 mOsm/kg in controls (23). Patients with sickle trait may have a diminished urinary concentrating capacity (24). This concentrating defect in children with SCD may result in enuresis (1) and an increased risk of dehydration during water deprivation.

The ability to concentrate urine depends on an intact collecting duct. The collecting ducts of juxtamedullary nephrons extend deepest into the medulla and are capable of generating the highest urine concentration. Poor sodium reabsorption in the collecting duct can result if the sluggish blood flow does not remove the reabsorbed sodium (21). The continued low-grade sickling and medullary congestion result in loss of the normal medullary concentration necessary for water reabsorption. This defective urine concentration can be transiently reversed by transfusion (25, 26). The permanent destruction of collecting ducts by medullary fibrosis in humans with SCD (12) and rats (22) results in an irreversible concentrating defect.

Vasopressin generation is normal in SCD, and the concentrating defect is not responsive to vasopressin. The concentrating defect is unique to sickling hemoglobinopathies; there is no concentrating defect in other anemias (27).

Diluting Capacity

Urinary dilution depends on the solute reabsorption in the ascending loop of Henle of cortical nephrons, which are not involved in SCD patients. They can usually dilute the urine normally (23, 25).

Hydrogen Ion and Potassium Excretion

A proton gradient from tubular cell to lumen underlies acid excretion. Proton secretion is associated with the "intercalated" collecting duct cells, most prominent in the cortical segment of the collecting duct (21). Damage to the papillary segment is therefore unlikely to cause a severe acidification defect. However, juxtamedullary nephrons, which reabsorb HCO_3^-, are also severely

involved in SCD. On this basis, some defect in acid excretion may occur (21).

An incomplete distal renal tubular acidosis (RTA) may complicate SCD, but it is usually not a clinical problem (25). The minimum urine pH achieved in response to NH_4Cl loading is not as low as in controls (5.8 vs. 5.1), but total NH_4 excretion is normal. Consequently, titratable acidity is reduced (28). Kurtzman has also described a "type IV RTA" in SCD, with a reduced ability to lower urine pH in response to Na_2SO_4, and inadequate K^+ secretion, especially in patients with decreased renal function (29). In one series, six of nine nephrotic SCD patients were reported to have type IV RTA (30).

Plasma renin and aldosterone may be increased in the face of medullary fibrosis (25). A protective mechanism likely exists: In the presence of inadequate K^+ secretion, a shift of K^+ to intracellular compartments probably occurs. Because this shift is under β_2 stimulation, β-blockers or ACE inhibition may result in hyperkalemia (25). The electrolyte abnormalities resemble those in type IV renal tubular acidosis but actually result from an aldosterone independent end organ failure secondary to medullary fibrosis.

Proximal Tubular Reabsorption

There is an increased capacity for sodium reabsorption and a decreased sodium excretion with loop diuretics in SCD (23,31). De Jong and van Eps have proposed that the alterations in renal cortical function are adaptive, compensating for defects in medullary sodium and water conservation (12). The increased proximal sodium reabsorption results in decreased distal sodium delivery. Diuretic response is poor, because it depends on this more distal sodium delivery. Proximal tubular phosphate reabsorption, which usually parallels sodium reabsorption, is also increased. This may cause hyperphosphatemia, especially in the presence of an increased phosphate load generated by hemolysis (25).

Tubular Secretion

There is a significant disparity between creatinine clearance (CCr) and inulin clearance (CIn) in SCD. For example, in a small group of patients with SCD and an increased glomerular filtration rate (GFR) measured by CIn (119 vs. normals 97 mL/min), CCr was significantly higher than CIn in SCD (154 vs. 119) but not in normals (114 vs. 97) (23). This is an expression of increased tubular secretion of creatinine in SCD (25). It is partly responsible for a difficulty in accurately assessing GFR in SCD (see below).

Uric acid secretion is similarly increased and is a functional adaptation to high uric acid generation (7). In patients with decreasing total GFR (and increasing GFR per nephron), fractional excretion of urate is further increased by decreased reabsorption (32).

Experimental Models of SCD Tubular Dysfunction

The rat model of RPN induced by bromoethylene-HBr exemplifies distal tubule physiologic disturbances (22). As a baseline, these rats have a more dilute urine than controls. The juxtamedullary nephrons are nonfunctional. Sodium excretion changes little in response to hypervolemia. Although the measured atrial natriuretic peptide (ANP) increases appropriately, an additional high-dose infusion of ANP can generate a normal response. The normal response to hypervolemia is likely mediated through inner medullary interstitial pressure, damaged in this model (and in SCD). In contrast, the response to salt loading generates a normal increased sodium excretion in experimental RPN, regulated by a more cortical mechanism (22).

Role of Prostaglandins in SCD Tubular Dysfunction

A series of studies begun by de Jong and van Eps explored the effects of prostaglandin (PG) inhibition on renal function (12,33). The effect of PG inhibition on tubular function is especially revealing: There was a greater fall (42%) in the fractional excretion of Na (FENa) in response to PG inhibition by indomethacin in SCD patients than in normals (16%). This reflects a greater than normal effect of PGs on the delivery of sodium to the distal diluting segment. Although normal urinary dilution is not affected by PG inhibition, PG inhibition decreases urinary dilution in SCD (23).

PGs increase proximal sodium reabsorption. Under PG inhibition, more solute is delivered to and then reabsorbed by the thick ascending limb of the loop of Henle, thereby increasing interstitial hypertonicity. More free water is then absorbed in the relatively solute-impermeable descending limb, resulting in a decreased

response to water loading (23). Thus, although urinary diluting capacity is normal in SCD, it is being maintained only by PG and will be decreased by indomethacin (12). PG inhibition increases distal delivery, preventing the appropriate effect of vasopressin suppression during water diuresis in SCD (12). Increased proximal sodium reabsorption in SCD results in a decreased natriuretic response to loop diuretics (22), and PG inhibition restores that response.

In contrast to normals, SCD patients fail to increase net acid excretion in response to inhibition of PG synthesis by indomethacin (28) because of decreased NH_4^+ excretion. It is likely that NH_4^+ excretion is maintained at maximum by endogenous PGs.

In summary, the tubular dysfunction of SCD manifests a defect in urine concentration, while dilution is maintained. Hydrogen ion and potassium secretion functions are only mildly affected, and proximal tubular mechanisms are exaggerated.

Treatment

Treatment of tubular disorders in SCD is usually unnecessary if renal function is normal. The risk of dehydration caused by decreased urinary concentrating ability requires earlier treatment of diarrhea or vomiting. There should be a cautious approach to volume expansion as treatment for sickle crises; administration of large volumes of standard sodium-containing fluids to significantly anemic patients with increased sodium reabsorption may result in congestive heart failure. Acidosis may require earlier treatment in the patient with SCD. Hyperuricemia, resulting from increased urate production, may be aggravated by diuretics (especially thiazides) that inhibit urate secretion. The edema accompanying severe anemia may be difficult to treat because the response to diuretics is diminished. Severe hemolysis may exceed the patient's ability to excrete potassium, especially if there is renal insufficiency. β-Blockers or ACE inhibition can aggravate hyperkalemia (25), especially in the presence of some degree of renal impairment (29).

Sickle Cell Glomerulopathy

Clinical

The association of significant proteinuria with SCD has been recognized sporadically and usually described as a nephritic process. As early as 1959 (34), nephrotic syndrome was recognized in SCD. Proteinuria was identified in 17 of 54 patients in 1978 (26). A population study found proteinuria in 20% of 284 patients in a single center, with a prevalence of 29% in adults but only 5% in children less than 10 years of age (35). Bakir et al. recognized nephrotic syndrome in 12 of 240 SCD adults, with 2–20 g protein per 24 h (30). In our series, 87 (26%) of 381 adult patients had significant proteinuria, with 12 in the nephrotic range (more than 2.5 g/24 h) (36). In a more recent study, of 34 adult patients with SCD, 7 had albuminuria with normal GFR and 17 had chronic renal failure, with glomerular injury and loss of ultrafiltration coefficient (37). In this series, GFR was related to hematocrit (38).

Lonsdorfer et al. (39) found that 40–45% of 31 SS patients aged 16–40 years had abnormal proteinuria, mostly selective (albumin). Twelve percent of the 17 patients who were under age 16 had abnormal proteinuria. Of 52 SA patients over age 16, approximately 18% had abnormal proteinuria. Those with S/Thal were similar to SS patients, those with SC disease were between SS and SA. Alvarex et al. (40) more recently noted that far more SS patients had microalbuminuria, but that the those with overt with proteinura tended (2 of 8) to progress in both proteinuria and decreased renal function, as noted by more sensitive cystatin-C. The 2006 study of 300 sickle hemoblobinpathy subjects by Guasch et al. (41) confirmed the progressive and expected development of albuminuria, and common (20%) development of renal insufficiency. The recent and most important long-term study by Powars (42) of over 1,000 SCD patients found an 12% incidence of chronic renal failure, at a median age of 37, as well as an even greater number with pulmonary hypertension.

The definition of sickle cell nephropathy that is most accepted is associated with nephrotic range proteinuria. While long-term studies have not been done, it appears to have a more rapid course than other causes of nephrotic syndrome. In the experience of Bakir (30) 2/3 developed renal failure within 2 years. The onset of renal failure was heralded by increasingly inadequate erythropoiesis (42), and carried a survival time of 4 years.

Pathology

The usual finding in sickle cell nephropathy is focal segmental glomerulosclerosis (FSGS), which is intimately associated with glomerular hypertrophy. Glomerular engorgement and hypertrophy were recognized as part of SCD, as well as FSGS in a 9-year-old nephrotic SCD patient in 1959 (34). Ten of our adult HbSS patients

without significant renal impairment underwent renal biopsy because of proteinuria (36). Eight had FSGS involving a mean of 27% of glomeruli, and the other two had focal global sclerosis. There was focal tubulointerstitial fibrosis adjacent to sclerotic glomeruli. The nonsclerotic glomeruli were all enlarged, with diameters of 186 ± 14.5 μm versus 137.9 ± 19.3 μm in 10 control biopsies. Immunofluorescence was positive only for IgM, C3, and C1q irregularly in sclerotic segments. Electron microscopy confirmed the absence of immune complex-type dense deposits. There was focal electron-lucent expansion of the subendothelial zone in six specimens, with occasional mesangial cell interposition. No new mesangial matrix material was observed to suggest membranoproliferative glomerulonephritis (MPGN).

These findings agree with the description by Churg (43). Glomerular hypertrophy was documented previously in adults with measured glomerular diameter (median 257 μ, range 220–316) greater than in controls (median 193 μ, range 142–253) (44). Bakir et al. described both the nonimmune MPGN-like lesion in nine patients, and FSGS (in 8% of glomeruli) or global sclerosis (in 14% of glomeruli) (30). In addition to FSGS, focal cortical infarcts have been described as a late finding in sickle cell nephropathy (10).

A report on the renal pathology in six HbSS patients with proteinuria described two patterns of FSGS: a "collapsing" and an "expansive" sclerosis (45). Glomeruli were significantly hypertrophied, with diameters of 233 ± 25.3 μ, compared with 158 ± 12.7 μ in normals. HbSS glomeruli were more hypertrophied than in idiopathic FSGS (188.2 ± 17.9 μm). The glomeruli from HbSS patients without clinical evidence of renal disease (243.5 ± 12.5 μm) were the same size as those with proteinuria.

Renal Function in Sickle Cell Glomerulopathy

Hyperfiltration

An increased GFR has been a recognized feature of SCD, especially in children, in studies dating from the 1950s (12). Renal plasma flow, as estimated by p-aminohippurate (PAH) clearance, is elevated in excess of GFR, resulting in a lower than normal filtration fraction (12.9 vs. 17.5) (23). The extraction ratio of PAH (normally 90–95%) is also lower (26). The cause and mechanism of this physiologic alteration is unclear, although it is likely that increased cortical blood flow itself can cause decreased secretion by limiting diffusion from rapidly flowing plasma (26). A more recent analysis suggests a distinctive pattern of increased glomerular permeability (to dextrans) in SCD nephropathy, an increase in pore radius, which is not explained by purely hemodynamic changes. When chronic renal failure develops, the total number of membrane pores is reduced and a size-selectivity defect occurs.

Role of Prostaglandins or Kinins in Glomerulopathy

It has been suggested that hyperfiltration and proximal tubular "hyperfunction" in SCD are a compensation for the distal tubular injury, mediated by the PG systems (12,22). In the studies by de Jong and van Eps (see previous discussion on tubular dysfunction) (12), indomethacin decreased GFR and the estimated renal plasma flow (ERPF) in SCD, without altering that of controls, suggesting that the PG system might be responsible for maintaining the GFR in SCD. Measured PGE_1 excretion did not differ from controls, but PGF_2 was lower (46), thus increasing the ratio of the vasodilator PGE_1 to the vasoconstrictor PGF_2. Allon et al. (23) found that indomethacin decreased PGF_1 (a metabolute and prostacyclin, a vasodilator hormone) more in SCD (46%) than in controls (15%). GFR was decreased 16% by indomethacin in SCD but was unchanged in normals. The more dramatically increased ERPF and decreased filtration fraction of SCD were returned toward normal, and the net effect of PG inhibition was to reverse hyperfiltration.

Overall renal Kallikrein excretion is altered, and while not significantly different from controls, could be related to microalbuminuria. Kinin influence could be hypothetically related to an effect upon vascular smooth muscle control in the presence of denuded endothelium, incapable of generating NO (47).

Pathogenesis

Immunopathogenic Mechanisms

In 1975, Strauss et al. (48) described an SCD membranoproliferative (MPGN) nephropathy in seven patients, four with nephrotic syndrome, three of these under 15 years, with immunopathologic studies suggesting an immunocomplex disease. One proposed cause for the sickle cell nephropathy has been immunologic reaction to renal tubular epithelial cell complexes (12). Most now agree that evidence of immunocomplex deposition is usually lacking in SCD patients with heavy proteinuria.

Relationship to Other Glomerulopathies

The relationship of the pathologic findings of SCD to other nephropathies is unclear. Glomerular hypertrophy with FSGS is also found in the setting of reduced renal mass. Examples are reflux nephropathy, severe obesity (49), and rat models of renoprival glomerulosclerosis (50). In FSGS associated with idiopathic nephrotic syndrome, the glomeruli that develop sclerosis are hypertrophied (53). The nephropathy associated with type I glycogen storage disease (GSD-1) includes both hyperfiltration and FSGS (53) (see Chapter 51). The nephropathy associated with cyanotic congenital heart disease may arise from mechanisms similar to those operating in SCD: low oxygenation and increased blood viscosity from polycythemia (25,52).

Possible Mechanisms of SCD Nephropathy

Hyperfiltration, in concert with direct endothelial damage by occlusion with sickled cells might lead to endothelial hyperplasia and ultimately fibrosis (12,25). The iron deposited in tubular cells as hemosiderin has been suspected to have a role in the chronic nephropathy of SCD (10). The nonimmune complex deposits found in SCD might derive from iron-protein complexes (10). Experimentally, saturated-iron complexes can induce a nephrotic syndrome in rabbits (26). Lande et al. found decreased renal cortical spin-echo signal by magnetic resonance imaging in SCD, suggesting an abnormal renal cortical iron metabolism (55). This does not occur in β-thalassemia, despite similar iron overload. Nevertheless, the iron overload from multiple transfusions in both SCD and β-thalassemia is appropriately treated with deferoxamine, or the more recent oral desferasirox (54).

It is possible that FSGS is the consequence rather than the cause of interstitial fibrosis, which might obstruct the efferent glomerular capillaries, raising intraglomerular pressure and resulting in progressive (reactive) sclerosis (53). In SCD, in which medullary fibrosis is most prominent, the vasa rectae supplying the juxtamedullary nephrons would be most affected by FSGS. Bhathena et al. (45) suggested that the "collapsing" pattern of FSGS, superimposed on already maximally hypertrophied glomeruli, might be a consequence of sickling and ischemic collapse, similar to glomerular "microinfarcts" suggested by Chauhan et al. (10). The "expansive" form of FSGS is viewed as a mesangial cell reaction to capillary collapse.

Both the pathophysiologic and pathologic findings in sickle cell nephropathy resemble those in the rodent model of glomerular hypertension induced by renal mass reduction (56). Thus hyperfiltration, glomerular enlargement, and focal and segmental glomerulosclerosis could be a result of an increase in intraglomerular pressure as a consequence of efferent arteriolar vasoconstriction. In that model, and in others, the glomerular hypertension and the pathologic consequences are attenuated by angiotensin II-converting enzyme (ACE) inhibition.

Any hypothesis should take into account the glomerular hypertrophy that is always present in SCD, probably related to the anemia itself. Proteinuria is not invariable in SCD and appears to be unrelated to the number and severity of SCD crises or the presence of hematuria, RPN, etc. Therefore some other factors are likely to be operative in those at greatest risk of proteinuria and FSGS. Systemic hypertension is notably absent in these patients (36). The presence of hyperfiltration, glomerular hypertrophy, and FSGS does not imply that these findings are sequential or causative. A common stimulus may be operative, such as the growth promoting hormones and cytokines (57,58), to which the glomerulus may be sensitive.

Diagnosis

Hyperfiltration

The upper limit of the normal range for GFR is not certain, even with CIn, the "gold standard." The reliability of the clearance methods that can substitute for CIn have not been validated in the elevated range. In adult SCD subjects aged 40–75 years, CCr correlated well with the clearance of ^{51}Cr-EDTA when clearance did not exceed 110 mL/min, although ^{51}Cr-EDTA exceeded CCr by almost 30% (58). In normals, the urinary clearance of ^{51}Cr-EDTA is 85–95% that of CIn, while the clearances of ^{99m}Tc-DTPA and ^{125}I-iothalamate are nearly identical to that of inulin (59). Because CCr usually exceeds CIn in normals, its validity in SCD is uncertain. The simplest estimation of GFR, by several formulas, uses PCr alone (60), but an additional problem in the use of these formulas is the greater overestimation of GFR by CCr in SCD patients than in normals. The plasma clearance of ^{51}Cr-EDTA following bolus injection approximates CIn in normals (61) but at high clearances is far less accurate.

The attempt to simply and correctly assess GFR is an important recent effort, to detect early, and possibly preventable, decreases in GFR in a population with an intrinsically high GFR. A decrease in GFR, particularly when accompanied by proteinuria, is ominous (63). However, the effects of treatment are difficult to assess in other than a clinical research environment, although simplified methods for non-isotopic iothalamate and PAH measurement are now available (64).

As a further development, in a study of sickle-B-Thallasemia patients, Voscaridou et al. (65) found that plasma cystatin-C (Cys-C) measurement correlated well with CCr in patients with decreased CCR, and in those with demonstrable proteinuria, but not in those with CCr >90 ml/min. In the normal and elevated range, in obese type II diabetic Pima Indians, Perkins et al. (66) were able to show good correlation between Cys-C and iothalamate clearance, and could show that a progressive decrease in iothalamate GFR was best demonstrated by Cys-C, rather than Cr-based formulae. Alvarez et al. (67) were able to study CCr, Cr, Cys-C and microalbumuria and frank proteinuria in children with SCD, and found that while Cr, and CrCl did not distinguish this population, Cys-C showed progressive decreases in clearance with microalbuminuria and proteinuria.

We (Scheinman JI and Belmont JM, unpublished) examined a database of multiply-transfused SCD and β-thal patients, with eGFR (Cr) over 85 mL/min, in a control period before an experimental iron removal intervention, measuring simultaneous Cr (Schwartz (60) or Cockroft-Gault (68)) and Cys-C (Filler (69)) estimates of GFR. For all 169 SCD patients, the eGFR (Cr) and eGFR (Cys-C) were well correlated ($r = 0.35$, $p = 0.001$). For the 71 SCD patients with eGFR (Cr) >200 the correlation was poor ($r = 0.06$), but was strong for the 98 SCD patients with eGFR (Cr) <200 ($r = 0.36$), and the 35 with eGFR (Cr) <150 ($r = 0.43$). When the analysis was restricted to the 120 patients whose repeated measures varied by no more than 20%, a similar correlation was found when eGFR (Cr) <200 ($r = 0.35$), and the relationship was even stronger with eGFR (Cr) <150 ($r = 0.59$). The correlation of the two estimates of GFR in the 662 β-thal patients was poor at all ranges ($r <= 0.20$). Therefore our preliminary impression is that if an appropriate eGFR (Cr) formula yields a value <200 mL/min, that same formula may very well be able to adequately estimate a change in GFR within the normal to elevated ranges.

Proteinuria detected by dipstick in a patient with SCD should be quantified and renal function assessed. Diseases other than sickle cell glomerulopathy should be considered. If hematuria is present, RBC casts may point to pathology other than sickle cell glomerulopathy. Hypertension, hypocomplementemia, and antinuclear antibodies also suggest other diagnoses. Judging from the relatively uniform findings in our series (36), few other additional studies are indicated.

Treatment

The course of the progression of FSGS to chronic renal insufficiency (CRI) in SCD remains difficult to assess, in part because of the difficulties in quantitation of renal function described above (64). Without a known cause, it is difficult to prescribe a treatment for the nephropathy of SCD. The patients with proteinuria in our series (36) were not those with most frequent sickle crises, nor the severest anemia. Nevertheless, some factors in the sickle cell condition must predispose to the nephropathy. Therefore it is reasonable to attempt to minimize sickling and those factors known to promote FSGS in other primary diseases or in animal models, but it is controversial whether hemodynamic alterations can alter the progression of FSGS to CRI (57).

Protein Restriction. A high protein intake accelerates the development of FSGS in uninephrectomized rats, without necessarily causing glomerular hyperperfusion (53). It is therefore attractive to consider protein restriction in the management of SCD nephropathy, as is being tried in several forms of renal disease. In children, restriction of protein intake may carry unreasonable risks (71). Delayed growth and development is already a particular risk in the SCD patient. Therefore, we advise only the avoidance of an unusually high protein intake (greater than the recommended dietary allowance).

Angiotensin-Converting Enzyme Inhibition. Glomerular hyperperfusion and proteinuria could be mediated through increased glomerular capillary pressure, reduction of which by ACE inhibition might protect the glomerulus from FSGS.

In our 2-week trial of enalapril therapy of 10 patients with mild SCD nephropathy, BP, GFR (CIn), and ERPF (PAH clearance) did not change significantly, whereas proteinuria diminished by 57%, rebounding after treatment withdrawal (36). A more recent 6-month controlled trial of enalapril in 22 SCD patients with microalbuminuria showed a significant decrease in the treatment group, while the control group increased (72). Whether long-term ACE inhibitor therapy has a salutary effect in preventing renal insufficiency is untested.

Chronic Renal Insufficiency

Clinical

Renal failure is one of the major organ failures that occur in SCD, almost certainly the consequence of the progression of FSGS. Of 22 patients with nephrotic syndrome, 68% developed CRI (30). Population studies of SCD have all shown a significant incidence of CRI. Of 368 patients in one sickle cell center, 4.6% had CRI (73) associated with proteinuria and increased age. In our series of 375 patients, 6.7% had CRI (36). In a series of 785 patients from California, 33 had CRI (4.2%) (74). Overall, 5–18% of SCD patients develop CRI (75).

The epidemiology of renal involvement in SCD may depend upon other genetic factors that effect the levels of fetal hemoglobin (HbF), the tendency to sickle, and other contibutions. Powars (76) initially reported the association of β-globin gene cluster haplotypes with renal involvement: The Central African Republics (CAR) haplotypes (Bantu, Cameroon) or Benin (intermediate involvement) versus non-CAR (Senegalese and Arab/Indian) phenotypes. Guasch (77) found no association with these haplotypes, but instead, with microdeletions in the α- globin gene: Microalbuminuria was found in 22 of 76 (29%) adult SCD patients, but only 13% with the micro-deletions, versus 40% without those micro-deletions ($p = <0.01$). Those factors that effect the interactions of RBCs with endothelium may be equally important (see below).

Treatment

Specific treatment of the patient with SCD and renal failure has been poorly explored, and the problems of CRI are only magnified by SCD. Patients with renal failure, even if it is mild, sometimes have symptomatic anemia requiring transfusion. In some of these patients, treatment with erythropoietin can variably restore hemoglobin concentrations to higher levels (78). A few patients have been treated with hydroxyurea plus erythropoietin, with apparent benefit (79).

Dialysis and Transplantation. The U.S. Renal Data System reported the causes of renal failure for 255,573 patients treated from 1989 through 1993 (62). Overall, 235 were SCD patients. This is far less than the 1% expected from the incidence of HbSS in the total population. It is possible that physicians do not offer treatment to many SCD patients who develop CRI, assuming that the other problems are insurmountable.

Ojo (80) reviewed the transplant results in SCD and found 82 patients. There was no difference in the 1-year cadaveric graft survival (SCN: 78% vs. Other-ESRD: 77%), and the multivariable adjusted 1-year risk of graft loss indicated no significant effect of SCN (relative risk [RR] = 1.39, P = 0.149). However, the 3-year cadaveric graft survival tended to be lower in the SCN group (48% vs. 60%, P = 0.055) and their adjusted 3-year risk of graft loss was significantly greater (RR = 1.60, P = 0.003). There was a trend toward improved survival in the SCN transplant recipients compared to their dialysis-treated, wait-listed counterparts (RR = 0.14, P = 0.056). In comparison to the Other-ESRD (relative risk (RR) = 1.00), the adjusted mortality risk in the SCN group was higher both at 1 year (RR = 2.95, P = 0.001) and at 3 years (RR = 2.82, P = 0.0001) after renal transplantation. They also found a trend toward better patient survival with renal transplantation relative to dialysis in end-stage sickle cell nephropathy.

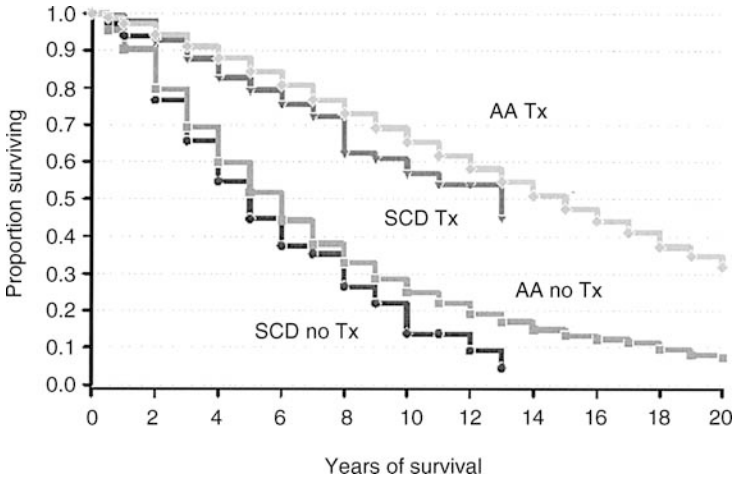
In an analysis of the UNOS registry from 1987 to 1996 (81), 54 patients were found with SCD, renal failure, and data on 1st renal transplant outcome. Patient survival for SCD was 90% at 1 year and (of 30 patients) 75% at 3 years. The proportional risk ratio (compared to IgA nephropathy) was 7.8, the greatest for any condition. First graft survival for SCD was 82.5% at 3 years, 54% at 3 years. The proportional risk ratio for graft survival was 1.77, but after correction for patient deaths with functioning graft, only 1.06 (barely an increased risk).

We have explored (unpublished) the USRDS Data files 2000, for evidence of the effect of SCD on the outcome of chronic renal failure. While the diagnostic code (assigned at the time of renal failure) for sickle cell nephropathy yielded 904 patients, further exploration of patients discovered by hospitalization codes to have SS disease yielded a total of 1,656 patients, 237 transplanted and 1,419 not transplanted, and even after eliminating other causes of renal failure (diabetes mellitus, etc.). SCD patients were compared to all other African American (AA) patients, for life-table survival. Without transplant, they significantly differed, with vastly different numbers, and projected 10-year survival was poor for both groups, 25% for AA, and 15% for SCD.

Further, the 36,264 transplanted AA patients had better survival than the 210 SCD patients, but the lifetable projected survivals were still quite close, approximately 50% survival at 15 years (*Fig. 49-4*). Using an age-adjusted cohort of AA patients as controls, the difference statistically disappeared ($p < 0.19$). Comparing the non-transplanted AA patients with the SCD patients was different, but both extremely poor: 25% for AA patient, and 14% for SCD patients at 10 years.

Figure 49-4

Lifetable analysis of patient survival, of end-stage renal failure patients having SCD or age-matched African-American (AA) controls, treated with kidney transplantation or dialysis alone. All curves differ significantly, due to the vastly greater numbers of AA patients (data unpublished).



A comparison of the 153 transplanted SCD patients with those who received no transplant showed a far better survival curve, 56% versus 14% at 10 years, even when compared to those 133 SCD patients who were placed on the transplant waiting list but never got a transplant. This was similar to the difference between all transplanted AA patients and Non-transplanted AA patients assigned to the transplant waiting list. Of 957 patients since 1991, essentially the cyclosporin era, only 53 SCD patients received transplants, versus 898 not Transplanted, and survival is projected at 67% at 7 years, versus 83% for the AA cohort.

These results continue to make the case that transplantation is a better option for the SCD patient with renal failure. However, results may be less satisfactory than for other AA patients, and grafts have been lost due to demonstrable massive sickling events.

The problems that have occurred in SCD patients after transplantation, especially the recurrent sickle crises, could have been aggravated by the increased blood viscosity associated with a rising hematocrit. These have been and treated with frequent partial exchange transfusions (82). In one early series, seven of eight had frequent sickle crises after transplantation (83). Renal venous thrombosis and infarction have been reported (84). In one patient with HbAS, the transplanted kidney was unfortunately removed for an apparently irreversible acute rejection that was actually intrarenal sickling (83).

The management of renal transplantation in SCD should then attend to potential immediate problems; it is reasonable to warm the kidney with 37°C saline, to infuse dopamine at 4 g/kg per min during and immediately postoperatively, and to provide 40% oxygen, with intravenous fluids to decrease viscosity. Partial exchange transfusion may be provided at 4-week intervals (83). It is possible that before an adequate erythropoietin (EPO) response occurs from the transplanted kidney, recombinant EPO should be given.

Sickle cell nephropathy has been reported to recur in as little as 3½ years, although other factors contributed (85). Accelerated recurrence of native disease has been noted in the transplanted kidney in other diseases, notably diabetic nephropathy (86). It is possible that the use of hydroxyurea, in itself likely to interact with other immunosupressants, can be utilized to prevent this. Bone marrow transplantation can cure SCD, and the possibility of its coupling with other transplants will undoubtedly be explored. Caution will be needed, in view of the possible increase in parvovirus (87), a common agent of aplastic crisis that might be especially dangerous in the transplant patient.

Other Problems in SCD Related to Nephrology

Anemia

In the presence of continued hemolysis, the patient with SCD depends on continued erythropoiesis, which

determines the level of anemia. Erythropoiesis can be measured by "erythron transferrin uptake" (88), independent of iron or transferrin saturation. A heme protein in the kidney senses a decreased level of O_2 delivery to the kidney and stimulates erythropoietin production (89). The SCD patient with renal failure loses this normal homeostatic mechanism, becoming far more anemic. Even in the presence of a competent bone marrow and erythropoietic stimulus, adequate iron is necessary. Patients with ulcers may subtly lose iron (10).

The Hb level should probably be maintained at 50–60% of normal in SCD, in view of the serious cardiac consequences of more severe anemia (90). Although an intrinsic cardiomyopathy has been suspected in SCD, a careful autopsy study of 52 patients with a mean age of 17 years, concluded that anemia alone caused heart failure (90).

Sickle Crises – Clinical Features

Sickle cell crises (1) are painful episodes of vaso-occlusion, often accompanied in the 2nd or 3rd day by fever without documented infection. Neither abnormal blood viscosity nor the number of sickle cells absolutely predicts the frequency of crises (4). The incidence of pain is 0.8/year in SS and 1/year in S-β-thalassemia (91). With greater degrees of anemia, there is less pain, probably because of diminished blood viscosity. With increasing HbF, there are proportionally fewer painful crises, suggesting a beneficial effect of even modest increases in HbF (91).

Crises may occur in infants as the hand-foot syndrome because of poorly developed collateral circulation. The chest syndrome is accompanied by radiologic "white-out" and is life threatening (92). The abdominal crisis is like a "surgical" abdomen but usually without rebound tenderness. In the presence of hematuria, the origin of the pain could be assumed to be the kidney. Neuromeningitis, multi-infarct dementia, or large-vessel occlusion (stroke) can occur (93), especially in infants and children (10). Genetic factors, related to the inflammatory mechanisms, may help to predict the risk of stroke (94) and other complications of SCD. A sudden anemia may be caused by sequestration crises in the spleen in the first 2 years of life, and thereafter, after development of splenic fibrosis, occurs more often in the liver. Aplastic crises arise from vaso-occlusion and hemolysis without compensatory erythropoiesis, often induced by a parvovirus-like agent.

Priapism

Priapism is a specialized vaso-occlusion of the penis, which was found as often as 42% in one series (1). Painful, hot, tender erection, most often on waking, lasts up to 3 h. This can be preceded by days or weeks of "stuttering." The engorgement is of the corpus-cavernosa, usually not the spongiosum in children, with the glans flaccid (10). The pain is referred to the perineum and to the abdomen, requiring analgesia, often exchange transfusion. Few require surgical drainage or a shunt. Fibrosis may result, with consequent impotence (10).

Pathophysiology of Sickling

The clinical heterogeneity of SCD is further determined by recently described aggravators of sickling related to oxidative stress. Hb from RBCs of SCD patients has increased auto-oxidation, increased generation of superoxide and H_2O_2, OH^-, and lipid peroxidation products (95). In a transgenic model of SCD, increased oxidant stress extends renal sickling from a more limited area in the renal medulla, to a more extensive distribution to the cortex (96). The aggravating factors of sickling are likely to include the agents of microvasular inflammation and constriction, and inhibitors of relaxation. Reactive species can divert vasoactive NO from mechanisms of vascular relaxation to agents of inflammation and constriction (95). Integrins (α4β1) of the sickled RBC bind to both fibronectin (an acute phase reactant) and vascular cell adhesion molecule 1 (VCAM-1) on endothelial cells, which itself is increased by inflammatory cytokines such as TNFα.

Hemoxygenase (HO-1) defends against and degrades heme, a lipophilic pro-oxidant, formed from the splitting of Hb (96), studied in a transgenic sickle mouse. In the HO-1 knockout model, administration of heme protein induces monocyte chemoattractive protein (MCP-1), mediated by NF-kB, and results in renal interstitial cell inflammation (97).

Thus increased induced oxidative stress from the sickling process precipitates acute vaso-occlusive disease, by mechanisms that affect the endothelium, and adhesion to it, as well as vasoconstriction.

The distorted sickle cell is partly explained by dehydration of the cell, by enhanced KCl co-transport, induced by cell swelling and acidification (98). Further, K+ and water efflux are enhanced by transiently increased SS cell cytosolic Ca, induced by the membrane distortion.

Acute Renal Failure

A doubling or more of PCr was found in 12 of 116 hospital admissions of patients with SCD. It was seen most often with infections and evidence of rhabdomyolysis, and in patients with lower Hb (mean 6.4 vs. 8.7 g/dL) (99). Volume depletion was the most common precipitating cause. In that study there was an 83% patient survival with recovery of function in all patients who survived, without progression to ESRD. It is likely that non-steroidal anti-inflammatory agents are partly responsible for some episodes of acute renal failure (100), in view of the likely maintenance of GFR in SCD by prostaglandin mechanisms.

Rhabdomyolysis with acute renal failure and disseminated intravascular coagulation has been seen (rarely) in sickle trait with rigorous military training (101). Similarly, there is an apparently increased risk of sudden unexplained death in patients with sickle trait (102).

Hypertension

Hypertension is unusual in SCD (2–6%) compared to the published incidence for the black population in the United States of 28%, as found by the Cooperative Study of Sickle Cell Disease (103), at all age ranges.

In our series (36), hypertension was rare, only found in 1 patient with nephrotic-range proteinuria, without renal insufficiency. The 1/3 incidence of hypertension in Powar's patients with renal insufficiency is unexplained (104). Nevertheless, she found a positive association between BP, stroke, and increased mortality in SCD (105).

Infections

Infections occur in HbSS as a result of vascular sequestration with tissue necrosis (e.g., osteomyelitis associated with avascular necrosis, or *Salmonella* infection with cholelithiasis) and the immune susceptibility of the splenectomized patient (1). The organisms most commonly found are *Diplococcus pneumoniae, Haemophilus influenzae, Mycoplasma,* and *Parvovirus* (10). Prophylactic penicillin is essential, as for any splenectomized patient. *H. influenza B* vaccination is also appropriate.

Urinary infections are uncommon in SCD, except perhaps in pregnancy. ^{99m}Tc-diphosphonate scan shows an increased renal localization in HbSS. This is apparently not caused by infection but more likely by regional stasis or tubular ischemia, causing peritubular extravasation (106).

Endocrine Abnormalities in SCD

Several clinical problem found in SCD can interact with both SCD itself, and with the nephrologic problems. Growth failure, hypogonadism, delayed puberty, and others may be independent endocrine defects or linked to iron overload (107).

General Treatment Strategies for Sickle Cell Disease

Institution of frequent transfusion can reduce the risk of recurrence of cerebral vasculopathy (93) by decreasing the proportion of rheologically abnormal cells. The risks of transfusion should be reserved for patients at greatest risk of sickling sequelae. Most recently, Doppler ultrasonography has helped identify cerebral vessels at risk of stroke (108). In turn, the long-term effects of iron overload are important with or without frequent transfusions, so that the use of new oral iron chelation agents is fortuitous (109).

Other hemoglobins modify the tendency of HbS RBCs to sickle. Fetal Hb (HbF) inhibits sickling. 5-Azacitidine can increase HbF, probably by recruitment of HbF cells, but may be a carcinogen (110). The only agent able to significantly decrease sickling by myelosuppression of HbSS cells, thus increasing HbF, is hydroxyurea. Hydroxyurea is a cytotoxic agent that inhibits growth of burst forming unit-erythroid (BFU-E) (111). In SCD, it is the SS cells that are suppressed, allowing an increase in HbF.

Charache et al. (112) achieved 8–18% HbF levels maintained over 2 years. It has been deduced that the level of HbF likely needed to produce a definitive clinical change is more than 20%, for either hemolysis or vasoocclusion (113). A trial in 59 patients (114) suggested that a mean HbF of 15% could be achieved. Trials of hydroxyurea in adults and children (115) have shown a safe reduction in acute episodes, and allowed normal growth and development (116). In addition to its effect on RBC generation, it induces oxidative damage to SS cells greater than to AA cells, even more than to HbF (117). Antioxidants such as α-tocopherol and ascorbate are protective in vitro, and may enhance the safety of hydroxyurea.

Other mechanisms that can minimize the tendency to sickling and vaso-occlusion have been explored. Duffy

glycoprotein on RBCs may help to clear inflammatory cytokines, and therby protect from organ damage (118). Reversing the cellular dehydration in SCD has been explored by blocking cation-transport (Gardos) channels in erythrocyte membranes and can shift cellular cation content and cell density toward normal (119). Clotrimazole, an antifungal drug, reduced cellular dehydration in vitro in transgenic mice with sickle cell disease and in patients with sickle cell anemia. Magnesium salts also interfere with cation transport and cause cell rehydration (120). Zinc sulfate was shown in a well-designed controlled study to decrease sickle crises by this mechanism (121).

Whether the cellular changes induced by clotrimazole, magnesium salts, Gardos channel blockade or other approaches are of significant clinical value are not yet known. Combinations of hydroxyurea plus clotrimazole and erythropoietin to prevent sickling have been studied in transgenic mice.

Bone marrow transplantation can cure SCD, and the possibility of its coupling with other transplants will undoubtedly be explored. Less ablative conditioning for bone marrow transplant is being explored (122) Caution will be needed, in view of the possible increase in parvovirus (87), a common agent of aplastic crisis that might be especially dangerous in the transplant patient.

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50 Diabetic Nephropathy

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Introduction

Over the last decades, the incidence of childhood-onset type 1 diabetes has significantly increased, particularly in children under the age of 5 years (1). Microvascular and macrovascular diseases represent serious long-term complications, which contribute to the poor prognosis of children and adolescents with diabetes (2–4). Recent data from the USA have shown that the number of life-years lost among children diagnosed with diabetes at the age of 10 years is 18.7 for boys and 19.0 for girls, thereby underlining the burden of the disease (5).

This chapter offers an overview of diabetic nephropathy (DN) in children and adolescents with type 1 diabetes.

Epidemiology

DN affects about 20–40% of subjects with diabetes and is the leading cause of end-stage renal disease (ESRD) in developed countries (6). The number of patients with DN is also growing in many parts of the developing world (6). In the USA, DN accounts for about 44% of new cases of ESRD (7) and similar data have been reported in Europe and other western countries (8). DN is also a major determinant of morbidity and mortality related to the increased risk of cardiovascular disease (9).

DN is defined as persistent proteinuria, greater than 500 mg/24 h, or albuminuria greater than 300 mg/24 h (10). A progressive increase in blood pressure and decline in glomerular filtration rate (GFR) are generally associated with the development of DN and they contribute to the progression towards ESRD (10).

Although overt DN or kidney failure is rarely seen in children with either type 1 or type 2 diabetes (less than 1% of pediatric patients) (11, 12), early structural and functional renal alterations develop soon after diagnosis in children, and accelerate during puberty (13, 14). Microalbuminuria (MA), defined as an albumin excretion rate (AER) between 20 and 200 μg/min or 30–300 mg/24 h, is the earliest stage of clinical nephropathy and its persistence is highly predictive of progression to overt nephropathy and also of cardiovascular disease (15, 16). The cumulative prevalence of MA in people with childhood-onset type 1 diabetes is 12–25% after 5-10 year diabetes duration (11, 17–21). Recent data from a prospective observational study has shown a cumulative prevalence of MA of 25.7% after 10 years and of 50.7% after 19 years of duration (22). Longitudinal studies suggest that MA is persistent only in 50% of children and adolescents, whereas in the other 50% MA can revert to normoalbuminuria at the end of puberty (21, 22). However, in 20–30% of patients with transient MA, it recurs during follow-up. With regard to overt nephropathy, in a recent population-based study of childhood-onset type 1 diabetes (22), the cumulative prevalence of macroalbuminuria has been found to be 13.9% after 19 years of diabetes duration, but lower rates were detected in previous clinical-based studies (18–20).

In addition, in children and adolescents, as in adults, a progressive increase in albumin excretion within the normal range has been found to be an important predictor of future development of MA (23, 24). Therefore, detecting these early alterations might help in identifying children and adolescents with type 1 diabetes at risk of DN and to implement preventive and therapeutic strategies to reduce the burden associated with this complication.

Natural History

The changes occurring in the kidney in patients with type 1 diabetes are generally classified into five stages, reflecting specific alterations in renal morphology and function (25) (*Table 50-1*).

The first stage in the natural history of DN is characterized by functional anomalies, including hyperfiltration and hyperperfusion, which are associated with increased renal size (25). These alterations are present at the diagnosis of diabetes in 25–50% of patients (25). Blood pressure is generally normal during this period, whereas albumin excretion may be increased, but it is reversible (25). These early functional anomalies are related to hyperglycemia and they generally reverse after insulin treatment, even though they can persist in some patients (25). Apart from hyperglycemia, several other

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Table 50-1

Stages of Diabetic Nephropathy: structural alterations, changes in albumin excretion, glomerular filtration rate and blood pressure

	Main structural alterations	Albumin excretion	Glomerular filtration rate	Blood pressure
STAGE 1 (at diagnosis): Hyperfunction/Hypertrophy	Increased renal size	May be increased, but it becomes normal after starting insulin treatment	Increased by 20–50%	Normal
STAGE 2 (after 2–5 years): Normoalbuminuria/Silent phase	Basement membrane thickening	Normal with transient increases related to poor glycemic control or exercise	Normal/ increased by 20–50%	Normal
STAGE 3 (after 6–15 years): Incipient diabetic nephropathy/ Microalbuminuria	Further basement membrane thickening and mesangial expansion	Increased: 20–200 μg/min or 30–300 mg/24 h	Normal/ increased	Increasing
STAGE 4 (after 15–25 years): Overt diabetic nephropathy/ Macroalbuminuria	Marked renal abnormalities	Further increased:>200 μg/min or >300 mg/24 h	Decreased	Increased
STAGE 5 (after 25–30 years): End stage renal failure	Advanced glomerulopathy	Macroalbuminuria/Often decreased due to glomerular occlusion	Marked decreased	Increased

mechanisms have been implicated in the pathogenesis of increased GFR, such as angiotensin II, prostaglandins, kinins, growth hormone (GH) and insulin-like factor-I (IGF-I), nitric oxide, and glucagon (26). It is not clear yet whether the primary abnormality in this phase is hyperfiltration or renal hypertrophy. On the basis of the "vascular hypothesis," the first alteration is hyperfiltration, as a consequence of defects in vascular control, and this is then followed by renal hypertrophy (26). In contrast, the "tubular hypothesis" suggests that renal hypertrophy comes first, due to an increased production of growth factors and cytokines induced by hyperglycemia (26). Longitudinal studies in children and adolescents with type 1 diabetes have suggested hyperfiltration as an independent predictor for MA and DN (27–30). However, other studies have failed to detect such a predictive value for hyperfiltration (31, 32) and therefore this topic still remains controversial.

The second stage in the natural history of DN is represented by a "silent phase," which develops over 2–5 years after diagnosis and is characterized by subtle morphological changes, including basement membrane thickening, mesangial expansion, glomerular hypertrophy and a modest expansion of the tubulo-interstitium (24). During this phase, patients may occasionally present MA, particularly during periods of poor metabolic control or intensive exercise. There is increasing evidence suggesting that during this phase, there is already a progressive increase in albumin excretion within the normal range, which represents an important predictor for MA, DN and cardiovascular disease (23, 24).

A progressive increase in albumin excretion leads to the phase of MA (or incipient nephropathy), defined as a urinary AER of 20–200 μg/min or 30–300 mg/24 h (10). The development of persistent MA may be associated with increased arterial blood pressure and a progressive decline in renal function, with a high risk of developing ESRD and an increased risk for cardiovascular disease (10). At the stage of MA there are already well-established histological and functional renal alterations (33).

A progressive increase in AER leads to the fourth stage of DN, which corresponds to overt nephropathy or macroalbuminuria, as defined by albustix-positive proteinuria (AER > 200 μg/min or >300 mg/24 h). Overt nephropathy is characterized by proteinuria, hypertension and a progressive decline in GFR, which, without treatment, evolve to ESRD (fifth stage of DN), generally 5–10 years after the diagnosis of proteinuria (25).

Risk Factors

Several risk factors, both modifiable (glycemic control, hypertension, dyslipidemia, diet, smoking) and non-modifiable (diabetes duration, puberty, genes, constitutional factors), have been related to the development of DN.

Glycemic Control

Several observational studies have shown a strong correlation between glycaemic control, as assessed by HbA1c, and DN in adults and children with type 1 and type 2 diabetes (20, 22, 34). In addition, it has been demonstrated that the probability of reverting from MA to normoalbuminuria is significantly related to a better glycemic control (35). Further evidence for the fundamental role of hyperglycemia in the pathogenesis of DN in type 1 and type 2 diabetes comes from two landmark interventional studies, such as the Diabetes Control and Complication Trial (DCCT) (36) and the United Kingdom Prospective Diabetes Study (UKPDS) (37). The DCCT, a multicentre prospective controlled clinical trial involving 1,441 patients with type 1 diabetes, has proved the effect of maintaining low levels of HbA1c in reducing the development of MA by one third, during a mean follow-up of 6.5 years. The DCCT cohort included a group of 195 adolescents, aged 13–17 years, but not prepubertal children. In this adolescent cohort, intensive compared to conventional treatment reduced the risk and progression of MA by 54% (38). In addition, the DCCT follow-up study, the Epidemiology of Diabetes Interventions and Complications (EDIC) (39), highlighted the important phenomenon of "metabolic memory." In fact, even though 2 years after the end of the DCCT, HbA1c levels were already similar between the previously intensive and conventional treated groups, those who benefited in the past from a better metabolic control still had an advantage in terms of development of complications (39). With regard to the adolescent cohort, the EDIC study showed that, in the previously intensively treated group, the risk of MA and proteinuria decreased by 48 and 85%, respectively (39). Another interesting finding that emerged from both the DCCT and the UKPDS (36, 37) was the lack of a clear threshold for HbA1c below which the risk of complications is annulled; instead there was a continuous reduction in complication risk as glycemic control improved. In contrast, another study showed a threshold effect for the development of MA at an HbA1c value of about 8.1% (40).

Hyperglycemia contributes to the development of DN through several mechanisms: activation of the polyol and hexosamine pathways, activation of protein kinase C (PKC), increased oxidative stress, increased production of advanced glycation end-products (AGEs), increased synthesis of growth factors, cytokines and angiotensin II (41). These factors can induce relevant structural and functional changes in the kidney. Furthermore, many of these factors can also promote early alterations in endothelial cells, which are responsible of the development of endothelial dysfunction. Several studies have shown that endothelial dysfunction is associated with the development not only of macrovascular disease but also of microvascular complications, such as MA and DN (42).

Blood Pressure

Increased blood pressure in people with diabetes significantly increases the risk of progression towards ESRD (28). Evidence also exists of a link between increased blood pressure and earlier stages of DN, such as MA (10). A direct correlation between albumin excretion and increases in blood pressure is present, even when both these parameters are still in the normal range and, in patients with MA, improvements in albumin excretion are often seen after antihypertensive treatment (43, 44). However, the temporal relationship between MA and rises in blood pressure has not been clarified yet, as different studies have often reached different conclusions (45–48). On one hand, increases in blood pressure have been found to precede MA and therefore to influence its development (46, 48). On the other hand, other studies have not supported this hypothesis and suggest that the two phenomena could appear together (45, 49). Increases in blood pressure, particularly at night, are often associated with MA and DN (50). However, subtle alterations in blood pressure and an impairment of its circadian rhythm can often be undiagnosed through assessment of office blood pressure. In contrast, the use of ambulatory blood pressure monitoring has allowed the identification of early alterations in blood pressure, both in adults and children with diabetes (50). An impairment in the nocturnal fall in blood pressure has been suggested as a predictor of future development of MA (51). In a group of 75 adolescents followed for a mean of 5 years, an impaired circadian variation in blood pressure with loss of the nocturnal dipping was detected before the onset of MA (51). However, the predictive value of the non-dipping phenomenon has not been consistently reported in all studies. In particular, some studies have shown significant overlaps in the nocturnal fall in blood pressure between normoalbuminuric and microalbuminuric subjects (52). In addition, differences between these two groups often disappear after adjusting for potential confounders, such as diabetes duration (52).

Dyslipidemia

It has been suggested that measurement of plasma lipids can add to the prognostic value of albumin excretion rate

measure in the prediction of subjects at risk of developing DN (53). Based on adult studies, subjects developing MA have higher cholesterol levels than subjects who do not progress (54) and reduction in cholesterol levels predicts regression of MA to normoalbuminuria (54). Triglyceride levels have also been suggested as a predictor of progression or regression of MA (55, 56). However, there is a lack of longitudinal studies investigating the relationship between lipid levels and the development of MA and DN in children and adolescents. On the basis of available cross-sectional studies, it appears that total cholesterol and low density lipoprotein cholesterol levels can influence the development of DN (57, 58). Similarly, from a longitudinal study it has emerged that cholesterol levels could influence the development of MA in children and adolescents with type 1 diabetes (59).

Puberty

Puberty represents an important risk factor for DN and MA often develops during this period of life in children with type 1 diabetes (60). Pubertal development is characterized by many physiological changes, involving both hormonal and metabolic processes (61). These factors together with psychological issues are frequently responsible of a poor metabolic control (61). However, there is also clear evidence that puberty itself, independently of glycemic control, is a risk factor for the development of MA, with a significantly higher risk for female than for male subjects (62). Changes in sex hormones and in the GH-IGF axis have been shown to play an important role in this context (63). These changes can interact with the diabetic milieu and the genetic background and cause a higher predisposition to the development of complications, such as DN. The higher testosterone levels and free androgen index found in adolescent girls with MA when compared with matched controls without MA (63) could contribute to renal disease and also to the female predominance of this complication during puberty (22, 62, 63).

Puberty is generally associated with a decrease in insulin sensitivity, which reaches a peak at stages 3 and 4 (64, 65). Adolescents with type 1 diabetes are more insulin resistant when compared with healthy controls (61), and this has mainly been attributed to the effect of increased GH. Circulating GH levels are increased in adolescents with type 1 diabetes, whereas circulating IGF-I levels are decreased, due to reduced portal insulin and consequent lack of effect of insulin on the expression of GH receptors in the liver (66, 67). In contrast, as the expression of GH receptors seems to be insulin-independent in other peripheral organs and tissues, such as the kidney, an increased local production of IGF-I with an associated paracrine action could be implicated in renal disease (66, 67). In cross-sectional studies these high renal levels of IGF-I have been associated with the development of DN (68). Increased urinary GH and IGF-I levels have been found in adolescents with type 1 diabetes and correlated with albumin excretion (68). In addition, increased urinary GH has been related to increased renal size, which in turn is a risk factor for MA (68).

Age at Diagnosis and Duration of Diabetes

Duration of diabetes is another major factor associated with the risk of complications (4). In children, the relationship between diabetes duration and risk of MA has been found in some studies (14, 19, 62), but not confirmed in others (12). The role of prepubertal duration of diabetes has been the object of wide discussion, generated by discordant results that emerged from several studies. Although in some studies (14, 69–71) the risk of developing MA was higher in children with onset of diabetes at or after puberty, suggesting that prepubertal duration was not a main determinant, other studies have underlined the contribution of prepubertal duration to the risk of developing MA (72–75). Children with onset of diabetes before the age of 5 years may have a delayed onset of complications, but after puberty there is an acceleration in their development (62, 76). Furthermore, some children with an early prepubertal onset of diabetes can also develop MA before puberty (62). Similar data have been found also for the risk of retinopathy in children with prepubertal onset of diabetes (77). Therefore, it appears that prepubertal years contribute to the development of microvascular complications, even though to a lesser extent than post-pubertal years.

Genetic Factors

The finding that, irrespective of a good glycemic control, some patients still develop complications (78), suggests that other factors, either environmental or genetic, are implicated in their etiology.

Only 30–50% of subjects with type 1 diabetes are at risk for DN and epidemiological and family-based studies support the role of genetic factors in its pathogenesis. The increasing prevalence of DN during the first 20 years after

diagnosis, followed by a plateau (78), suggests that only a subset of patients is really susceptible to this complication. The DCCT and other studies have shown a familial cluster of DN, with an increased risk of developing nephropathy for siblings of a proband with DN when compared to siblings of a proband without DN (79).

A family history of hypertension has also been shown to be a risk factor for developing DN (80). Parents of patients developing DN have higher blood pressure when compared to parents of subjects free of this complication (81, 82), and the risk seems to be increased by three-four fold. In addition, a family history of dyslipidemia, insulin resistance, type 2 diabetes or of a cluster of these cardiovascular risk factors, significantly increases the risk of diabetic kidney disease (83, 84), suggesting a role for cardiovascular genes in predisposing to nephropathy.

DN appears to be a complex genetic trait, with the contribution of different genes and an effect of their interaction with environmental factors (85). Several genes have been suggested as potential candidates in the pathogenesis of DN, but there is no evidence for a major effect of a single gene so far (85). DN is the combination of albuminuria and reduced GFR and it has been shown that both these components are highly heritable traits and that they have a different genetic basis (85). However, the results of genetic studies have been often conflicting, due to differences in the populations studied, to the small sample size, and also to differences in the definition of the trait analyzed (86). Among the most common genes that have been investigated for their potential relationship with DN are those encoding for components of the renin-angiotensin system, and mainly the angiotensin converting enzyme (ACE) gene and the angiotensin receptor I gene. In particular, the insertion/deletion polymorphism in the ACE gene has been the object of intense investigations. However, as emerged from a meta-analysis, it appears to have a small effect on the risk for developing DN in European populations (87). Polymorphisms in the ACE gene have been related also to differences in the response to treatment with ACE inhibitors (ACEIs) (88), thus suggesting the importance of future pharmacogenomic approaches in this area.

Other candidate genes have been those encoding lipoproteins (in particular apolipoprotein E), aldose reductase and heparane sulfate (89). In addition, as it is well known that inflammation, glycation and oxidation pathways as well as growth factors play an important role in the development of vascular complications, other possible candidate genes might be those encoding for components of these systems (89).

Smoking

Smoking is an independent risk factor for the development and progression of DN in adults with diabetes (90, 91). In a large prospective study in young individuals with type 1 diabetes, smoking was associated with 2.8-fold increased risk of MA whereas smoking cessation caused a significant improvement in AER (92). The link between smoking and DN can be related to an increased oxidant burden caused by cigarette smoking (93). In addition, as smokers have also higher blood pressure then non-smokers (94), this can represents an additional connection between smoking and the risk of developing DN.

Diet

A high protein intake has been suggested as a potential risk factor for the development of DN, with a normalization of GFR associated to a reduction of protein intake (95, 96). It seems that animal proteins exert a stronger effect than vegetable proteins (97), whereas a high intake of fish proteins has emerged as a factor able to reduce the risk of DN (98). The exact mechanisms to explain the association between protein intake and DN are not completely clear, although glucagon, prostaglandin and the renin-angiotensin system have been suggested as potential mediators (97, 99).

Other factors investigated in relation to DN are fat, minerals, vitamins and fibers (100–102). In particular, as DN is associated with an increased oxidant burden, the potential effect of treatment with anti-oxidants has been investigated, but the results have been inconclusive (103–105).

Other Factors

Recently, it has been shown that in adolescents with type 1 diabetes, clinical markers of insulin resistance, such as body mass index, are associated with the development of MA, in addition to glycemic control (106).

Another factor that has been related to DN is low birth weight (107). Intrauterine growth retardation, due to intrauterine or genetic factors, might lead to reduced nephron number and a decreased renal functional reserve with increased susceptibility to DN, in response to other environmental factors (107). This theory could also explain the association of short stature and risk of DN (107, 108).

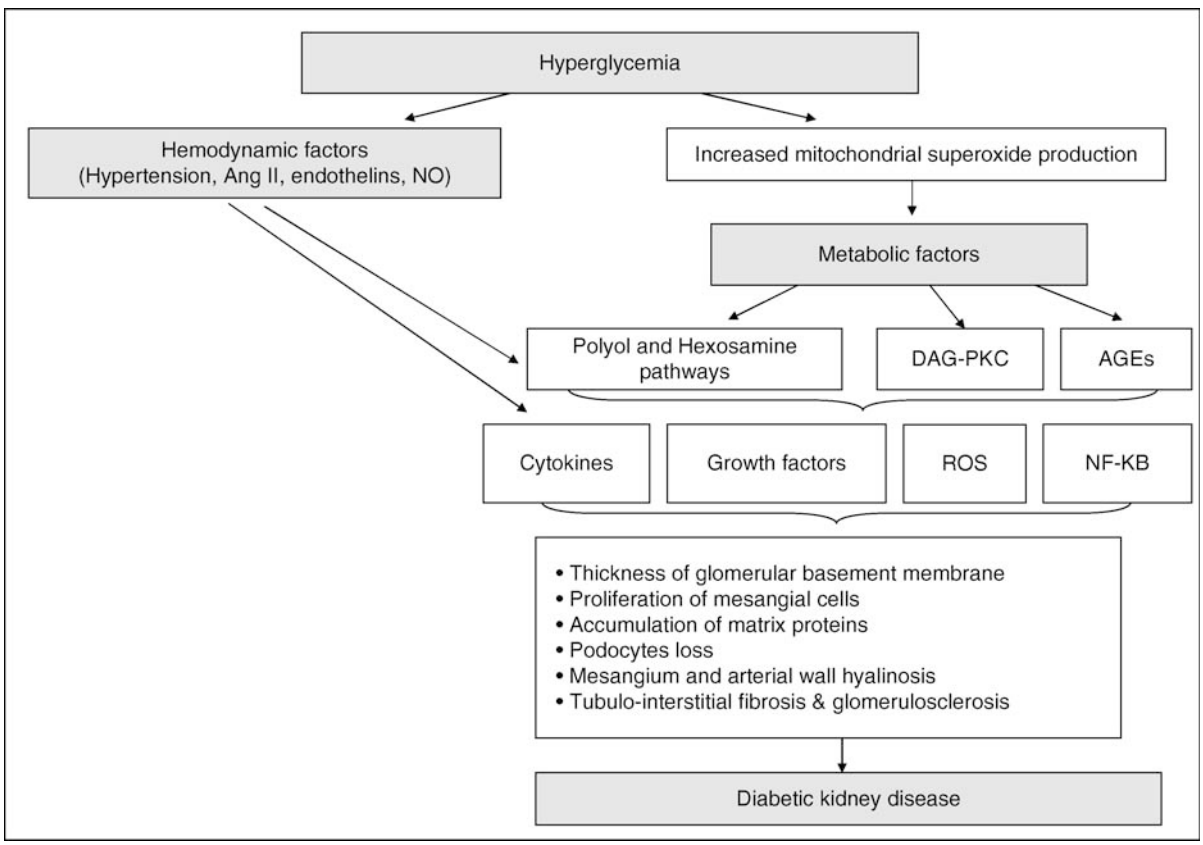
Pathogenesis

DN is the result of an interplay between hemodynamic and metabolic factors in the renal microcirculation (109), and the consequent activation of common intermediate pathways, associated with increased synthesis and release of growth factors, cytokines, chemokines and oxidant species, which are all final mediators of renal damage (109) (*Fig. 50-1*). Typical morphological changes in the diabetic kidney are represented by diffuse glomerular basement membrane thickening, mesangial expansion, hyalinosis of the mesangium and arteriolar walls, broadening and effacement of podocyte foot processes, reduction in podocyte number, glomerulosclerosis and tubulointerstitial fibrosis (110). These morphological changes in the kidney develop years before the clinical appearance of MA and overt proteinuria and this is an alarming aspect of DN, given that when the disease is clinically evident, some of the structural damage is already irreversible (33).

Thickening of the basement membrane is a common bioptic finding related to DN; it is associated with loss of glycosaminoglycans and therefore of negative charges, with consequent increased loss of anionic albumin (111, 112). A subsequent increase in the size of membrane pores leads to the development of non-selective proteinuria. An imbalance between the production and the degradation of mesangial matrix proteins, together with an increase in mesangial cells number, is responsible for mesangial expansion in DN (112). Hyperglycemia and renal hypertension together with other factors can activate pathways leading to an increased synthesis and deposition of matrix proteins, particularly by stimulating local production of cytokines and growth factors (113).

Figure 50-1

Schematic representation of metabolic and hemodynamic factors implicated in the pathogenesis of diabetic nephropathy. *NO* nitric oxide; *Ang II* angiotensin II; *ROS* reactive oxidative species; *DAG-PCK* diacylglycerol-protein kinase C; *AGEs* advanced glycosylated-end products; *NF-KB* nuclear factor-kappa B



Furthermore, the same factors can inhibit proteins and enzymes implicated in the degradation of the extracellular matrix, for example by a non-enzymatic glycation of these components (113).

As in other progressive glomerulopathies, changes in podocyte characteristics are a key feature of DN (114). Podocytes are highly specialized epithelial cells, interconnected by foot processes, which delimit the slit diaphragm, the main size-selective barrier in the glomerulus. The diabetic milieu can induce several morphological changes in these epithelial cells, as emerged from both human and experimental studies (111). The first detectable alteration in podocytes in the context of DN is a broadening and effacement of their foot processes (111). These progressive podocyte changes lead to a decrease in their density and number and a detachment from the glomerular basement membrane. The last phenomenon is directly correlated to levels of AER and to the decline in GFR (111). Podocytes can also undergo hypertrophy, apoptosis and increased synthesis of collagen IV, whereas there is a decreased synthesis of proteins such as nephrin (111).

Another characteristic of DN is the hyalinosis of the afferent and efferent arterioles in the glomerulus, due to the accumulation of complement components, fibrinogen, immunoglobulins, albumin and other plasma proteins (115). Hypertrophy and sclerosis of the iuxtaglomerular apparatus is another frequent finding in DN (116).

Hemodynamic Factors

Hemodynamic factors implicated in the pathogenesis of DN include systemic and intraglomerular pressure and activation of vaso-active mediators (109). Glomerular hypertension is the result of reduced resistance in both the afferent and efferent arterioles, an effect which is predominant in the former. On the basis of several experimental studies it has been suggested that a subset of patients with diabetes could be predisposed to intraglomerular hypertension, due to defects in the renal mechanisms of flow autoregulation (117). The lack of the normal vasoconstriction in the afferent arteriole in response to increased systemic hypertension, associated with the constriction of the efferent arteriole, contribute to the increase in intraglomerular blood pressure (113). A potential mediator of the afferent arteriole dilation is an increased oxidative burden. It has been hypothesized that some reactive oxygen species (ROS) might affect K+ channels in the afferent arteriole with consequent hyperpolarization and increased Ca+ influx and vasodilatation. K+ channels have a dominant role in the electromechanical function of the afferent arteriole but only a minor effect on the efferent arteriole (118). Glomerular hypertension can in turn induce the activation of several pathways and molecules, which can mediate renal injury. In particular, it has been shown that it can, for example, activate GLUT-1 in glomerular cells, thereby inducing an intracellular hyperglycemic milieu (113).

Angiotensin II is a key mediator of DN, exerting both hemodynamic and non-hemodynamic effects (109). The hemodynamic effects of angiotensin II include both systemic and renal vasoconstriction (119). In the kidney, angiotensin II also increases glomerular capillary pressure and permeability. Non-hemodynamic effects of angiotensin II include activation of transforming growth factor-beta 1 (TGF-ß1) and other cytokines, activation of ROS production in mesangial cells, stimulation of extracellular matrix and inhibition of its degradation, activation of the intracellular NF-kB and reduction in podocyte nephrin expression (119, 120). Other factors that have been implicated in the hemodynamic changes related to DN are vasoactive factors, which can influence intrarenal circulation by acting on the afferent and efferent arterioles. In particular, endothelin and angiotensin II are the most relevant vasoconstrictors in this context, whereas nitric oxide, bradykinin and prostaglandins are the main vasodilators (109).

Metabolic Factors

Growth Factors

Several growth factors have been implicated in the pathogenesis of DN, through complex intra-renal systems (121–123). GH/IGF, TGF-ß, vascular endothelial growth factor (VEGF), epidermal growth factor, and connective tissue growth factor (CTGF) are among those better known and investigated (121–123).

The implication of the GH-IGF system in the pathogenesis of DN is well established (121, 122). Diabetes is associated with decreased hepatic production of IGF-I related to portal insulinopenia, with consequent lack of the inhibitory feedback of IGF-I on the anterior pituitary and therefore GH hypersecretion (66, 67). The integrity of GH receptors in peripheral tissues, other than the liver, can cause a local increased production of IGF-I, which can in turn exert paracrine effects (66, 67). Antagonist of the GH/IGF-I system, including somatostatin analogues, GH and IGF-I receptors antagonists as well as antagonists of downstream factors activated by GH/IGF-I, such as the

ACE and AGEs systems, have been found to have beneficial effects on the diabetic kidney in animal models (121).

TGF-ß is considered a crucial factor in the development of renal kidney disease (121–123). It appears to be a key point of convergence of both hemodynamic and metabolic pathways activated in DN. In the diabetic milieu several factors can induce increased expression of this growth factor, such as PKC, hyperglycemia and hypertension, and mechanical strains. TGF-ß is a profibrotic cytokine, thus having an important role in the expansion of the extracellular matrix, by stimulating the production of several components of the matrix and, in the meantime, by altering extracellular matrix composition (121–123).

VEGF is another relevant growth factor implicated in the pathogenesis of DN, even though its role in this context is not as well defined as for diabetic retinopathy (121–123). In particular, VEGF is a key angiogenic factor, which influences the proliferation of endothelial cells and exerts a pivotal role in vascular integrity (123). Anoxia is an important stimulus for its production as well as AGEs, angiotensin II and oxidative stress (123). VEGF is expressed in different cells in the kidney, including podocytes and tubular cells. Increased VEGF levels have been reported in both type 1 and type 2 diabetes and have been related to DN (124). In children with type 1 diabetes developing MA, VEGF levels are increased when compared with diabetic children with normoalbuminuria, and they have an independent predictive value for future development of DN (125).

CTGF is one of the most recent identified growth factors with a role in DN. Increased concentrations of CTGF have been detected in the glomerulus of diabetic patients and animals (126–128). In adult patients with type 1 diabetes, increased levels of CTGF are strongly correlated with the severity of DN (129, 130) and are an independent predictor of ESRD and mortality (131). CTGF is considered the downstream mediator of TGF-ß in extracellular matrix synthesis. However, there is also evidence of a TGF-ß-independent regulation of CTGF, which is related to a direct activation of its synthesis by hyperglycemia, AGEs and static pressure (128, 132, 133).

Cytokines

DN is considered an inflammatory disease and several cytokines have been implicated in its pathogenesis: interleukin (IL)-1, IL-6, IL-18, and tumor necrosis factor-alpha (TNF-α) (134). In the kidney, inflammatory cytokines are synthesized and released by endothelial, mesangial, glomerular and tubular cells (134). IL-1 has been implicated in increased vascular permeability, proliferation of extracellular matrix and abnormalities in microcirculation (134). IL-6 can also induce changes in the extracellular matrix (134). TNF-α has been shown to have a cytotoxic effect on glomerular, mesangial and epithelial cells in the kidney (123, 134).

Chemokines and cytokines implicated in the recruitment of monocytes/macrophages have been related to the development and progress of DN (135). Monocytes chemoattractant protein-1 (MCP-1) is a chemokine with the highest chemotactic activity towards monocytes. A causative role in the development of DN has been suggested for MCP-1, as a result of the recruitment of monocyte/macrophage in the kidney (136). Furthermore, in vitro studies have shown that MCP-1, by interacting with its receptor (CCR2), can promote fibronectin deposition in the diabetic glomerulus (137). Blocking the MCP-1/CCR2 pathway ameliorates glomerulosclerosis, indicating that the MCP-1/CCR2 pathway could play a crucial role in the progression of DN (138). Increased MCP-1 levels have been reported in subjects with diabetes when compared with controls and it has been shown that plasma and urine MCP-1 levels can be used to assess renal inflammation in this disease (139).

AGEs and their Receptors

Another link between elevated glucose levels and DN is the direct effect of non-enzymatic glycosilation on cellular macromolecules, causing alterations in their structural and functional properties (140–143). AGEs have been implicated in several biologic activities, mostly by binding to the AGE-specific receptors (RAGEs) on many cells (141, 142). In particular, they can enhance oxidative stress and stimulate the release of cytokines and growth factors, which in turn accelerate chronic inflammation and endothelial dysfunction (141). Increased serum levels of AGEs have been detected in children and adolescents with type 1 diabetes, even before the development of clinically evident signs of microvascular complications (144).

Animal studies support the role of AGE in the pathogenesis of DN. In fact, blocking of AGE, with aminoguanidine, significantly reduces renal changes (145).

There is also a large body of evidence of a predictive role for the soluble form of the RAGE (sRAGE) in the development of vascular diabetic complications, including DN (146). sRAGE can be measured in peripheral blood and it seems to result from the expression of a RAGE splice gene variant that encodes an amino-terminally

truncated form of the receptor and/or from the cleavage of the native membranous receptor (146). Serum sRAGE levels are significantly higher in type 2 diabetic patients than in non-diabetic subjects and positively associated with the presence of coronary artery disease (147) and also independently related to albumin excretion (146). A circulating C-truncated form of RAGE (esRAGE) exists and seems to work as a scavenger for AGEs and in this way could exert a protective influence for diabetic complications. Reduced circulating levels of esRAGE have been found in patients with type 1 diabetes, and related to the severity of vascular complications (148).

Oxidative Stress

Oxidative stress is one of the most important factors involved in the pathogenesis of DN (113, 143). Several pathways have been related to an increased production of oxidative stress in the context of DN: increased mitochondrial electron transport, the AGEs-RAGE system, an increased activity of cytochrome P-450, xantine oxidase, cyclooxigenase and lipoxigenase, increased glucose autooxidation and an impaired activity of endothelial nitric oxide synthase (149). Many of the pathways activated by hyperglycemia can induce mitochondrial superoxide overproduction and blocking of this effect can reduce damage related to high glucose levels (150, 151). This increased mitochondrial production of ROS can in turn stimulate different processes, including PKC activity, synthesis of growth factors and cytokines, and stimulation of NF-kB. Oxidative stress seems to play an important role also in the context of the so-called "metabolic memory" (41). In fact, increased mitocondrial superoxide production consequent to hyperglycemia could induce not only immediate effects, such as activation of PKC or other pathways, but might also damage mitocondrial DNA (41). This, in turn, could lead to synthesis of altered subunits of the electron-transport system, which could produce increased amounts of superoxide, even in the presence of physiological glucose levels (41).

Intracellular Factors

Polyol and Hexosamine Pathways

The polyol pathway has been implicated in the pathogenesis of DN, through the action of aldose reductase, the first and rate-limiting enzyme in this pathway (41). Aldose reductase reduces the aldeide form of glucose to sorbitol in a reaction which consumes NADPH. In physiological situations sorbitol is then oxidized to fructose by sorbitol dehydrogenase and therefore addressed again into the glycolisis. In the presence of hyperglycemia, the production of sorbitol overcomes the potential of its oxidation by sorbitol dehydrogenase, with accumulation in several cells, including renal tubular and glomerular cells. Several mechanisms have been suggested to link the polyol pathway to the development of diabetic complications. These include a dysregulation of the cellular osmotic status, reduction of Na+/K+-ATPase activity, cytosolic increase in NADH/NAD+ and decrease in NADPH (41). The depletion of NADPH appears to be the most important mediator, as it is associated with an impairment of several enzymatic reactions requiring this enzyme, such as nitric oxide synthase, cytochrome P450, and glutathione reductase, thereby altering the intracellular oxidant-antioxidant status and inducing vasoconstriction and poor blood supply (41).

The hexosamine pathway converts fructose-6 phosphate in N-acetyl glucosamine, which is a substrate for reactions such as proteoglycan synthesis and generation of *O*-linked glycoprotein (41, 152). N-acetyl-glucosamine has been implicated in the activation of the transcriptional factor Sp1, which is associated with increased synthesis of factors, such as TGF-ß1 and plasminogen activator inhbitor-1, which in turn are associated with the development of vascular complications (41, 152). In addition, the hexosamine pathway is also associated with increased oxidative stress and the effects of the activation of this pathway can be prevented by overexpression of antioxidants, such as superoxide dismutase (150).

Diacylglycerol (DAG)-PKC Pathway

The DAG-PKC system can induce several alterations, which can contribute to DN (41, 153, 154). These mechanisms include changes in endothelial permeability, vasoconstriction, increased synthesis of extracellular matrix and stimulation of cytokine synthesis, cell growth, angiogenesis, and leukocyte adhesion (41, 153, 154).

Hyperglycemia can stimulate de novo synthesis of DAG, followed by the activation of PKC (41). PKC modulates the activity of various enzymes, including phospholipase A2 and Na+/K+ ATPase, as well as the expression of genes related to components of the extracellular matrix (41, 153). PKC-beta is the major isoform induced in the kidney by hyperglycemia and ruboxistaurin, a PKC-beta isoform selective inhibitor, has been shown to have a beneficial effect on microvascular complications, by

normalizing endothelial dysfunction and GFR and reducing loss of visual function (145).

Screening and Diagnosis

Assessment of Albumin Excretion and GFR

Albumin excretion. Measurement of albumin excretion is the basis for the diagnosis of DN (25). MA is the earliest detectable marker of DN and represents an important risk factor for the development of DN and cardiovascular disease (53). MA is not only a phenomenon related to renal damage, but is associated with a more generalized sieving of albumin from the blood bed, due to a diffuse endothelial dysfunction (42).

Screening for MA can be performed in three ways: (1) 24-h collection (2) timed collection (e.g. 4-h or overnight); (3) a spot urinary sample (155). 24-h or timed urine collections are often difficult to be performed with accuracy, particularly in children. Therefore, the easiest way is the assessment of the albumin-creatinine ratio (ACR) on a spot urine sample, preferably using the first voided urine in the morning, in order to avoid bias related to the known diurnal variation in albumin excretion. The correct interpretation of albumin excretion requires that other factors and conditions which could influence it are excluded. Exercise, urinary tract infections, acute febrile illness, immunoglobulin A or other forms of nephritis, marked hypertension, and menstrual cycle in women can all cause transient increase in albumin excretion. Given that the intra-individual daily variation in albumin excretion may fluctuate by 40–50%, multiple measurements of albumin excretion are required (155).

The definition of MA can be: (1) AER between 20 and 200 μg/min or 30–300 mg in 24-h urine collection; (2) ACR 2.5–25 mg/mmol in males or 3.5–25 mg/mmol in females; (3) albumin concentration 30–300 mg/l in early morning urine sample (155). Persistent MA is defined as 2 out of 3 abnormal samples collected over a period of 3–6 months (155). Values above the upper limit for MA definition are diagnostic of macroalbuminuria or overt nephropathy.

Based on the guidelines of the International Society for Pediatric and Adolescent Diabetes, screening should be performed from the age of 11 years with 2 years of diabetes duration and from the age of 9 years with 5 years diabetes duration (155) (*Table 50-2*). The American Diabetes Association recommends that annual screening should start once the child is 10 years of age and has had a diabetes duration of 5 years and to test more frequently if the values are increasing (156).

Table 50-2

Screening recommendations based on the International Society for Pediatric and Adolescent Diabetes (ISPAD) guidelines 2006–2007

Screening recommendation for MA
• Annually assessment of albumin excretion from age 11 years with 2 years of diabetes duration and from age 9 years with 5 years of duration
• Method: Urinary albumin/creatinine ratio
• Exclude other causes of increased albumin excretion: strenuous exercise, orthostatic proteinuria, hypertension, acute febrile illnesses, smoking, urinary infections, nephritis, menstruation

GFR. The National Kidney Foundation (NKF) strongly recommends measuring GFR in subjects at risk of kidney disease, such as diabetic patients (157). Alterations in GFR can be predictive of future deterioration of renal disease and, in addition, are an important predictor of cardiovascular events (158). A 10-year follow up study has clearly shown the role of reduced GFR in identifying adult patients with diabetes at high risk for cardiovascular mortality (159). Evaluation of GFR has an important role in identifying alteration in renal function, even in the presence of albumin excretion in the normal range (160, 161). A recent study performed in patients with type 1 diabetes has shown a progressive decline in renal function as an early event in patients with MA (162). Furthermore, an increased GFR is a common finding during the early stages of diabetes and is associated with hyperperfusion and increased renal size (27, 163, 164). In human studies, this early stage of hyperfiltration has been found to be an important predictor of future development of nephropathy (27–30, 165), independent from glycemic control, even though this has not been confirmed in all studies.

The direct evaluation of GFR is difficult to perform, being time-consuming and requiring expertise and specific laboratory techniques for the analysis (166). The evaluation of creatinine clearance is a possible alternative but it requires timed urine collection and consequently it can be difficult to be performed accurately, particularly in children. Plasma creatinine should be an easier alternative (167). However, it is influenced by several factors, particularly in children and adolescents. In fact, plasma creatinine levels are influenced not only by renal filtration rate but also by tubular secretion, age, gender, changes in muscle mass related to growth and puberty (167).

Formulae based on creatinine levels and allowing for confounding factors, such as body composition, have been developed and used for a long time in children. The NKF recommends the use of the Schwartz or the Counahan-Barratt formulae (168, 169) for the estimation of GFR in subjects below 18 years of age (157). These formulae represent a simple mathematical calculation applicable for the day-to-day clinical evaluation of GFR in children. However, the determination of height at the time of creatinine measurement is a fundamental point in applying these formulae. This represents a relevant limitation because height is not easily available or verifiable in the laboratory where creatinine is measured and GFR calculated. Another important point in the application of these formulae is that, as they include a constant k, each laboratory needs to derive its own constant to increase the accuracy of the GFR estimation (170). Furthermore, creatinine-based formulae as well as creatinine clearance are unreliable in the hyperfiltration state. Therefore, the search for a new endogenous surrogate marker of GFR to be applied instead of the cumbersome direct assessment of GFR is highly required and different plasma markers, such as cystatin C or symmetric dimethylarginine, are under consideration in both adult and pediatric populations (171).

Emerging Roles of Proteomic and Genomic Approaches in the Prediction, Detection and Monitoring of Diabetic Nephropathy

DN is a complex disease, with different mechanisms implicated in its pathogenesis and progression (109). The disease is often silent for years, even though during this period of time significant morphological alterations have already occurred in the kidney (111). The characterization of predisposing genetic factors and of blood or urinary markers is particularly important for the identification of subjects at risk and the implementation of preventive and therapeutic strategies, ideally by personalizing them on the basis of the patient risk profile (172). This is the basis for the role of a genomic and proteomic approach in DN (173, 174). In particular, genome-wide association studies represent an important attempt at the identification of the genetic basis of DN and the development of clinical proteomic investigations to possibly highlight new molecular markers diagnostic and/or prognostic of DN (174). Several large multicenter studies such as the Genetics of Kidneys in Diabetes, the Family Investigation of Nephropathy and Diabetes, the Epidemiology of Diabetes Interventions and Complications, and the European rational approach for the genetics of diabetic complications study, are currently underway, searching for DN susceptibility genes (175). Genomic and proteomic technologies could be used to assess gene expression and specific protein expression directly in kidney tissues, even at the time of onset of diabetes to characterize the patient's risk profile. Several experimental studies have already explored the protein expression profile in the kidney (173). However, in humans, a potential limitation for this application of genomics and proteomics is represented by the need for kidney biopsies. A valid alternative is the application of these new technologies on urinary and blood samples. Initial studies in humans have applied proteomics to assess the potential urinary protein profile, which could help in identifying subjects at risk of DN (174). A pilot study in adolescents with type 1 diabetes has detected specific urinary polipeptides associated with diabetes and, even more interestingly, a specific pattern of peptides associated with early nephropathy (176). Similarly, in adults with type 2 diabetes, specific urinary polypeptides have been related to the risk of developing DN (177). Therefore, the application of proteomics for the identification of the pattern of urinary or plasma proteins appears to be a reliable and feasible way to predict and monitor DN.

Prevention and Treatment (◉ *Table 50-3*)

Intensive Blood Glucose Control

The DCCT and the EDIC have provided strong evidence for the important role of strict glycemic control for the reduction of risk of micro- and macro-vascular complications in subjects with type 1 diabetes, including adolescents (36, 38, 39). In the adolescent cohort of the DCCT, beneficial effects on complications were obtained, even though the mean HbA1c was significantly higher, by about 1%, when compared to the adult cohort (38). This

Table 50-3

Preventive and treatment strategies for DN in children and adolescents

Preventive and treatment strategies
• Optimizing glycemic control
• Control blood pressure
• Control lipid levels
• Moderate decrease in protein intake
• Avoid smoking

underlines an important issue in achieving good metabolic control during puberty. Psychological issues, together with the effect of the physiological insulin resistance (64, 65) that occurs during puberty and the numerous changes in the hormonal milieu (61), give rise to several problems in managing adolescents with type 1 diabetes (61). Other studies have also shown the difficulties in achieving good glycemic control and avoiding the risk of episodes of hypoglycemia and weight gain in the meantime (178). The issue of weight gain is of particular relevance for adolescent girls, who often drop their insulin injection to avoid overweight (179). Therefore, other strategies need to be implemented to improve glycemic control, particularly during adolescence.

Blood Pressure Control

In adults with type 1 diabetes and MA, treatment with ACEIs or angiotensin receptor blockers (ARBs) is recommended, based on the evidence of a positive effect in reducing the rate of progression and promoting regression of MA (10). A beneficial effect of ACEIs has been shown in microalbuminuric normotensive patients, in whom the ACE inhibition arrests the increase, and even reduces, AER (180). Furthermore, in patients with diabetes but with albumin excretion within the normal range, ACEIs have been proved to be effective in reducing the risk of developing MA (43). This effect appears to be independent of baseline blood pressure, renal function and type of diabetes (181). Both ACEIs and ARBs have been shown to be effective, but the use of ARBs has been associated with increased risk of all-cause mortality when compared to placebo (182).

However, there is no guidance for the use of ACEIs or ARBs in the pediatric population in the context of MA. In fact, these drugs have been approved and used in the treatment of hypertension, but there is no indication regarding MA. Four small studies have been performed and confirmed the likely efficacy of ACEIs in adolescents with MA but there have been no formal randomized controlled trials (RCT) (183–186). Overall these studies have shown that ACEIs lead to a reduction in albumin excretion. However, it is difficult to draw definite conclusions from these studies and the issue of the potential long-term use of ACEIs in individuals with MA also raises the problem of potential side effects of these drugs.

The ADA recommends starting treatment with ACEIs for persistent MA (156). Similarly the recent ISPAD guidelines suggest using ACEIs or ARB for persistent MA to prevent progress to proteinuria, even though they acknowledge the lack of evidence in this context (155).

Management of Dyslipidemia

Many large-scale interventional trials have demonstrated that treatment with statins, the most effective lipid lowering drug class, significantly reduces the risk of coronary heart disease events and total mortality (187). People with diabetes and DN often present with dyslipidemia and statin therapy has been associated with a significant reduction in the risk of macrovascular complications (187). Statins have effects other than the reduction in cholesterol levels (188). In fact, they also have other beneficial properties, such as inhibition of arterial smooth muscle cell proliferation, prevention of oxidation of LDL cholesterol, plaque stabilization, effect on macrophages, improvement of endothelial dysfunction, and anti-inflammatory and anti-thrombotic effects (188). These actions might be beneficial at the renal side. Even though dyslipidemia is often found in children and adolescents with diabetes (59, 189–191), there is no consensus on the role of statin treatment in this age group, mainly because no RCTs have been conducted.

Diet Intervention and Smoking Cessation

A low protein diet seems to reduce the increase in albumin excretion rate and the decline in GFR in adults with type 1 diabetes. A meta-analysis of studies investigating the effect of protein intake has shown that a diet restriction to 0.5–0.8 g/kg/day reduces the risk of progression of DN (192). However, there are no specific data for children and adolescents, where generally a minimum of protein intake of 1 g/kg is sufficient for normal growth, but it is not clear whether this reduces the risk of DN (193).

As smoking is common among adolescents with type 1 diabetes (194) and it is related to the development of DN (90), it is important to discourage young people as early as possible from smoking.

New Potential Therapeutic Strategies

New potential therapeutic possibilities for the treatment of DN are emerging and they include drugs targetting

specific pathways implicated in the pathogenesis of DN (inhibitors of advanced glycation, PKC inhibitors, and glycosaminoglycans) (145, 195).

Glycosaminoglycans are among the biochemical components of the glomerular basement membrane matrix structure (196). Sulodexide, an oral formulation of the natural glycosaminoglycan, composed of 80% fast-moving heparan sulphate and 20% dermatan sulphate, has been shown to have potential nephroprotective effects, through several mechanisms, which could explain its remodeling effect (196). Several studies have analyzed its effects on albumin excretion and two RCTs in particular have shown a significant anti-albuminuric action (196). However, till now there are no available studies on this new drug in children and adolescents with diabetes.

Ruboxistaurin, a selective inhibitor of PKC–beta, has been shown to have beneficial effects in experimental models of diabetes, with reduction of glomerular hyperfiltration and albuminuria (145). Drug development with ruboxistaurin has reached the stage of phase II evaluation with uncertain results (145).

Another potential new therapeutic strategy is targeted toward AGE inhibition (145). Pyridoxamine, in particular, is an AGE inhibitor, with positive effects on animal models of nephropathy. A phase II study in patients with DN has shown mixed efficacy with a good safety profile (145).

Future studies are required for a better evaluation of these drugs as well as for the development of new therapies, which could target other specific metabolic or hemodynamic pathways implicated in the pathogenesis of DN.

Conclusions

DN represents a serious complication of childhood-onset diabetes associated with significant morbidity due to progressive loss in renal function and the associated risk for cardiovascular disease. Even though kidney failure and overt nephropathy are not common in children and adolescents, important structural and functional alterations at the renal level occur even during childhood and accelerate during puberty. Several risk factors have been associated with the development of DN, but a lot still needs to be clarified to develop better preventive and therapeutic strategies, which could reduce the burden associated with DN and thereby improve the prognosis for young people with type 1 diabetes.

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51 Renal Manifestations of Metabolic Disorders

William G. van't Hoff

Introduction

While the majority of children with renal dysfunction have a structural, immunological or infective disorder, there are some who have a metabolic defect arising from an abnormality in the biochemical pathways of cell metabolism. In many metabolic disorders, substrate accumulation or an enzyme deficiency can be documented in the kidney but without evidence of renal dysfunction (e.g., some of the lysosomal storage disorders). This review concentrates on those disorders in which there is a clear renal functional or structural abnormality. Several of these conditions are described in detail elsewhere in this book, this chapter highlights a number of other metabolic disorders and provides, in tabular form of the various metabolic causes of renal symptomatology (see *Tables 51-1–51-7*). Many metabolic disorders can cause acute or chronic renal failure however it is rare for this to be the sole manifestation therefore these conditions are listed under the most important renal feature.

The proximal renal tubule has a very high-energy expenditure and is therefore sensitive to metabolic disorders that interfere with the generation of ATP. Mitochondrial dysfunction, alterations in glutathione metabolism and reduced ATP levels are felt to be important in the pathogenesis of the Fanconi syndrome which is one of the commonest renal manifestations of metabolic disease (*Table 51-1*), a fuller description is given in Chapter 42. Nephrocalcinosis and urolithiasis may also be manifestations of metabolic dysfunction, usually arising from defective reabsorption of a solute (e.g., cystine in cystinuria) or as a result of the urinary excretion of abnormally elevated plasma constituents (e.g., oxalate in hyperoxaluria). Renal glomerular involvement is less common but can occur as a result of abnormal deposition of storage material or due to a defect in the synthesis of the glomerular components (see *Table 51-4*). As a result of improvements in the care of children with metabolic disorders, longer term manifestations are becoming more apparent. Cystinosis was once described as a renal disorder, but as a result of successful renal transplantation, patients survive into adulthood and in the long term develop extra-renal problems (1). In addition, in some disorders increased survival has led to the development of renal dysfunction (e.g., methylmalonic acidemia). Thus pediatric nephrologists need to remain vigilant to the possibility of a metabolic disorder especially in children with extra-renal symptoms.

Methylmalonic Acidemia

Methylmalonic acidemia (MMA) is an autosomal recessive disorder of the metabolism of methylmalonyl Coenzyme A caused either by a defect of methylmalonyl Coenzyme A mutase or of the cofactor, adenosyl cobalamin (2). Patients usually present in the neonatal period or early infancy with lethargy, vomiting, poor feeding, failure to thrive and recurrent metabolic acidosis. Patients deficient in the cofactor respond favorably to cobalamin supplements but for those who have a partial or complete deficiency of the apoenzyme, the prognosis is poor with a mortality of approximately 50% within the first decade. In the cobalamin-unresponsive patients, there is a significant incidence of neurological and renal manifestations.

Renal Tubular and Glomerular Dysfunction

Children with methylmalonic academia have evidence of renal tubular dysfunction which becomes very profound during episodes of metabolic decompensation (usually triggered by intercurrent infection) with massive renal salt and bicarbonate losses. In a study of seven cobalmin-unresponsive patients, five had defects in urine concentration, two had impaired urine acidification and several had hyporeninemic hypoaldosteronism (3). Chronic renal impairment has also been documented in cobalamin-unresponsive MMA patients. Walter et al. documented a low GFR in eight of 12 such children, five of whom had a GFR < 40 ml/min/1.73 m^2 (4). In a retrospective survey of patients with all forms of MMA, Baumgartner and

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Table 51-1

Fanconi syndrome (See Chapter 42 for full description)

Disorder	Main features	Renal features	References
Cystinosis	Poor growth, vomiting, rickets, often blond, fair hair, corneal cystine crystals, multisystem involvement	Fanconi syndrome, chronic renal failure, rarely urinary tract dilatation, calculi (urate/calcium oxalate)	See Chapter 42 (111)
Tyrosinaemia type 1	Hepatomegaly, liver disease, rickets, neurological crises	Fanconi syndrome, nephromegaly, nephrocalcinosis, calculi, chronic renal impairment	See Chapter 42 (112, 113)
Mitochondrial disorders	Myopathy, poor growth, cardiomyopathy, liver dysfunction, neuropathy, retinopathy, ophthalmoplegia, endocrinopathy	Fanconi syndrome, Bartter-like features	See Chapter 42 (49–70)
		Proteinuria	
		Tubulo-interstitial nephritis	
		Cysts	
Galactosemia	Vomiting, diarrhea, poor growth, jaundice, liver disease, hypotonia, cataracts	Fanconi syndrome	See Chapter 42
Dent's disease	Hypercalciuria, tubular proteinuria, X-linked	Partial Fanconi syndrome (low molecular weight proteinuria), nephrocalcinosis/ nephrolithiasis, chronic renal failure	(114, 115) See Chapter 42
Oculo-cerebro-renal (Lowe's) syndrome	Congenital cataracts, other ocular abnormalities, hypotonia, delayed development, X linked	Partial Fanconi syndrome (low molecular weight proteinuria), chronic renal failure, nephrocalcinosis, rarely calculi	(116) See Chapter 42
Wilson's disease	Liver disease, neurological symptoms, Kayser-Fleischer rings, cardiomyopathy	Fanconi syndrome, proteinuria, rarely acute renal failure, distal RTA, hypercalciuria, calculi	See Chapter 42 (117, 118)
Glycogen storage disease type 1	Hepatomegaly, hypoglycemia, seizures, poor growth, lactic acidosis	Fanconi syndrome, hypercalciuria, hyperfiltration, rarely distal tubular acidosis, renal calculi, proteinuria, focal segmental glomerulosclerosis, chronic renal impairment	See Chapter 42 (30–43)
Fanconi Bickel syndrome	Hepatomegaly, rickets, poor growth, hypoglycemia	Fanconi syndrome, hyperfiltration, "diabetic-like" nephropathy	See Chapter 42 (44–48)
Fructosemia	Acute onset after fructose ingestion: poor feeding, vomiting, poor growth, hypoglycemia, liver failure	Fanconi syndrome, rarely acute renal failure	See Chapter 42 (119)
Lysinuric Protein Intolerance	Failure to thrive, growth retardation, hypotonia, hepatosplenomegaly	One report of Fanconi syndrome, proteinuria, hematuria, chronic kidney disease, hypertension	(109, 110)
GRACILE syndrome	Severe growth retardation, neonatal lactic acidosis, cholestasis, liver hemosiderosis	Fanconi syndrome	(120)

Viardot found that 20% of patients had a GFR < 60 ml/min/1.73 m^2 (5) although this may be an underestimate of the incidence of renal impairment because, in some patients, GFR had to be estimated from plasma creatinine concentrations. A review of long term renal function in children with early-onset cobalamin-unresponsive MMA in London, studied by single injection slope clearance chromium-EDTA GFR, indicated that virtually every GFR value was below the normal range and every child surviving to 12 years had a GFR < 40 ml/min/1.73 m^2 (6). In a review of 30 French patients, chronic kidney disease occurred in 47% with a median onset of 6.8 years (7). The mainstay of treatment is a low protein, high calorie diet and many such children have poor growth and reduced muscle bulk. Consequently neither plasma creatinine concentrations nor GFR estimated by Schwartz-Haycock formula, accurately reflect glomerular function in MMA (4, 8). Proteinuria and hematuria are absent and renal histopathology shows changes of tubulo-interstitial nephritis (4, 11, 12). It is likely that the tubular dysfunction noted

Table 51-2

Other tubular dysfunction

Disorder	Main features	Renal features	References
Carbonic anhydrase II deficiency	Osteopetrosis, fractures, cerebral calcification, mental retardation, poor growth,	Mixed type of RTA	(121, 122)
Methylmalonic acidemia	Vomiting, acidosis, hypotonia, poor growth	Type IV RTA, tubulo-interstitial changes, chronic renal failure	(3)
Pyruvate carboxylase deficiency (some forms)	Metabolic acidosis, neurological symptoms, hepatomegaly	Tubular acidosis, renal impairment	(123)
Ehlers-Danlos syndrome	Skin fragility, joint hypermobility, visceral or vascular dilatation/rupture	Renal tubular acidosis, medullary sponge kidney, diverticulae, hypoplasia	(124, 125)
Carnitine palmitoyl transferase deficiency type 1	Fasting/illness leading to encephalopathy, seizures, hepatomegaly	Renal tubular acidosis	(126)
Adenosine deaminase deficiency	Severe combined immunodeficiency	Transient RTA, proteinuria, mesangial sclerosis	(127)
Lysinuric protein intolerance	See *Table 51-4*	Renal tubular acidosis, aminoaciduria, glomerulo-nephritis, renal failure	(109, 110)
Nephrogenic diabetes insipidus	Polyuria, polydipsia, hyposthenuria	Hydronephrosis, hydroureter, megacystis (in long term)	See Chapter 40
Kearns Sayre syndrome	Ophthalmoplegia, retinal degeneration, heart block, ataxia, hyperparathyroidism	Bartter-like syndrome, hypomagnesemia	(57–61)
Fabry's disease	See text	Hyposthenuria, renal tubular acidosis, proteinuria, chronic renal failure	(93–99)
Metachromatic leucodystrophy	Neurologic and intellectual regression, spasticity, incontinence	Renal tubular acidosis, mild aminoaciduria, mild renal impairment	(128)
Transaldolase deficiency	Liver failure, cirrhosis. Neonatal multi-system form (hydrops)	Undefined tubulopathy Genitourinary malformations	(129)
Fatty acid oxidation disorders	Liver disease (steatosis, hypoketotic hypoglycemia, hepatomegaly), acute neonatal illness, cardiomyopathy, myopathy, encephalopathy	Renal tubulopathy, acute renal failure	(130)

in MMA is related to hyporeninemic hypoaldosteronism observed in patients with tubulo-interstitial nephritis and renal impairment (3).

The exact mechanisms of nephrotoxicity in MMA are not yet known. Methylmalonic acid administration to rats causes tubular (but not glomerular) injury and leads to proteinuria (9). There is structural similarity between methylmalonate (which accumulates as a result of the enzyme deficiency) and maleic acid, used experimentally in animals to create a model of the renal Fanconi syndrome. However the tubular manifestations of MMA do not correspond to generalized proximal tubular dysfunction. More recent work on the pathophysiology of MMA has focused on the interaction of methylmalonate on transporter proteins in the kidney. The tubular uptake of dicarboxylic and tricarboxylic acid intermediates of the TCA cycle is mediated by Na^+-coupled carboxylate transporters (hNACs). Both methylmalonate and 2-methylciytrate (which also accumulates in MMA) share structural similarities to dicarboxylates that are known to inhibit hNACs and this could lead to energy depletion in the tubular cells (10). Likewise accumulation of methylmalonate and 2-methylcitrate may also disrupt glutathione transport into the mitochondrion. (10) It seems likely

Table 51-3

Nephrocalcinosis and urolithiasis

Disorder	Main features	Other renal features	References
Cystinuria	Calculi		See Chapter 41
Primary Hyperoxaluria	Calculi/nephrocalcinosis	Acute/chronic renal failure (presentation in infants)	See Chapter 43
Familial hyperuricemic nephropathy	Gout, chronic renal failure		(89–92)
Hypoxanthine-guanine phosphoribosyl transferase (HGPRT) deficiency	Lesch Nyhan syndrome (complete deficiency): developmental delay, choreo-athetoid movements, self mutilation	Acute/chronic renal failure	(81–83)
	Partial deficiency: gouty arthritis		
Adenine phosphoribosyl-transferase deficiency	Calculi 2,8-dihydroxyadenine (2,8-DHA)	Acute/chronic renal failure	(82)
Hereditary renal hypouricemia	Urolithiasis (urate/calcium oxalate), hyperuricemia, hypercalciuria,	Uric acid nephropathy/acute renal failure	(86–88, 131)
Blue diaper syndrome	Hypercalcemia, hypercalciuria, indicanuria → blue discolouration urine	Nephrocalcinosis	(132)
Xanthinuria	Calculi, myopathy, arthropathy	Acute/chronic renal failure	See Chapter 58 (145)
		Renal cysts (rare)	
Hereditary orotic aciduria	Megaloblastic anemia, orotic acid crystalluria, hematuria, immunodeficiency	Crystalluria, obstructive uropathy	(133–135)
Glycogen storage disease type 1	See *Table 51-1*	Tubular dysfunction, nephrocalcinosis, proteinuria, chronic renal impairment	(30, 31)
Wilson's disease	See *Table 51-1*	Acute renal failure, tubular dysfunction	(117)
Congenital lactase deficiency	Watery diarrhea, poor weight gain	Nephrocalcinosis, hypercalciuria, hypercalcemia	(136)
Glucose-galactose malabsorption	Watery diarrhea	Calculi, nephrocalcinosis, hypercalcemia	(137)
Cystinosis	See *Table 51-1*	Rarely calculi	See Chapter 41, (111)
Dent's disease/X-linked nephrolihthiasis	See *Table 51-1*	Nephrocalcinosis, aminoaciduria, partial Fanconi syndrome, chronic kidney disease	(114)
Hypophosphatasia (infantile form)	Rickets, developmental delay, hypercalcemia, craniosynostosis	Hypercalciuria, nephrocalcinosis	(138)
Cystic fibrosis	Chronic lung disease, poor growth, pancreatic dysfunction	Microscopic nephrocalcinosis, calculi, proteinuria, rarely nephrotic syndrome (amyloidosis), chronic renal failure	(139–142)
Tyrosinemia type 1	See *Table 51-1*	Nephrocalcinosis, Fanconi syndrome, nephromegaly, chronic renal impairment	See Chapter 42 (112, 113)
McCune-Albright syndrome	Fibrous bony dysplasia, café-au-lait pigmentation, precocious puberty	Nephrocalcinosis, some have hypophosphatemic rickets	(143)
Apparent mineralocorticoid syndrome	Hypertension, hypokalaemia, alkalosis, reduced plasma renin	Nephrocalcinosis, medullary cysts	(144)

Table 51-4

Proteinuria/glomerulonephritis

Disorder	Main features	Renal features	References
Fabry's disease	See text Sensory changes in extremities, angiokeratoma, hyperhidrosis, corneal, lens opacities	Hyposthenuria, renal tubular acidosis, proteinuria, chronic renal failure	(93–99)
Imerslund-Grasbeck syndrome	See text	Proteinuria, urinary tract abnormalities	(26)
Lecithin: cholesterol acyltransferase deficiency	See text	Proteinuria, hematuria, renal failure	(104, 105)
Galactosialidosis (early infantile form)	Hydrops, edema, coarse facies, inguinal hernias, visceromegaly, spinal involvement, corneal and fundal abnormalities, cardiomyopathy, developmental delay, telangiectasias	Proteinuria, nephrotic syndrome	(146–149)
Glutamyl ribose-5-phosphate glycoproteinosis	Coarse facies, optic atrophy, muscle wasting, failure to thrive, seizures, neurological deterioration	Proteinuria, chronic renal failure	(150)
Mucopolysaccharidosis type I (Hurler syndrome)	Coarse facies, corneal clouding, visceromergaly, dysostosis multiplex, developmental delay	Nephrotic syndrome, hypertension (due to aortic luminal narrowing)	(151)
Congenital disorder of glycosylation type 1	See text and *Table 51-5*	Proteinuria (congenital nephrotic syndrome), microcysts	(71–77)
Infantile sialic acid storage disease	Hydrops, edema, dysmorphism, hepatosplenomegaly, hypotonia, poor growth	Proteinuria, nephrotic syndrome	(152)
Cystic fibrosis	See *Table 51-3*	Proteinuria, nephrotic syndrome (amyloidosis), microscopic nephrocalcinosis, calculi, chronic renal failure	(139–142)
α_1-antitrypsin deficiency	Liver and lung disease	Hematuria, membranoproliferative GN (only seen with advanced liver disease), chronic renal failure	(153, 154)
Lipoprotein glomerulopathy	Elevated apolipoprotein E	Nephrotic syndrome (resistant to treatment), hematuria, hypertension, renal failure. Glomerular lipoprotein thrombi	(155, 156)
Lysinuric protein intolerance	Vomiting, diarrhea after weaning, poor appetite and growth, hypotonia, visceromegaly, interstitial pneumonitis, encephalopathy	Proteinuria, hematuria, nephrotic syndrome, immune complex glomerulonephritis, tubulo-interstitial nephritis, renal tubular acidosis, renal failure	(109, 110)
Prolidase deficiency	Rash, dysmorphism, anemia, splenomegaly, recurrent infections	Systemic lupus erythematosus	(157)
Cobalamin C deficiency	See text	Hemolytic uremic syndrome, glomerulopathy	(23–25)
Mitochondrial disorders	See text	Fanconi syndrome Rarely proteinuria, nephrotic syndrome, tubulo-interstitial nephritis	(49–70)
Gaucher's disease	Hepatosplenomegaly, hypersplenism	Proteinuria, hematuria, acute glomerulonephritis Calculi (personal communication)	(158, 159)

Table 51-5

Metabolic causes of Renal cysts

Disorder	Main features	Renal features	References
Zellweger's syndrome	Facial dysmorphism, hypotonia, severe developmental delay, hepatomegaly	Microcysts/large cortical cysts	(160)
Alagille's syndrome	Intrahepatic cholestasis, dysmorphism, posterior embryotoxin, butterfly vertebrae, hypogonadism, peripheral pulmonary stenosis	Cysts, multicystic dysplastic kidney, unilateral agenesis, hypoplasia, urinary concentration defect, calculi, glomerular mesangiolipidosis, renal failure	(161, 162)
Congenital disorders of glycosylation	See text and *Table 51-4*	Cysts, proteinuria	(71–77)
Glutaric aciduria type II (Neonatal onset)	Prematurity, hepatomegaly, dysmorphism, genitourinary abnormalities, hypotonia, odor, metabolic acidosis, hypoglycemia	Cystic change (microcysts/polycystic kidneys), nephromegaly, renal dysgenesis	(163–166)
Nephronophthisis	Can be associated abnormalities: (Leber's amaurosis, retinopathy, liver disease, neurological and skeletal abnormalities)	Hyposthenuria, chronic renal failure.	See Chapter 35
Smith-Lemli-Opitz syndrome	Facial dysmorphism, hypotonia, mental retardation, abnormalities limbs, brain, genitalia. Cholesterol biosynthesis defect	Cystic dysplasia, hypoplasia, agenesis, duplication, PUJ obstruction, VUR	(167, 168)
Cystinosis	See *Table 51-1*	Cysts are very rare	(169)
Pearson's syndrome	Pancytopaenia, pancreatic dysfunction, tubulopathy See text (mitochondrial disorders)	Cortical cysts	(51, 54)
Fabry's disease	See text Sensory changes in extremities, angiokeratoma, hyperhidrosis, corneal, lens opacities	Renal sinus parapelvic cysts, hyposthenuria, proteinuria, chronic kidney disease	(94, 95, 99)
Apparent mineralocorticoid syndrome	Hypertension, hypokalaemia, alkalosis, reduced plasma renin	Nephrocalcinosis, medullary cysts	(144)

that disruption of mitochondrial function underpins the nephrotoxicity (2, 10).

Patients with MMA have hyperuricemia which might also be a factor in causing renal damage. Urate crystals have not however been observed in renal biopsy material and plasma urate is easily controlled in these patients with allopurinol (3).

Management of ESRF in Methylmalonic Acidemia

There are relatively few reports of the management of end-stage renal failure in MMA patients. Plasma methylmalonate appears to rise in association with plasma creatinine either due to reduced filtration or because it is nephrotoxic (10, 11, 13, 14). Hemodialysis clearly reduces plasma methylmalonate and may improve metabolic and nutritional status (10, 13). Since the deficient methylmalonyl CoA mutase is expressed in liver, a liver transplant should provide replacement enzyme. Several patients with MMA have now received liver transplants, usually as an isolated organ in young children in an attempt to prevent multisystem damage. (15). However there remains a significant mortality and morbidity rate with a number of survivors demonstrating chronic kidney disease and neurological dysfunction.

The first patient who received a combined liver-kidney transplant (10, 13) remains well with good renal and hepatic function 13 years after surgery (W. van't Hoff, personal communication). While here have been other successes (16), other patients have died shortly after the procedure and, worryingly, liver transplantation does not seem to protect the child from further neurological toxicity (12, 13, 17–19).There have been a few reports of isolated renal transplant in MMA. A 24 year old patient

Table 51-6

Abnormal Urine (with acknowledgment to Timo Kouri)

Disorder	Main features	Renal features	References
Alkaptonuria	Ochronotic pigmentation of cartilage and collagen, arthritis	Urine turns black on standing if alkaline, calculi (adults)	(170)
Porphyria (Acute intermittent)	Abdominal pain, constipation, neurologic features (usually after puberty)	Urine may turn red/purple	(171–173)
		Acute or chronic renal failure, urinary retention	
		Acute hypertension	
Some Organic acidemias	Progressive infantile encephalopathy, weight loss	Characteristic urine smell	(174)
		Maple syrup urine disease	(175)
	Recurrent acidosis	"Sweaty feet" in isovaleric/glutaric acidemia	
Some aminoacidurias	Developmental delay (untreated), light pigmentation, abnormal gait, eczema, epilepsy	Characteristic urine smell Musty/ mousy in phenylketonuria	(176)
			(112, 113)
	Hepatomegaly, liver disease, rickets, neurological crises	Rancid smell in tyrosinemia	
Blue diaper syndrome	Hypercalcemia, hypercalciuria	Nephrocalcinosis Indicanuria → blue discoloration urine	(132)

Table 51-7

Other manifestations

Disorder	Main features	Renal features	References
Menke's syndrome/ Ehlers-Danlos type IX/ Occipital Horn Syndrome (OHS)	X-linked disorders, abnormal hair and facies, developmental delay and regression. OHS have lax skin, herniae, no neurological features	Bladder or ureteric diverticulae	(177, 178)
Homocystinuria	Ectopia lentis, skeletal abnormalities, neuropsychiatric symptoms, thromboembolism	Renal infarction, hypertension	(179)
Carnitine palmitoyl transferase deficiency type II	Neonatal onset encephalopathy, hepatomegaly, cardiomyopathy	Renal dysgenesis	(180)
Glucose-6-phosphatase deficiency	Hemolytic anemia, jaundice	Acute renal failure	(181)
Exercise intolerance and myoglobinuria syndrome (various causes)	Recurrent hemolysis, muscle cramps, weakness, exercise intolerance, vomiting	Myoglobinuria, oligo/anuric acute renal failure (McArdle disease and carnitine palmitoyl transferase type II deficiency can also present in this way in adults).	(182–184)

demonstrated improved clinical and metabolic control after an isolated renal transplant although she developed diabetes mellitus and suffered marked cyclosporin toxicity (14, 20). A similarly favorable outcome, 10 years after transplant, with an improvement in urine methylmalonate excretion has been demonstrated in a 17 year old patient after renal transplantation (15, 21). Several other cases of renal transplantation in MMA have not been reported (personal communication) but in the author's view, this modality of treatment appears, at this time, to have a safer outcome at least in the short-term. The long term prognosis for any transplanted patient with MMA remains uncertain (16, 22).

Cobalamin Defects

Early-onset hemolytic uremic syndrome has been reported in a number of patients with cobalamin C or G deficiency (17, 18, 23, 24), in which there are defects in the synthesis of adenosylcobalamin and methylcobalamin (cobalamin C defect) or of methionine synthase (cobalamin G defect). Patients with cobalamin C deficiency have defective function of the two enzymes dependent on these cofactors (methylmalonyl CoA mutase and *N*-methyl tetrahydrofolate: homocysteine methyltransferase). These children therefore have homocystinuria, hypomethioninemia and cystathioninuria in addition to methylmalonic aciduria. Most present in early infancy with poor feeding, failure to thrive, hypotonia, retinitis, respiratory distress and cardiomyopathy. Investigation shows a megaloblastic anemia, pancytopenia and liver dysfunction. Generally the prognosis has been poor. There have been rare case reports of older children with cobalamin C deficiency who developed hypertension, proteinuria and chronic renal impairment, in association with FSGS or an unclassified glomerulopathy (18, 25).

A number of patients with defective absorption of the cobalamin-Intrinsic factor (IF) complex (Imerslund-Grasbeck syndrome) have persistent proteinuria (20, 26). The Cobalamin-IF complex binds to cubilin in the intestinal brush border. Cubilin is also expressed in the renal proximal tubule where it interacts with megalin to form a tandem endocytic receptor complex (21, 27). Cubilin is the major receptor for proximal tubular albumin reabsorption, thus patients with Imerslund-Grasbeck syndrome in which the cubilin gene is defective, and have albuminuria (22, 28). Other manifestations include megaloblastic anemia and biochemically, homocystinuria, homocystinemi, and methylmalonic aciduria (20, 26). In addition to the metabolic abnormalities, two children with this disorder were reported as suffering from recurrent urinary tract infections secondary to neuropathic bladders (23, 29).

Glycogen Storage Diseases

Clinical

The glycogen storage diseases (GSD) are genetic disorders of the metabolism and regulation of glycogen. Some forms of GSD have significant renal manifestations: GSD type 1 can lead to a tubulopathy and chronioc kidney disease, Fanconi-Bickel syndrome has a characteristic tubulaopathy and Tarui disease (type VII) can lead to acute rhabdomyolysis and renal failure. GSD type 1 is characterized by a deficiency of glucose-6-phosphatase in the liver, kidne, and intestine, causing excessive glycogen storage, hepatomegaly, hypoglycemia, lactic acidosis, hyperuricemia, hyperlipidemia, and poor growth. Long term complications include delayed puberty, hepatic adenomata and renal disease. Chen and colleagues drew attention to the complication of ESRF in older GSD patients (24, 30). In their review in 1988, 18 children under 10 years had normal renal function, while 4 of 20 patients aged 13–47 years, had renal dysfunction (proteinuria, hypertension or chronic renal failure). Subsequent investigators have demonstrated renal tubular and glomerular abnormalities. Ultrasonography demonstrates the renal enlargement secondary to glycogen deposition (25–28, 31–33).

The mechanisms of renal damage are not fully understood although there may be important compasisons with diabetic nephropathy (34). Both diabetes mellitus and GSD I involve increased flux through the pentose phosphate pathway, increasing triose phosphates and diacylglycerol and thereby stimulating protein kinase C and the renin-angiotensin system. Yiu and colleagues demonstrated upregulation of angiotensin in a mouse model of GSD 1a (35).

Renal Tubular Dysfunction

Proximal tubular dysfunction occurs as an early feature in GSD 1 but is generally sub-clinical and a Frank Fanconi syndrome is rare. Although tubular proteinuria (RBP, β2 M) and enzymuria (NAG) is significantly elevated in GSD 1 patients, plasma electrolytes are less disturbed (26, 27, 32, 33). Distal tubular function is also perturbed with hypercalciuria and hypocitraturia, predisposing GSD 1 patients to nephrolithiasis (29, 30, 36, 37). A Gitelman-like syndrome of hypomagnesemia and hypocalciuria has also been described in a patient with glycogen storage disease type II (31, 38).

Glomerular Dysfunction

Patients with GSD 1 develop albuminuria and hyperfiltration (26, 27, 30, 32, 33). In a Dutch study, mean GFR and ERPF were 188 and 927 ml/min/1.73 m^2, respectively (normal values for adult controls 90–145 and 327–697, respectively) in a group of 23 GSD 1 patients, aged 2–22 years (26, 32). Persistent hyperfiltration leads to focal and global glomerulosclerosis and a decline in GFR

(32, 33, 39, 40). Other histologic abnormalities include glycogen deposition in proximal tubules, glomerular enlargement and thickening and lamellation of GBM (34, 35, 41). Management of the metabolic abnormalities, including frequent feeds and the use of uncooked cornstarch are the mainstays of treatment of GSD nephropathy. Anti-proteinuric and lipid lowering agents may have a role (42). Liver transplantation has been performed to prevent malignant change in hepatic adenomata and combined liver-kidney transplantation has also been successful (43).

Fanconi Bickel Syndrome

The Fanconi Bickel syndrome is a very rare autosomal recessive disorder of monosaccharide transport, presenting in the first year of life with hepatomegaly (due to glycogen storage), hypoglycemia and a severe generalized proximal tubulopathy leading to rickets. The extent of the Fanconi syndrome can be equal in magnitude to that in disorders such as cystinosis or tyrosinemia, but is particularly characterized by heavy glycosuria and galactosuria (37–39, 44–46). Treatment is directed towards frequent feeds (and the use of uncooked cornstarch) together with management of the tubulopathy. The condition arises due to mutations in the GLUT2 facilitative glucose transporter, which is expressed in hepatocytes, pancreatic beta cells and the basolateral membranes of intestinal and renal tubular epithelial cells (40, 47). Decreased monosaccharide uptake by the liver explains the post-prandial hyperglycemia and hypergalactosemia (which is exacerbated by inappropriately low insulin secretion due to abnormal glucose-sensing by pancreatic beta cells). The inability of the liver to transport glucose, together with heavy losses of glucose from the renal tubule, contributes to pre-prandial hypoglycemia (40, 47). Renal glomerular hyperfiltration, microalbuminuri, and diffuse mesangial expansion have been reported in one child (41, 48) and reduced GFR has been observed in some adults.

Mitochondrial Disorders

Introduction

Mitochondrial disorders are caused by defects in the respiratory chain enzymes, which are located in this organelle. The respiratory chain is responsible for the process of oxidative phosphorylation in which electrons are transferred to oxygen generating a proton gradient. The flow of protons back through the mitochondrial membrane releases energy, which allows for the formation of ATP. The respiratory chain enzyme complexes are encoded partly by mitochondrial and partly by nuclear DNA. Mitochondrial DNA (mtDNA) differs from nuclear DNA: it is much smaller, has a unique genetic code, exists as a double stranded loop and is inherited exclusively from the mother. In contrast to nuclear DNA, mtDNA is randomly segregated during cell division. The proportion of mutant and wild-type mtDNA can therefore vary from tissue to tissue and can also alter over time, explaining the enormous heterogeneity of mitochondrial disorders (42, 49). The classification of mitochondrial disorders has been based on clinical, biochemica, and molecular phenotypes. The clinical features are extremely heterogeneous and often change with time but virtually all patients have neurological symptoms at some point. Frequent manifestations include myopathy, encephalopathy, seizures, developmental delay, ophthalmoplegia, retinal degeneration, cardiomyopathy, endocrinopathy, and liver disease.

Renal Manifestations

In a review of 42 patients with mitochondrial disorders, 21 had renal involvement (50). Varying degrees of tubular dysfunction may be seen but the commonest distinct renal phenotype is a Fanconi syndrome, usually occurring in an infant with multisystem dysfunction in which case the prognosis is poor (43, 44, 51, 52). Generalized proximal tubular dysfunction has also been reported in children with other mitochondrial syndromes including Kearns-Sayre, Pearson's, Leigh's encephalopathy and Coenzyme Q (CoQ(10)) deficiency (43, 45–49, 51, 53–56). In some children, hypomagnesemia and hypocalcemia are the predominant electrolyte abnormalities (48–50, 57–59). Other tubular manifestations include a Bartter-like syndrome (51, 60, 61), renal tubular acidosis, and hypercalciuria (52, 53, 62, 63). Renal glomerular involvement with proteinuria, congenital nephrotic syndrome, focal segmental glomerulosclerosis, nephronophthisis, tubulo-interstitial nephritis or chronic renal impairment are rarer associations of mitochondrial disorders (43, 51, 54–56, 64–67). An Alport-like phenotype of sensorineural deafness, nephropathy and diabetes has been reported in an adult pedigree subsequently found to have a mitochondrial mutation (68). Renal cysts have been reported in both adults and children with mitochondrial disorders (57, 58, 69, 70). In most children the renal lesion is part of multisystem dysfunction, but there are occasional reports of patients presenting with FSGS and later developing

other manifestations of mitochondrial disease (54, 59, 65, 70). A high index of suspicion is therefore required to diagnose a mitochondrial disorder. Unfortunately measurement of the plasma lactate/pyruvate ratio which is the first investigation of a child with mitochondrial dysfunction may give a normal result in a patient with a renal tubulopathy due to loss of lactate in the urine (43, 51). In such a case, careful delineation of other system involvement, biopsy and detailed biochemical and molecular studies may be required to confirm a defect in mitochondrial function.

Congenital Disorders of Glycosylation

The congenital disorders of glycosylation (CDG) are a group of inherited multisytem disorders in which there is defective *N*- and/or *O*-glycosylation of proteins (60, 71–73). Many sub-types have been described and the presentation is very heterogeneous, ranging from multiple exostoses, progeria, developmental delay in older children to a fatal multisystem disorder in infancy (72). Children with the commonest form, CDG Ia may have dysmorphism, hypotonia, failure to thrive, diarrhea, abnormal fat pads, inverted nipples, abnormal eye movements, hepatomegal, and cardiomyopathy. Investigations often show hypothyroidism and olivoponto-cerebellar atrophy (72). Diagnostically, abnormal isoelectric focusing of serum transferrin is the first screening test (although will not identify some rarer forms), and if abnormal, should lead to more detailed enzymatic and other tests (72).

The commonest renal manifestation is the presence of microcysts which produce a hyperechoic picture on ultrasonography, most commonly found in children with multisystem variants (61, 62, 74–76). The cysts are located in the cortex and probably arise from the tubules (63, 77). The kidneys may be enlarged and single cysts have also been reported (62, 75). Microcysts are not seen in all patients; even within a pair of affected siblings, only one had renal cysts (61, 74). Proteinuria has been recorded in several cases and may contribute to the severe edema and ascites that these infants can develop (62, 75). There have also been reports of nephrotic syndrome, both occurring within the first two months of life (63–65, 77, 78). One of these infants also developed renal failure and at post mortem the kidneys showed features of diffuse mesangial sclerosis (64, 78). Transferrin isoelectric focusing should be added to the list of investigations required to exclude a metabolic cause of nephrotic syndrome in infants (the test is not affected by the nephrotic state or by renal failure (64, 72)).

Disorders of Purine Metabolism and Transport

Children who develop renal calculi, acute renal failure in the neonatal period or crystal nephropathy require investigation of their purine metabolism. Purines are involved in the synthesis of nucleotides and coenzymes, in signal transduction (e.g., cAMP) and in the generation of ATP. The metabolic end product, uric acid, and its immediate precursor (xanthine) are insoluble in urine, so that overproduction can predispose to the development of crystal formation. Urate is normally extensively reabsorbed in the proximal tubule. The process is age- and sex-dependent with children reabsorbing less of the filtered urate and consequently having lower plasma urate concentrations (66, 79). As glomerular filtration rate declines, the fractional excretion of urate increases (67, 80).

Urolithiasis is the commonest renal manifestation of a disorder of purine production (e.g., in adenine phosphoribosyl transferase (APRT) deficiency and hypoxanthine-guanine phosphoribosyl transferase (HGPRT) deficiency; see chapter XX Urolithiasis etc.). Children may or may not have typical features of calculi (pain, hematuria, infection). In some the diagnosis is made following family studies or during investigation of crystalluria. Very rarely, these disorders present with oliguric or anuric acute renal failure, either due to bilateral obstructive calculi (81) or due to crystal nephropathy which can sometimes occur within the neonatal period (82, 83). Lesch-Nyhan syndrome is inherited in an X-linked recessive manner and nearly always presents in males. However very rarely heterozygous females can manifest equally severe symptoms (84). In both APRT and HGPRT deficiencies the urinary urate/creatinine ratio is normal, the plasma urate is also normal in APRT deficiency but is grossly elevated in HGPRT deficiency (79). In these cases detailed investigation of purine metabolism is required. Both conditions are treated with a high fluid intake, restriction of dietary purines and with allopurinol, which inhibits xanthine oxidase. Rasburicase, a urate oxidase enzyme, has also been used to control hyperuricemia in a neonate with Lesch-Nyhan nephropathy (85).

Hyperuricemia with subsequent hyperuricosuria is also a feature of glycogen storage disease type I. Calculi and renal failure can also occur as a result of disorders of urate transport in the renal tubule. Hyperuricosuria and hypouricemia can occur as a result of generalized proximal tubular dysfunction (e.g., Fanconi syndrome) but has also been described as an isolated transport defect associated with hypercalciuria, decreased bone density and

the development of calculi composed of a mixture of calcium oxalate and urate (69–72, 86–88).

Familial juvenile hyperuricemic nephropathy (FJHN) is an autosomal dominant disorder leading to hyperuricemia, gou, and progressive renal failure (66, 67, 79, 89). The onset is frequently in childhood or early adult life. The disorder is characterized biochemically by hyperuricemia due to a reduced fractional excretion of urate (<12%). There is evidence that hyperuricemia predates renal impairment and it is known that allopurinol reduces the progression of the nephropathy suggesting that renal damage is related to hyperuricemia (73, 74, 89, 90). There is a considerable overlap in this phenotype with that of medullary cystic kidney disease type 2, an autosomal dominant disorder causing a urinary concentrating defect, salt wasting, polyuria, and also associated with hyperuricemia and gout. Despite its name, renal cysts are not necessarily seen. The genetic bases of these disorders are complex. Four mutations in the gene encoding Uromodulin (UMOD) have been identified in three families with MDCK2 and one with FJHN (91). Further studies have demonstrated the clinical and genetic overlap and variability in these disorders (92).

Fabry's Disease

Fabry's disease is an X-linked disorder in which glycosphingolipids (predominantly globotriaosylceramide) accumulate in plasma and tissues as a result of a deficiency of α-galactosidase A (75, 93, 94). Affected males usually present in childhood (median age about 9 years) with recurrent painful crises of the hands and feet, a characteristic skin rash (angiokeratoma corporis) and hypohidrosis but the diagnosis is often delayed for years. Slit lamp examination reveals corneal and lenticular opacities. Progressive glycosphingolipid deposition in the heart, blood vessels and kidneys leads to the development of valvular and conduction abnormalities, angina, cerebrovascular disease, and progressive renal damage, usually in adult life. Overt renal disease is rare in children but renal histological changes can be demonstrated in children even without proteinuria (95). However, proteinuria, chronic renal failure, and even ESRF can occur by 15 years of age (76, 77, 94, 96). The urine may contain casts and desquamated cells containing lipid globules (78, 98). Subsequently, polyuria and polydipsia indicate defective tubular function and end-stage renal failure occurs typically around the fourth decade (76, 77, 96). Histological examination of the kidney will demonstrate inclusions with a characteristic "onion skin" appearance, in tubular epithelia, glomerular cells (especially podocytes) and endothelium (98). Glycosphingolipid deposits are seen in endothelial and epithelial cells of the glomerulus, Bowman's capsule and in the distal tubules (98). In an adult review of 24 patients, mean age 38 years, 50% had renal sinus cysts compared to 7% in healthy matched controls, leading the authors to suggest that multiple renal sinus cysts in a patient with kidney disease should raise suspicion of Fabry's (99).

The renal prognosis may bear some relationship to the level of detectable α-galactosidase A activity: onset of CRF was significantly later in those with > 1% activity compared to those with undetectable activity (77, 94). Recent studies indicate that approximately 1% adult males with undiagnosed causes of ESRF, have Fabry disease (80, 81, 100). Affected females (heterozygotes) generally have milder or delayed manifestations although as a result of random X-inactivation, these can be variable (76, 94). In an adult review, proteinuria was found in most patients, 28% males and 13% of females had chronic kidney disease with eGFR < 60 ml/min/1.73 m^2 generally with heavier proteinuria (101). There is good evidence in both adults and children, that treatment with recombinant human alpha-galactosidase A can ameliorate symptoms, maintain renal function and lead to good clearance of GL-3 in plasma, kidney, skin, and heart (102, 103, 104).

Lecithin-Cholesterol Acyl Transferase (LCAT) Deficiency

LCAT is required for the esterification of cholesterol with unsaturated fatty acid derived from lecithin. Patients with LCAT deficiency accumulate unesterified cholesterol and phosphatidylcholine in plasma and tissues (104). Sequelae of tissue accumulation occur within childhood and include grayish corneal opacities, a hemolytic anemia and proteinuria (sometimes in the nephrotic range) leading to progressive renal failure in adulthood (104, 105). Tendon xanthomata and atherosclerosis have been described in a few cases. Biochemically, LCAT deficiency is characterized by a low total cholesterol concentration, variable triglyceride concentration and abnormalities of lipoprotein structure and composition. The histology of the kidney shows mesangial hypercellularity and expansion with foam lipid deposits, holes and vacuolization in the glomerular basement membrane, arteriolar narrowing due to intimal thickening and subendothelial lipid deposits (105).

Lysinuric Protein Intolerance

Lysinuric protein intolerance (LPI) is a rare autosomal recessive disorder, prevalent in Finland and characterized by defective gastrointestinal and renal transport of the amino acids ornithine, arginine, and lysine. Low plasma concentrations of these amino acids impair urea cycle function, causing reduced nitrogen tolerance and hyperammonemia. Patients self-select a protein poor diet, but as a consequence are nutritionally deficient in many other substrates (106). LPI is due to mutations in the SLC7A7 gene, which encodes the y(+)LAT-1 protein, the catalytic light chain subunit of the heterodimeric amino acid transporter (107, 108). In a review of 39 patients, 74% had proteinuria, 38% hematuria, 36% hypertension, 38% a raised plasma creatinine and 4 patients required dialysis (109). A renal Fanconi syndrome has also been described (110).

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52 Infectious Diseases and the Kidney

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The kidney is involved in a wide range of bacterial, viral, fungal, and parasitic diseases. In most systemic infections, renal involvement is a minor component of the illness, but in some, renal failure may be the presenting feature and the major problem in management. Although individual infectious processes may have a predilection to involve the renal vasculature, glomeruli, interstitium, or collecting systems, a purely anatomic approach to the classification of infectious diseases affecting the kidney is rarely helpful because most infections may involve several different aspects of renal function. In this chapter, a microbiologic classification of the organisms affecting the kidney is adopted. Although they are important causes of renal dysfunction in infectious diseases, urinary tract infections and hemolytic uremic syndrome (HUS) are not discussed in detail because they are considered separately in Chapters 54 and 48 respectively.

Elucidation of the cause of renal involvement in a child with evidence of infection must be based on a careful consideration of the geographic distribution of infectious diseases in different countries. A history of foreign travel; exposure to animals, insects, or unusual foods or drinks; outdoor activities such as swimming or hiking; and contact with infectious diseases must be sought in every case. The clinical examination should include a careful assessment of skin and mucous membranes and a search for insect bites, lymphadenopathy, and involvement of other organs. A close collaboration with a pediatric infectious disease specialist and hospital microbiologist will aid the diagnosis and management of the underlying infection.

A tantalizing clue to the pathogenesis of glomerular disease is the marked difference in the incidence of nephrosis and nephritis in developed and underdeveloped areas of the world. In several tropical countries, glomerulonephritis (GN) accounts for up to 4% of pediatric hospital admissions; the incidence in temperate climates is 10- to 100-fold less. This difference might be explained by a complex interaction of several different factors, including nutrition, racial and genetically determined differences in immune responses, and exposure to infectious diseases. A growing body of evidence, however, suggests that long-term exposure to infectious agents is a major factor in the increased prevalence of glomerular diseases in developing countries.

Renal involvement in infectious diseases may occur by a variety of mechanisms: direct microbial invasion of the renal tissues or collecting system may take place in conditions such as staphylococcal abscess of the kidney as a result of septicemic spread of the organism or as a consequence of ascending infection; damage to the kidney may be caused by the systemic release of endotoxin or other toxins and activation of the inflammatory cascade during septicemia or by a focus of infection distant from the kidney; ischemic damage may result from inadequate perfusion induced by septic shock; the kidney may be damaged by activation of the immunologic pathways or by immune complexes resulting from the infectious process. In many conditions, a combination of these mechanisms may be operative. In the assessment of renal complications occurring in infectious diseases, the possibility of drug-induced nephrotoxicity caused by antimicrobial therapy should always be considered. The nephrotoxic effects of antibiotics and other antimicrobial agents are not addressed in this chapter but are covered in Chapter 53.

Bacterial Infections

Bacterial infections associated with renal disease and the likely mechanisms causing renal dysfunction are shown in *Table 52-1.*

Systemic Sepsis and Septic Shock

Impaired renal function is a common occurrence in systemic sepsis (1). Depending on the severity of the infection and the organism responsible, the renal involvement may vary from insignificant proteinuria to acute renal failure requiring dialysis. The organisms causing acute renal failure as part of systemic sepsis vary with age and geographic location and also differ in normal and immunocompromised children. In the neonatal period, group B streptococci, coliforms, *Staphylococcus aureus*, and *Listeria monocytogenes* are the organisms usually

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Table 52-1

Likely mechanisms causing renal dysfunction in bacterial infections

Organism	Site/infection	Infection localized to kidney	Systemic infection; toxin/ inflammation	Ischemia/ hypoperfusion; vasomotor nephropathy	Distant infection; "immunologic/ delayed"	Others
Neisseria meningitidis	Septicemia		++	++		
	Chronic meningococcemia				+	
Staphylococcus aureus	Renal abscess	++				
	Distant abscess/ endocarditis (see *Table 52-2*)				++	
	Sepsis		++	++		
	Toxic shock		++	++		
Staphylococcus epidermidis	Shunt infection (see *Table 52-2*)				++	
Group A *Streptococcus*	Sepsis		++	++		
	Toxic shock		++	++		
	APSGN				++	
Haemophilus influenzae (new biotype)	Brazilian purpuric fever		++	++		
Leptospira interrogans	Leptospirosis, Weil's disease	++		++		
Streptococcus pneumoniae	Sepsis		++	++		
	Pneumonia					+ (HUS)
Escherichia coli and *Shigella*	Sepsis		++	++		
	Diarrhea			++		
	Colitis				++ (HUS)	
Salmonella species	Sepsis		++	++	+	
	Diarrhea			++		+ (HUS)
Vibrio species	Cholera			++		
Klebsiella	Sepsis		++	++		
Yersinia species	Enteritis				+	+ (HUS)
Campylobacter jejuni	Enteritis				+	
Mycobacteria tuberculosis	Tuberculosis	+			+	
Treponema pallidum	Syphilis				+	

Table 52-1 (Continued)

Organism	Site/infection	Infection localized to kidney	Systemic infection; toxin/ inflammation	Ischemia/ hypoperfusion; vasomotor nephropathy	Distant infection; "immunologic/ delayed"	Others
Mycoplasma pneumoniae	Pneumonia				+	+ (HUS)
Legionella	Pneumonia	+				Rhabdomyolysis
Rickettsia rickettsii	Rocky mountain spotted fever	+		+		
Coxiella burnetii	Q fever				+	

++, frequent complication of infection; +, uncommon but recognized complication; APSGN, acute post-streptococcal glomerulonephritis; HUS, hemolytic uremic syndrome

responsible. In older children, *Neisseria meningitidis*, *Streptococcus pneumoniae*, and *S. aureus* account for most of the infections. In people who are immunocompromised, a wide range of bacteria are seen, and, similarly, in tropical countries other pathogens, including *Haemophilus influenzae*, Salmonella species, and *Pseudomonas pseudomallei*, must be considered. Where *H. influenzae* type B vaccine has been introduced, however, the incidence of severe systemic infections due to this organism has shown a sharp fall.

Systemic sepsis usually presents with nonspecific features: fever, tachypnea, tachycardia, and evidence of skin and organ underperfusion. The pathophysiology of renal involvement in systemic sepsis is multifactorial (1, 2). Hypovolemia with diminished renal perfusion is the earliest event and is a consequence of the increased vascular permeability and loss of plasma from the intravascular space. Hypovolemia commonly coexists with depressed myocardial function because of the myocardial depressant effects of endotoxin or other toxins. The renal vasoconstrictor response to diminished circulating volume and reduced cardiac output further reduces glomerular filtration, and oliguria is thus a consistent and early event in severe sepsis (1, 3). A number of vasodilator pathways are activated in sepsis, including Nitric Oxide and the Kinin pathways. This may lead to inappropriate dilatation of vascular beds. Vasodilation of capillary beds leading to warm shock is common in adults with sepsis due to Gram-negative organisms but is less commonly seen in children, in whom intense vasoconstriction is the usual response to sepsis. If renal underperfusion and vasoconstriction are persistent and severe, the reversible prerenal failure is followed by established renal failure with the characteristic features of vasomotor nephropathy or acute tubular necrosis. Other mechanisms of renal damage in systemic sepsis include direct effects of endotoxin and other toxins on the kidney, and release of inflammatory mediators such as tumor necrosis factor (TNF) and other cytokines, arachidonic acid metabolites, and proteolytic enzymes (3). Nitric oxide (NO) is postulated to play a key role in the pathophysiology of renal failure in sepsis. Whether the renal effects of increased NO are beneficial or harmful remains unclear. Trials of selective NO synthetase inhibition did not offer any advantages over saline resuscitation (4). NO in endotoxemia is possibly beneficial because it maintains renal blood flow and glomerular filtration. Activation of coagulation is an important component of the pathophysiology of septic shock and may contribute to renal impairment. Activation of multiple prothrombotic and antifibrinolytic pathways occurs, together with downregulation of antithrombotic mechanisms such as the protein C pathway. Treatment with Activated protein C has been shown to improve outcome of adult septic shock, but has not been confirmed to have benefit in pediatric sepsis, and may carry a risk of bleeding particularly in infants (5).

The renal findings early in septic shock are oliguria, with high urine/plasma urea and creatinine ratios, low urine sodium concentration, and a high urine/plasma osmolarity ratio. Once established, renal failure supervenes, and the urine is of poor quality with low urine/plasma urea and creatinine ratios, elevated urine sodium concentration, and low urine osmolarity. Proteinuria is usually present, and the urine sediment may contain red cells and small numbers of white cells (1).

Management of Acute Renal Failure in Systemic Sepsis

The management of acute renal failure in systemic sepsis depends on early diagnosis and administration of appropriate antibiotics to cover the expected pathogens.

In addition, management is directed at improving renal perfusion and oxygenation. Volume replacement with crystalloid or colloid should be undertaken to optimize preload. Central venous pressure or pulmonary wedge pressure monitoring is essential to guide volume replacement in children in severe shock (1, 2). The use of low-dose (2–5 pg/kg/min) dopamine to reduce renal vasoconstriction together with administration of inotropic agents such as dobutamine or epinephrine to improve cardiac output may reverse prerenal failure. Early elective ventilation should be undertaken in patients with severe shock. If oliguria persists despite volume replacement and inotropic therapy, dialysis should be instituted early, because septic and catabolic patients may rapidly develop hyperkalemia and severe electrolyte imbalance.

In most children who develop acute renal failure as part of systemic sepsis or septic shock, the renal failure is of short duration, and recovery can be expected within a few days of achieving cardiovascular stability and eradication of the underlying infection. Occasionally, renal cortical necrosis or infarction of the kidney may result in prolonged or permanent loss of renal function.

Specific Infections Causing the Systemic Sepsis Syndrome

Meningococcus

N. meningitidis continues to be a major cause of systemic sepsis and meningitis in both developed and underdeveloped parts of the world (6). In developed countries, most cases are caused by group B and Y strains, particularly after introduction of meningococcal C vaccination, whereas epidemics of Meningococcus groups A, C and W135 continue to occur in many underdeveloped regions of the world (6, 7). Infants and young adults are most commonly affected, but cases in adolescents and young children are also common. There are two major presentations of meningococcal disease (6): *Meningococcal meningitis* presents with features indistinguishable from those of other forms of meningitis, including headache, stiff neck, and photophobia. Lumbar puncture is required to identify the causative agent and distinguish this from other forms of meningitis. Despite the acute nature of the illness, the prognosis is good, and most patients with the purely meningitic form of the illness recover without sequelae.

Meningococcemia with purpuric rash and shock is the second and more devastating form of the illness. Affected patients present with nonspecific symptoms of fever, vomiting, abdominal pain, and muscle ache. The diagnosis is only obvious once the characteristic petechial or purpuric rash appears. Patients with a rapidly progressive purpuric rash, hypotension, and evidence of skin and organ underperfusion have a poor prognosis, with a mortality of 10–30%. Adverse prognostic features include hypotension, a low white cell count, absence of meningeal inflammation, thrombocytopenia, and disturbed coagulation indices (8).

Renal failure was seldom reported in early series of patients with meningococcemia, perhaps because most patients died rapidly of uncontrolled septic shock. With advances in intensive care, however, more children are surviving the initial period of profound hemodynamic derangement, and renal failure is more often seen as a major management problem. Approximately 10% of children with fulminant meningococcemia develop renal failure, which usually occurs 24–48 h after the onset of illness (9).

The pathophysiology of meningococcal septicemia involves the activation of cytokines and inflammatory cells by endotoxin (6, 7). Mortality is directly related to both the plasma endotoxin concentration and the intensity of the inflammatory response, as indicated by levels of TNF and other inflammatory markers (10). Patients with meningococcemia have a profound capillary leak leading to severe hypovolemia. Loss of plasma proteins from the intravascular space is probably the major cause of shock (11), but myocardial suppression secondary to IL-6 production is also important (12). Intense vasoconstriction further impairs tissue and organ perfusion, and vasculitis with intravascular thrombosis and consumption of platelets and coagulation factors is also present (6).

Oliguria is invariably present in children with meningococcemia during the initial phase of the disorder. This is prerenal in origin and may respond to volume replacement and inotropic support. If cardiac output cannot be improved and renal underperfusion persists, established renal failure supervenes. Occasionally, cortical necrosis or infarction of the kidneys occurs. Children with meningococcemia should be aggressively managed in a pediatric intensive care unit, with early administration of antibiotics (penicillin or a third-generation cephalosporin), volume replacement, hemodynamic monitoring, and the use of inotropic agents and vasodilators. If oliguria persists despite measures to improve cardiac output, elective ventilation and dialysis should be instituted early (6, 7). Because activation of coagulation pathways occurs, severe acquired protein-C deficiency may result and is usually associated with substantial mortality (13). Protein C is a natural anticoagulant which also has important anti-inflammatory activity. Despite evidence for impaired function of the activated protein C pathway in meningococcal diseases (14), and adult trials suggesting benefit of

activated protein C administration in Septic shock (PROWESS trial) (15), pediatric trials of activated protein C showed no clear benefit, and were associated with increased risk of intracranial bleeding in very young infants (5). The role of aPC therapy in pediatric sepsis remains unclear. Most patients who survive the initial 24–48 h of the illness and regain hemodynamic stability will ultimately recover renal function even if dialysis is required for several weeks.

The least common presentation of meningococcal sepsis is chronic meningococcemia. Patients with this form of the illness present insidiously with a vasculitic rash, arthritis, and evidence of multiorgan involvement. The features may overlap those of Henoch-Schonlein purpura or subacute bacterial endocarditis (SBE), and the diagnosis must be considered in patients presenting with fever, arthritis, and vasculitic rash, often accompanied by proteinuria or hematuria. Response to antibiotic treatment is good, but some patients may have persistent symptoms for many days resulting from an immune-complex vasculitis.

Staphylococcus Aureus

Staphylococcal infections may affect the kidneys by direct focal invasion during staphylococcal septicemia, forming a renal abscess; by causing staphylococcal bacteremia; or by toxin-mediated mechanisms, as in the staphylococcal toxic shock syndrome.

Staphylococcal Abscess. Staphylococcal renal abscess presents with fever, loin pain and tenderness, and abnormal urine sediment, as do abscesses caused by other organisms (16). The illness often follows either septicemia or pyelonephritis. The diagnosis is usually considered only when a patient with clinical pyelonephritis shows an inadequate response to antibiotic treatment. The diagnosis is confirmed by ultrasonography or computed tomographic scan, which shows swelling of the kidney and intrarenal collections of fluid. Antibiotic therapy alone may result in cure, but if the patient remains unwell with evidence of persistent inflammation despite use of appropriate antibiotics, surgical intervention may be required. Percutaneous drainage under ultrasonographic or computed tomographic scan guidance is often effective and may avoid the need for a more direct surgical approach (16, 17).

Staphylococcal Toxic Shock Syndrome. The staphylococcal toxic shock syndrome is a systemic illness characterized by fever, shock, erythematous rash, diarrhea, confusion, and renal failure. The disorder was first described by Todd et al. in 1978 in a series of seven children (18). During the 1980s, thousands of cases were reported in the United States. Most cases were in menstruating women, in associated with tampon use. Although most cases worldwide are seen in women and are associated with menstruation, children of both sexes and of all ages are affected (19).

The illness usually begins suddenly with high fever, diarrhea, and hypotension, together with a diffuse erythroderma (20). Mucous membrane involvement with hyperemia and ulceration of the lips and oral mucosa or vaginal mucosa, strawberry tongue, and conjunctival injection are usually seen. Desquamation of the rash occurs in the convalescent phase of the illness. Confusion is often present in the early stages of the illness and may progress to coma in severe cases. Multiple organ failure with evidence of impaired renal function, elevated levels of hepatic transaminases, thrombocytopenia, elevated CPK and disseminated intravascular coagulation (DIC) is often seen.

According to CDC criteria, the diagnosis is made on the basis of the clinical features of fever, rash, hypotension, and subsequent desquamation along with deranged function of three or more of the following organ systems: gastrointestinal (GI), mucous membranes, renal, hepatic, hematologic, central nervous system, and muscle. Other disorders causing a similar picture, such as Rocky Mountain spotted fever, leptospirosis, measles, and streptococcal infection, must be excluded.

The staphylococcal toxic shock syndrome is now known to be due to infection or colonization with strains of *S. aureus* that produce one or more protein exotoxins (21). Most cases in adults are associated with toxic shock toxin I; in children, many of the isolates associated with the syndrome produce other enterotoxins (A to F). The staphylococcal enterotoxins appear to induce disease by acting as superantigens (22), which activate T cells bearing specific V beta regions of the T-cell receptor; this causes proliferation and cytokine release (23). The systemic illness and toxicity are believed to result largely from an intense inflammatory response induced by the toxin. The site of toxin production is often a trivial focus of infection or simple colonization, and bacteremia is rarely observed.

Renal failure in toxic shock syndrome is usually caused by shock and renal hypoperfusion. In the early stages of the illness, oliguria and renal impairment are usually prerenal and respond to treatment of shock and measures to improve perfusion. In severe cases and in patients in whom treatment is delayed, acute renal failure develops as a consequence of prolonged renal underperfusion, and dialysis may be required. In addition to underperfusion, direct effects of the toxin or inflammatory

mediators may also contribute to the renal damage. Recovery of renal function usually occurs, but in severe cases with cortical necrosis or intense renal vasculitis, prolonged dialysis may be required.

The management of staphylococcal toxic shock syndrome depends on early diagnosis and aggressive cardiovascular support with volume replacement, inotropic support, and, in severe cases, elective ventilation. If oliguria persists despite optimization of intravascular volume and administration of inotropic agents, dialysis should be commenced early (20).

Anti-staphylococcal antibiotics should be started as soon as the diagnosis is suspected and the site of infection identified. Initial empiric antimicrobial therapy should include an anti-staphylococcal antibiotic effective against betalactamase-resistant organisms and a protein synthesis-inhibiting antibiotic such as clindamycin to stop further toxin production (24). If there is a focus of infection such as a vaginal tampon, surgical wound, or infected sinuses, the site should be drained early to prevent continued toxin release into the circulation. The intravenous administration of immune globulins may be considered when infection is refractory to several hours of aggressive therapy, an undrainable focus is present, or persistent oliguria with pulmonary edema occurs (24). With aggressive intensive care, most affected patients survive, and renal recovery is usual, even in patients who have had severe shock and multiorgan failure. Relapses and recurrences of staphylococcal toxic shock syndrome occur in a proportion of affected patients because immune responses to the toxin are ineffective in some individuals.

Panton Valentine Leucocidin (PVL) producing staphylococcal infection: In recent years there have been increasing reports of severe staphylococcal disease, associated with shock and multiorgan failure, caused by strains of staphylococci producing the PVL toxin. Panton-Valentine Leukocidin (PVL) is a phage-encoded toxin, which profoundly impairs the host response due to its toxic effect on leucocytes (see review (25)). PVL producing strains are associated with tissue necrosis and increased propensity to cause abscesses in lung, bone, joint, and soft tissue infections. Perinephric abscesses have been reported (26). There are increasing numbers of children and adults admitted with fulminant sepsis, and shock due to PVL producing strains, and renal failure is a significant component of the multiorgan failure. In addition to intensive care support, antibiotic treatment of PVL strains should include antibiotics which reduce toxin production, such as clindamycin, linezolid or rifampicin, as well as vancomycin if the strain is resistant to methicillin. Beta-lactam antibiotics should be avoided, as there is some data to suggest that PVL toxin production can increased by these antibiotics under some conditions (27). Immunoglobulin infusion may also be of benefit. Aggressive surgical drainage of all collections requires close consultation with orthopedic and surgical teams.

Streptococcus Pyogenes

The group A streptococci (GAS) are a major worldwide cause of renal disease, usually as poststreptococcal nephritis. However, in addition to this post-infection immunologically mediated disorder, in recent years there have been increasing reports of GAS causing acute renal failure as part of an invasive infection with many features of the staphylococcal toxic shock syndrome (28).

Acute Poststreptococcal Glomerulonephritis. Acute poststreptococcal GN (APSGN) is a delayed complication of pharyngeal infection or impetigo with certain nephritogenic strains of GAS. Different strains can be serotyped according to the antigenic properties of the M protein found in the outer portion of the bacterial wall. APSGN after pharyngeal infection is most commonly associated with serotype M12. In contrast, in APSGN after impetigo, serotype M49 is most commonly identified (29). On occasions, other serotypes and non-typeable strains have been described as causing GN.

The pathology and pathogenesis of the disorder is discussed in detail in Chapter 30. APSGN has a worldwide distribution. Epidemiologic differences are observed between pharyngitis-associated and impetigo-associated streptococcal infections. Pharyngitis-associated APSGN is most common during school age and has an unexplained male/female ratio of 2:1. It occurs more often in the cooler months, and familial occurrences are commonly described. The latent period is 1–2 weeks, in notable contrast to impetigo-associated cases, which have a latent period of 2–6 weeks. In many developing countries, children have chronic skin infections, and it may be difficult to establish the latent period with accuracy. Impetigo-associated cases are more common in the warmer months, sex distribution is equal, and children tend to be younger. Introduction of a nephritogenic strain into a family often results in the occurrence of several cases within that family, and in some cases, attack rates of up to 20% have been described (30). The incidence is linked to poor socioeconomic conditions.

Renal involvement in APSGN can be mild, and in many patients, the disease may not be manifested clinically. Studies of epidemics with nephritogenic strains of streptococci have shown that up to 50% of those infected had subclinical evidence of renal disease (30, 31). In a typical case a sudden onset of facial or generalized edema

occurs. Hypertension is usually modest but is severe in 5% of cases, and occasionally may lead to encephalopathy or left ventricular failure. The urine is smoky or tea colored in 30–50% of cases. Pallor, headache, backache, lethargy, malaise, anorexia, and weakness are all common nonspecific features.

The urine volume is decreased. Proteinuria is present (up to 100 mg/dL), and microscopy shows white cells, red cells, and granular and hyaline casts. Urea, electrolyte, and creatinine levels are normal in subclinical cases but show features of acute renal failure in severe cases. It may be possible to culture GAS from the skin or the throat in some patients. Other evidence of infection with a GAS can be obtained through the antistreptolysin-O titer (ASOT), which is increased in 60–80% of cases. Early antibiotic treatment can reduce the proportion of cases with elevated ASOT to 30%. Anti-deoxyribonuclease B and anti-hyaluronidase testing has been shown to be of more value than ASOT in confirming group A streptococcal infection in impetigo-associated cases. Measurement of anti-M protein antibodies is of more value for epidemiologic purposes than for the diagnosis of individual cases (31). Decreased C3 and total hemolytic complement levels are found in 90% of cases during the first 2 weeks of illness and return to normal after 4–6 weeks.

Penicillin should be given to eradicate the GAS organisms. Erythromycin, clindamycin, or a first-generation cephalosporin can be given to patients allergic to penicillin. Antibiotic treatment probably has no influence on the course of renal disease but will prevent the spread of a nephritogenic strain (32). Close contacts and family members who are culture-positive for GAS should also be given penicillin, although antibiotic treatment is not always effective in eliminating secondary cases. Recurrent episodes are rare, and immunity to the particular nephritogenic strain that caused the disease is probably lifelong. Antibiotic prophylaxis is therefore unnecessary.

Most studies suggest that the prognosis for children with APSGN is good, with more than 90% making a complete recovery. However, 10% of cases may have a prolonged and more serious course with long-term chronic renal failure (33).

Other Streptococci. APSGN has also been described after outbreaks of group C Streptococcus infection (34). This has occurred after consumption of unpasteurized milk from cattle with mastitis. Patients developed pharyngitis followed by APSGN. Endostreptosin was found in the cytoplasm of these group C strains, and during the course of the illness, patients developed anti-endostreptosin antibodies. This antigen has been postulated to be the nephritogenic component of GAS.

In addition, strains of group G streptococci have been implicated in occasional cases of APSGN (35). Isolates possessed the type M12 protein antigen identical to the nephritogenic type M12 antigen of some group A streptococcal strains.

Streptococcal Toxic Shock Syndrome and Invasive Group A Streptococcal Infection. Since 1988, there have been several reports of an illness with many similarities to the staphylococcal toxic shock syndrome, occurring in both children (36) and adults, associated with invasive group A streptococcal disease (32, 37, 38). Patients with this syndrome present acutely with high fever, erythematous rash, mucous membrane involvement, hypotension, and multiorgan failure. Unlike staphylococcal toxic shock syndrome, in which the focus of infection is usually trivial and bacteremia is seldom seen, the streptococcal toxic shock syndrome is usually associated with bacteremia or a serious focus of infection such as septic arthritis, myositis, or osteomyelitis (36, 38). Laboratory findings of anemia, neutrophil leukocytosis, thrombocytopenia, and DIC are often present, together with impaired renal function, hepatic derangement, and acidosis. Acute renal failure requiring dialysis occurs in a significant proportion of cases.

It is not clear why there are increasing numbers of cases with invasive disease caused by GAS, nor why there has been an emergence of streptococcal toxic shock syndrome, and indeed a similar syndrome caused by some pseudomonas and klebsiella strains. The most common antecedent of invasive GAS disease is varicella infection, with the streptococcal infection developing after the initial vesicular phase of the disease is subsiding. Strains causing toxic shock syndrome and invasive disease appear to differ from common isolates of GAS in producing large amounts of pyrogenic toxins that may have superantigen-like activity. Another important mechanism is the production by invasive GAS of an IL8 protease. IL8 serves as a molecular bridge between receptors on neutrophils and the vascular endothelium. Cleavage of this protein prevents neutrophil attachment to the endothelium, and results in uncontrolled spread of the bacteria through the tissues (39). In severe cases necrotizing fasciitis occurs with extensive destruction of the subcutaneous tissues, and is often associated with multiorgan failure. The pathophysiology of streptococcal toxic shock syndrome and that of invasive disease is similar in that superantigen toxins that induce release of cytokines and other inflammatory mediators play a role in both conditions. However GAS toxic shock is usually more severe, carries a higher mortality, and is more often associated with focal collections or necrotizing fasciitis.

Treatment of streptococcal toxic shock syndrome depends on the administration of appropriate antibiotics, aggressive circulatory support, and treatment of any multiorgan failure. Surgical intervention to drain the infective focus in muscle, bone, joint, or body cavities is often required. Antibiotic therapy with beta-lactams should be supplemented by treatment with a protein synthesis-inhibiting antibiotic, such as clindamycin, and it is suggested that this limits new toxin production (40, 41).

A number of new therapies are in development. Firstly, pooled intravenous immunoglobulins are now in widespread use in the treatment of toxic shock, particularly when caused by streptococcus (42, 43).

The role of steroids remains unclear, with their hemodynamic benefit set against the detrimental effects of hyperglycemia secondary to gluconeogenesis. (44). The benefit of insulin therapy to control hyperglycemia is unclear. A recent study in adults found that intensive insulin therapy increased the risk of serious adverse events (45). In contrast to adult patients, in children with severe sepsis, the use of activated protein C (Drotrecogin) cannot be recommended, as in a multicenter trial, fatality was increased in the treatment group (5). Recovery of renal function occurs in patients who respond to treatment of shock and the eradication of the infection.

Leptospira

Leptospirosis is an acute generalized infectious disease caused by spirochetes of the genus Leptospira (46). It is primarily a disease of wild and domestic animals, and humans are infected only occasionally through contact with animals. Most human cases occur in summer or autumn and are associated with exposure to leptospira-contaminated water or soil during recreational activities such as swimming or camping. In adolescents and adults, occupational exposure through farming or other contact with animals is the route of infection.

The spirochete penetrates intact mucous membranes or abraded skin and disseminates to all parts of the body, including the cerebrospinal fluid (CSF). Although leptospires do not contain classic endotoxins, the pathophysiology of the disorder has many similarities to that of endotoxemia. In severe cases, jaundice occurs because of hepatocellular dysfunction and cholestasis. Renal functional abnormalities may be profound and out of proportion to the histologic changes in the kidney (47). Renal involvement is predominantly a result of tubular damage, and spirochetes are commonly seen in the tubular lesions. The inflammatory changes in the kidney may result from either a direct toxic effect of the organism or immune-complex nephritis. However, hypovolemia, hypotension, and reduced cardiac output caused by myocarditis may contribute to the development of renal failure. In severe cases, a hemorrhagic disorder caused by widespread vasculitis and capillary injury also occurs (47, 48).

The clinical manifestations of leptospirosis are variable. Of affected patients, 90% have the milder anicteric form of the disorder, and only 5–10% have severe leptospirosis with jaundice. The illness may follow a biphasic course. After an incubation period of 7–12 days, a nonspecific flu-like illness lasting 4–7 days occurs, associated with septicemic spread of the spirochete. The fever then subsides, only to recur for the second, "immune," phase of the illness. During this phase, the fever is low grade and there may be headache and delirium caused by meningeal involvement, as well as intense muscular aching. Nausea and vomiting are common. Examination usually reveals conjunctival suffusion, erythematous rash, lymphadenopathy, and meningism.

The severe form of the disease (Weil's disease) presents with fever, impaired renal and hepatic function, hemorrhage, vascular collapse, and altered consciousness. In one series the most common organs involved were the liver (71%) and kidney (63%). Cardiovascular (31%), pulmonary (26%), neurologic (5%), and hematologic (21%) involvements were less common (49). Vasculitis, thrombocytopenia, and uremia are considered important factors in the pathogenesis of hemorrhagic disturbances and the main cause of death in severe leptospirosis (50). Urinalysis results are abnormal during the leptospiremic phase with proteinuria, hematuria, and casts. Uremia usually appears in the second week, and acute renal failure may develop once cardiovascular collapse and DIC are present (48).

The clinical features of leptospirosis overlap with those of several other acute infectious diseases, including Rocky Mountain spotted fever, toxic shock syndrome, and streptococcal sepsis. The diagnosis of leptospirosis should be considered in febrile patients with evidence of renal, hepatic, and mucous membrane changes and rash, particularly if a history of exposure to fresh water is found. Diagnosis can be confirmed by isolation of the spirochetes from blood or CSF in the first 10 days of the illness or from urine in the second week (48). The organism may be seen in biopsy specimens of the kidney or skin or in the CSF by dark-field microscopy or silver staining. Serologic tests to detect leptospirosis are now sensitive and considerably aid the diagnosis. Immunoglobulin M (IgM) antibody may be detected as early as 6–10 days into the illness, and antibody titers rise progressively over the next 2–4 weeks. Some patients remain seronegative, and negative serologic test results do not completely exclude the

diagnosis. In one series levels of IgM and IgG anticardiolipin concentrations were significantly increased in leptospirosis patients with acute renal failure (50). Leptospirosis is treated with intravenous penicillin or other beta-lactam antibiotics. The severity of leptospirosis is reduced by antibiotic treatment, even if started late in the course of the illness (51). Supportive treatment with volume replacement to correct hypovolemia, administration of inotropes, and correction of coagulopathy is essential in severe cases. Dialysis may be required in severe cases and may be needed for prolonged periods until recovery occurs.

Streptococcus Pneumoniae

Infection with *S. pneumoniae* is one of the most common infections in humans and causes a wide spectrum of disease, including pneumonia, otitis media, sinusitis, septicemia, and meningitis. Despite the prevalence of the organism, significant renal involvement is relatively rare but is seen in two situations: pneumococcal septicemia in asplenic individuals or in those with other immune deficiencies presents with fulminant septic shock in which renal failure may occur as part of a multisystem derangement. The mortality from pneumococcal sepsis in asplenic patients is high, even with early antibiotic treatment and intensive support.

The second nephrologi syndrome associated with *S. pneumoniae* is a rare form of HUS. In 1955, Gasser and colleagues described HUS as a clinical entity in children, and they included two infants with pneumonia among the five patients they described (52). HUS associated with pneumococcal infection is induced by the enzyme neuraminidase released from *S. pneumoniae* (53, 54). Thomsen-Friedenrich antigen (T antigen) is present on the surface of red blood cells, platelets, and glomerular capillary endothelia against which antibodies are present in normal serum. Neuraminidase causes desialation of red blood cells, and possibly other blood cells and endothelium, by the removal of terminal neuraminic acid, which leads to unmasking of the T antigen. The resultant widespread agglutination of blood cells causes intravascular obstruction, hemolysis, thrombocytopenia, and renal failure. Results of the direct Coombs test are frequently positive, either from bound anti-T IgM or from anti-T antibodies. The diagnosis of Thomsen-Friedenrich antibody-induced HUS should be suspected in patients with acute renal failure, thrombocytopenia and hemolysis after an episode of pneumonia or bacteremia caused by *S. pneumoniae*. Fragmented red blood cells will usually be present on blood film.

Association with *S. pneumoniae* is defined by culture of pneumococci from a normally sterile site within a week before or after onset of signs of HUS. Clues to a pneumococcal cause, in addition to culture results, include severe clinical disease, especially pneumonia, empyema, pleural effusion, or meningitis; hemolytic anemia without a reticulocyte response; positive results on a direct Coombs test; and difficulties in ABO crossmatching or a positive minor crossmatch incompatibility (55). However, when renal disease is seen in the context of severe pneumococcal infection, it is important to maintain a broad diagnostic perspective, because the occurrence of acute tubular necrosis due to septic shock and DIC is well described (56, 57).

Therapy for this syndrome should be with supportive treatment and antibiotics (usually a third generation cephalosporin); dialysis may be required if renal failure occurs. Because normal serum contains antibodies against the Thomsen-Friedenrich antigen, blood transfusion should be undertaken with washed red blood cells resuspended in albumin rather than plasma (53, 54). Exchange transfusion and plasmapheresis have been used in some patients, with the rationale that these procedures may improve outcome by eliminating circulating neuraminidase (53, 57, 58). Intravenous IgG has been used in a patient and was shown to neutralize neuraminidase present in the patient's serum (59).

In comparison to patients with the more common diarrhea-associated HUS, *S. pneumoniae*-induced HUS patients have a more severe renal disease. They are more likely to require dialysis. Their long-term outcome maybe affected by the severity of the invasive streptococcal disease itself, and a significant proportion of surviving patients (30–70%) develop end-stage renal failure (60, 61). A recent review of UK cases found an eightfold increase in early mortality as compared to diarrhoea-induced HUS (62).

Gastrointestinal Infections (*Escherichia coli*, Salmonella, Campylobacter, Yersinia, Shigella, *Vibrio cholerae*)

The diarrheal diseases caused by *Escherichia coli*, Salmonella, Shigella, Campylobacter, vibrios, and Yersinia remain important and common bacterial infections of humans. Although improvements in hygiene and living conditions have reduced the incidence of bacterial gastroenteritis in developed countries, these infections remain common in underdeveloped areas of the world, and outbreaks and epidemics continue to occur in both developed and underdeveloped countries. Renal involvement in the enteric infections may result from any of four possible mechanisms.

Severe Diarrhea and Dehydration

Regardless of the causative organism, diarrhea results in hypovolemia, abnormalities of plasma electrolyte composition, and renal underperfusion. If severe dehydration occurs and is persistent, oliguria from prerenal failure is followed by vasomotor nephropathy and established renal failure.

Systemic Sepsis and Endotoxemia

E. coli, Shigella, and Salmonella (particularly *Salmonella typhi*) may invade the bloodstream and induce septicemia or septic shock. Acute renal failure is commonly seen in infants with *E. coli* sepsis but is also reported with Klebsiella, Salmonella, and Shigella infections. Its pathophysiology and treatment were discussed previously.

Enteric Pathogen-Associated Nephritis

Enteric infections with *E. coli*, Yersinia, Campylobacter, and Salmonella have been associated with several different forms of GN, including membranoproliferative GN (MPGN), interstitial nephritis, diffuse proliferative GN, and IgA nephropathy (63–65).

In typhoid fever, GN ranging from mild asymptomatic proteinuria and hematuria to acute renal failure may occur (64, 66–68). Renal biopsy findings show focal proliferation of mesangial cells, hypertrophy of endothelial cells, and congested capillary lumina. Immunofluorescent studies show IgM, IgG, and C3 deposition in the glomeruli, with Salmonella antigens detected within the granular deposits in the mesangial areas. In the IgA nephropathy after typhoid fever, Salmonella vi antigens have been demonstrated within the glomeruli.

Yersinia infection has been reported as a precipitant of GN in several studies (65, 69). Transient proteinuria and hematuria are found in 24% of patients with acute yersinia infection, and elevated creatinine levels in 10%. Renal biopsy reveals mild mesangial GN or IgA nephropathy. Yersinia antigens, immunoglobulin, and complement have been detected in the glomeruli. *Yersinia pseudotuberculosis* is well recognized as one of the causes of acute tubulointerstitial nephritis causing acute renal failure, especially in children; patients have histories of drinking untreated water in endemic areas (70–72). The illness begins with the sudden onset of high fever, skin rash, and GI symptoms. Later in the course, periungual desquamation develops, mimicking Kawasaki disease. Elevated erythrocyte sedimentation rate, C-reactive protein level, and thrombocytosis are noticeable, and mild degrees of proteinuria, glycosuria, and sterile pyuria are common. Acute renal failure, which typically develops 1–3 weeks after the onset of fever, follows a benign course with complete recovery. Renal biopsy mainly reveals findings of acute tubulointerstitial nephritis. Antibiotic therapy, although recommended, does not alter the clinical course, but reduces the fecal excretion of the organism (73, 74).

Enteric Pathogen-Induced Hemolytic Uremic Syndrome

HUS is characterized by three distinct clinical signs: acute renal failure, thrombocytopenia, and microangiopathic hemolytic anemia. It was first described in 1955 and was associated with infection by Shiga toxin-producing *Shigella dysenteriae*. A major breakthrough in the search for the cause of HUS occurred in the 1980s when Karmali *et al.* reported that 11 of 15 children with diarrhea-associated HUS had evidence of infection with a strain of *E. coli* that produced a toxin active on Vero cells (75). In diarrhea-associated HUS in the United States and most of Europe, *E. coli* 0157:H7 is the most important of these strains. *E. coli* 0157:H7 occurs naturally in the GI tract of cattle and other animals, and humans become infected through contaminated food products. Most outbreaks have been associated with consumption of undercooked meat, but unpasteurized milk and cider, drinking water, and poorly chlorinated water for recreational use have also been implicated as vehicles for bacterial spread. HUS is discussed in detail in Chapter 67.

Mycobacterium Tuberculosis

The global epidemic of Mycobacterium tuberculosis is growing. Several factors have contributed to this increase, including the emergence of the human immunodeficiency virus (HIV) infection epidemic, large influxes of immigrants from countries in which tuberculosis (TB) is common, the emergence of multiple-drug-resistant *M. tuberculosis*, and breakdown of the health services for effective control of TB in various countries. It is generally estimated that, overall, one-third of the world's population is currently infected with the TB bacillus. There are more than 8 million cases of TB, which result in the death of approximately 2 million people each year. Furthermore, 5–10% of people who are infected with the TB bacillus develop TB disease or become infectious at some time during their lives (76, 77). After respiratory illness in children, mycobacteria are widely distributed to many organs of the body during the lymphohematogenous phase of childhood TB (78). Tubercle bacilli can be recovered from the urine in many cases of miliary TB. Hematogenously-spread tuberculomata develop in the

glomeruli, which results in caseating, sloughing lesions that discharge bacilli into the tubules. In most cases, the renal lesions are asymptomatic and manifest as mycobacteria in the urine or as sterile pyuria.

Tuberculomata in the cortex may calcify and cavitate or may rupture into the pelvis, discharging infective organisms into the tubules, urethra, and bladder. Dysuria, loin pain, hematuria, and pyuria are the presenting features of this complication, but in many cases, the renal involvement is asymptomatic, even when radiologic and pathologic abnormalities are very extensive. Continuing tuberculous bacilluria may cause cystitis with urinary frequency and, in late cases, a contracted bladder (79). The intravenous urogram is abnormal in most cases. Early findings are pyelonephritis with calyceal blunting and calyceal-interstitial reflux. Later, papillary cavities may be seen, indicating papillary necrosis. Ureteric strictures, focal calcification, hydronephrosis, and cavitation may also be seen. Renal function is usually well preserved, and hypertension is uncommon. In some cases, either the infection itself or reactions to the chemotherapeutic agents may result in renal failure with evidence of an interstitial nephritis (79–81).

Classic symptomatic renal TB is a late and uncommon complication in children, rarely occurring less than 4 or 5 years after the primary infection, and therefore is most commonly diagnosed after adolescence (78, 80). Adult studies have shown that 26–75% of renal TB coexists with active pulmonary TB and 6–10% of screened sputum-positive pulmonary TB patients have renal involvement.

The diagnosis is established by isolation of mycobacteria from the urine or by the presence of the characteristic clinical and radiographic features in a child with current or previous TB. Renal TB is treated with drug regimens similar to those used for other forms of TB, with isoniazid, rifampicin, pyrazinamide and ethambutol administered initially for 2 months, and isoniazid and rifampicin then continued for a further 7–10 months. Late scarring and urinary obstruction may occur in cases with extensive renal involvement, and such patients should be followed by ultrasonography or intravenous urogram.

Mycobacteria, both *M. tuberculosis* and atypical mycobacteria, have also emerged as important causes of opportunistic infection in immunocompromised patients undergoing dialysis and in patients undergoing renal transplantation. The possibility of mycobacterial disease must be considered in patients with fever of unknown origin or unexplained disease in the lungs or other organs. Results of the Mantoux test are often negative, and diagnosis depends on maintaining a high index of suspicion and isolating the organism from the infected site.

Treponema Pallidum

Renal involvement has been well documented in both congenital and acquired syphilis, with an estimated occurrence of 0.3% in patients with secondary syphilis and up to 5% in those with congenital syphilis (82, 83). The most common manifestation of renal disease in congenital syphilis is the nephrotic syndrome, with proteinuria, hypoalbuminemia, and edema. In some patients, hematuria, uremia, and hypertension may be seen. The renal disease is usually associated with other manifestations of congenital syphilis, including hepatosplenomegaly, rash, and mucous membrane findings.

Nephritis in congenital syphilis is usually associated with evidence of complement activation, with depressed levels of Clq, C4, C3, and C5. Histologic findings are a diffuse proliferative GN or a membranous nephropathy. The interstitium shows a cellular infiltrate of polymorphonuclear and mononuclear cells (84). Immunofluorescent microscopy reveals diffuse granular deposits of IgG and C3 along the glomerular basement membrane (GBM). Mesangial deposits may also contain IgM. On electron microscopy, scattered subepithelial electron-dense deposits are seen, with fusion of epithelial cell foot processes (84).

Good evidence exists that renal disease is due to an immunologically mediated reaction to treponemal antigens. Antibodies reactive against treponemal antigens can be eluted from the glomerular deposits, and treponemal antigens are present in the immune deposits. Treatment of both congenital and acquired syphilis with antibiotics results in rapid improvement in the renal manifestations (82, 84).

Mycoplasma Pneumoniae

Renal involvement is surprisingly rare in *Mycoplasma pneumoniae* infection considering the prevalence of this organism and its propensity to trigger immunologically mediated diseases such as erythema multiforme, arthritis, and hemolysis. Acute nephritis associated with Mycoplasma infection may occur 10–40 days after the respiratory tract infection (85, 86). Renal histopathologic findings include type 1 MPGN, proliferative endocapillary GN, and minimal change disease (87). Antibiotic treatment of the infection does not appear to affect the renal disease, which is self-limited in most cases (85, 86).

Legionnaires' Disease

Since its recognition in 1976, Legionnaires' disease, caused by *Legionella pneumophila*, has emerged as an important

cause of pneumonia. The disease most commonly affects the elderly but has been reported in both normal and immunocompromised children (97, 98). Renal dysfunction occurs in a minority of patients (98). Patients who develop renal impairment present with oliguria and rising urea and creatinine levels. They are usually severely ill, with bilateral pulmonary infiltrates, fever, and leukocytosis. Shock may be present, and the renal impairment has been associated with acute rhabdomyolysis with high levels of creatine phosphokinase and myoglobinuria. Renal histologic examination usually shows a tubulointerstitial nephritis or acute tubular necrosis (98, 99). The pathogenesis of the renal impairment is uncertain, but the organism has been detected within the kidney on electron microscopy and immunofluorescent studies, which suggests a direct toxic effect. Myoglobinuria and decreased perfusion may also be contributing factors, however. Mortality has been high in reported cases of Legionnaires' disease complicated by renal failure. Treatment is based on dialysis, intensive care, and antimicrobial therapy with erythromycin (98). Steroid therapy may be effective for tubulointerstitial nephritis (99).

Rickettsial Diseases

The rickettsial diseases are caused by a family of microorganisms that have characteristics common to both bacteria and viruses and that cause acute febrile illnesses associated with widespread vasculitis. With the exception of Q fever, all are associated with erythematous rashes. There are four groups of rickettsial diseases:

1. The typhus group includes louse-borne and murine typhus, spread by lice and fleas, respectively.
2. The spotted fever group includes Rocky Mountain spotted fever, tick typhus and related Mediterranean spotted fever and rickettsial pox, which are spread by ticks and mites, with rodents as the natural reservoir.
3. Scrub typhus, which is spread by mites.
4. Q fever, which is spread by inhalation of infected particles from infected animals.

Rickettsial diseases have a worldwide distribution and vary widely in severity, from self-limited infections to fulminant and often fatal illnesses (88). In view of the widespread vasculitis associated with these infections, subclinical renal involvement probably occurs in many of the rickettsial diseases. However, in Rocky Mountain spotted fever, tick typhus, and Q fever, the renal involvement may be an important component of the illness.

Rocky Mountain Spotted Fever

Rocky Mountain spotted fever is the most severe of the rickettsial diseases (89, 90). The onset occurs 2–8 days after the bite of an infected tick. High fever develops initially, followed by the pathognomonic rash, which occurs between the second and sixth days of the illness. The rash initially consists of small erythematous macules, but later these become maculopapular and petechial, and in untreated patients, confluent hemorrhagic areas may be seen. The rash first appears at the periphery and spreads up the trunk. Involvement of the palms and soles is a characteristic feature (88).

Headache, restlessness, meningism, and confusion may occur together with other neurologic signs. Cardiac involvement with congestive heart failure and arrhythmia are common. Pulmonary involvement occurs in 10–40% of cases. Infection is associated with an initial leucopenia, followed by neutrophil leukocytosis. Thrombocytopenia occurs in most cases.

Histopathologically, the predominant lesions are in the vascular system (91). Rickettsiae multiply in the endothelial cells, which results in focal areas of endothelial cell proliferation, perivascular mononuclear cell infiltration, thrombosis, and leakage of red cells into the tissues. The renal lesions involve both blood vessels and interstitium, and acute tubular necrosis may occur. Acute GN with immune-complex deposition has been reported (92), but in most cases the pathology appears to be a direct consequence of the invading organism on the renal vasculature (90, 93).

Renal dysfunction is an important complication of Rocky Mountain spotted fever. Elevation of urea and creatinine levels occurs in a significant proportion of cases, and acidosis is common. Prerenal renal failure caused by hypovolemia and impaired cardiac function may respond to volume replacement and inotropic support, but acute renal failure may subsequently occur, necessitating dialysis.

Rocky Mountain spotted fever is diagnosed by the characteristic clinical picture, the exclusion of disorders with similar manifestations (e.g., measles, meningococcal disease, and leptospirosis), and detection of specific antibodies in convalescence. Culture of *Rickettsia rickettsii*, immunofluorescent staining, and polymerase chain reaction (PCR) testing of blood and skin biopsy specimens are available only in reference laboratories. Antibiotics should be administered in suspected cases without awaiting confirmation of the diagnosis (93). Doxycycline is the drug of choice for children of any age. Chloramphenicol is also effective (94). Intensive support of shock and multiorgan

failure may be required in severe cases, and peritoneal dialysis or hemodialysis may be required until renal function returns. Before the advent of specific therapy, mortality was 25%. Today the overall mortality in the United States is still 5–7%. Death predominantly occurs in cases in which the diagnosis is delayed.

Q Fever

Q fever is caused by *Coxiella burnetii* and has a worldwide distribution, with the animal reservoir being cattle, sheep, and goats. Human infection follows inhalation of infected particles from the environment. The clinical manifestations range from an acute self-limited febrile illness with atypical pneumonia to involvement of specific organs that causes endocarditis, hepatitis, osteomyelitis, and central nervous system disease (95).

Proliferative GN may be associated with either Q fever endocarditis, rhabdomyolysis or a chronic infection elsewhere in the body (96). Renal manifestations range from asymptomatic proteinuria and hematuria to acute renal failure, hypertension, and nephrotic syndrome. Renal histologic findings are those of a diffuse proliferative GN, focal segmental GN, or mesangial GN. Immunofluorescent studies reveal diffuse glomerular deposits of IgM in the mesangium, together with C3 and fibrin. *C. burnetii* antigen has not been identified within the renal lesions.

Treatment of the underlying infection may result in remission of the renal disease, but prolonged treatment may be required for endocarditis. Tetracycline has been used in conjunction with rifampicin, co-trimoxazole, or a fluoroquinolone.

Intravascular and Focal Infections

Nephritis has been reported in association with the presence of a wide range of microorganisms that cause chronic or persistent infection (*Table 52-2*) (63, 100). It is likely that any infectious agent that releases foreign antigens into the circulation, including those of very low virulence, can cause renal injury either by deposition of foreign antigens in the kidney or by the formation of immune complexes in the circulation, which are then deposited within the kidney. Nephritis is most commonly seen in association with intravascular infections such as SBE or infected ventriculoatrial shunts, but it is also seen after focal extravascular infections; ear, nose, and throat infections; and abscesses.

Bacterial Endocarditis

Renal involvement is one of the diagnostic features of bacterial endocarditis. Virtually all organisms that cause endocarditis also produce renal involvement (*Table 52-2*). Although endocarditis caused by bacteria is the most common and is readily diagnosed by blood culture (100), unusual but important causes of culture-negative endocarditis include Q fever (101) and Legionella infection (102). In the immunocompromised individual, opportunistic pathogens such as fungi and mycobacteria are important causes.

The usual renal manifestations of SBE are asymptomatic proteinuria, hematuria, and pyuria. Loin pain, hypertension, nephrotic syndrome, and renal failure may occur in more severe cases.

The renal lesions occurring in endocarditis are variable, and focal embolic and immune-complex-mediated features may coexist (100, 103, 104). Embolic foci may be evident as areas of infarction, intracapillary thrombosis, or hemorrhage. More commonly, there is a focal necrotizing or diffuse proliferative GN. Immunofluorescent studies show glomerular deposits of IgG, IgM, IgA, and C3 along the GBM and within the mesangium. Electron microscopy reveals typical electron-dense deposits along the GBM and within the mesangium (100, 103, 104).

Early reports suggested that the renal lesions were caused by microemboli from infected vegetations depositing in the kidney, a hypothesis supported by the occasional presence of bacteria within the renal lesions. Most subsequent evidence, however, indicates that immunologic mechanisms rather than emboli are involved in the pathogenesis in most cases: bacteria are rarely found within the kidney, and renal involvement occurs with lesions of the right side of the heart, which would not be likely to embolize to the kidney. Immune complexes containing bacterial antigens are present in the circulation, and both bacterial antigens and bacteria-specific antibodies can be demonstrated within the immune deposits in the kidney. Serum C3 level is usually low, and complement can be found within both the circulating and the deposited immune complexes. These features all support an immune-complex-mediated pathogenesis of the renal injury (100, 103, 104).

Treatment of the endocarditis with antibiotics usually results in resolution of the GN and is associated with the disappearance of immune complexes from the circulation and return of C3 levels to normal. The prognosis of the renal lesions in SBE generally depends on the response of the underlying endocarditis to antibiotics or, in cases of antibiotic failure, to surgical removal of the infective vegetations (105).

Shunt Nephritis

In patients previously treated by shunting for hydrocephalus, there is a well-documented association of GN with infected ventriculoatrial shunts. This condition is another example of an immune-complex nephritis similar to that seen in endocarditis (106). Coagulase-negative staphylococci are the causative organisms in 75% of cases. The clinical and pathologic findings are similar to those in SBE. Presenting features are proteinuria, hematuria, and pyuria, and they may progress to renal failure. Immune complexes containing the bacterial antigens and complement are present in the serum, and C3 is depressed. Histologic findings are those of a diffuse mesangiocapillary GN. Immunofluorescent microscopy demonstrates deposits of immunoglobulin and C3 along the GBM, and bacterial antigen can be demonstrated in the renal lesions (107).

The prognosis for the renal lesion is good if the infection is treated early. This usually involves removal of the infected shunt and administration of appropriate antibiotics (106, 108). The possible progression to end-stage renal disease requires frequent nephrologic monitoring of patients with ventriculoatrial shunts (106). There are a few reports in the literature of a similar renal complication occurring in chronic infection of ventriculoperitoneal shunts.

Other Focal Infections

GN has been reported after chronic abscesses (63), osteomyelitis, otitis media, pneumonia, and other focal infections (*Table 52-2*). Acute renal failure has been the presenting feature of focal infections in various sites, including the lung, pleura, abdominal cavity, sinuses, and pelvis. Many different organisms have been responsible, including *S. aureus, Pseudomonas, E. coli,* and *Proteus* species. This is probably another example of immune-complex GN. C3 level is decreased in approximately one-third of reported cases, and immunofluorescent studies reveal diffuse granular deposits of C3 in the glomeruli of all reported instances, with a variable presence of immunoglobulin. The renal lesion is that of MPGN and crescentic nephritis. The renal outcome is reported to be good with successful early treatment of the underlying infection.

Viral Infections

The role of viral infections in the causation of renal disease has been less well defined than that of bacterial infections. Clearly defined associations of renal disease have been made with hepatitis B virus (HBV), hepatitis C virus (HCV), HIV, and hantaviruses, but the role of most other viruses in the pathogenesis of renal disease is not clearly defined. Most viruses causing systemic infection may trigger immunologically mediated renal injury. With increasing application of molecular techniques, it may be that a significant proportion of GNs currently considered to be idiopathic will ultimately be shown to be virus induced. In children with immunodeficiency states and those undergoing renal transplantation, viruses such as cytomegalovirus (CMV) and polyoma virus have been recognized to be associated with nephropathy.

Hepatitis B Virus

Since the discovery of hepatitis B surface antigen (HBsAg) in 1964, hepatitis virus has been shown to infect more than 5% of the world's population and is a major cause of chronic hepatitis, cirrhosis, and hepatocellular carcinoma worldwide. Some 350 million people have HBsAg in the circulation (WHO figures). The infection is most common in Africa and the Orient, where it is acquired in childhood by vertical transmission from infected mothers or by horizontal transmission from other children or adults. In developed countries, transmission in adults occurs more often by blood product exposure, sexual contact, or intravenous drug use. The epidemiology of HBV infection in children is changing following the widespread use of effective vaccination at birth, in both developed and developing countries.

HBV is a complex DNA virus with an outer surface envelope (HBsAg) and an inner nucleocapsid core containing the hepatitis B core antigen (HBcAg), DNA polymerase, protein kinase activity, and viral DNA. Incomplete spherical and filamentous viral particles consisting solely of HBsAg are the major viral products in the circulation and may be present in concentrations of up to 1014 particles per mL of serum. Hepatitis B e antigen (HBeAg) can be released from HBcAg by proteolytic treatment and may be found in the circulation either free or complexed to albumin or IgG antibodies. The presence of HBeAg correlates with the presence of complete viral particles and the infectivity of the individual (109).

Infection with HBV may result in either a self-limited infectious hepatitis followed by clearance of the virus and complete recovery, or a chronic or persistent infection in which the immune response is ineffective in eliminating the virus. Chronic HBV infection with continued presence of viral antigens in the circulation caused by an ineffective

Table 52-2
Focal infections causing glomerulonephritis

Site of infection	Organism
Infective endocarditis	Coagulase-negative staphylococci
	Staphylococcus aureus
	Pneumococci
	Viridans streptococci
	Enterococci
	Anaerobic streptococci
	Diphtheroids
	Haemophilus influenzae
	Coliforms
	Bacteroides
	Coxiella burnetii
	Legionella
	Candida albicans
Shunt infections	Coagulase-negative staphylococci
	S. aureus
	Diphtheroids
	Gram-negative bacilli
	Anaerobes
Focal abscess	*S. aureus*
	Gram-negative bacilli
Osteomyelitis	*S. aureus/streptococci*
Pyelonephritis	Coliforms
Pneumonia	*Streptococcus pneumoniae*
	Klebsiella
	S. aureus
	Mycoplasma
Otitis media	Pneumococcus/*S. aureus*
Gastrointestinal infection	*Yersinia, Campylobacter*
	Salmonella, Shigella

host immune response provides the best-documented example of immunologically mediated renal injury caused by persistent infection (110). Development of chronic HBV infection is positively associated with infection at a younger age, particularly in infancy, where the rate of chronic infection is up to 90%. In contrast, the likelihood of an acute, symptomatic illness increases with age, to a level where approximately 40% of infections are symptomatic in children above 15 years.

Patterns of Hepatitis B Virus Immunologically Mediated Renal Disease

Prodromal illness

In the early prodromal phase of HBV hepatitis, before the onset of jaundice, some patients develop fever, maculopapular or urticarial rash, and transient arthralgias or arthritis. Occasionally, proteinuria, hematuria, or sterile pyuria are observed. The syndrome usually lasts 3–10 days and often resolves before the onset of jaundice (110, 111). There have been no histologic studies of the renal changes during this prodromal period.

Hepatitis B Virus-Associated Polyarteritis Nodosa

Since 1970, numerous reports have linked HBV infection with polyarteritis nodosa (PAN). Most of these cases have been in adults, but the disorder has also been reported in children (112, 113), where it is estimated that approximately one third of PAN cases are caused by HBV (114). HBV PAN appears to be uncommon in Africa and the Orient, where infection is usually acquired in childhood, and has declined in incidence following the introduction of HBV vaccination (115).

HBV PAN presents weeks to months after a clinically mild hepatitis but may occasionally predate the hepatitis. After a prodromal illness, frank vasculitis affecting virtually any organ appears. Abdominal pain, fever, mononeuritis multiplex, and pulmonary and renal involvement may occur. The renal involvement may appear as hypertension, hematuria, proteinuria, or renal failure (see Chapter 61). Laboratory investigations reveal a florid acute-phase response, leukocytosis, and anemia. Transaminase levels are usually elevated, and HBsAg is present in the circulation. The pathology consists of focal inflammation of small and medium-sized arteries, with fibrinoid necrosis, leukocyte infiltration, and fibrin deposition. Renal pathology may be limited to the medium-sized arteries or may coexist with GN (110, 116).

Circulating immune complexes containing HBsAg and anti-HBs antibodies are usually present in the circulation (110, 116). C3, C4, and total hemolytic complement levels are depressed. HBsAg, IgG, and IgM antibodies to HBV and C3 have been identified by immunofluorescence in the blood vessels (116). A positive ANCA excludes HBV-PAN (115). Although most evidence suggests that the pathogenesis involves an immune-complex-mediated vasculitis, autoantibody or cell-mediated vascular injury may coexist. If the condition is untreated, the mortality is high (112). Most studies suggest that steroids or immunosuppressants help to suppress the vasculitis but potentially predispose to chronic infection

or progressive liver disease (110, 112). Successful treatment of hepatitis B-associated PAN with nucleoside analogues such as lamivudine or newer anti-viral drugs, either alone or in combination with interferon-alpha and conventional immunosuppressive therapy, has been reported (117–119).

Hepatitis B Virus-Associated Membranous Glomerulonephritis

HBV is now the major cause of membranous GN (MGN) in children worldwide. The proportion of patients with MGN caused by HBV is directly related to the incidence of HBsAg in the population, with 80–100% of all cases of MGN in some African and Oriental countries being associated with HBV (110, 120) (see Chapter 26).

HBV MGN usually presents in children aged 2–12. There is a striking male predominance; in the United States, 80% of patients are males (121). The virus is usually acquired by vertical transmission from infected mothers or horizontally from infected family members. Unlike adults with HBV MGN, children do not usually have a history of hepatitis or of active liver disease, but liver function test results are generally mildly abnormal. Liver biopsy specimens may show minimal abnormalities, chronic persistent hepatitis, or (occasionally) more severe changes (121).

The renal manifestations are usually of proteinuria, nephrotic syndrome, microscopic hematuria, or (rarely) macroscopic hematuria. Hypertension occurs in less than 25% of cases, and renal insufficiency is rare.

HBsAg and HBcAg are usually present in the circulation, and HBe antigenemia is seen in a high proportion of cases. Occasionally, HBsAg may be found in the glomeruli but is absent from the circulation. C3 and C4 levels are often low, and circulating immune complexes are found in most cases.

Immunohistologic study reveals deposits of IgG and C3 and (less commonly) IgM and IgA in subepithelial, subendothelial, or mesangial tissue. HBV particles may be seen on electron microscopy, and all the major hepatitis B antigens, including HBsAg, HBcAg, and HBeAg, have been localized in the glomerular capillary wall on immunofluorescence.

Immunologic deposition of HBV and antibody in the glomerular capillary wall is clearly involved in the glomerular injury, but the underlying immunologic events are incompletely understood (110, 122). Passive trapping of circulating immune complexes may be involved, but the circulating immune complexes containing HBsAg are usually larger than would be expected to penetrate the basement membrane. HBsAg and HBcAg are anionic and are therefore unlikely to penetrate the glomerular capillary wall. In contrast, HBeAg forms smaller complexes with anti-HBe antibodies and may readily penetrate the GBM. This may explain the observation that HBeAg in the circulation frequently correlates with the severity of the disease (110). An alternative mechanism for immune-mediated glomerular injury is the trapping of HBV antigens by antibody previously deposited in the kidney. Anti-HBe antibodies are cationic and may readily localize in the glomerulus and subsequently bind circulating antigen and complement. The third possibility is that the depositions of HBV and antibodies are consequences of glomerular injury by cellular mechanisms or autoantibodies. Little evidence supports this view at present (110). A transgenic mouse model of HBV-associated nephropathy has been developed, in which HBsAg and HBcAg is expressed in liver and kidney, particularly tubular epithelial cells, without viral replication. In these mice, gene expression analysis revealed upregulation of acute-phase proteins, particularly C3, although measurable serum C3 levels were reduced. This supports the notion that local persistent expression of HBV viral proteins contributes to HBV-associated nephropathy (123).

Other Hepatitis B Virus Glomerulonephritides

HBV infection has been associated with a variety of other forms of GN in both adults and children. In one small series in children, MPGN was found to be equal in incidence to MGN in the spectrum of HBV-associated GNs (124). Both MPGN and mesangial proliferative GN may be triggered by HBV. In several countries where HBV is common, the proportion of patients with these forms of nephritides who test positive for HBV greatly exceeds the incidence of positivity in the general population (122). As with MGN and HBV-associated PAN, circulating immune complexes and localization of HBV antigens in the glomeruli have been reported in both MPGN and mesangial proliferative GN, and it is likely that similar mechanisms are occurring (110, 125). Several other forms of GN have been associated with HBV, including IgA nephropathy, focal glomerulosclerosis, crescentic nephritis, and systemic lupus erythematosus, but the evidence for these associations is less consistent than for the entities discussed earlier (125).

Treatment of Hepatitis B Virus Glomerulonephritis

HBV is normally cleared as a result of cell-mediated responses in which cytotoxic T cells and natural killer

cells eliminate infected hepatocytes. It is not surprising, therefore, that the administration of steroids and immunosuppressive agents either may have no effect on HBV disease or may increase the risk of progressive disease (126). Children with HBV MGN have a good prognosis, and two-thirds undergo spontaneous remission within 3 years of diagnosis. Steroid therapy does not appear to provide any additional benefit (110, 120, 110). Antiviral therapy with pegylated interferon-alpha and lamivudine shows promise in facilitating clearance of HBV, and in some cases, elimination of the infection with antiviral therapy in both children and adults is associated with improvement or resolution of the coexisting renal disease. There is considerable effort being put into the development of newer anti-viral agents which avoid the common problems of resistance associated with lamivudine (127–129).

Hepatitis C Virus

HCV is an enveloped, single-stranded RNA virus of approximately 9.4 kb in the Flaviviridae family. There are six major HCV genotypes. Hepatitis C is a common disease affecting approximately 400 million people worldwide. In the United States, 4.1 million persons are estimated to be anti-HCV positive, and 3.2 million may be chronically infected (130). An estimated 240,000 children in the United States have antibody to HCV and 68,000–100,000 are chronically infected (131). Children become infected through receipt of contaminated blood products or through vertical transmission. The risk of vertical transmission increases with higher maternal viremia and maternal co-infection with HIV.

Acute HCV infection is rarely recognized in children outside of special circumstances such as a known exposure from an HCV-infected mother or after blood transfusion. Most chronically infected children are asymptomatic and have normal or only mildly abnormal alanine aminotransferase levels. Although the natural history of HCV infection during childhood seems benign in the majority of instances, the infection can take an aggressive course in a proportion of children, leading to cirrhosis and end-stage liver disease during childhood. The factors responsible for this more aggressive course are unidentified (131). Even in adults, the natural history of HCV infection has a variable course, but a significant proportion of patients will develop some degree of liver dysfunction, and 20–30% will eventually have end-stage liver disease as a result of cirrhosis. The risk of hepatocellular carcinoma is significant for those who have established cirrhosis. Hepatitis C is currently the most common condition leading to liver transplantation in adults in the "Western world."

GN has been described as an important complication of chronic infection with HCV in adults. The clinical presentation is usually of nephrotic syndrome or proteinuria, hypertension, or hematuria, with or without azotemia (132). MPGN, with or without cryoglobulinemia, and MGN are most commonly described. Isolated case reports of other, more unusual patterns of glomerular injury, including IgA nephropathy, focal segmental glomerulosclerosis, crescentic GN, fibrillary GN, and thrombotic microangiopathy, have also been associated with HCV infection (132, 133). Glomerular deposition of hepatitis antigens and antibodies has been described and is believed to play a role in pathogenesis. Cryoglobulinemia is a common accompaniment of GN that is associated with the depression of serum complement levels (132). Renal failure may develop in 40–100% of patients who have MPGN (132, 134). The presence of virus-like particles as well as viral RNA within the kidney sections of patients with HCV-associated glomerulopathies has been reported (135).

The diagnosis should be suspected if glomerular disease is associated with chronic hepatitis, particularly with the presence of cryoglobulins, but renal biopsy is necessary to establish a definitive diagnosis.

HCV infection is relatively common in children with end-stage renal disease and is an important cause of liver disease in this population. Acquisition of HCV infection continues to occur in dialysis patients because of nosocomial spread (136). Elevation of transaminase level is not a sensitive marker of infection in children and HCV enzyme-linked immunosorbent assay or PCR testing should be used to increase sensitivity (137). HCV-infected renal transplant recipients had higher mortality and hospitalization rates than other transplant recipients (138), and HCV infection has been reported to be associated with de novo immune-mediated GN, especially type 1 MPGN, in renal allografts, resulting in accelerated loss of graft function (139, 140).

No large randomized, controlled trials of treatment of children with chronic hepatitis C have been performed, although one study (PEDS-C) is currently recruiting patients into a trial of pegylated interferon +/− ribavirin (141). Small heterogeneous studies of interferon monotherapy have reported sustained virologic response rates of 35–40% (131). In adults, improvement of proteinuria and renal function often follows interferon-alpha treatment (132, 134), but relapses are common after cessation of treatment. Combination of interferon with ribavirin in

patients with chronic liver disease has been shown to increase the rate of sustained response in these patients (142). As yet, however, there are few data regarding the use of combination therapy with interferon and ribavirin in children. Moreover, interferon-alpha therapy is associated with acute or subacute renal failure in more than one-third of the patients with renal transplants (143).

Hepatitis C may be complicated by systemic mixed cryoglobulinemic (MC) vasculitis, and in some cases by a polyarteritis nodosa (PAN)-type non-cryoglobulinemic vasculitis (144). Treatment with interferon-α (IFN-α) and ribavirin mostly is associated with an improvement of vasculitic symptoms. In some cases, exacerbation and rarely new onset of vasculitis of the peripheral nervous system have been described after this treatment. In fulminant cases immunosuppressive therapy with steroids, and cyclophosphamide, or rituximab may be needed to control life threatening vasculitis prior to antiviral treatment (144).

Herpes Viruses

Cytomegalovirus

CMV is one of the eight human herpes viruses. Transmission of the virus requires exposure to infected body fluids such as breast milk, saliva, urine, or blood. Individuals initially infected with CMV may be asymptomatic or display nonspecific flu-like symptoms. After the initial infection CMV, like all herpes viruses, establishes latency for life but will be periodically excreted by an asymptomatic host. CMV replicates within renal cells, and on biopsy samples from immunocompromised hosts, viral inclusions can be visualized by light microscopy in cells of the convoluted tubules and collecting ducts (145). Glomerular cells and shed renal tubular cells may have characteristic inclusions, but clinically evident renal disease is rare and is seen virtually only in immunocompromised or congenitally infected children (145, 146).

The clinical manifestations of CMV-induced renal disease in congenitally infected infants are variable and range from asymptomatic proteinuria to nephrotic syndrome and renal impairment. In congenital CMV infection, histologic changes of viral inclusions commonly occur in the tubules. In addition, proliferative GN has been reported, with evidence on electron microscopy of viral immune deposits in glomerular cells (146, 147). In CMV-infected immunocompromised patients, immune-complex GN has been documented with mesangial deposits of IgG, IgA, C3, and CMV antigens within glomeruli. Eluted glomerular immunoglobulins have been shown to contain CMV antigens (148).

CMV is the most common viral infection after kidney transplantation. Experience with pediatric kidney transplant recipients suggests a 67% incidence of CMV infection (149). The direct and indirect effects of CMV infection result in significant morbidity and mortality among kidney transplant recipients. CMV-negative patients who receive a CMV-positive allograft are at risk for primary infection and graft dysfunction. Patients who are CMV seropositive at the time of transplantation are also at risk of reactivation and superinfection. Tubulointerstitial nephritis is a well-characterized pathologic feature of renal allograft CMV disease, which can be difficult to distinguish from injury caused by rejection. Histologic evidence of endothelial cell injury and mononuclear cell infiltration in the glomeruli has been reported (148). CMV glomerular vasculopathy in the absence of tubulointerstitial disease, causing renal allograft dysfunction, has also been reported (150). Beyond the acute allograft nephropathy associated with CMV viremia, CMV is known to cause chronic vascular injury. This may adversely affect the long-term outcome of the allograft and may be the explanation for the observed association with chronic allograft nephropathy (151).

Newer techniques for rapidly diagnosing CMV infection are becoming widely available and include shell vial culture, pp65 antigenemia assay, PCR, and the hybrid-capture RNA-DNA hybridization assay for qualitative detection of CMV PCR. Quantitative plasma PCR testing (PCR viral load) is increasingly used for diagnosis and monitoring of CMV viremia in renal transplant recipients.

Antiviral agents that have been shown to be effective against CMV include ganciclovir, valganciclovir, foscarnet, and cidofovir. Ganciclovir remains the drug of choice for treating established disease. Intravenous ganciclovir therapy is preferred in children because of the erratic absorption of oral ganciclovir. Major limitations of ganciclovir therapy are the induction of renal tubular dysfunction and bone marrow toxicity, principally neutropenia and thrombocytopenia. Dosage adjustments are necessary for recipients with renal dysfunction. Oral valganciclovir is now used for CMV prophylaxis post-transplant (152). Use of other antiviral agents such as foscarnet and cidofovir is limited because of nephrotoxicity and difficulty of administration. A number of reports have demonstrated the effectiveness of high-titer CMV immune globulin therapy in reducing severe CMV-associated disease when used in combination with ganciclovir (149, 153).

Varicella-Zoster Virus

The association of varicella with nephritis has been known for more than 100 years since Henoch reported on four children with nephritis that occurred after the appearance of varicella vesicles. Varicella, however, is rarely associated with renal complications (154). In fatal cases with disseminated varicella and in the immunocompromised individual, renal involvement is more common. Cases in which varicella infection caused GN in renal transplant recipients have been reported (155). Histologic findings in fatal cases include congested hemorrhagic glomeruli, endothelial cell hyperplasia, and tubular necrosis. In mild and nonfatal cases and in non-immunocompromised individuals, varicella is occasionally associated with a variety of renal manifestations, ranging from mild nephritis to nephrotic syndrome and acute renal failure (156). Histologic findings include endocapillary cell proliferation, epithelial and endothelial cell hyperplasia, and inflammatory cell infiltration (154). Rapidly progressive nephritis has also been reported. Immunohistochemical studies reveal glomerular deposition of IgG, IgM, IgA, and C3. On electron microscopy, granular electron-dense deposits have been found in the paramesangial region, and varicella antigens may be deposited in the glomeruli. The features suggest an immune-complex nephritis. Elevated circulating levels of IgG and IgA immune complexes and depressed C3 and C4 levels support this possibility (154).

Fulminant disseminated varicella and varicella in immunocompromised patients should be treated with intravenous acyclovir.

Epstein-Barr Virus

Renal involvement is common during acute infectious mononucleosis, usually manifesting as an abnormal urine sediment, with hematuria in up to 60% of cases. Hematuria, either microscopic or macroscopic, usually appears within the first week of the illness and lasts for a few weeks to a few months. Proteinuria is usually absent or low grade. More severe renal involvement with proteinuria, nephrotic syndrome, or acute nephritis with renal failure is much less common. Acute renal failure may be seen during the course of fulminant infectious mononucleosis with associated hepatic failure, thrombocytopenia, and encephalitis. It is usually caused by interstitial nephritis that is likely the result of immunopathologic injury precipitated by Epstein-Barr virus (EBV) infection. However, the identification of EBV DNA in the kidney raises the possibility that direct infection might play a role (157). The renal involvement must be distinguished from myoglobinuria caused by rhabdomyolysis, which may occur in infectious mononucleosis, and from bleeding into the renal tract as a result of thrombocytopenia.

Renal histologic findings in EBV nephritis are an interstitial nephritis with mononuclear cell infiltration and foci of tubular necrosis. Glomeruli may show varying degrees of mesangial proliferation. On immunohistochemical study, EBV antigens are seen in glomerular and tubular deposits. The prognosis for complete recovery of renal function is good. Treatment with corticosteroids may have a role in the management of EBV-induced acute renal failure and may shorten the duration of renal failure (158).

EBV-associated post-transplantation lymphoproliferative disease is a recognized complication in renal transplant recipients. Latent infection of EBV in renal proximal tubular epithelial cells has recently been described as causing idiopathic chronic tubulointerstitial nephritis (159).

Herpes Simplex Virus

The herpes simplex virus (HSV) causes persistent infection characterized by asymptomatic latent periods interspersed with acute relapses. As with other chronic and persistent infections, immunologically mediated disorders triggered by HSV are well recognized, and it is perhaps surprising that HSV has rarely been linked to nephritis. Acute nephritis and nephrotic syndrome have been associated with herpes simplex encephalitis. Renal histology shows focal segmental GN with mesangial and segmental deposits of IgM, C3, and HSV antigens. As with other herpes viruses, HSV has been suggested as a trigger for IgA nephritis, MPGN, and membranous nephropathy. Elevated levels of HSV antibodies have been reported in patients with a variety of forms of GN, but no conclusive evidence exists of an etiologic role for HSV (160).

Adenovirus and Enterovirus

Adenovirus and enterovirus, are unrelated ubiquitous pathogens that infect large proportions of the population annually and yet are rarely associated with renal disease. The literature contains scattered reports of acute nephritis after infection with each of these viruses.

Adenovirus is a major cause of hemorrhagic cystitis and was implicated as the cause of hemorrhagic cystitis in 23–51% of children with this disorder (161). Boys are affected more often than girls, and hematuria persists for 3–5 days. Microscopic hematuria, dysuria, and frequency may occur for longer periods. Adenovirus types 11 and 21 are the usual strains isolated.

Picornaviruses, including enteroviruses, echovirus and coxsackieviruses, have been linked with acute nephritis and acute renal failure associated with rhabdomyolysis. Coxsackie B virus can be isolated in urine. Direct infection of kidney cells is supported by *in vitro* work demonstrating lytic infection of human podocyte and proximal tubular epithelial cell cultures, although different strains exhibit variable degrees of nephrotropism. Renal damage *in vivo* may have both a direct lytic mechanism and an immune-complex basis (162). In the newborn, enteroviruses cause fulminant disease with DIC, shock, and liver failure, and acute renal failure may occur.

Measles Virus

Renal involvement from measles virus is uncommon, although measles virus can be cultured from the kidney in fatal cases. An acute GN has been reported to follow measles with evidence of immune deposits containing measles virus antigen within the glomeruli. The nephritis is generally self-limiting (163).

Mumps Virus

Mild renal involvement is common during the acute phase of mumps infection. One-third of children with mumps have abnormal urinalysis results, with microscopic hematuria or proteinuria. Mumps virus may be isolated from urine during the first 5 days of the illness, at a time when urinalysis findings are abnormal. Plasma creatinine concentrations usually remain normal, despite the abnormal urine sediment, but more severe cases in adults have been associated with evidence of acute nephritis with impaired renal function. Renal biopsy specimens demonstrate an MPGN with deposition of IgA, IgM, C3, and mumps virus antigen in the glomeruli, which suggests an immune-complex-mediated process (164).

Human Immunodeficiency Virus

Despite the increasing availability of interventions to limit vertical HIV transmission, an estimated 1,500 children continue to acquire HIV each day. The World Health Organization estimated that there were 33.2 million people, including 2.1 million children, living with HIV infection at the end of 2007. Of 2.1 million AIDS deaths, 330,000 were in children. The number of children on anti-retroviral treatment (ART) rose from 75,000 in 2005 to almost 200,000 in 2007.

Renal involvement in HIV infection was first described in 1984 in adults (165–167) and in children (168), and renal involvement occurs in 2–15% of HIV-infected children in the United States (169–171). Since the development of highly active antiretroviral therapy (HAART), however, the incidence of end-stage renal disease in HIV infection in both adults and children in industrialized countries has declined, but it is predicted that the dramatic decline in AIDS-related deaths will lead to an ageing population of HIV-infected individuals who will be at risk of non-HIV related renal problems, such that the numbers of HIV-positive ESRD patients will increase in the United States (172).

HIV infection is associated with a number of renal pathologies. HIV-associated nephropathy (HIVAN) is a syndrome of glomerular and tubular dysfunction, which can progress to end-stage renal failure. It is discussed more fully below. Glomerular syndromes other than HIVAN include MGN that resembles lupus nephritis and immune-complex GN, with IgA nephropathy and HCV-associated MPGN being the most common forms. There have also been several case reports of amyloid kidney (171, 173, 174). The kidneys may be affected by various other mechanisms. Opportunistic infections with organisms such as BK virus (BKV) that give rise to nephropathy and hemorrhagic cystitis have been reported in association with HIV infection (175). Systemic infections accompanied by hypotension can cause prerenal failure leading to acute tubular necrosis. Acute tubular necrosis has also been reported in HIV patients after the use of nephrotoxic drugs such as pentamidine, foscarnet, cidofovir, amphotericin B, and aminoglycosides. Intratubular obstruction with crystal precipitation can occur with the use of sulfonamides and intravenous acyclovir. Indinavir is well recognized to cause nephropathy and renal calculi (176). MPGN associated with mixed cryoglobulinemia and thrombotic microangiopathy/atypical-cal HUS in association with HIV infections have been reported (177, 178).

HIV-Associated Nephropathy (HIVAN)

HIVAN is characterized by both glomerular and tubular dysfunction, the pathogenesis of which is not entirely known. HIVAN is a clinico-pathologic entity that includes proteinuria, azotemia, focal segmental glomerulosclerosis

or mesangial hyperplasia, and tubulointerstitial disease (171). In adults in the United States, there is a markedly increased risk of nephropathy among African American persons with HIV infection. This appears to be true in children as well, but the data are sparse. The spectrum of HIVAN seems to be coincident with the degree of AIDS symptomatology. It is thought that HIVAN can present at any point in HIV infection, but most patients with HIVAN have CD4 counts of less than 200×10^6/200 cells/mL, which suggests that it may be primarily a manifestation of late-stage disease (179).

Although a spectrum of clinicopathologic entities including mesangial hyperplasia, focal segmental glomerulosclerosis, minimal change disease, and systemic lupus erythematosus nephritis has been described, the classic pathologic feature of HIVAN is the collapsing form of focal and segmental glomerulosclerosis (180). In the affected glomeruli, visceral epithelial cells are hypertrophied and hyperplastic, and contain large cytoplasmic vacuoles and numerous protein resorption droplets. There is microcystic distortion of tubule segments, which contributes to increasing kidney size. Podocyte hyperplasia can become so marked that it causes obliteration of much of the urinary space, forming "pseudocrescents" (173). Capillary walls are wrinkled and collapsed with obliteration of the capillary lumina. The interstitium is edematous with a variable degree of T-cell infiltration (181). The Bowman capsule can also be dilated and filled with a precipitate of plasma protein that represents the glomerular ultrafiltrate. One of the most distinctive features of HIVAN, however, is the presence of numerous tubuloreticular inclusions within the cytoplasm of glomerular and peritubular capillary endothelial cells (173). Immunofluorescence testing is positive for IgM and C3 in capillary walls in a coarsely granular to amorphous pattern in a segmental distribution (180, 181).

The presence of the HIV genome in glomerular and tubular epithelium has been demonstrated using complementary DNA probes and in situ hybridization. Proviral DNA has been detected by PCR in the glomeruli, tubules, and interstitium of micro dissected kidneys from patients who had pathologic evidence of HIVAN, but it has also been detected in the kidneys of HIV-positive patients with other glomerulopathies (182). A combination of both proliferation and apoptosis of renal cells may cause the loss of nephron architecture. Apoptosis has been demonstrated in cells in the glomerulus, tubules, and interstitium of biopsy specimens from HIV-positive patients with focal segmental glomerulosclerosis. In addition, the role of various cytokines and growth factors, specifically transforming growth factor beta (TGF-beta), in the development of sclerosis has been studied (183, 184).

Transgenic murine models provide some of the strongest evidence for a direct role of HIV-1 in the induction of HIVAN. These mice do not produce infectious virus but express the HIV envelope and regulatory genes at levels sufficient to re-create the HIVAN that is seen in humans (183). Serial deletion experiments have concluded that the *nef* and *vpr* genes are necessary though not sufficient for HIVAN pathogenesis. Additional factors such as genetic predisposition are thought to explain the fact that African Americans have a far greater likelihood of developing HIVAN than other racial groups, and that HIVAN is more likely in patients with a family history of ESRD.

HIVAN can manifest as mild proteinuria, nephrotic syndrome, renal tubular acidosis, hematuria, and/or acute renal failure (168–171). Nephrotic syndrome and chronic renal insufficiency are late manifestations of HIVAN. Children with HIVAN are likely to develop transient electrolytic disorders, heavy proteinuria, and acute renal failure due to systemic infectious episodes or nephrotoxic drugs.

Early stages of HIVAN can be identified by the presence of proteinuria and "urine microcysts" along with renal sonograms showing enlarged echogenic kidneys. Urinary renal tubular epithelial cells are frequently grouped together to form these microcysts, which were found in the urine of children with HIVAN who had renal tubular injury (171). Advanced stages of HIVAN typically present with nephrotic syndrome with edema, heavy proteinuria, hypoalbuminemia, and few red or white blood cells in urinary sediments. Hypertension may be present, but usually blood pressure is within or below the normal range. HIVAN in adults follows a rapidly progressive course, with end-stage renal disease developing within 1–4 months, but in children this rapid progression does not necessarily occur. Definitive diagnosis of HIVAN should be based on biopsy results, and biopsy should be performed if significant proteinuria is present, because in approximately 50% of HIV-infected patients with azotemia and/or proteinuria (>1 g/24 h) who undergo renal biopsy, the specimen will have histologic features consistent with other renal diseases (179).

When available, HAART should be given to children with symptomatic HIV disease. Specific treatment of HIVAN remains controversial. Several studies have looked at the role of HAART, angiotensin I-converting enzyme (ACE) inhibitors, steroids, and even cyclosporin with somewhat encouraging results. However, as yet no randomized case-controlled trials have been undertaken. Most of the studies have been small and retrospective, and many have included patients both with and without renal biopsy-proven HIVAN. Cyclosporin has been used to treat HIVAN in children with remission of nephrotic

syndrome (169). Similar responses have been reported to treatment with corticosteroids in various studies (185–188). ACE inhibitors have been used with encouraging results (189).

The general regimen used to treat patients with HIV, including HAART, should be applied to children with HIVAN. The dosages of some medications must be adjusted to the patients glomerular filtration. There are reports of spontaneous regression of HIVAN with supportive management and treatment with HAART, particularly with regimes containing protease inhibitors (190–193). It should be emphasized that the improvement reported with other modalities of treatment such as corticosteroids, cyclosporin, and ACE inhibitors always occurs when these agents are given in conjunction with antiretroviral therapy.

The kidneys of transgenic mice have been found to have elevated levels of TGF-beta messenger RNA and protein (184). Furthermore, gene expression analysis on tubular epithelial cells from a patient with HIVAN found upregulation of several inflammatory mediator genes downstream of interleukin 6 and of the transcription factor NFkB (194). Several other therapeutic options have been suggested, aimed specifically at the presumed role of TGF-beta in the pathogenesis of HIVAN. Treatment directed at its synthesis using gene therapy to block TGF-beta gene expression is being explored. Therapy directed at decreasing the activity of TGF-beta using anti-TGF-beta antibodies or other inhibitory substances is also an area of investigation. In addition, blocking renal receptors for chemokines such as RANTES (regulated upon derivation, normal T cell expressed and secreted), interleukin-8, and monocyte-chemoattractant protein-1 has been proposed as another possible treatment alternative (195).

In the HAART era, the outlook for HIV patients with ESRD has improved, but these patients fare worse than ESRD patients without HIV (196). Most reports of HIV-infected patients on hemodialysis have shown poor prognosis, with mean patient survival times ranging from 14–47 months. Mortality is therefore still close to 50% within the first year of dialysis. In general, improved survival is associated with younger age at initiation of hemodialysis and with higher CD4 counts. Access complications such as infection and thrombosis tend to occur at a higher rate in HIV-infected hemodialysis patients. Cross infection with HIV in dialysis patients is very rare. No patient-to-patient HIV transmission has yet been reported in a hemodialysis unit in the United States, although several such cases have occurred in South America (195, 197).

Peritoneal dialysis is an alternative for HIV-infected patients. The incidence of peritonitis varies across studies, but some studies did report a higher incidence of Pseudomonas and fungal peritonitis in the HIV-positive population (195). Infections with unusual organisms such as *Pasteurella multocida*, *Trichosporon beigelii*, and *Mycobacterium avium intracellulare* complex have also been reported. Several studies, however, have suggested that there is no significant difference between the HIV-infected and non-HIV-infected populations. Of note is that virus capable of replication *in vitro* has been recovered from the peritoneal dialysis effluent, and it can be recoverable for up to 7 days in dialysis bags at room temperature and for up to 48 h in dry exchange tubing (195).

Previously, long-term dialysis had been thought to be preferable to renal transplantation, primarily because of the concern that the immunosuppressive therapy required after transplantation could promote progression of HIV/AIDS. A multicenter prospective study has been addressing these questions (198). Data so far indicate that the outcome for liver and kidney transplantation is not considerably different from patients without HIV, with good graft persistence, and a low rate of development of opportunistic infections in those with well-controlled HIV and relatively high CD4 counts (199).

Human Polyoma Viruses

The human polyoma viruses are members of the papovavirus family and have received increasing attention as pathogens in immunocompromised patients. They are nonenveloped viruses ranging in size from 45–55 nm, with a circular, double-stranded DNA genome that replicates in the host nucleus. The best-known species in this genus are the BKV, the JC virus (JCV), and the simian virus SV40. BKV was first isolated from the urine of a 39-year-old man who developed ureteral stenosis 4 months after renal transplantation (200). The name of the virus refers to the first patients initials, which is also true of JCV. BKV establishes infection in the kidney and the urinary tract, and its activation causes a number of disorders, including nephropathy and hemorrhagic cystitis. BKV-associated nephropathy has become an increasingly recognized cause of renal dysfunction in renal transplantation patients (201–205). JCV establishes latency mainly in the kidney, and its reactivation can result in the development of progressive multifocal leukoencephalopathy. There are a few reports of nephropathy in association with JCV infection (see references in (206)), but BKV poses a much bigger problem in this regard. Recent studies have reported SV40 in the allografts of children who received renal transplants and in the urine, blood, and kidneys of adults with focal segmental glomerulosclerosis,

which is a cause of end-stage renal disease and an indication for kidney transplantation (207).

BKV infection is endemic worldwide

Seroprevalence rates as high as 60–80% have been reported among adults in the United States and Europe. The peak incidence of primary infection (as measured by acquisition of antibody) occurs in children 2–5 years of age. BKV antibody may be detected in as many as 50% of children by 3 years of age, and in 60–100% of children by 9 or 10 years of age; antibodies wane thereafter. BKV infection may be particularly important in the pediatric transplantation population, in whom primary infection has a high probability of occurring while the children are immunosuppressed (208).

Primary infection with BKV in healthy children is rarely associated with clinical manifestations. Mild pyrexia, malaise, vomiting, respiratory illness, pericarditis, and transient hepatic dysfunction have been reported with primary infection. Investigators hypothesize that after an initial round of viral replication at the site of entry, viremia follows with dissemination of the virus to distant sites at which latent infection is established. The most frequently recognized secondary sites of latent infection are renal and uroepithelial cells. Secondary infection has been reported to cause tubulointerstitial nephritis and ureteral stenosis in renal transplantation patients. It may be that renal impairment in immunocompromised patients and in non renal solid organ transplant recipients is found to be frequently associated with BKV infection.

BK Virus Nephropathy in Patients Undergoing Renal Transplantation

The reported prevalence of BKV nephropathy in renal allografts is between 1 and 8% (201, 202, 205, 209, 210). Asymptomatic infection is characterized by viral shedding without any apparent clinical features. Viruria, resulting from either primary or secondary infection, can persist from several weeks to years. Tubulointerstitial nephritis associated with BKV in renal transplant recipients is accompanied by histopathologic changes, with or without functional impairment. "Infection" and "disease" must be differentiated carefully. BKV infection (either primary or reactivated) can progress to BKV disease, but will not always do so (208). Furthermore, not all cases of BKV disease lead to renal impairment. However, infection can progress to transplant dysfunction and graft loss, although the diagnosis may be complicated by the coexistence of active allograft rejection.

BKV nephritis is reported to have a bimodal distribution, with 50% of BKV-related interstitial nephritis cases occurring 4–8 weeks after transplantation and the remainder of patients developing disease months to years after transplantation (211). Allograft failure is due mainly to extensive viral replication in tubular epithelial cells leading to frank tubular necrosis (203). Although damage is potentially fully reversible early in the disease, persisting viral damage leads to irreversible interstitial fibrosis. Tubular atrophy and allograft loss has been observed in 45% of affected patients (203, 212).

In most cases, BKV nephropathy in adult renal transplant recipients represents a secondary infection associated with rejection and its treatment. In children, however, primary BKV infection giving rise to allograft dysfunction may occur (208).

The definitive diagnosis of BKV nephropathy requires renal biopsy. Histopathologic features include severe tubular injury with cellular enlargement, marked nuclear atypia, epithelial necrosis, denudation of tubular basement membranes, focal intratubular neutrophilic infiltration, and mononuclear interstitial infiltration, with or without concurrent tubulins. This constellation of histologic features, particularly severe tubulitis, is often misinterpreted as rejection, even by the experienced pathologist. The presence of well-demarcated basophilic or amphophilic intranuclear viral inclusions, primarily within the tubular and parietal epithelium of the Bowman capsule, can help distinguish BKV disease from rejection (202, 203, 205). Additional tests such as immunohistochemistry, PCR analysis, or electron microscopy of biopsied tissue aimed at the identification of BKV may be required.

A practical diagnostic approach for identifying BKV in renal transplant patients is summarized in *Table 52-3*.

Other Implications of BK Virus in Renal Transplantation

BKV infection may cause ureteral obstruction due to ureteral ulceration and stenosis at the ureteric anastomosis. BKV-associated ureteral stenosis has been reported in 3% of renal transplant patients and usually occurs between 50 and 300 days after transplantation. Ulceration due to inflammation, proliferation of the transitional epithelial cells, and smooth muscle proliferation may lead to partial or total obstruction.

High-level BKV replication is implicated in acute, late-onset, long-duration hemorrhagic cystitis after bone marrow transplantation (213).

There are two case reports in children of renal carcinomas arising in the transplanted kidney in association with BK virus nephropathy. It remains unclear whether

BK virus itself has oncogenic potential in the transplant setting, but this is possible given that the big T antigen (T-Ag) expressed by polyomavirus family viruses has been shown to have the ability to disrupt chromosomal integrity (214, 215).

Treatment

Whether patients with asymptomatic viremia or viruria need specific therapeutic intervention is not certain. Review of the literature suggests that careful reduction of immune suppression, combined with active surveillance for rejection, will result in clinical improvement. Reduction in immunosuppression may precipitate episodes of acute cellular rejection, which need to be judiciously treated with corticosteroids. The outcome of BKV nephropathy is unpredictable, and stabilization of renal function may occur regardless of whether maintenance immunotherapy is altered or not (216).

Some reports favor the use of cidofovir. Cidofovir has important nephrotoxic side effects in the usual therapeutic dosage recommended for the treatment of CMV infection, and for BKV nephropathy a reduced dosage regime is generally used. The efficacy of cidofovir in reducing viremia has been demonstrated (see review in (210)). However, spontaneous clearance of viral infection after reduction of immunosuppression (without cidofovir) has also been reported. There are also case studies of the use of leflunamide.

Presence of BKV by PCR or decoy cells in urine signifies BKV replication. Decoy cells are caused by infection of the urinary epithelial cells with human polyoma viruses. The nuclei are enlarged and nuclear chromatin is completely homogenized by viral cytopathic effect. Positive PCR results for BKV viruria and presence of decoy cells have poor predictive value. Specificity is increased if >10 cells/Cytospin along with presence of inflammatory cells.

Presence of antibody is usually indicative of previous infection; however, positive results for BKV DNA PCR on serum signifies BK viremia. BKV PCR testing of plasma has proven to be a sensitive (100%) and specific (88%) means to identify BKV-associated nephropathy in adults. Viral load has also been used to monitor infection and clearance. However, because primary infection occurs in childhood, it might not be applicable to the pediatric population.

The definitive diagnosis of BKV nephropathy requires renal biopsy. Histopathology might mimic rejection or drug toxicity. However, characteristic findings have been described. Electron microscopy and immune staining are helpful in confirming the diagnosis. PCR assays of viral load in tubular cells have been reported to be a sensitive marker for diagnosis and monitoring.

Viral Hemorrhagic Fever

Viral hemorrhagic fever involves at least 12 distinct RNA viruses that share the propensity to cause severe disease with prominent hemorrhagic manifestations (*Table 52-4*). The viral hemorrhagic fevers, widely distributed throughout both temperate and tropical regions of the world, are important causes of mortality and morbidity in many countries. Most viral hemorrhagic fevers are zoonoses (with the possible exception of dengue virus), in which the virus is endemic in animals and human infection is acquired through the bite of an insect vector. Aerosol and nosocomial transmissions from infected patients are important for Lassa, Junin, Machupo, and Congo-Crimean hemorrhagic fevers, and Marburg and Ebola viruses (217).

Viral hemorrhagic fevers have many clinical similarities but also important differences in their severity, major organs affected, prognosis, and response to treatment. In all viral hemorrhagic fevers, severe cases occur in only a minority of those affected; subclinical infection or nonspecific febrile illness occurs in the majority. Fever, myalgia, headache, conjunctival suffusion, and erythematous rash occur in all the viral hemorrhagic fevers (218). Hemorrhagic manifestations range from petechiae and bleeding from venepuncture sites to severe hemorrhage into the GI tract, kidney, and other organs. A capillary leak syndrome, with evidence of hemoconcentration, pulmonary edema, oliguria, and ultimately shock, occurs in the most severely affected patients (218). Renal involvement occurs in all the viral hemorrhagic fevers, proteinuria is common, and prerenal failure is seen in all severe cases complicated by shock. However, in Congo-Crimean hemorrhagic fever and hemorrhagic fever with renal syndrome (HFRS), an interstitial nephritis, which may be hemorrhagic, is characteristic, and renal impairment is a major component of the illness.

Dengue

Dengue is caused by a flavivirus that is endemic and epidemic in tropical America, Africa, and Asia, where the mosquito vector *Aedes aegypti* is present (219). Classic dengue is a self-limited nonfatal disease; dengue hemorrhagic fever and dengue shock syndrome, which occur in a minority of patients, have a high mortality if not aggressively treated with fluids. After an incubation period of 5–8 days, the illness begins with fever, headache, arthralgia, weakness, vomiting, and hyperesthesia. In uncomplicated dengue the fever usually lasts 5–7 days. Shortly after

Table 52-3

Diagnosis of bk virus (bkv) in renal transplant recipients

Tests	Comments
Urine	
Cytology: decoy cells BKV PCR	Presence of BKV by PCR or decoy cells in urine signifies BKV replication. Decoy cells are caused by infection of the urinary epithelial cells with HPV. The nuclei are enlarged and nuclear chromatin is completely homogenized by viral cytopathic effect. Positive PCR results for BKV viruria and presence of decoy cells have poor predictive value. Specificity is increased if >10 cells/Cytospin along with presence of inflammatory cells.
Serum	
Antibody assays BKV BKV PCR	Presence of antibody is usually indicative of previous infection; however, positive results for BKV DNA PCR on serum signifies BK viremia. BKV PCR testing of plasma has proven to be a sensitive (100%) and specific (88%) means to identify BKV-associated nephropathy in adults. Viral load has also been used to monitor infection and clearance. However, because primary infection occurs in childhood, it might not be applicable to the pediatric population.
Tissue	
Histopathology, electron microscopy, BKV PCR	The definitive diagnosis of BKV nephropathy requires renal biopsy. Histopathology might mimic rejection or drug toxicity. However, characteristic findings have been described. Electron microscopy and immune staining are helpful in confirming the diagnosis. PCR assays of viral load in tubular cells have been reported to be a sensitive marker for diagnosis and monitoring.

HPV, human polyoma virus; PCR, polymerase chain reaction

onset a maculopapular rash appears, sparing the palms and the soles, and is occasionally followed by desquamation. Fever may reappear at the onset of the rash.

In dengue hemorrhagic fever and dengue shock syndrome, the typical febrile illness is complicated by hemorrhagic manifestations, ranging from a positive tourniquet test result or petechiae to purpura, epistaxis, and GI bleeding with thrombocytopenia and evidence of a consumptive coagulopathy. Increased capillary permeability is suggested by hemoconcentration, edema, and pleural effusions (219). In severe cases, hypotension and shock supervene, largely as a result of hypovolemia. Renal manifestations include oliguria, proteinuria, hematuria, and rising urea and creatinine. Acute renal failure occurs in patients with severe shock, primarily as a result of renal underperfusion. However, glomerular inflammatory changes may also occur. Children with dengue hemorrhagic fever show hypertrophy of endothelial and mesangial cells, mononuclear cell infiltrate, thinning of basement membranes, and deposition of IgG, IgM, and C3. Electron microscopy shows viral particles within glomerular mononuclear cells (220).

The diagnosis of dengue is made by isolation of the virus from blood or by serologic testing. There is no specific antiviral treatment, and management of patients with dengue shock syndrome or dengue hemorrhagic fever depends on aggressive circulatory support and volume replacement with colloid and crystalloid (221, 222). With correction of hypovolemia, renal impairment is usually reversible, but dialysis may be required in patients with established acute renal failure.

Yellow Fever

Yellow fever is caused by a flavivirus, and is transmitted by mosquito bites, typically *Aedes* species. It remains an important public health problem in Africa and South America. Renal manifestations are common and include albuminuria and oliguria. Over the next few days after first manifestation of infection, shock, delirium, coma, and renal failure develop, and death occurs 7–10 days after onset of symptoms. Laboratory findings include thrombocytopenia and evidence of hemoconcentration, rising urea and creatinine levels, hyponatremia, and deranged liver function test results. Pathologic findings include necrosis of liver lobules, cloudy swelling and fatty degeneration of the proximal renal tubules, and, often, petechiae in other organs. The oliguria appears to be prerenal and is due to hypovolemia; later, acute tubular necrosis supervenes. At present, there is no effective antiviral agent for yellow fever.

Table 52-4

Viral hemorrhagic fevers (hf)

Virus	Geographic distribution	Source of human infection	Incubation period (days)	Renal involvement	Renal pathology	Treatment
Dengue	Tropical Africa, South America, Asia	Mosquito	5–8	Rare, only in Dengue HF or shock	Vasomotor nephropathy, immune complex	Supportive
Yellow fever	Africa, South America	Mosquito	3–6	Common	Vasomotor nephropathy	Supportive
Congo-Crimean	Eastern Europe, Africa, Asia	Tick, nosocomial	2–7	In severe cases	Vasomotor nephropathy	Supportive ? Ribavirin
Hantaan viruses (Puumala, Seoul strains)	Europe, Asia, Africa, America	Rodents	4–42	HF and renal syndrome (Korean, Chinese, and Japanese epidemic HF, nephropathia epidemica)	Vasomotor nephropathy Interstitial nephropathy	Supportive ? Ribavirin
Rift Valley fever	Sub-Saharan Africa	Mosquito, contact with infected animals	3–7	Rare, only in fulminant cases	Vasomotor nephropathy	Supportive
Lassa fever	West Africa	Rodents, nosocomial	3–16	In cases with shock	Vasomotor nephropathy	Supportive and ribavirin
Junin, Argentine HF	Argentina	Rodents, nosocomial	5–19	In cases with shock	Vasomotor nephropathy	Supportive
Machupo, Bolivian HF	Bolivia	Rodents, nosocomial	5–19	In cases with shock	Vasomotor nephropathy	Supportive
Marburg	Africa	?, nosocomial	5–10	In cases with shock	Vasomotor nephropathy	Supportive
Ebola	Africa	?, nosocomial	5–10	In cases with shock	Vasomotor nephropathy	Supportive
Omsk	Eastern Europe	Tick	2–4	In cases with shock	Vasomotor nephropathy	Supportive
Kyasanur forest	India	Tick	3–8	In cases with shock	Vasomotor nephropathy	Supportive

Congo-Crimean Hemorrhagic Fever

Congo-Crimean hemorrhagic fever, first recognized in the Soviet Union, is now an important human disease in Eastern Europe, Asia, and Africa (223). Severely affected patients become stuporous or comatose 5–7 days into the illness, with evidence of hepatic and renal failure and shock. Proteinuria and hematuria are often present. The disease is fatal in 15–50% of cases. The virus is sensitive to ribavirin, but in one small trial of i.v. ribavirin versus supportive treatment only, there was no significant improvement in outcome in the treatment group (224).

Rift Valley Fever

Rift Valley fever is found in many areas of sub-Saharan Africa. In humans, most infections follow mosquito bites or animal exposure. The infection may present as an uncomplicated febrile illness, with muscle aches and

headaches. In 10% of patients, encephalitis or retinal vasculitis occurs as a complication. In a small proportion of cases, a fulminant and often fatal hemorrhagic illness occurs with hematemesis, melena, epistaxis, and evidence of profound DIC. Severe hepatic derangement, renal failure, and encephalopathy are often present. Despite intensive care, mortality is high.

Hemorrhagic Fever and Renal Syndrome (Hantavirus)

The viruses causing HFRS all belong to the Hantavirus genus in the Bunyaviridae family. The hantaviruses are distributed worldwide and are maintained in nature through chronic infection of rodents and small mammals. Transmission to humans is by aerosolized infectious excreta. Human disease usually occurs in summer among rural populations with exposure to rodent-infested barns or grain stores. Urban transmission can occur, however. At least five hantaviruses are known to cause HFRS: Hantaan, Seoul, Puumala, Porogia, and Belgrade viruses (225). HFRS is endemic in a belt from Norway in the west through Sweden, Finland, the Soviet Union, China, and Korea to Japan in the east. The clinical severity of HFRS varies throughout this belt (226). Clinical entities include Korean hemorrhagic fever, nephropathia epidemica in Scandinavia, and epidemic hemorrhagic fever in Japan and China.

In general, HFRS due to Hantaan, Porogia, and Belgrade viruses is more severe and has higher mortality than that due to Puumala virus (*nephropathia epidemica*) or Seoul virus. Hantaan is predominant in the Far East, Porogia and Belgrade in the Balkans, and Puumala in Western Europe; Seoul has a worldwide distribution (225). The clinical features of the disease vary. The incubation period is 4–42 days. Although HFRS occurs with the same clinical picture in children as in adults, both incidence rates and antibody prevalence rates are very low in children under 10 years of age. Men of working age make up the bulk of clinical cases (226). Mild cases are indistinguishable from other febrile illnesses. In more severe cases, fever, headache, myalgia, abdominal pain, and dizziness are associated with the development of periorbital edema, proteinuria, and hematuria. There is often conjunctival injection, pharyngeal injection, petechiae, and epistaxis or GI bleeding. The most severely affected patients develop shock and renal failure. The disease usually passes through five phases: febrile, hypotensive, oliguric, diuretic, and convalescent. Laboratory findings include anemia, lymphocytosis, thrombocytopenia, prolonged prothrombin and bleeding times, and elevated levels of fibrin degradation products. Liver enzyme levels are elevated, and urea and creatinine levels are elevated during the oliguric phase. Proteinuria and hematuria are consistent findings.

The renal histopathologic findings are those of an interstitial nephritis with prominent hemorrhages in the renal medullary interstitium and renal cortex. Acute tubular necrosis may also be seen. Immunohistochemical analysis reveals deposition of IgG and C3, and the GBM, mesangial, and subendothelial deposits may be seen on electron microscopy (227).

Recovery from Hantavirus-associated disease is generally complete, although chronic renal insufficiency is a rare sequela of HFRS. In mildly affected patients, the disease is self-limiting and spontaneous recovery occurs. However, in severe cases, with shock, bleeding, and renal failure, dialysis and intensive circulatory support may be required (228). Mortality rates vary depending on the strain of virus; rates are 5–15% for hemorrhagic fever and renal syndrome in China and significantly lower for the milder Finnish form associated with the Puumala virus strain.

Ribavirin is active against Hantaan viruses *in vitro*, and clinical trials indicate that both mortality and morbidity can be reduced by treatment with this antiviral agent if it is administered early in the course of illness. Dosages of 33 mg/kg followed by 16 mg/kg every 6 h for 4 days and then 8 mg/kg every 8 h for 3 days have been used (229).

Lassa Fever

Lassa fever is a common infection in West Africa, caused by an arenavirus, and usually manifests as a nonspecific febrile illness. In 10% of cases, a fulminant hemorrhagic disease occurs. In severe cases, proteinuria and hematuria are usually present, and renal failure may occur. Ribavirin is effective in decreasing mortality. As in other hemorrhagic fevers, intensive hemodynamic support and correction of the hemostatic derangements are important components of therapy (230).

Argentine and Bolivian Hemorrhagic Fevers

Junin and Machupo viruses, the agents of Argentine and Bolivian hemorrhagic fever, respectively, cause hemorrhagic fevers with prominent neurologic features and systemic and hemorrhagic features similar to those of Lassa fever. Oliguria, shock, and renal failure occur in the most severe cases.

Marburg Disease and Ebola Hemorrhagic Fever

Marburg and Ebola viruses have been associated with outbreaks of nosocomially transmitted hemorrhagic fever. Both viruses cause fulminant hemorrhagic fever. Onset is with high fever, headache, sore throat, myalgia, and profound prostration. An erythematous rash on the trunk is followed by hemorrhagic conjunctivitis, bleeding, impaired renal function, shock, and respiratory failure. The mortality rate is high. Renal histopathologic findings in fatal cases are of tubular necrosis, with fibrin deposition in the glomeruli. There is no specific treatment for these disorders.

Emerging Viral Infections

The important role played by a number of other recently characterized viruses is only now being recognized, as improved molecular diagnostic techniques allow identification of hitherto unrecognized viruses. Two examples of recently described viruses are metapneumovirus (237) and bocavirus (238). While both have significant prevalence, and may make an important contribution to the burden of childhood viral infection, as yet there are no reports indicative of significant renal pathology in association with these infections.

Influenza H5N1

Influenza virus has been linked with nephritis and acute renal failure. An emerging infectious disease is avian flu, caused by highly pathogenic H5N1 strains which have hitherto been confined to an avian reservoir, and there have been several outbreaks of infection in humans, particularly in the first part of this decade. Commonly, these patients develop a flu-like illness with prominent respiratory and gastrointestinal symptoms. Renal failure may develop alongside multi-organ failure in the context of acute respiratory distress syndrome (231). As yet, there is no clear correlation of degree of initial renal insufficiency, and outcome (232). There is little data available on treatment, but based on the known resistance patterns of H5N1 strains, oseltamivir and zanamivir are the preferred agents to be used for treatment of infection with H5N1.

SARS

Severe Acute Respiratory Syndrome (SARS) is a newly-emerged infectious disease which was first seen in South China in 2002. It is caused by a SARS coronavirus (SARS CoV). Predominantly, it causes a viral pneumonia, with diffuse alveolar damage; it has considerable mortality (233). Renal effects are not generally significant in the pathophysiology of SARS. However, SARS CoV has been found in kidney tissue at post-mortem (234) (235). SARS CoV enters cells via Angiotensin Converting Enzyme 2 (ACE2) (236), and it is thought that the invasion of kidney tissue reflects the virus' tropism for ACE2, which is expressed on kidney cells.

Parasitic Infections

Chronic exposure to infectious agents is a major factor in the increased prevalence of glomerular diseases in developing countries. Malaria is the best-documented parasitic infection associated with glomerular disease, but other parasitic infections including schistosomiasis, filariasis, leishmaniasis, and possibly helminth infections may also induce nephritis or nephrosis.

Malaria and Renal Disease

Malaria is estimated to cause up to 500 million clinical cases of illness and more than 1 million deaths each year (239). The association of quartan malaria and nephritis has been well known in both temperate and tropical zones since the end of the nineteenth century.

Epidemiologic studies provide the most conclusive evidence for a role of *Plasmodium malariae* in glomerular disease (240, 241). Chronic renal disease was a major cause of morbidity and mortality in British Guiana in the 1920s. The frequent occurrence of *P. malariae* in the blood of these patients led to detailed epidemiologic studies that implicated malaria as a cause of the nephrosis. After the eradication of malaria from British Guiana, chronic renal disease ceased to be a major cause of death in that country (240).

The link between malaria and nephrotic syndrome was strengthened by studies in West Africa in the 1950s and 1960s that demonstrated a high prevalence of nephrotic syndrome in the Nigerian population (242). The pattern of nephrotic syndrome differed from that in temperate climates, with an older peak age, extremely poor prognosis, and unusual histologic features. The incidence of *P. malariae* parasitemia in patients with the nephrotic syndrome in Nigeria was vastly in excess of that occurring in the general population, whereas the incidence of *Plasmodium falciparum* parasitemia was similar

to that in the general population. The age distribution of nephrotic syndrome also closely paralleled that of *P. malariae* infection (242). In some affected patients, circulating immune complexes and immunoglobulin, complement, and antigens were present in the glomeruli that were recognized by *P. malariae*-species antisera.

There is now a view that the patterns of childhood renal disease described in the last century may no longer be representative of the current situation. The variable patterns of renal disease throughout Africa may no longer reflect a dominant role for "malarial glomerulopathy," and the relative causative role of tropical infections in nephropathy remains an unanswered question (243).

Clinical and Histopathologic Features of Quartan Malaria Nephropathy

Most patients have poorly selective proteinuria and are unresponsive to treatment with steroids or immunosuppressive agents. The characteristic lesions of *P. malariae* nephropathy are capillary wall thickening and segmental glomerular sclerosis, which lead to progressive glomerular changes and secondary tubular atrophy (242). Cellular proliferation is conspicuously absent. Electron microscopy shows foot-process fusion, thickening of the basement membrane, and increase in subendothelial basement membrane-like material. Immunofluorescent studies show granular deposits of immunoglobulin, complement, and *P. malariae* antigen in approximately one-third of patients.

In addition to the histologic pattern, termed quartan malaria nephropathy, *P. malariae* infection is associated with a variety of other forms of histologic appearance, including proliferative GN and MGN (244).

Although quartan malaria nephropathy has been clearly linked to *P. malariae* infection in Nigeria, a number of studies from other regions in Africa have not revealed the typical histopathologic findings described in the Nigerian studies (245). Furthermore, quartan malaria nephropathy may be seen in children with no evidence of *P. malariae* infection or deposition of malaria antigens in the kidney. This, together with the fact that antimalarial treatment does not affect the progression of the disorder, raises the possibility that factors other than malaria might be involved in the initiation and perpetuation of the disorder. Although there is undoubtedly a strong association between *P. malariae* infection and nephrotic syndrome on epidemiologic grounds, the direct causal link is not proven. Most likely, a number of different infectious processes, including malaria, hepatitis B, schistosomiasis, and perhaps other parasitic infections that cause chronic or persistent infections and often occur concurrently in malaria areas, may all result in glomerular injury and a range of overlapping histopathologic features. The prognosis for the nephrotic syndrome in most African studies has been poor, regardless of whether the histologic findings were typical of quartan malaria nephropathy or whether *P. malariae* parasitemia was implicated. Treatment with steroids and azathioprine is generally ineffective, and a significant proportion of patients progress to renal failure.

Disease Associated with *Plasmodium Falciparum* Infection

P. falciparum appears to be much less likely to cause significant glomerular pathology. Epidemiologic studies have failed to show a clear association between *P. falciparum* parasitemia and the nephrotic syndrome. Whereas renal failure appears to be a common complication of severe malaria in adults, it seldom occurs in children.

Renal biopsy specimens from adult patients with acute *P. falciparum* infections who have proteinuria or hematuria show evidence of glomerular changes, including hypercellularity, thickening of basement membranes, and hyperplasia and hypertrophy of endothelial cells (246). Electron microscopy reveals electron-dense deposits in the subendothelial and paramesangial areas. Deposits of IgM, with or without IgG, are localized mainly in the mesangial areas. *P. falciparum* antigens can be demonstrated in the mesangial areas and along the capillary wall, which suggests an immune-complex GN. The changes, generally mild and transient, are probably unrelated to the acute renal failure that may complicate severe *P. falciparum* infection (246). Heavily parasitized erythrocytes play a central role in the various pathologic factors (247).

Renal failure occurring in severe *P. falciparum* malaria is usually associated with acidosis, volume depletion, acute intravascular hemolysis or heavy parasitic infection that leads to acute tubular necrosis. Recent studies have confirmed an important role for volume depletion in children with severe falciparum malaria, who characteristically have evidence of tachycardia, tachypnoea, poor perfusion and in severe cases hypotension (248). Volume expansion with either colloid or crystalloid results in improvement in hemodynamic indices and reduction in acidosis (249). There is growing evidence that volume expansion with albumin is associated with a better outcome than saline or synthetic colloids (250, 251). Treatment with antimalarials, correction of hypoglycemia and

electrolyte imbalance, and volume expansion reduces mortality to less than 5%. Although renal failure is usually associated with infection by *P. falciparum*, acute renal failure has been described with *Plasmodium vivax* infection and mixed infections (252).

Blackwater Fever

The term blackwater fever refers to the combination of severe hemolysis, hemoglobinuria, and renal failure. It was more common at the start of the twentieth century in nonimmune individuals receiving intermittent quinine therapy for *P. falciparum* malaria. Blackwater fever has become rare since 1950, when quinine was replaced by chloroquine. However, the disease reappeared in the 1990s, after the reuse of quinine because of the development of chloroquine-resistant organisms. Since then, several cases have been described after therapy with halofantrine and mefloquine, two new molecules similar to quinine (amino-alcohol family) (253). Renal failure generally occurred in the context of severe hemolytic anemia, hemoglobinuria, and jaundice. The pathophysiology of the disorder is unclear; however, it appears that a double sensitization of the red blood cells to the *P. falciparum* and to the amino-alcohols is necessary to provoke the hemolysis. Histopathologic findings include swelling and vacuolization of proximal tubules, necrosis and degeneration of more distal tubules, and hemoglobin deposition in the renal tubules. Recent studies indicates a better outcome with earlier initiation of intensive care and dialysis combined with necessary changes in antimalarial medications.

Schistosomiasis

Schistosomiasis affects 200 million people living in endemic areas of Asia, Africa, and South America (254). The infection is usually acquired in childhood, but repeated infections occur throughout life. *Schistosoma japonicum* is found only in the Orient, whereas *Schistosoma haematobium* occurs throughout Africa, the Middle East, and areas of southwest Asia. *Schistosoma mansoni* is widespread in Africa, South America, and southwest Asia.

Human infection begins when the cercarial forms invade through the skin, develop into schistosomula, and move to the lungs via the lymphatics or blood. They then migrate to the liver and mature in the intrahepatic portal venules, where male:female pairing takes place. The adult worm pairs then migrate to their final resting site - the venules of the mesenteric venous system of the large intestine (*S. mansoni*) or in the venules of the urinary tract (*S. haematobium*). The females release large numbers of eggs, which may remain embedded in the tissues, embolize to the liver or lungs, or pass into the feces or urine.

Clinical manifestations may occur at any stage of the infection. Cercarial invasion may cause an intense itchy papular rash. Katayama fever is an acute serum sickness-like illness that occurs several weeks after infection, as eggs are being deposited in the tissues. Deposition of the eggs in tissues results in inflammation of the intestines, fibrosis of the liver, and portal hypertension. With *S. haematobium*, chronic inflammation and fibrosis of the ureters and bladder may lead to obstructive uropathy (255).

Renal manifestations of schistosomiasis occur most commonly in *S. mansoni* infection. Schistosomal nephropathy usually presents with symptoms including granulomatous inflammation in the ureters and bladder, but glomerular disease (probably on an immune-complex basis) may also occur. Renal disease usually occurs in older children or young adults with long-term infection, but serious disease may also occur in young children (255).

The early renal tract manifestations of schistosomiasis are suprapubic discomfort, frequency, dysuria, and terminal hematuria. In more severe cases, evidence of urinary obstruction appears. Poor urinary stream, straining on micturition, a feeling of incomplete bladder emptying, and a constant urge to urinate may be severely disabling symptoms. The fibrosis and inflammation of ureters, urethra, and bladder may be followed by calcification and may result in hydroureter, hydronephrosis, and bladder neck obstruction. Renal failure may ultimately develop, and there is a suspicion that squamous cell carcinoma of the bladder may be linked to the chronic infective and inflammatory process. Secondary bacterial infection is common within the obstructed and inflamed urinary tract (254).

The hepatosplenic form of *S. mansoni* infection may be accompanied by a glomerulopathy in 12–15% of cases, manifested in the majority as nephrotic syndrome (256). Histopathologic findings include mesangioproliferative GN, focal segmental glomerulosclerosis, mesangiocapillary GN, MGN, and focal segmental hyalinosis (257). Immune complexes may be detected in the circulation of these patients, and glomerular granular deposition of IgM, C3, and schistosomal antigens are seen on immunofluorescence. Usually Schistosoma-specific nephropathy is a progressive disease and is not influenced by antiparasitic or immunosuppressive therapy (258), but isolated case reports of remission after treatment with praziquantel have been reported (259).

The diagnosis is confirmed by the detection of Schistosoma eggs in feces, urine, or biopsy specimens. Eggs are shed into the urine with a diurnal rhythm, and urine collected between 11 AM and 1 PM is the most useful. Urinary sediment obtained by centrifugation or filtration through a Nuclepore membrane should be examined.

In cases in which studies of urine and feces yield negative results in patients in whom the diagnosis is suspected, rectal biopsy specimens taken approximately 9 cm from the anus have a high diagnostic yield for both *S. mansoni* and *S. haematobium* infection. Biopsy of liver or bladder may be required to establish the diagnosis.

Antibodies indicating previous infection can be detected using enzyme-linked immunosorbent assay or radioimmunoassay. The tests are sensitive but lack specificity and may not differentiate between past exposure and current infection.

Praziquantel is the drug of choice for treatment of schistosomiasis. A single oral dose of 40 mg/kg is effective in *S.haematobium* and *S. mansoni* infection and is usually well tolerated. The alternative drug for *S. mansoni* infection is oxamniquine. Complete remission of urinary symptoms may occur in renal disease of short duration, but in late disease with extensive fibrosis, scarring, and calcification, obstructive uropathy and renal failure may persist after the infection has been eradicated. There are reports of a drastic decrease in the number of severe hepatosplenic forms of *S. mansoni* infection after mass treatment of the population in endemic areas with oxamniquine. This also reduced schistosomal nephropathy (256).

Leishmaniasis

Visceral leishmaniasis is a chronic protozoon infection characterized by fever, hepatosplenomegaly, anemia, leukopenia, and hyperglobulinemia. Proteinuria and/or microscopic hematuria or pyuria have been reported in 50% of patients with visceral leishmaniasis (260). Acute renal failure in association with interstitial nephritis has also been reported (261). Renal histologic analysis in patients with visceral leishmaniasis reveals glomerular changes, with features of a mesangial proliferative GN or a focal proliferative GN, or a generalized interstitial nephritis with interstitial edema, mononuclear cell infiltration, and focal tubular degeneration. Immunofluorescence reveals deposition of IgG, IgM, and C3 within the glomeruli, as well as electron-dense deposits in the basement membrane and mesangium on electron microscopy (260). Circulating immune complexes together with immunoglobulin and complement deposition in the glomeruli suggests an immune-complex cause.

Renal disease in leishmaniasis is usually mild and may resolve after treatment of the infection. Renal dysfunction may be associated with treatment for visceral leishmaniasis with antimony compounds.

Filariasis

Proteinuria is more common in filarial hyperendemic regions of West Africa than in nonfilarial areas. Renal histologic analysis has shown a variety of different histopathologic appearances; the most common is diffuse mesangial proliferative GN with C3 deposition in the glomeruli (262). Renal biopsy specimens also demonstrate large numbers of eosinophils in the glomeruli, and microfilariae may be seen in the lumen of glomerular capillaries. Filarial antigens have been detected within immune deposits within the glomeruli.

Hydatid Disease

Echinococcus granulosus causes chronic cysts within a variety of organs. In addition, nephrotic syndrome in association with hydatid disease has been reported. Membranous nephropathy, minimal change lesions, and mesangiocapillary GN have been described in association with hydatid disease (263, 264). Immunofluorescence reveals deposits of immunoglobulin, complement, and hydatid antigens within the glomeruli. Remission of nephrotic syndrome has been reported with treatment by antiparasitic agents such as albendazole (263, 264).

Trypanosomiasis

Few reports have been published of renal disease occurring in patients with trypanosomiasis. The trypanosomal antigens can induce GN in a variety of experimental animals (265).

Toxoplasmosis

Nephrotic syndrome has occasionally been reported as a manifestation of congenital toxoplasmosis.

Dissemination of previously latent toxoplasma infection in patients undergoing treatment with immunosuppressive drugs has been increasingly recognized in recent years. Reactivation of toxoplasmosis or progression of recently acquired primary infection should be considered in patients undergoing renal transplantation or immunotherapy for renal disease who develop unexplained inflammation of any organ.

Fungal Infections

Fungal infections of the kidneys and urinary tract occur most commonly as part of systemic fungal infections in patients with underlying immunodeficiency, as focal urinary tract infections in patients with obstructive lesions, or as a result of indwelling catheters. Although Candida infection is the most common fungal infection in both immunocompromised and non immunocompromised hosts, virtually all other fungal pathogens may invade the renal tract during severe immunocompromise.

Urinary infection with *Candida albicans* is most commonly a component of systemic candidiasis in patients who are severely immunocompromised. Systemic candidiasis is also seen in premature and term infants with perinatally acquired invasive candidiasis. Presentation is usually with systemic sepsis, fever or hypothermia, hepatosplenomegaly, erythematous rash, and thrombocytopenia. Systemic candidiasis may be seen on ophthalmologic investigation as microemboli in the retina. The first clue to the underlying diagnosis may be the presence of yeasts in the urine (266).

Candida involvement of the urinary tract may affect all structures including the glomeruli, tubules, collecting system, ureters, and bladder. Microabscesses may form within the renal parenchyma, and large balls of fungi may completely obstruct the urinary tract at any level. Acute renal failure caused by systemic candidiasis or obstruction of the renal tracts with fungal hyphae is a well-recognized complication of systemic candidal infection (266, 267). Indwelling catheters (which form a nidus for persistent infection) should be removed. Successful treatment of non-obstructing bilateral renal fungal balls by fluconazole either alone or in combination with liposomal amphotericin B has been reported (268, 269). In the presence of obstruction, however, percutaneous nephrostomy to relieve the obstruction with antegrade amphotericin B irrigation, coupled with systemic antifungal therapy, is the mainstay of treatment (267). Amphotericin B is the most effective antifungal agent, but it is not excreted in the urine. Local irrigation via nephrostomy provides good results, however. For treatment of urinary tract candidiasis, it is usually combined with fluconazole or 5-flucytosine, both of which are excreted in high concentrations in the urine. Treatment is required for weeks to months to ensure complete elimination of the fungus, and the ultimate outcome is largely dependent on whether there is a permanent defect in immunity.

Miscellaneous Conditions

Hemorrhagic Shock and Encephalopathy

In 1983, Levin et al. first described hemorrhagic shock and encephalopathy, which appeared to be distinct from previously recognized pediatric disorders (270). Other cases have subsequently been reported from several centers in the United Kingdom, Europe, Israel, the United States, and Australia, and the syndrome is now recognized as a new and relatively common severe childhood disorder (271).

Hemorrhagic shock and encephalopathy usually affects infants in the first year of life, with a peak onset at 3–4 months of age. A prodromal illness with fever, irritability, diarrhea, or upper respiratory infection occurs 2–5 days before the onset in two-thirds of cases. Affected infants develop profound shock, coma, convulsions, bleeding and evidence of DIC, diarrhea, and oliguria. Laboratory findings include acidosis, falling hemoglobin and platelet levels, elevated urea and creatinine levels, and elevated levels of hepatic transaminases. Despite vigorous intensive care, the prognosis is poor, and most affected infants die or are left severely neurologically damaged (271, 272). A small number of patients have been reported to survive without residual sequelae.

The renal impairment appears to be largely prerenal in origin, and when aggressive volume replacement and treatment of the shock results in improved renal perfusion, rapid improvement in renal function is usually observed. In patients with profound shock unresponsive to initial resuscitation, vasomotor nephropathy supervenes and dialysis may be required. Myoglobinuria in association with hemorrhagic shock and encephalopathy has been reported.

Kawasaki Disease

Following the description of the mucocutaneous lymph node syndrome by Kawasaki in 1968, Kawasaki disease has been recognized as a common and serious childhood

illness with a worldwide distribution. Although the etiology remains unknown, epidemiologic features clearly suggest an infective cause. The disease occurs in epidemics, and wavelike spread has been demonstrated during outbreaks in Japan.

Amyloidosis

Deposition of amyloid within the kidney is an important complication of chronic and persistent infection. Amyloidosis is most common in patients with chronic osteomyelitis and chronic pulmonary infections such as bronchiectasis and is seen occasionally in those with persistent infections such as leprosy or malaria (273–275).

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53 Nephrotoxins

Deborah P. Jones · Russell W. Chesney

The kidney is responsible for the elimination and metabolism of many foreign organic compounds, including pharmacologic agents. Ultimately these agents reach the urinary space for excretion via glomerular filtration or tubular secretion (*Fig. 53-1*). In some instances drugs must undergo transformation into metabolites, a reaction that may actually take place within tubular cells before secretion into the tubular lumen. While performing its role as an excretory and metabolic organ, the kidney may be at risk for drug-induced toxicity.

Factors that contribute to the susceptibility of the kidney to injury include the magnitude of blood flow through glomerular and peritubular vessels; the large surface area available for uptake of potentially harmful compounds; the concentration of specific substrates within tubular fluid which may induce injury or promote accumulation of the toxin within the renal tubular cell; dependence of the renal tubular cell on a high metabolic rate to maintain function; and the distribution of unique transport systems that concentrate drugs or toxins within the tubular cell (1).

Nephrotoxins may preferentially damage specific nephron segments, depending on the area of maximal exposure, the location of specific uptake systems, and the tubular location of specific intracellular sites that are susceptible to the toxin (1). The concentration of a drug within the renal tubular cell may be affected by the dissociation constant of the compound, its lipid solubility, tubular secretory or reabsorptive activity, water handling, competitive tubular transport mechanisms, urinary pH, and urinary flow rate. The concentration also may be affected by the distribution of enzymes that metabolize individual drugs and intracellular protective systems important in the maintenance of normal cell metabolism.

Potential mechanisms for drug-induced renal dysfunction include alterations in renal perfusion and glomerular filtration rate, tubular cell damage, and tubular obstruction (1). Toxic nephropathy, or drug-induced renal dysfunction, is primarily a disorder of the renal tubule; however, significant tubular damage eventually results in alterations in glomerular function. In addition, drugs or their metabolites may alter glomerular blood flow through vasoconstriction of the glomerular capillaries (1). This may be a primary event through actions on the endothelial cell or a secondary event via activation of tubuloglomerular feedback after tubular damage allows increased distal delivery of solute and fluid.

Drugs may undergo redox recycling, which results in oxidative stress within the cell and alters the normal antioxidant defense mechanisms. The elaboration of reactive oxygen species by the cytochrome P-450 system may enable reactive intermediates to initiate damage directly or to combine with glutathione (GSH) to deplete available GSH (2). GSH protects cells from oxidant stress as it participates in detoxification reactions during vital cell processes such as protein and nucleic acid synthesis. Depletion of cellular levels of GSH is a common pathway for nephrotoxic injury (2). Drugs or metabolites may either directly conjugate GSH or be metabolized to compounds that are reactive and may bind to GSH. Ultimately, reactive species promote injury, which interferes with normal cellular functions such as energy production, detoxification, transport, and macromolecular synthesis. Although they may come from widely different chemical classes and have different renal distributions, toxic compounds may ultimately affect renal integrity and function through inhibition of cellular energy production, disruption of membrane integrity, perturbation of vital cell functions such as protein or nucleic acid synthesis, or alteration in intracellular calcium homeostasis.

This chapter focuses on the clinical characteristics, and mechanisms of injury, as well as methods to reduce or prevent drug-associated renal damage. The most common offending agents have been chosen; this discussion is not meant to be all inclusive.

Organic Anion Transporters

Organic anions comprise a chemically heterogeneous group of compounds including drugs, environmental chemicals, including plant and animal toxins, as well as metabolites of endogenous and exogenous substances. A list of drugs which are organic anions is found in *Table 53-1* (3). Organic anions are primarily eliminated by the kidneys and liver via a family of organic anion transporters

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Figure 53-1

Renal drug elimination is s combination of glomerular filtration and tubular secretion. Many factors have an impact on renal cell accumulation and net excretion.

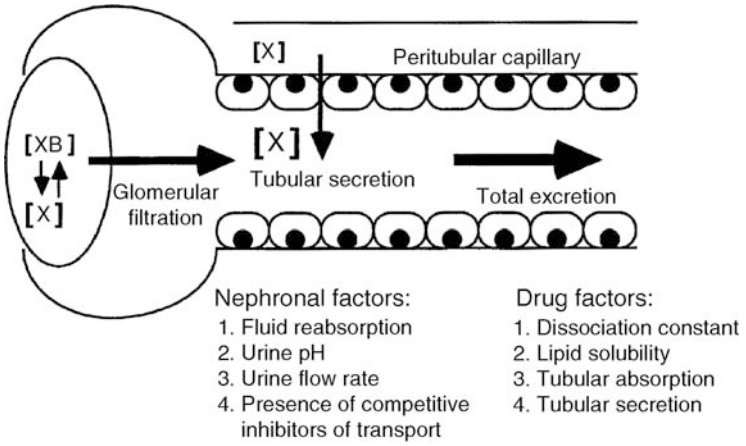

Table 53-1

Substances transported by OAT1 (from reference (5))

Endogenous
cAMP, cGMP, PGE1, PGE2, 6-ketoPGF1a, urate, folate, neurotransmitter metabolites
Drugs
Antibiotics: penicillin antibiotics, cephem antibiotics, carbapenem antibiotics
Antiviral: acyclovir, amantidine, adefovir, cidofovir
NSAIDs: salicylates, indomethacin, ibuprofen, diclofenac, naproxen, sulindac
Diuretics: loop diuretics, thiazide diuretics
Antihypertensives: ACE inhibitors, angiotensin receptor antagonists
Anti-neoplastic: methotrexate, azathioprine, Ara-C, 5-FU, cyclophosphamide, chlorambucil, anti-epileptic: valproate
Uricosuric: probenecid

known as the OAT. This family of transporters is essential to the renal elimination of numerous substrates; altered function of the OAT family may be responsible for interindividual variation in the toxicity of its substrates (3). The first OAT transporter characterized was OAT1, which is also known as the p-aminohippurate (PAH) transporter since PAH is the classic substrate for this transport system. Five structurally similar OATs belong to this family of transporters: OAT2, OAT3, OAT4, URAT1 and OAT5. OAT1 and OAT3 are localized to the basolateral membrane of the proximal tubule, where they function as the major pathway for organic anion entry into the proximal tubular cell (*Fig. 53-2*) (4). One of the main features of the OAT family of transporters is that substrate specificity is quite broad. In contrast to OAT1 and OAT2, OAT4 is localized to the apical membrane of the proximal tubular cell. These transporters are anion exchangers, as uptake of substrate is stimulated by an outwardly directed concentration gradient of dicarboxylate compounds.

The OAT1 transporter interacts with numerous drugs including β-lactam antibiotics, antiviral agents, diuretics, antineoplastic compounds, angiotensin converting enzyme inhibitors and nonsteroidal antiinflammatory drugs (NSAIDs) (4). OAT3 has somewhat different substrate specificity and has been found to interact with histamine II receptor antagonists, estrone sulfate, β-lactam antibiotics, antiviral agents, diuretics, antineoplastic compounds, angiotensin converting enzyme inhibitors and NSAIDs (5). Coadministration of substrates which also rely upon this transport system for elimination results in reduced accumulation of both substrates; this interaction can markedly influence the proximal tubular cell accumulation and ultimately the renal elimination of these compounds. Examples include the increased half-life noted for penicillin when it is given with probenecid. Similarly, increased toxicity secondary to increased plasma drug levels is observed when methotrexate is coadministered with NSAIDs or β-lactam antibiotics. Similar interactions have been reported for antiviral agents, diuretics and nucleoside derivatives (5).

Multidrug Resistance Proteins

The multidrug resistance protein (MRP) family is comprised of active transporters with ATP-binding cassette motifs (*Fig. 53-2*). The main renal MRP is MRP4, which is located on the apical membrane of the proximal tubular cell where is facilitates efflux of a wide variety of organic acids, both endogenous and exogenous, including numerous drugs (*Table 53-2*) (6). Interaction among substrates for MRP4 is complex, as evidenced by allosteric stimulation of MRP4 mediated transport by some substrates but not others. Current evidence supports the presence of two independent transport sites that can be modified by multiple allosteric binding sites, such that binding of certain substrates is dependent upon the presence of others (7).

Figure 53-2

Proximal tubular transport systems important to drug excretion. The organic anion transporters (OAT) and multidrug resistance-associated proteins (MRP) in the proximal tubular cell. Uptake of organic anions across the basolateral membrane is mediated by anion exchange via OAT 1 and OAT3. MRP2 and MRP 4 mediate basolateral efflux. The luminal organic anion exchanger, OAT 4, may also allow luminal efflux of organic anions. From reference (5).

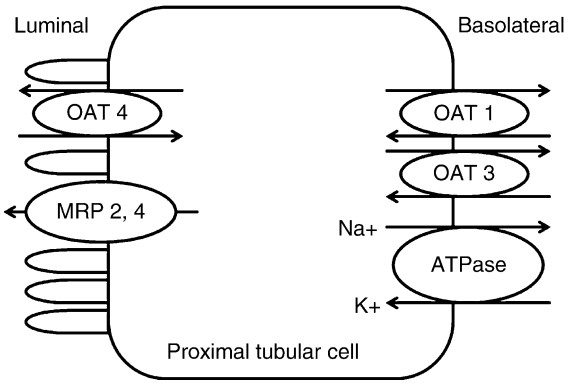

Table 53-2

Selected endogenous and exogenous substrates which interact with MRP4 (from reference (7))

Endogenous substrates
cAMP, cGMP, urate, PGE1, PGE2, folate, conjugated steroids
Exogenous substrates
Methotrexate, leucovorin, topotecan, adefovir, tenofovir, ceftizoxime, cefazolin, cefotaxime, hydrochlorothiazide, furosemide, olmesartan, PAH
Inhibitors of MRP4
Dipyridamole, sildenafil, indomethacin, sulindac, losartan, probenecid

Nephrotoxicity from Antibiotics

The concentration of antibiotics by renal tissue exposes the kidney to toxicity that other organs do not experience (8). Nephrotoxic clinical syndromes include acute tubular necrosis (ATN), acute interstitial nephritis (AIN), direct tubular effects on transport systems (electrolytes, protons leading to acidosis, concentration defects, or the full-blown Fanconi syndrome), and tubular obstruction from drug precipitation.

Aminoglycosides

Aminoglycoside antibiotics are widely used antimicrobials that are particularly nephrotoxic; these compounds are concentrated within the renal tubular cell (9). Aminoglycosides are excreted primarily by glomerular filtration. Reabsorption by the proximal tubule cell occurs after charge interaction and binding to the brushborder membrane of the proximal tubule, followed by transport into the cell by pinocytosis via vesicles directed to lysosomes (10, 11). Although less significant, reabsorption from the basolateral surface of the tubular cell may also contribute to total intracellular uptake (12). The molecular mechanism for aminoglycoside uptake by the brush border of the proximal tubular cell involves binding to megalin, a multiligand endocytic receptor (13). Depletion of megalin or coadministration of ligands that bind megalin in animal models of aminoglycoside nephrotoxicity result in reduced accumulation of the drug within the proximal tubular cell, and prevention of cell damage (14). Accumulation of the drug within the lysosome results in phospholipid hydrolysis and the formation of electron-dense myeloid bodies, which are membranous structures found within lysosomes (9). Aminoglycosides appear to have a generalized effect on phospholipid membranes, resulting in decreased surface charge and alterations in fluidity and permeability of tubular cell membranes (8, 9).

Pathologic alterations have been reported after a single dose of aminoglycoside, but most animal models of aminoglycoside toxicity use multiple doses over several days to produce a model of toxic nephropathy. Once appreciable injury occurs, lysosomal membranes release sequestered aminoglycosides as well as lysosomal

enzymes, which cause generalized cell injury and eventual necrosis (15, 16).

The main pathologic finding in aminoglycoside nephrotoxicity is tubular cell necrosis, involving principally the most proximal segments of the nephron: the convoluted tubule and pars recta (S1 and S2) (1, 17). The earliest pathologic finding is an increased number of lysosomes, followed by alterations in the brush-border architecture and mitochondrial swelling (18). This process may be focal or diffuse. Of interest, even during continued aminoglycoside administration, tubular cell regeneration can be evident. Gentamicin also interferes with mitochondrial respiration by enhancing hydrogen peroxide generation, which includes iron mobilization from the mitochondria. Hydroxyl-radical scavengers and iron chelators are protective in the rat model of aminoglycoside toxicity (2).

Clinical features of aminoglycoside nephrotoxicity include subtle perturbation of tubular and/or glomerular function; nephrotoxicity occasionally progresses to renal failure. The earliest clinically detectable abnormality is enzymuria, which results from loss of brushborder membrane segments bearing the enzymes (19). Tubular damage may rarely produce a Fanconi syndrome with proximal tubular wasting of glucose, phosphate, amino acids, and bicarbonate as well as numerous ions including magnesium (9, 17). Initially, urine volume may be increased because of a vasopressin-resistant concentrating defect. Renal dysfunction is most commonly detected after a rise in serum creatinine concentration or elevated aminoglycoside trough levels. Although it is rare, severe, sustained renal dysfunction has been described with peak serum creatinine levels at 3–10 days after cessation of aminoglycoside therapy and full recovery in weeks (20).

Glomerular filtration rate (GFR) may decrease after gentamicin administration in the absence of tubular necrosis as a result of angiotensin II–mediated afferent arteriolar vasoconstriction. Prostaglandin synthetase inhibitors potentiate the vasoconstriction and hypofiltration; this provides indirect evidence of a palliative role of locally produced vasodilatory prostaglandins in this form of nephrotoxic injury (21). In addition to the microvascular and tubular changes, K_f (glomerular capillary ultrafiltration coefficient) is decreased because of a decrease in the number and density of glomerular capillary wall fenestrae (22, 23).

The relative toxicity of the aminoglycosides, in order from most toxic to least, is neomycin, gentamicin, tobramycin, and amikacin (17). This order is dependent on the number of ionizable amino groups, which affects brushborder membrane binding (24). Several factors may contribute to or predispose to aminoglycoside nephrotoxicity. Drug dosage and duration of therapy, particularly repeated and prolonged courses, determine the severity of the tubular toxicity. Concomitant administration of other nephrotoxic drugs, concomitant administration of cationic amino acids, volume depletion, potassium depletion, endotoxemia, hypokalemia, and hyperphosphatemia further potentiate renal injury (10, 17, 25) Prevention of aminoglycoside toxicity involves the prudent use of aminoglycosides along with planned monitoring for early nephrotoxicity.

β-Lactam Antibiotics

The β-lactam antibiotics are related to the penicillins, and include the cephalosporins and the penems. Structurally, they are bicyclic compounds that acylate bacterial wall proteins and penetrate the bacterium to inactivate vital enzymes. Whereas most penicillin-like compounds are not nephrotoxic, certain cephalosporins and carbapenem compounds are associated with renal toxicity (26).

The β-lactams are extracted from renal venous blood by the organic acid transport system (OAT 1 and OAT3) located on the basolateral surface of the tubular cell. Intracellular accumulation results because of the limited ability of the proximal tubular cell to secrete the β-lactam at the luminal membrane into the luminal fluid (urine). Differences in organic acid transporter characteristics between basolateral and apical membranes result in marked accumulation with high intracellular levels, sometimes a thousand times higher than the levels found in plasma or other types of cells (26). Because of the very specific distribution of the organic acid transporters, renal injury in the form of tubular cell necrosis is limited to the proximal tubule (26). The initial event is thought to be lipid peroxidation with particular damage to the mitochondrial membrane, possibly through acylation of important membrane-associated transporters. Mitochondrial damage interferes with energy production, which leads to decreased levels of adenosine triphosphate and subsequent cell damage and death. Replacement of deficient cellular substrates can reverse toxicity (16). Damage secondary to β-lactams is related to decreased mitochondrial respiration and decreased succinate uptake by mitochondria within hours after drug administration (26).

The original compounds cephaloridine and cephaloglycin are no longer used; the new compound imipenem, which is a carbapenem, is nephrotoxic and is used only in combination with cilastin, an inhibitor of brushborder dehydropeptidase I. Cilastin inhibits the metabolism of imipenem to metabolites with structural similarity to cephaloridine (27). Meropenem, another agent in the

carbapenem class, is not metabolized to an appreciable degree and therefore does not require coadministration with cilastin. Cilastatin was designed as a nephroprotective OAT inhibitor, allowing higher serum levels and lower proximal renal tubular intracellular levels of the penems (28).

Clinical characteristics of β-lactam–associated nephrotoxicity include ATN with or without oliguria, which lasts days to weeks. Drug dosage should be adjusted according to the patient's renal function. Administration of organic anion transporter inhibitors (probenecid) may help to prevent injury. However, because these agents also impair tubular secretion, and thus drug elimination, blood levels of the β-lactam would be expected to be higher in the presence of organic anion inhibitors.

Renal toxicity from β-lactams can also result from hypersensitivity reactions. The molecular weights of all β-lactams are insufficient to be directly immunogenic. These antibiotics or their metabolites can bind to larger molecules, which then act as haptens. Although exposure to many penicillins and cephalosporins can result in acute interstitial nephritis (AIN), AIN is most common with methicillin (29, 30). This syndrome consists of fever, rash, oliguria, and, rarely, arthralgias. Laboratory findings are prominent in the urine with pyuria, eosinophiluria, and proteinuria with azotemia. The main therapy is discontinuation of the β-lactam, but glucocorticoids have been used and may enhance recovery (31).

Antifungal Agents

Amphotericin B

Amphotericin B is an antifungal agent that exerts its effect by interaction with membrane sterols. This chemical interaction is the basis for its antifungal action as well as renal toxicity (17). Amphotericin B has two major pathophysiologic effects on the kidney: renal tubular cell injury, which is manifest as hypokalemia, hypomagnesemia, and acidosis; and decreased GFR secondary to afferent arteriolar vasoconstriction (9, 32).

Unlike most other drug-associated renal tubular toxicity, the primary tubular site of amphotericin B–induced injury is the distal tubule (17). The interaction of this drug with membrane cholesterol leads to the formation of aqueous pores, which greatly increase the permeability of the normally tight epithelium of the distal tubule; this results first in a backleak of H^+, Na^+, and Cl^-, and then to a vasopressin-resistant polyuria (17, 33). Use of this agent will result in the formation of pores in all patients exposed. Increased permeability to Cl^- may stimulate increased tubuloglomerular feedback and reduce GFR, which ultimately results in renal ischemia and azotemia. In a canine model, amphotericin administration led to a decrease in urine flow and GFR within 1 h, accompanied by increased renal vascular resistance as a result of afferent arteriolar constriction (34). Aminophylline blocks amphotericin B–induced vasoconstriction, which suggests a role for adenosine as a potential mediator (35). Verapamil also protects the kidney from vasoconstriction, which indicates that increased calcium in vascular smooth muscle may mediate the acute effects (36).

Amphotericin B nephrotoxicity may become clinically evident as a reduction in GFR, usually detected as an increase in plasma creatinine level or as distal tubular dysfunction with metabolic acidosis (secondary to the distal type of renal tubular acidification defect), salt wasting, polyuria, hypomagnesemia, or hypokalemia (10, 17). Nephrotoxicity is predictable and usually reversible, although irreversible azotemia has been observed at large dosages. The severity of tubular toxicity and glomerular dysfunction is proportional to the cumulative dose. In adults, a cumulative dose exceeding 5 g was associated with significant renal dysfunction, whereas patients who received less than a 600 mg cumulative dose rarely suffered renal toxicity (37). Concomitant use of diuretics, cyclosporine, or aminoglycoside antibiotics and abnormal pretreatment renal function increase the risk of amphotericin toxicity (17, 37).

Sodium loading appears to have a beneficial effect on amphotericin B–induced nephrotoxicity by maintaining intravascular volume and reducing renal vasoconstriction (17, 37). Mannitol has been shown to ameliorate nephrotoxicity in animal studies, but the benefit of this agent in humans has not been consistently demonstrated (38, 39).

Lipid formulations of amphotericin B have been developed in an attempt to reduce renal toxicity. The aim of the lipid preparations of amphotericin B is to direct the drug to preferentially bind to fungal membranes rather than mammalian membranes (40). In one study of HIV patients with invasive histoplasmosis, nephrotoxicity occurred in 37% of patients treated with amphotericin B compared to 9% of patients treated with liposomal amphotericin B (41). However, as noted above, nephrotoxicity can occur with the liposomal preparation, an effect which appears to be dose-related. In a pediatric study which assessed the change in serum creatinine before and after amphotericin B lipid complex, the mean serum creatinine did not change, however, approximately 15% of patients had higher posttreatment creatinine compared to pretreatment levels (42).

Nonsteroidal Antiinflammatory Drugs

Nonsteroidal antiinflammatory drugs (NSAIDs) inhibit cyclooxygenase and thereby reduce prostaglandin synthesis from arachidonic acid. Prostaglandin production can be stimulated by angiotensin II, arginine vasopressin, catecholamines, and renal sympathetic nerve stimulation. Stimulation of prostaglandin synthesis occurs by hormone-mediated activation of phospholipases, which release arachidonic acid from membrane-associated phospholipids (43).

Under conditions such as impaired GFR, alterations in renal hemodynamics, and sodium depletion, however, the compensatory release of vasodilatory prostaglandins I_2 and E_2 helps to maintain renal blood flow and GFR, and modifies the effect of local vasoconstrictive substances such as angiotensin II (43). Therefore, it is in the presence of such altered physiologic states that renal functional alterations associated with NSAIDs are likely to occur, because the normal compensatory mechanisms are blocked. NSAIDs potentiate prerenal ischemia, increasing sodium reabsorption by the proximal tubule and the ascending limb of the loop of Henle. NSAIDs also increase the sensitivity of the collecting tubule to vasopressin, which further adds to salt and water retention and may produce a hyporeninemic hypoaldosteronism through reduction of renin release (43).

The organic anion transporter family (OAT) is likely to play a significant role in the pathogenesis of NSAID-induced acute kidney injury, as this basolateral transport system regulates the proximal tubular cell accumulation of this class of drugs. In addition, concomitant administration of NSAIDs with other drugs that are substrates for the basolaterally located OAT1 or OAT3 may affect toxicity induced by either agent (28).

Acute renal failure secondary to NSAIDs results from excessive vasoconstriction at the level of the afferent arteriole. An acute phenomenon often associated with oliguria, NSAID-induced acute renal failure is usually reversible. Risk factors include volume depletion, diabetes mellitus, congestive heart failure, hepatic failure with ascites, and hypoalbuminemia (43).

In addition to having physiologic actions that produce renal functional changes, NSAIDs may cause tubulointerstitial nephritis. Arising after 2–23 months of therapy, this syndrome is associated with an interstitial mononuclear cell infiltrate and occasionally epithelial cell podocyte fusion on electron microscopy (44). Renal functional recovery typically occurs when the agent is discontinued.

Long-term use of NSAIDs may cause papillary necrosis as well as chronic renal insufficiency through chronic ischemia (44). The risk of chronic renal disease (serum creatinine level above 1.5 mg/dL) in daily users of NSAIDs was 2.1% (43). Although most epidemiologic studies were performed in adults, children also are at risk for renal failure (45). Prenatal exposure may lead to renal failure-associated deaths after administration of 140–400 mg of indomethacin per day for anywhere between 2 and 11 weeks of therapy (46). Risk factors for renal functional changes are underlying volume depletion and renal disease (43). Acute administration of NSAIDs to patients with chronic renal insufficiency results in decreased renal plasma flow, decreased GFR, decreased fractional excretion of sodium, and decreased fractional free water clearance (47). Most healthy, normal subjects who take NSAIDs for a limited duration tolerate these agents without adverse events, but a subset of individuals appears to be at risk. Because ibuprofen is now commercially available for treatment of fever in children, the incidence of NSAID-related renal disease may increase. Febrile children often have reduced fluid intake in the face of increased insensible fluid losses with clinically mild but significant hypovolemia, which places them at risk for NSAID toxicity.

Antiviral Agents

Acyclovir and Ganciclovir

Acyclovir and ganciclovir are active against herpes viruses and cytomegalovirus (CMV). Acyclovir is excreted primarily by the kidney. Anywhere from 30 to 90% of the drug is excreted unchanged in the urine in patients with normal glomerular filtration. Acyclovir is probably excreted by both glomerular filtration and tubular secretion (8). Pretreatment with probenecid decreases renal clearance of the drug by 32%, which supports a role for tubular secretion as a partial mechanism of excretion. The dosing interval must be adjusted in the setting of a decreased GFR.

Acyclovir administration is complicated by renal, hepatic, and neurologic dysfunction (8). Renal toxicity is associated with plasma concentrations of acyclovir exceeding 20 mg/mL. Renal levels of acyclovir can be 20 times the plasma levels. Crystals form within the renal tubule, which leads to intratubular obstruction followed by a rise in the serum creatinine concentration within 24–48 h (48, 49). Needle-shaped crystals may form in the urine even in those who do not have clinical evidence of renal dysfunction. In most cases, renal failure is not associated with oliguria and GFR returns to normal within 1 week. Urinary findings include hematuria, pyuria, proteinuria, and

crystalluria. Histologically, ATN may be observed along with patchy interstitial infiltration (49).

Renal toxicity was noted in rats during preclinical toxicology studies. An obstructive nephropathy developed, with associated drug crystallization within the collecting ducts. The development of crystalluria was dosage-related. Acyclovir administration to rats was accompanied by a reduction in GFR and significant increase in total renal vascular resistance without changes in urinary flow rate (50). Ganciclovir administration did not induce a decrease in GFR or change in renal vascular resistance in this rat model. In a large series of patients who received acyclovir for serious infections, 16% of adult patients experienced elevations of the serum creatinine level (49). Among children given acyclovir, 12% had elevated serum creatinine concentrations (51). Some of the elevations were transient, with improvement in the creatinine levels during continued administration of the drug. In most patients, renal function returns to normal after discontinuation of the drug. Most importantly, one must adjust dosing intervals in the face of decreased GFR.

Risk factors for renal toxicity include high peak concentrations after rapid intravenous administration, dehydration (or conditions in which urine flow is diminished), coadministration of other nephrotoxic drugs and preexisting renal disease (51). Therefore, consideration of the individual's renal function, adequate hydration, and slow infusion rates (over a minimum of 1 h) should lower the risk for renal toxicity.

Ganciclovir is excreted entirely by glomerular filtration, with 99% of the dose appearing in the urine (8). In addition, plasma clearance is highly correlated with creatinine clearance and is approximately twice that of creatinine clearance, which indicates significant elimination by proximal tubular secretion. Ganciclovir is seldom associated with renal insufficiency or damage except in the setting of impaired GFR and concomitant administration of nephrotoxic agents.

Foscarnet

Foscarnet is an antiviral agent with promising results against herpes viruses. Foscarnet is a pyrophosphate analogue that interferes with viral replication by binding to a site on the herpes virus DNA polymerase or human immunodeficiency virus (HIV) reverse transcriptase (52, 53). Foscarnet is used to treat CMV infection alone or in combination with ganciclovir, usually in the setting of CMV prophylaxis, CMV retinitis, or disease after bonemarrow transplantation or in immunodeficient states.

Foscarnet appears to be excreted primarily by glomerular filtration, with little contribution by tubular secretion (52, 53). Foscarnet causes reversible decreases in GFR in 20–60% of patients. Two mechanisms for renal damage have been reported: tubular necrosis and crystallization within glomerular capillaries resulting in crescentic glomerulonephritis. Hydration at the time of administration is thought to reduce the incidence of acute renal failure considerably.

Functional renal tubular effects of foscarnet include hypophosphatemia, hypokalemia, renal tubular acidosis, and vasopressin-resistant diabetes insipidus (54). Alterations in mineral metabolism associated with foscarnet therapy include hyperphosphatemia, hypocalcemia, and transient increases in parathyroid hormone level (55). Explanation of these alterations is difficult because animal studies demonstrated inhibition of proximal tubular reabsorption of phosphate and would have predicted hypophosphatemia. One group of investigators postulated that direct binding of foscarnet with ionized calcium is responsible for the acute hypocalcemia and that reducing the dosage may allow continued therapy.

In a series of bone marrow transplant recipients, increased creatinine levels occurred in 17 of 19 patients, with four requiring hemodialysis (56). Hypocalcemia was reported in 16 and hypophosphatemia in 17. When 26 courses of foscarnet were given to patients with HIV infection, two patients experienced a creatinine level higher than 3.0 mg/dL, six had hyperphosphatemia, three had hypophosphatemia, three had hypocalcemia, and two had hypokalemia (57). Acute renal failure associated with foscarnet also has been reported (58). Hydration with saline may reduce the risk of nephrotoxicity. An increase in the serum creatinine level (defined as more than 25% above baseline) occurred in 66% of nonhydrated adults but only 13% of hydrated adults. Even those with abnormal pretreatment GFR tolerated foscarnet therapy if the drug was given along with hydration (59). Twenty-five percent of pediatric allogeneic stem cell recipients given prophylaxis against CMV required discontinuation of foscarnet due to renal dysfunction or electrolyte imbalance (60).

Cidofovir

Cidofovir, a nucleoside analogue of cytosine, is an antiviral agent with activity against herpes viruses and the BK virus (polyoma virus). The drug is filtered in its parent state without significant biotransformation. In addition, cidofovir interacts with OAT1 at the basolateral

membrane of the proximal tubular cell and exits the luminal membrane via OAT 4 or the MRP4. (28) It causes increased creatinine concentration and proteinuria by direct proximal tubular toxicity characterized by proximal tubular necrosis. Although the precise mechanism of renal damage is not known, one theory proposed that cidofovir may interfere with synthesis or degradation of membrane phospholipids (61). Cidofovir (as well as adefovir) uses the basolateral organic anion transporter for tubular secretory elimination. Renal toxicity of cidofovir is dependent on its uptake into the proximal tubular cells via this organic acid exchanger (61).

Administration of saline and probenecid are thought to reduce renal toxicity. Renal damage is occasionally irreversible (62). In the days before the administration of protective treatment, acute renal insufficiency was reported in one-third of patients.

Tenofovir

Tenofovir is an acyclic nucleoside phosphonate used for treatment of HIV. The compound is administered as a prodrug, tenofovir disoproxil fumarate (TDF). Tenofovir is excreted by glomerular filtration and tubular secretion. In the proximal tubule, tenofovir enters the basolateral cell membrane via the organic anion transporter, OAT-1, and is secreted into the proximal tubular lumen by the multidrug resistance protein (MRP-2) (63).

Tenofovir has been associated with nephrotoxicity in the form of reduced GFR with or without tubular toxicity (63). Occasionally Fanconi syndrome has been reported. Administration of other potentially nephrotoxic medications with TDF increases the risk for renal toxicity; this is likely related to changes in GFR which are followed by delayed renal clearance. In addition, the choice of other antiretroviral therapy appears to affect the degree of renal toxicity attributed to tenofovir. Regimens that include ritonavir in combination with TDF are accompanied by a greater decrement and a greater rate of decline in GFR (based upon estimated creatinine clearance). Those HIV patient receiving TDF in combination with ritonavir were 3.7 times more likely to experience a significant decline in GFR (>15%) as compared to those not receiving the drug (64). Reduced renal clearance of tenofovir is postulated as the mechanism for the increased risk for renal toxicity with the combination of drugs. Furthermore, there is evidence that polymorphisms in the apical transport system used by tenofovir may affect the susceptibility to nephrotoxicity (65). Ritonavir inhibits the multidrug-resistant transporter and may allow increased accumulation of tenofovir within the proximal tubular cell, thus accounting for the reduced renal clearance and site of toxicity (64).

Indinavir

The antiretroviral protease inhibitor indinavir is associated with renal toxicity in the form of acute renal failure, crystalluria leading to urinary colic, dysuria or/and nephrolithiasis and chronic renal dysfunction (63, 66). Indinavir is metabolized primarily by the liver through the P-450 system and approximately 20% of the drug is excreted unchanged in the urine (66). Drugs which inhibit the hepatic metabolic pathway or the presence of chronic liver disease result in increased plasma drug levels. The increased filtered load of drug in addition to factors such as urinary flow rate, GFR and urine pH (solubility decreases with increasing urine pH) may predispose to crystallization of indinavir in the urine. (66) Urinary crystals were identified in 20% of adults receiving the drug in a prospective study; the crystals were described as flat rectangular plates that may be found in fan-shaped or starburst aggregates (67). Chemical analysis confirmed that the crystals were composed of indinavir. Acute changes in serum creatinine have been observed in 13–26% of individuals taking indinavir (68–70). Crystalluria-related urologic symptoms, including loin pain, dysuria and nephrolithiasis (known as the indinavir-associated renal complication, or IRC) were identified in 7.3% of adults receiving the drug which translates into 6.7 events per 100 person years of indinavir exposure (70). Children appear to have a greater risk for IRC with a reported incidence of 29% as compared to an overall incidence rate of 19% among adults (71). Risk factors indentified for acute renal damage and IRC are longer duration of treatment, concomitant use of acyclovir, trimethoprim/sulfamethoxazole, lower body mass index, and presence of chronic hepatitis (68, 70, 72). Often a potential precipitating event was identified such as increased fluid loss or decreased oral intake (70). A third clinical syndrome associated with indinavir is that of a slow, asymptomatic rise in the serum creatinine (72). Urinary findings include low grade proteinuria, and leukocyturia/eosinophilluria (72, 73). When performed, the renal biopsy shows interstitial inflammation with fibrosis, and in some cases deposition of crystals within the renal parenchyma surrounded by an inflammatory infiltrate. Improvement in renal function was observed in most cases after continuation of the drug. (72) Recommendations for prevention of renal damage include liberal intake

of oral fluids, recognition of drugs and/or concomitant liver disease which may impair metabolism, and surveillance for chronic leukocyturia which may indicate chronic renal interstitial inflammation. Individuals taking indinavir who present with urinary complaints should be hydrated while imaging is undertaken to evaluate for nephrolithiasis. As indinavir stones are radiolucent, they will not be identified by routine radiographic studies (63).

Calcineurin Inhibitors

Cyclosporine and tacrolimus, the most widely used calcineurin inhibitors (CNI) are immunosuppressive agents used in posttransplantation combination therapy, treatment of nephrotic syndrome and autoimmune disease through their action to selectively target IL-2 dependent T-cell proliferation (74). However, the major dose limiting side effect is nephrotoxicity. Since cyclosporine has been in use longer than tacrolimus, there are many studies in animal models aimed at elucidating potential mechanisms for its nephrotoxicity. However, one may assume that the mechanism for toxicity is common to both agents (75). Both inhibit the enzymatic activity of calcineurin, a vital enzyme linking cytokine signaling in T cells to nuclear transcription factors such as the nuclear factor of activated T cells. Cyclosporine may also alter the transcription of other genes such as nitric oxide synthetase, transforming growth factor β, endothelin-1, and collagen proteins.

Acute toxicity associated with CNI is characterized by dose-dependent and reversible reduction in glomerular filtration rate and afferent arteriolar constriction (75). In addition, hypertrophy of the juxtaglomerular apparatus and increased renin production are found in kidneys exposed to CNI. Several mechanisms have been implicated in the hemodynamic alterations observed with CNI. These include activation of the renin-angiotensin system, activation of the sympathetic nervous system, increased release of endothelin-1, a potent vasoconstrictor, perturbation of the prostaglandin synthetic pathways favoring elaboration of vasoconstrictor species, and impaired generation of nitric oxide (74, 76). The summary of all of these potential mechanisms is renal vascular constriction. Studies using animal models of cyclosporine toxicity have found that inhibition of the above pathways will at least partially reverse the physiologic effects on the renal vasculature.

The chronic nephrotoxicity of CNI is characterized by progressive loss of renal function which is not dose-dependent, nor reversible. On biopsy, tubulointerstitial fibrosis, and arteriolar changes may be seen (77). In a meta-analysis of patients with autoimmune disease whose treatment regimens did or did not include cyclosporine, a risk difference of 21% was attributed to cyclosporine use (78). Furthermore, the risk for chronic renal insufficiency increases over time; this is the result of progressive renal arteriolar hyalinosis and tubulointerstitial fibrosis (76). The mechanisms underlying the chronic nephrotoxicity include angiotensin, transforming growth factor β, vascular endothelial growth factor and other compounds generated by infiltrating macrophages/mononuclear cells (77).

Tubular cell toxicity also accompanies the renal toxicity. Hyperkalemia, hyperuricemia and hypomagnesemia are common electrolyte disturbances associated with CNI use. In addition, CNI are associated with thrombotic microangiopathy, or a hemolytic-uremic syndrome-like picture characterized by extensive thrombosis of the renal microcirculation (77, 79). Endothelial injury is the primary event; however, the mechanisms have not been elucidated. One case report found decreased activity of the von Willebrand factor-cleaving metalloprotease ADAMTS13 in a renal allograft recipient; interestingly, the activity increased after cessation of cyclosporine (80).

Numerous potential drug interactions may occur with CNI. Because they are metabolized by the cytochrome P-450 system; concomitant administration of compounds that stimulate the hepatic cytochrome P-450 system can result in increased plasma clearance, whereas those that inhibit cytochrome P-450 increase cyclosporine plasma levels and potentiate toxicity (81).

Radiocontrast Agents

Radiographic contrast agents are freely filtered at the glomerulus and are excreted primarily by the kidney. Some concentration of the drug may occur within the renal tubular cell, but most of a bolus of contrast material is excreted directly and rapidly into the urine (17).

The incidence of nephrotoxicity after administration of radiocontrast agents is difficult to assess accurately because of differences in the definition of contrast nephropathy and the populations included in the clinical studies. Studies of adults indicate that between 1 and 20% of the general population may be at risk for acute renal failure after radiocontrast administration, with a somewhat higher incidence among hospitalized or critically ill patients (82). Risk factors for nephrotoxicity include preexisting renal insufficiency, diabetes mellitus, multiple myeloma, dehydration, and hyperuricemia (82).

Preexisting renal dysfunction is associated with a much greater risk of acute renal failure. Of patients who have renal insufficiency after radiocontrast administration, 50–75% had preexisting renal dysfunction, defined as a serum creatinine level of 2–3 mg/dL (17).

Nephrotoxicity may be mild, with minimal clinical symptoms. Most cases are manifested as nonoliguric acute renal dysfunction, although renal injury may be more severe with typical oliguric renal failure. The peak rise in the serum creatinine level usually occurs 3–5 days after contrast administration, with return to baseline by 10–14 days. If nephrotoxicity is severe, pronounced oliguria may be evident within 24 h of the injection (17). Pathologic changes are localized to the proximal tubular cell in the form of pronounced vacuolization, termed *osmotic nephrosis* (17). This form of acute tubular toxicity is characterized by a low fractional excretion of sodium.

Mechanisms responsible for the development of radiocontrast-induced renal dysfunction are not entirely understood. Several factors may interact to induce injury: tubular obstruction, direct tubular toxicity, and ischemia (82, 83). Contrast agents induce the precipitation of intratubular proteins such as the myeloma proteins and urinary mucoproteins (Tamm-Horsfall protein), which potentially leads to tubular obstruction by casts (84). Although this theory often has been proposed as a mechanism for renal toxicity after contrast agent administration, studies have failed to support a significant role of cast formation and secondary tubular obstruction in the development of contrast nephropathy (85). Of note, contrast agents are uricosuric and lead to urate crystal formation within renal tubular lumens, especially if urine flow or pH is reduced (86).

In support of a direct tubular toxicity, pathologic changes after radiocontrast administration include vacuolization of the proximal tubules, interstitial edema and inflammation, and tubular cell necrosis (82). Many studies demonstrate increased urinary excretion of enzymes and tubular proteins after administration of contrast agents. This is indicative of nonspecific renal tubular damage. Exposure of isolated proximal tubule segments to diatrizoate-containing contrast media resulted in cellular injury, and this effect was potentiated by hypoxia and addition of meglumine, a cationic compound often combined with diatrizoate (82). In addition, selected studies suggest altered renal tubular cell function secondary to the inhibition of tubular cell metabolism (17, 87). Both organic acid transport and sodium transport are reduced in experimental forms of contrast nephropathy. A direct tubulotoxic effect was found when renal tubular cells in culture were incubated in various concentrations of contrast agent (87).

The renal hemodynamic response to intravenous radiocontrast administration is transient vasodilation (increased renal blood flow) followed by vasoconstriction and a decrease in the renal blood flow and GFR (82, 88). The vasoconstriction occurs within minutes after injection of contrast agents and lasts for 10–20 min. This biphasic response in blood flow appears to be unique to the renal vasculature. The diminished filtration rate observed after administration of contrast agents cannot be mediated entirely by hypoperfusion because GFR remains decreased even after normalization of renal blood flow. Vasoconstriction is mediated by increased lipid peroxidation from the increased production and local release of oxygen free radicals and adenosine, a potent vasoconstrictor (82, 88). If there is a role for the renin-angiotensin system, it has not been consistently demonstrated. Reduced renal production of prostacyclin is associated with contrast-induced injury (88). Additional vasoconstrictive forces may involve endothelin, because plasma and urinary levels of endothelin are increased after administration of high-osmolar contrast media. Contrast agents may induce structural and functional alterations in the erythrocyte, which results in hyperviscosity with sludging in small blood vessels and hypoxemia (89). This phenomenon could explain the preferential distribution of ischemia to the medulla.

Prevention of contrast nephropathy requires identification of potential risk factors. These include dehydration, NSAID use, diabetes, and conditions associated with decreased effective plasma volume, such as congestive heart failure, nephrosis, or cirrhosis, in which renal perfusion may already be compromised (82, 83). Hydration has been said to be completely effective in prevention of injury. Administration of furosemide did not appear to offer protection. Mannitol administration was protective in nondiabetic patients but may have potentiated injury in diabetic patients (82). Likewise, dopamine afforded some renal protection in nondiabetic patients, yet offered no benefit in diabetic patients (82). *N*-acetyl cysteine given by intravenous and/or oral route has become a popular method for prevention of contrast-associated nephrotoxicity in adults. Unfortunately, its use remains controversial due to conflicting findings of the available randomized trials, insufficient number of study participants and heterogenetity of study design (90).

Recommendations from one consensus panel include (1) evaluation of all patients for risk factors, (2) optimization of intravascular volume status prior to contrast administration, (3) use of low osmolality contrast in all patients, (4) discontinuation of all drugs that may adversely alter renal function for at least 24 h prior to

contrast, (5) pharmacologic prophylaxis for high risk patients with the caveat that only therapies supported by clinical evidence should be used, (6) measurement of serum creatinine within 24–72 h after contrast exposure (91).

Cancer Chemotherapeutic Agents

Ifosfamide

Ifosfamide is a cancer chemotherapeutic agent with impressive activity against a wide variety of solid tumors in children, including neuroblastoma, osteosarcoma, rhabdomyosarcoma, soft tissue sarcomas, testicular tumors, and Wilms' tumor (92). Ifosfamide is a structural isomer of cyclophosphamide. The tumoricidal action of these compounds is related to the alkylation and cross-linking of DNA to inhibit cell replication. Although both ifosfamide and cyclophosphamide are associated with hemorrhagic cystitis as well as myelosuppression, alopecia, and gastrointestinal symptoms, ifosfamide is associated with significant renal toxicity, which is not observed after administration of cyclophosphamide.

Ifosfamide may induce tubular wasting of glucose, amino acids, protein, and phosphate as well as decreased GFR (92, 93). Although there is a great deal of interpatient variability in the pattern of ifosfamide-induced renal toxicity, in general ifosfamide toxicity is initially manifest as subclinical tubular damage with increased urinary excretion of brushborder enzymes from cell membrane loss, and a transient increase in urinary excretion of amino acids, phosphate, glucose, and protein. A progressive increase in renal tubular toxicity was observed after the initial three or four courses of ifosfamide treatment, with return to normal in between courses (93). Subsequent courses were characterized by failure to return to pretreatment levels between courses, with fixed tubular functional changes. In addition, glomerular damage and decreased GFR (presumably secondary to chronic tubular damage and fibrosis) were observed in five of seven patients at the end of therapy (92). Although less commonly observed, distal tubular dysfunction may occur after ifosfamide administration. Skinner reported a concentrating defect in 50% of the patients in his series, and hypokalemia occurred in patients reported by Husband et al. (94). In some patients evidence that ifosfamide has significantly affected renal function is a delay in the clearance of drugs such as methotrexate. Tubulopathy may become persistent, often in association with impaired glomerular function. One should be reminded that, even in patients with obvious renal toxicity, the plasma creatinine level is often within normal limits because it is an insensitive marker of glomerular function in these patients. In general, patients do not manifest reductions in GFR in the absence of established tubular injury.

Not only are there acute effects of ifosfamide administration, but a subset of patients experience continued renal dysfunction, manifest as tubular wasting syndromes, or reduced GFR. The incidence of chronic renal failure secondary to ifosfamide therapy is reported to be 1–4% (93). Another late complication of ifosfamide administration is the occurrence of rickets caused by chronic hypophosphatemia (95–97). Of 12 children on whom Skinner reported, eight had what was considered to be significant renal damage, six had reduced GFR, four had hypophosphatemia, five had glycosuria, 10 had generalized aminoaciduria, and three had radiographic evidence of rickets (97).

The incidence of ifosfamide-associated renal injury is highly variable, depending on the cumulative dosage, rapidity of administration, previous or concomitant administration of platinums or other nephrotoxic drugs or radiation, presence of a unilateral kidney, and age. Ifosfamide nephrotoxicity seems to be dose dependent and is more likely to occur in younger children or those who have undergone nephrectomy (92, 95, 98). Previous or sequential administration of other nephrotoxic agents increases the likelihood that renal toxicity will be observed with ifosfamide treatment. Most notably, patients who have previously been treated with cisplatin are at a greater risk for ifosfamide nephrotoxicity (98, 99). Carboplatin administration also increases the risk of tubular toxicity after ifosfamide therapy (100). This phenomenon is interesting, because the major site of injury after the platinums is the late proximal and collecting duct, whereas that after ifosfamide appears to be the early proximal tubule.

The clinical pattern of renal injury is one of initially transient, reversible tubular wasting of glucose, potassium, bicarbonate, phosphate, and amino acids as well as low-molecular-weight proteins such as β2-microglobulin. With early toxicity, tubular function tends to return nearly to baseline at the time of the next course. A few long-term follow-up studies have been published. These suggest that the renal defect in many of these children is chronic and may be progressive in nature. This is in contrast with results of long-term follow-up of cisplatin injury, in which GFR may improve over time although tubular defects may persist.

Numerous studies have looked at the incidence and pattern of renal injury associated with ifosfamide. Findings are highly variable, and partly this reflects the

aggressiveness with which the studies of renal tubular and glomerular function were performed. If serum creatinine or creatinine clearance is used to assess the presence of decreased GFR, the incidence of glomerular dysfunction is underestimated, as evidenced by the study of Ashraf et al., who found that 7 of 20 patients had abnormal GFR values as assessed by chromium ethylenediaminetetra-acetic acid (Cr-EDTA) clearance, yet none of these had elevated plasma creatinine levels compared to age-related controls (101). In addition, a subset of children demonstrated increased fractional excretion of phosphate, yet fewer had overt hypophosphatemia.

The activation of ifosfamide to its tumoricidal metabolites, 4-hydroxyifosfamide (4-OH-IF) and aldoifosfamide, occurs in the liver, with further metabolism to either isophosphoramide or carboxyifosfamide. The parent drug and its metabolites are excreted in the urine (102). When attempts are made to contrast the metabolites of cyclophosphamide and ifosfamide to incriminate the nephrotoxic species, ifosfamide metabolism is found to produce significantly greater quantities of chloracetaldehyde than cyclophosphamide. Because this compound has been noted to be toxic to cells and is found in high concentration in both the urine and plasma, it may play a role in the renal toxicity after ifosfamide therapy.

The mechanism of the renal tubular injury secondary to ifosfamide administration is unknown. Studies in which cultured proximal tubular cells are incubated in the parent drug or metabolites have been performed (103). The parent drug, ifosfamide, did not have an effect on amino acid or glucose transport, but the metabolite 4-OH-IF clearly inhibited transport of both substrates after 24 h in 100 μmol/L. Another potentially active metabolite, chloracetaldehyde, stimulated uptake at low concentrations (<200 μmol/L) and shorter incubations but inhibited uptake at higher concentrations and longer exposure times. In low concentrations, 4-OH-IF stimulated phosphate transport, but transport was decreased at higher dosages (100 μmol/L). At even higher concentrations (300–400 μmol/L), Na^+–K^+-adenosine triphosphatase (Na^+–K^+-ATPase) activity was inhibited. Chloracetaldehyde (at concentrations greater than 175 μmol/L), reduced phosphate transport and the activity of the mitochondrial enzyme succinate cytochrome *c* oxidoreductase.

The bladder toxicity of the oxazaphosphorine compounds results primarily from the metabolite acrolein, although 4-OH-IF has also been implicated (92). Mesna, sodium-2 mercaptoethane sulfonate, has been used to reduce ifosfamide- and cyclophosphamide-related bladder toxicity without alteration of tumoricidal effects. Mesna binds to acrolein, chloracetaldehyde, and 4-OH-IF (104). Unfortunately, mesna does not seem to offer protection against tubular toxicity, although its failure to do so has been postulated to be related to the availability of mesna at the site of tubular injury (105). Mesna is oxidized to dimesna, which has no protective action. Dimesna can be converted back to mesna by renal tubular cells, but at the dosages currently used in clinical practice, free mesna levels may not be adequate or may be highly variable and thus inadequate for tubular protection.

Platinum Compounds

Cisplatin (*cis*-diaminedichloroplatinum II) is an inorganic platinum compound that may induce both acute and chronic renal toxicity with magnesium, sodium, and potassium wasting as well as decreased GFR (106). The drug contains a platinum atom surrounded by two chloride atoms and two ammonia molecules in the *cis* position. Carboplatin (*cis*-diammine-1, 1-cyclobutane-dicarboxylato) platinum (II) has been introduced as a less nephrotoxic form of platinum, although it is now known that this compound also may cause renal injury. Cisplatin and carboplatin may undergo aquation with substitution of the chloride ions with hydroxyl groups, a reaction favored by low chloride concentrations such as are found in the cell, to form positively charged complexes that are likely to be the active form of the drug. Platinum compounds are excreted by the kidney via glomerular filtration. Platinum is concentrated in the renal cortex, with levels higher than in plasma, and appears to be accumulated via some transport process. Data suggest that elimination of the drug is delayed in the setting of a decreased GFR.

Proximal tubular cells are believed to accumulate platinum compounds by an energy-requiring mechanism or possibly by the organic base transport system (106). Differences in uptake of the platinum compounds might be related to stereospecificity or relative solubility and reactivity. Shortly after intravenous injection of cisplatin into rats, accumulation can be observed in the juxtamedullary region and outer stripe. This pattern is consistent with localization within the S3 portion of the proximal convoluted tubule, which is thought to be particularly susceptible to platinum. Acute changes in renal function occur after cisplatin administration, and these appear to be hemodynamically mediated, with reduced renal blood flow as well as tubular injury. Histologically, platinum-induced renal toxicity is characterized by tubular dilation, cytoplasmic vacuolization, nuclear pyknosis, and hydropic degeneration of cells in the corticomedullary region or S3 segment of the proximal tubule.

In a series of 22 children receiving cisplatin, 18 demonstrated GFR of less than 80 mL/min/1.73 m^2 as assessed by Cr-EDTA clearance (107). There was a mean decrease in GFR of 8% per course, but a great deal of individual variability was seen. In addition, the authors noted that neither plasma creatinine nor creatinine clearance was reliable in screening patients with significant reductions in GFR.

Long-term outcome for children who received cisplatin as part of their anticancer chemotherapy was studied by Brock et al. (108). Of 40 children available for study at the completion of therapy, 16 had a GFR above 80 mL/min/1.73 m^2, 13 had a GFR of 60–80 mL/min/1.73 m^2, and 11 had a GFR below 60 mL/min/1.73 m^2. Clearance measurements were repeated a mean of 2 years and 6 months from the end of therapy. A statistically significant improvement in GFR was found. Children with a GFR between 60 and 80 mL/min/1.73 m^2 at the end of treatment had a better chance of regaining a normal GFR; however, all of the 11 children with a GFR below 60 mL/min/1.73 m^2 improved, and three had normal GFR at last follow-up. Hypomagnesemia was found in 6 of 21 children at last follow-up.

The mechanism of platinum-related renal injury is controversial. It has been suggested that cisplatin is a direct tubular toxin and must be present in the tubular lumen at a minimum concentration of 1 mmol/L or 200 mg/mL (106). Several cellular perturbations have been identified in renal proximal tubular cells. These include inactivation of Na^+–K^+-ATPase, impaired mitochondrial function, altered gluconeogenesis, impaired substrate transport, lipid peroxidation, oxidation of sulfhydryl groups, and inhibition of nucleic acid and protein synthesis (109). The nephrotoxicity of cisplatin, transplatin, and carboplatin was examined in two continuous proximal tubular cell lines: primary cultures of rabbit cortical cells and OK cells (a continuous proximal tubular cell line). OK cells were spared from injury, probably because of decreased cellular uptake of the compounds. The rabbit proximal cells exhibited reduced viability at concentrations of cisplatin approximating 100 fmol/L, similar to peak levels in cancer patients. Toxicity was related to intracellular levels of platinum (109).

Isolated S3 segments of proximal tubules from rats given either transplatin or cisplatin were examined for alterations in oxygen consumption and respiration. No consistent abnormalities were detected, which makes mitochondria unlikely candidates for early nephrotoxicity (106). Other studies noted changes in mitochondrial function. Cisplatin also increased the oxidation of mitochondrial sulfhydryl groups, depleting GSH and leading to perturbations in mitochondrial calcium uptake and membrane potential (110). Antioxidants did not prevent the associated nephrotoxicity, which indicates that platinum probably exerts its effect by interacting directly with thiol groups rather than by inducing lipid peroxidation. Unfortunately, the concentrations of cisplatin used were considerably higher than would be expected from *in vivo* dosing. DNA synthesis was significantly inhibited by small concentrations of cisplatin, whereas greater concentrations of the other compounds were required. Cisplatin in the concentration range of 30–100 mmol/L induced a decrease in the activity of Na^+–K^+-ATPase as well as glucose uptake; the effect on glucose transport is probably secondary to a reduction in Na^+–K^+-ATPase activity because the sodium gradient would be decreased. Other experiments have shown that cisplatin may decrease glucose as well as phosphate transport. Similar findings were observed with carboplatin, but concentrations 20 times higher were necessary to induce the same alterations in cell functions (109).

Many favor inhibition of DNA synthesis as the primary biochemical effect. Cisplatin damages DNA by inducing interstrand and intrastrand cross-links and preventing normal replication. The amount of platinum cross-linkage correlates with toxicity as well as mutagenicity (106). Intrastrand cross-links are not found with transplatin, which is a much less toxic form of this class of agent.

Polyuria is a common finding after cisplatin administration and appears in two phases: urine osmolality falls over the first 24–48 h after administration, and the second phase of increased urine volume and decreased osmolality occurs between 72 and 96 h and is accompanied by decreased GFR as well (106). During the early phase, blood vasopressin levels are low and the kidney may concentrate urine in response to large doses of vasopressin. The latter phase of polyuria is thought to be secondary to the dilution of the medullary urea gradient, possibly related to abnormal urea recycling in the loop of Henle. In a model of chronic cisplatin nephrotoxicity, rats received three doses over 3 weeks. Polyuria, increased urinary sodium excretion, and decreased GFR along with decreased urinary cyclic adenosine monophosphate (cAMP) excretion were observed in the cisplatin-treated animals (106). Production of cAMP by inner medullary collecting duct cells was decreased after vasopressin stimulation in cisplatin-treated rats. Forskolin, a direct activator of adenyl cyclase, induced similar cAMP production in treated and control rats. In contrast, cAMP production after administration of sodium fluorescein, an agent that stimulates cAMP production via the guanine nucleotide

regulatory protein, was significantly lower in cisplatin-treated animals than in controls. These results led the investigators to conclude that the polyuria associated with cisplatin is caused by an abnormality in signal transduction at the level of the G-protein component of the vasopressin receptor (111).

Hypomagnesemia is another common manifestation of cisplatin-induced renal toxicity and commonly persists long after therapy ceases (112). Rats receiving intraperitoneal cisplatin (2.5 mg/kg) for 3 weeks developed hypomagnesemia by the second week, which persisted to the eighth week after therapy (113). Urinary excretion of magnesium was inappropriately elevated. In addition, gut absorption of magnesium was decreased. Calcium and magnesium transport by the superficial proximal and distal tubules appeared to be unaffected when measured at 7 weeks, even though urinary excretion was elevated. Bone and muscle magnesium levels were significantly reduced at the end of the eighth week, which suggests depletion of mineral stores. Cisplatin increases distal tubular sodium conductance. Infusion of cisplatin into rat distal tubular segments caused an attenuation of the normally negative luminal charge generated by active sodium reabsorption in this segment (104). The effect of cisplatin on the luminal charge was reversed by addition of amiloride, a diuretic that blocks sodium channels. The authors conclude that cisplatin may interact with distal tubular Na^+ channels to increase potassium and magnesium excretion and that this is attenuated by coadministration of amiloride (114).

Therapeutic intervention to reduce renal toxicity associated with cisplatin has been aimed at reduced production and enhanced excretion of highly reactive metabolites (114, 115). Infusion of mannitol and saline is the clinical intervention most commonly used to reduce cisplatin nephrotoxicity. In addition to providing optimal hydration before, during, and after administration, the coadministration of saline may reduce the spontaneous aquation reaction. In addition, these maneuvers may enhance excretion of the drug and minimize the period of contact between the renal tubule and toxin. The compound sodium thiosulfate reverses cisplatin-induced DNA cross-links and has been reported to reduce cisplatin injury in animals and humans (116, 117). Amifostine is a thiol prodrug which is converted to its active form by nontumor cells; it scavenges freeradicals and protects cell membranes and DNA from damage. It has been approved by the FDA for use with cisplatin to prevent cumulative toxicity (118).

Another potential method for minimizing renal toxicity is to avoid increasing the exposure to renal toxins by detecting reduced GFR early and by taking the GFR into account in dosing. Dosing of carboplatin based on the patient's GFR has been practiced by many centers and may potentially prevent toxicity by allowing dosage modifications in patients who have experienced otherwise undetectable reductions in GFR.

The combination of ifosfamide and cisplatin potentiates renal injury (119). One study compared glomerular function (unfortunately, creatinine clearance was used as the measure) and tubular function after either low-dose ifosfamide or a combination of ifosfamide and cisplatin and then high-dose cisplatin. Tubular functions such as amino acid excretion and albuminuria were comparable for the high-dose ifosfamide and combination groups, but phosphate wasting was more pronounced in the combination group (119). Despite delivering a much lower cumulative ifosfamide dose, combination therapy resulted in lower phosphate reabsorption in significantly more patients and a trend toward more frequent occurrence of tubular dysfunction. The aminoglycoside gentamicin did not appear to contribute significantly to observed renal toxicity. Of the 14 children with overt renal tubular wasting syndrome, eight had received combination therapy. Experience with combination therapy reveals that acute toxicity from ifosfamide is higher when carboplatin is administered within the same week than when a period of months elapses between these toxic therapies (100).

In conclusion, renal dysfunction as a result of drug administration may be manifest as a reduction of GFR or tubular dysfunction, most commonly of the proximal tubule. In most cases, this form of renal injury is reversible. Occasionally, there may be long-term renal dysfunction, as in the case of amphotericin B, cisplatin, and ifosfamide. The clinician must be aware of the potential for renal toxicity and must be prepared to make adjustments in the dosage or discontinue the offending agent. Anticipation of potential renal toxicity allows for maneuvers that may prevent renal damage and subsequent functional changes. Drug-induced renal dysfunction provides a model for acute renal failure and provides insight into renal injury in the general sense, which gives hope that in the future it will be possible to prevent injury or enhance recovery.

Methotrexate

Methotrexate (MTX) is a folic acid antagonist that is used in the treatment of approximately 60% of all malignancies in children, including acute lymphoblastic

leukemia (ALL), non-Hodgkins lymphoma, osteosarcoma, and several malignant brain tumors (120). High-dose MTX (HDMTX), in which doses in the range of 1,000–33,000 mg/m^2 are used in combination with leucovorin, is associated with acute renal dysfunction in 0–12.4% of patients, for an overall incidence rate of 1.8% (121). MTX-induced renal dysfunction results in delayed elimination of the drug and its metabolites. Prolonged exposure to even relatively low levels of MTX markedly increases liver, gut, bone marrow and mucus membrane toxicities (122). There is evidence to support a role for the organic anion transporters 1 and 3 in the renal elimination of MTX. Furthermore, coadministration of agents that also interact with this transport system (such as NSAIDs or probenecid) results in delayed MTX clearance (3). Renal clearance of MTX accounts for 90% of its plasma elimination; urinary flow rate and urinary pH affect renal MTX clearance. Thus, aggressive hydration and urinary alkalinization are routine clinical measures employed to optimize MTX elimination. The mechanism for MTX nephrotoxicity is postulated to be precipitation of the drug and metabolites within the renal tubular lumen, although direct tubular toxicity may also be involved. Most studies assess acute changes in renal function associated with MTX by measurement of plasma creatinine with various definitions of renal dysfunction. However, a single center study of children who were given HDMTX for ALL noted a significant decline in GFR as measured by inulin clearance over the 3 days after administration of HDMTX (120). Mean GFR returned to baseline by 7 days posttreatment. Only 2 of the 58 patients had clinical evidence of renal dysfunction, with a doubling of their baseline serum creatinine levels. MTX-related nephrotoxicity appears to be entirely reversible, with a median time to recovery of renal function of 16 days (range 4–48 days) (121). Furthermore, subsequent doses of HDMTX have been successfully given to patients who previously experienced renal dysfunction without recurrence of acute renal failure. When acute renal failure occurs in the setting of HDMTX administration, removal of the remaining drug becomes an additional goal of therapy due to the potential for severe and possibly fatal toxicity. Dialytic methods including high flux hemodialysis may remove plasma MTX, however, a marked rebound in plasma MTX concentrations occurs with cessation of dialysis (122). Administration of carboxypeptidase-G2 metabolizes circulating MTX to the inactive metabolite 2, 4-diamino-N10-methylpteroic acid (DAMPA), which uses a nonrenal path of elimination, allowing for a >98% reduction in plasma MTX levels within 15 min of administration (122). There is a paucity of information regarding the long-term renal sequelae associated with MTX administration or in those individuals who experienced acute renal failure associated with HDMTX.

Bevacizumab

Bevacizumab is a humanized monoclonal antibody directed against vascular endothelial growth factor (VEGF) which has been added to chemotherapy against breast, colon, lung and renal cancers. Its use in the pediatric cancer patient is in the treatment of solid tumors such as osteosarcoma, neuroblastoma and some brain tumors. Its utility in such regimens is as an inhibitor of angiogenesis. Administration of bevacizumab is associated with proteinuria and hypertension (123). Unlike the vast majority of nephrotoxic agents discussed in this chapter, in which the major site of injury is the renal tubule, VEGF inhibitors primarily affect the integrity and function of the glomerulus. A review of all randomized clinical trials that used either bevacizumab in combination with standard chemotherapy or standard chemotherapy alone was conducted to ascertain the risk of the renal side effects of proteinuria and hypertension (123). Proteinuria occurred in 21–63%, but heavy proteinuria occurred in 1.0–1.8%. Overall the relative risk for developing proteinuria with treatment regimens which include bevacizumab was 40% (RR 1.4, CI 1.1, 1.7) higher as compared to control. This risk was higher among those who were given high-dose bevacizumab with a relative risk of 2.2 (CI 1.6, 2.9). The incidence of hypertension was 2.7–36%. Severe hypertension developed in 8.7% and 16% with relative risk of 3.0, and 7.5 of those on low dose and high dose bevacizumab, respectively, compared to those not given the anti-VEGF drug. Interestingly, a case series reported features of thrombotic microangiopathy in renal biopsies from patients who developed renal toxicity after bevacizumab administration (124). A mouse model developed to simulate the therapeutic effects of anti-VEGF by targeted podocyte VEGF ablation revealed that loss of VEGF production by the glomerular podocyte was accompanied by histopathologic changes characteristic of thrombotic microangiopathy (124). The animals developed hypertension which was preceded by the development of proteinuria and glomerular disease. The production of VEGF is necessary for normal maintenance and function of the endothelium; thus, inhibition of VEGF production by the glomerulus may result in endothelial damage and glomerular disease. Use of bevacizumab as an adjunctive therapy is in its early stages in childhood cancer, thus the risk for renal effects have not been reported in children.

Natural Nephrotoxins

Marine animals, reptiles, and insects produce a number of biologic nephrotoxins (111) (*Table 53-3*).

Many of these toxins result in acute renal failure either directly or indirectly. The main indirect mechanism of nephrotoxicity is biologictoxin-induced rhabdomyolysis (112). Although these natural nephrotoxins are uncommon causes of acute renal failure, they are of considerable interest.

Marine Animal Nephrotoxins

Fish poisons include at least three forms of biotoxins that result in ATN. First, there is an association between the ingestion of raw bile from the gall bladder of carp and acute renal failure. The belief that the raw bile of carp improves vision and rheumatism is found in Asia (125). Many of these Asian carp species have been introduced into the United States, and at least five species of carp have been implicated (126). All five carp species have ichthyogallotoxin in their bile, and this may represent the toxin. Alternatively, A C-27 bile alcohol, termed *cyprinol* and found in carp bile, is nephrotoxic in animals (127).

Table 53-3

Common biologic nephrotoxins produced by animals and plants

Snake
Phospholipase A_2
Myotoxins
Procoagulant activating factors V and X
Spider
Sphingomyelinase D *(Loxosceles)*
Neurotoxins *(Latrodectus)*
Bee
Melittin
Phospholipase A_2
Mast cell degranulating protein
Wasp
Antigen 5
Mastoparans
Carp
Ichhthyogallotoxin
Cyprinol

A second toxin as yet unidentified is responsible for Haff disease. Haff disease, characterized by acute rhabdomyolysis, can occur in humans and other animals that eat eels and burbot fish. Rhabdomyolysis can be found in subjects who ingest buffalo fish, a member of the carp family (128). These myotoxins or icthyosarcotoxins are unidentified.

A third agent is the palytoxin of the blue humphead parrotfish. Ingestion of this toxin can also result in cardiac muscle damage (129). Factors contributing to the pathogenesis of acute renal failure from rhabdomyolysis include the quality of myoglobin in nephrons, an acidic, highly concentrated urine, and delayed recognition of the disease.

Envenomation-Induced Acute Renal Failure

Snake Venoms

Four families of venomous snake are responsible for 40,000 deaths/year, and 5–30% of subjects bitten by these snakes develop acute renal failure. Two families are responsible for most deaths and cases of renal failure: (1) the cobras, African mambas, and coral snakes, and (2) the vipers, adders, moccasins, and rattlesnakes (130). Approximately 90% of venoms are proteins that are proteolytic enzymes, phospholipases, and hyaluronidases. Other enzymes include ribonucleases, deoxyribonucleases, nucleotidases, and acidic and basic phosphatases. Procoagulants that activate the clotting cascade via factors V and X are also present. Myotoxins can also lead to rhabdomyolysis and myocardial damage, which reduce cardiac contractility, and pigment nephropathy.

Patients may show ATN, hypersensitivity reactions, extracapillary glomerulonephritis, AIN, and necrotizing arteritis of interlobular arteries on histologic studies (127, 131). Laboratory investigations show evidence of intravascular hemolysis, disseminated intravascular coagulation, and azotemia (132). A number of snakebites lead mainly to rhabdomyolysis, including those of the king brown snake, sea snakes, diamondback rattler, canebrake rattler, and several vipers (133).

Spider Venoms

Two groups of spiders produce venom responsible for acute renal failure: the recluse spiders and the widow spiders (133). The mechanism of toxicity of the venom of the 56 recluse species is the presence of sphingomyelinase D,

which leads to necrotic skin lesions, edema, erythema, chills, nausea, and myalgias (134). The main recluse spider in the United States is the brown recluse. These bites are generally more serious in young children.

The widow spiders excrete neurotoxins that produce cramping and muscle spasms. These toxins can lead to a neurogenic bladder with urinary obstruction. The venom of the black widow spider of the United States can also lead to rhabdomyolysis.

Bee and Wasp Venoms

Acute renal failure due to bee and wasp venoms requires massive numbers of stings, usually more than 100, which generally follows disturbance of a hive or nest (127). The toxins include melittin and other hemolysins, hyaluronidase, and others (*Table 53-3*). The toxins in wasps differ and consist of active amines, kinins, and antigen 5. The mechanisms of renal failure include hemolysis, rhabdomyolysis, direct toxic effects, and dehydration. The venom of the dark scorpion of the southwest United States may also cause rhabdomyolysis (133). Hypersensitivity reactions to bee and wasp venoms may also occur. The treatment of acute renal failure is symptomatic and dialysis may be required.

Chinese Herb Nephropathy

Chinese herb nephropathy, initially described in the early 1990s by Vanherweghem et al. in Belgium, is characterized by a tubulointerstitial disease (135). Proximal tubular dysfunction with a Fanconi syndrome or, more commonly, insidious chronic renal failure has been described (136). Aristolochic acids are the likely etiologic agent. Substitution of *Aristolochia fangchi* for *Stephania tetrandra*, herbs which differ in name in Chinese by only one character, is thought to have been the historical event leading to the increased incidence of this indolent renal disorder. These herbs were generally taken for the purpose of weight loss.

Aristolochic acids are naturally occurring compounds with carcinogenic and nephrotoxic effects. Although there is still debate as to whether they are solely responsible for all of the reported cases of Chinese herbal nephropathy (137), they have been shown to cause an interstitial nephropathy in the rabbit (138) but not the rat (139). Isolated case reports also describe proximal tubular dysfunction with hypokalemia, abnormal urinary excretion of low-molecular-weight proteins, and glycosuria as the presenting features in several adults (140–142).

Pathologic evidence of renal injury was demonstrated in the rabbit model. As with human pathologic changes, there was a distinct corticomedullary gradient of acellular interstitial fibrosis. Three patterns of injury were described: (1) restriction of fibrosis to the medullary ray (S3 segments), (2) extension to the outer cortical labyrinth (convoluted proximal tubules, S1 and S2 segments), and (3) extension to the inner cortical labyrinth and outer medulla (proximal tubules of deep nephrons) (138). The exact mechanism of aristolochic acid-induced injury is not known.

Renal function continues to deteriorate despite cessation of consumption of herbal remedies. Oral corticosteroid therapy may slow the progression of renal insufficiency. A group of 81 adults with Chinese herb nephropathy were subdivided according to pattern of renal failure (143). Thirty-nine demonstrated moderate renal failure with progression. Of these, 14 received oral prednisone initiated at 1 mg/kg for 1 month and then tapered to 0.15 mg/kg. The rate of increase in the serum creatinine level was less in the steroid-treated group.

Pediatric cases have not been reported.

Environmental Toxins

Organic Solvents: Ethylene Glycol, Diethylene Glycol, Propylene Glycol

Ethylene glycol is a major constituent of antifreeze and may be accidentally ingested because it has a sweet taste. It is metabolized initially by alcohol dehydrogenase to glycoaldehyde and eventually to oxalate. Intermediate metabolites include glycolic acid and glyoxylic acid, which contribute to the associated metabolic acidosis. A change in the ratio of the unreduced form of nicotinamide adenine dinucleotide to the reduced form favors the accumulation of lactic acid. Central nervous system symptoms and cardiovascular instability precede the onset of renal disease. Renal toxicity takes two forms: crystallization of oxalate within the renal tubules and direct tubular damage resulting in acute renal failure. Metabolic acidosis with elevated anion gap and altered mental status should prompt the consideration of this poisoning. Examination of the urine sediment early in the course may reveal either needle-shaped calcium oxalate monohydrate crystals or octahedral calcium oxalate dihydrate crystals. An elevated plasma osmolar gap may also be an indirect clue. Treatment is aimed at clearance of the toxic metabolites and may include hemodialysis in the case of renal failure or severe metabolic acidosis.

Diethylene glycol has been responsible for accidental poisoning associated with acute renal failure in the form of medication contamination. Recently, an outbreak of renal failure in Haiti resulted after two acetaminophen preparations were inadvertently contaminated with diethylene glycol during manufacture using contaminated glycerin (144). The mechanism of renal toxicity is thought to be proximal tubular damage and necrosis. Other organ systems, including the liver, pancreas, and brain, are often severely injured and contribute to the overall mortality of this accidental poisoning.

Propylene glycol (PG) may be responsible for ATN. The renal failure is usually the result of intravenous administration of medication solubilized in PG (144). Parenteral medications that contain PG include diazepam, digoxin, esmolol, hydralazine, nitroglycerin, pentobarbital, phenobarbital, phenytoin, and sulfamethoxazole/trimethoprim. Vitamin preparations may also contain PG. Clinical manifestations are similar to those of ethylene glycol toxicity, including metabolic acidosis with increased anion gap. A potential mechanism for toxicity has been postulated to be the inhibition of proximal tubular transport processes and membrane disruption (145).

Heavy Metals (Excluding Platinum)

Lead, mercury, gold, bismuth, and copper are uncommon but serious causes of nephrotoxicity. They have common mechanisms of renal tubular toxicity, and chronic exposure may lead to presentation with an acute syndrome or dysfunction. The primary site of injury is the proximal renal tubule, where heavy metals cause impaired mitochondrial function and secondary alterations in membrane integrity. Exposure to mercury and lead is generally accidental due to environmental contamination and is much less common now than several decades ago. The reader is referred to previously published comprehensive reviews of heavy metal renal toxicity (146, 147).

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Urinary Tract Disorders

54 Urinary Tract Infections

Albert Bensman · Olivier Dunand · Tim Ulinski

Introduction

Urinary tract infection (UTI) is defined by the presence of bacteria in bladder urine. The term "urinary tract infection" lacks precision. Once the diagnosis of UTI is made, it is important to classify the location and severity of tissue invasion. This determination has major implications for therapy and attendant morbidity (and in rare cases, mortality).

Acute pyelonephritis is an infection which involves the bacterial invasion of renal parenchyma. Acute cystitis is an infection limited to superficial invasion of the bladder. The term "asymptomatic bacteriuria" refers to the presence of infected urine which produces no clinical symptoms. In most instances, asymptomatic bacteriuria is diagnosed when urine cultures are obtained as part of a routine checkup; or obtained in "at risk" children to prevent the development of serious infection...

UTIs are relatively common in infants and young children. The risk before puberty is 3–5% in girls and 1–2% in boys (1, 2). In young febrile infants aged less than 24 months the prevalence is 3–5%. Prevalence is different depending on age and gender. Four subgroups of patients can be identified: males younger than 1 year (3%), males older than 1 year (2%), females younger than 1 year (7%) and females older than 1 year (8%) (3). Data from Ginsburg (4) and Wiswell (5, 6) show a strong risk reduction among circumcised males: the prevalence of UTI among febrile male circumcised infants is 0.2%.

In children, UTIs may serve as an important marker of structural or functional urinary tract abnormalities. Although the most common abnormality heralded by UTIs is vesico ureteral reflux; UTIs may be the first symptom of obstructive uropathy or bladder dysfunction.

Major morbidity, in terms of renal scarring, is the most significant sequelae of recurrent pyelonephritis. Extensive work from Jodal et al. (7) has demonstrated a significantly increased risk of renal scarring is with repeated episodes of pyelonephritis.

Diagnosis and Therapy of Childhood UTIs

There are four main steps in the clinical management of UTIs in childhood:

- Diagnosis of UTI
- Determination of the site of the infection
- Search for the cause of the UTI
- Treatment

Diagnosis of UTI

The definitive diagnosis of UTI is critical and involves a number of important considerations. False positive diagnosis may lead to unnecessary treatment, as well as invasive and expensive clinical and radiographic examinations. False-positive diagnoses are frequent in infants and small children because reliable collection of urine specimens without contamination is difficult. On the other hand, false negative diagnosis dramatically increases the risks of renal scarring and its attendant morbidity: hypertension, complications of pregnancy in women, and end-stage renal disease.

Methods to Obtain Urine Specimens

The gold standard for obtaining urine in an infant is by suprapubic aspiration. By using this method the risk of contamination is very low. Complications are rare with the use of ultrasound guidance. Further, experienced pediatricians have a low complication rate with aspiration following clinical palpation of the bladder (8). Urinary catheterization is also a very reliable method for obtaining urine without contamination, particularly in female infants. In some centers physicians and the nurses are reluctant to use this technique for fear of negative psychological effects or causing pain. No actual data support

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such concerns. Clean-catch mid-stream urine specimens can be collected in toilet-trained children. Ramage et al. (9) pointed out the accuracy of clean-catch urine (C.C.U.) collection in infancy. Using this simple technique, these authors demonstrated strong correlation with urine obtained by supra-pubic aspiration. Most would concur with standard use of the C.C.U. technique. The huge advantage of this method is that it is readily performed in private pediatric offices. The collection of urine in "collection bags" adhesively attached to the the perineal area *has no role in the diagnosis of childhood UTIs*. The high contamination rate, with "false positive" rates as high as 86% (10) may lead to unnecessary hospitalizations, and/or inappropriate clinical and radiological testing. This conclusion, has recently been endorsed by a subcommittee of the American Academy of Pediatrics (3). A new technique suitable for office use, "the midstream urinary collector," may be a valuable non-invasive method of obtaining children's urine for culture. With this apparatus, a bag collects urine except for the very last portion (which is free of contaminants from the urethra) and is caught in a test tube. The test tube is separated from the collector, sealed with a lid and sent to the laboratory (11). Additional large scale studies are awaited regarding the ultimate value of this interesting approach.

Urine Culture

It is very important to prevent the growth of contaminating bacteria. In order to achieve this goal, the urine should be maintained at 4°C, until it arrives at the bacteriologic laboratory (12). The standard culturing technique involves streaking on blood agar and MacConkey media. A simple dipslide culture system (suitable for office or outpatient clinic use) has approximately the same sensitivity. The studies of dipslide culture report sensitivities between 87 and 100% and specificities of approximately 92% (3). It is important to recognize that some uncommon bacteria do not grow on all media but require specially enriched culture media.

Bacteria that Cause UTI in Childhood

In the pediatric age group, 60–80% of the UTI are caused by uropathogenic E. coli (13, 14). The other common bacteria are Proteus, Klebsiella, Staphylococcus saprophyticus, Enterococcus, and Enterobacter (15, 16). Uncommon in uncomplicated infections, but reported with increased frequency with anatomical defects, following surgical manipulation of the genitourinary system, and following repeated courses of standard antimicrobials are Pseudomonas, Group B streptococcus, Staphyloccoccus aureus and epidermidis. Urinary tract infections caused by Haemophilus influenzae and parainfluenzae in children were studied recently by Hanson et al. (17). Over a 24 year period, only 36 children were identified. But the inability of this bacteria to grow on standard media suggests that the true frequency of these strains as a cause of UTI may be underestimated. These bacteria require blood agar or other rich culture media to grow, and are most commonly found in association with serious structural urinary tract abnormalities.

Interpretation of Urine Culture

Interpretation of culture results depends on the method of urine collection, and at times, the clinical setting. The Kass criteria are still used for midstream voided specimens (18): the cutoff level is 100,000 CFU/ml. By supra pubic aspiration, any growth is considered significant. In catheterized specimens, the cutoff level suggested by Hoberman et al. is 50,000 CFU/ml (19). Most of the time there is only one bacterial strain. The confluent growth of colonies of different types strongly indicates contamination.

Pyuria is usually present in children with UTI (80–90% of episodes of symptomatic UTI). A urine sample without pyuria does not exclude symptomatic UTI. Microscopy for leucocytes is variably sensitive (32–100%) and specific (45–97%) (3).

Urinalysis for Immediate Diagnostic Information

The various components include the reagent slide tests: nitrite, leucocytes (LE), protein and blood. Reagent strips (dipstick) simplify identification of these components. The LE test has a high sensitivity which ranges from 67 to 94% (3) but much lower specificity. The test for nitrite has a much higher specificity (90–100%) (3) but a lower sensitivity (16–82%). Nitrite testing may be useful for diagnosing a UTI when it is positive but it has less value when it is negative. Dipstick testing for blood or protein have poor sensitivity and specificity in the diagnosis of UTI. The microscopic examination of urine for bacteria is effective in diagnosis with experienced personnel (3, 20).

Localization of the UTI

The differentiation between upper (pyelonephritis) and lower (cystitis) UTI is very important. It particularly has major clinical implications in young children. The risk of renal scarring is significant with pyelonephritis, and not a concern with cystitis. Therefore the management (investigations, antibiotic used, length of therapy) is totally different for pyelonephritis and cystitis. The location of the site of infection is based on a combination of clinical, laboratory and imaging findings.

Clinical Signs

The main symptom of pyelonephritis is high fever. According to the American Academy of Pediatrics, UTI should be considered in any infant aged less than 2 years with unexplained fever (3, 21). This UTI should be managed as a pyelonephritis. Older children may complain of back or flank pain, chills. Renal tenderness may be found in these children. In cases of pyelonephritis, the clinical findings may include varied gastrointestinal symptoms: diarrhea, vomiting, and nausea which may confound the diagnosis of UTI. In addition, neurological symptoms such as irritability, and seizures (particularly with high fever) may exist. Acute focal bacterial nephritis is a severe form of pyelonephritis. It is an interstitial bacterial nephritis formely known as lobar nephronia. It is most often described in adults with diabetes but recent articles have described this condition in children (22). Signs described include flank pain, fever, and rapid deterioration into a "septic" picture (23).

In case of bacterial cystitis, there is rarely fever $>38°C$. Common findings include low grade abdominal pain and bladder/voiding symptoms such as frequency, pain with micturition, suprapublic discomfort, difficulty in voiding (retention) or hesitancy, urgency, and enuresis.

Specific Clinical Signs of UTIs in Neonates and Infants

The symptoms are nonspecific and require a high degree of clinical suspicion. They include fever, poor feeding, failure to thrive, abdominal pain, haematuria, and malodorous urine. Jaundice may be an early diagnostic sign of UTI in infancy. Garcia et al. (24) has shown that UTIs were found in 7.5% of asymptomatic, afebrile jaundiced infants younger than 8 weeks old. Infants with the onset of jaundice after 8 days of age were more likely to have a UTI in this study. The presence of UTI should be considered in all infants and young children 2 months to 2 years of age with unexplained fever (21). All UTIs in this age group (particularly with high fever) should be considered as pyelonephritis until proven otherwise.

Biological Tests

Most of these tests are not very specific. Winberg (25) described decreased renal concentrating capacity in pyelonephritis. A number tests were described in adults which differentiated the level of UTIs. Many studied the immune response, which reportedly was different according to the site of UTIs. Specific antibodies to the infecting bacteria were higher in cases of pyelonephritis (26, 27). This was confirmed in experimental models (28). The detection of antibody-coated bacteria in urine using fluorescein-labeled antiimmunoglobulin (29) was reported as diagnostic of pyelonephritis in adolescents and young adults. However, it was unreliable in children (30), presumably because there is a delay of several days before antibody is produced in younger age groups. An elevated erythrocyte sedimentation rate, a positive C-reactive protein, and an elevated peripheral WBC count with an increased absolute neutrophil counts have all been reported as are non-specific indicators of upper tract infection.

In children younger than 2 years of age Garin et al. (31) showed that patients with pyelonephritis had statistically higher white blood cell count (WBC), erythrocyte sedimentation rate (ESR) C-reactive protein (CRP) than those with cystitis. Although the sensitivity of the test was 80–100%, their specificity was $< 28\%$. In the NICE Clinical Guideline 54 (32), it was concluded that C-reactive protein alone should not be used to differentiate acute pyelonephritis from cystitis in infants and children. Recently a high procalcitonin concentration was described as a validated predictor of acute pyelonephritis in febrile UTI (33) (confirmed by early renal scintigraphy) and for late renal scars (34, 35). Such interesting findings await large scale confirmatory studies, particularly in infants and young children.

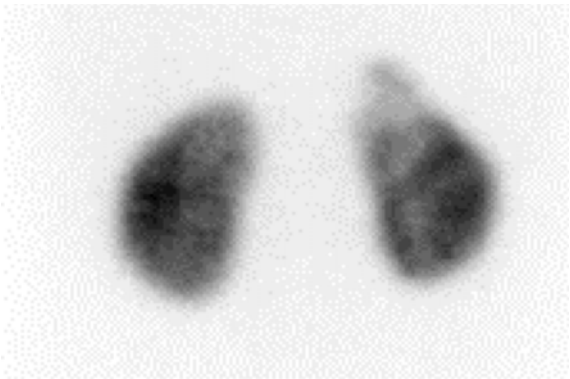
Imaging Tests in Localizing the Site of Infection

Renal cortical scintigraphy (with 99 m Tc-DMSA or 99 m Tc-glucoheptonate) is widely accepted for the diagnosis of pyelonephritis in all pediatric age groups. However, with this technique it is difficult to

distinguish between new, acute inflammatory changes and previously established renal scars. A meta analysis of animal studies of acute pyelonephritis showed an overall sensitivity of 86% and specificity of 91% (36) As seen in *Fig. 54-1* renal cortical scintigraphy shows decreased uptake of radiotracer in lesions of pyelonephritis (37). Despite a large body of published literature, the role of radionuclide renal scans in the clinical management of the child with UTI still is unclear (3). Most of the time such imaging has no role in the specific management of childhood UTIs. For that reason, the use of DMSA scintigraphy scanning is only recommended by the NICE clinical guidelines (32) in situations when it is clinically important to confirm or exclude acute pyelonephritis, and when the power Doppler ultrasound (see below) is not available or the diagnosis still cannot be confirmed.

Figure 54-1

Scintigraphy with 99 mTcDMSA. Decreased uptake of radionuclide in the upper pole of left kidney.



Renal Ultrasound

Most of the time, conventional renal ultrasound is insensitive for the diagnosis of pyelonephritis. Signs of pyelonephritis include focal or diffuse renal enlargement and abnormal cortical echogenicity mostly areas of increased echogenicity which may mimic a renal mass (*Fig. 54-2*). In case of hypoechoic area, it may presage a renal abscess in formation. D. Morin et al. described the abnormal sonogram renal changes (38) in children with acute pyelonephritis. Using a high frequency transducer they found abnormal sonogram changes in 87% of the cases including increased renal pelvis, focal hyper or hypoechogenicity, or diffuse hyperecogenicity. According to their experience, when performed by a trained radiologist this technic is nearly as sensitive as the DMSA scan in diagnosing renal involvement in children with unobstructed acute pyelonephritis.

Power Doppler ultrasound is more sensitive than a gray-scale ultrasound (*Fig. 54-3*).

Pyelonephritis is associated with renal ischemia. It is seen as a hypovascular zone in the renal cortex (39).

Computed Tomography

The features of pyelonephritis by CT have been well-described (40), and are shown in *Fig. 54-4*. After intravenous contrast, areas of infected renal parenchyma have decreased contrast enhancement due to the renal ischemia, whereas normal renal parenchyma becomes brighter. Despite its potential value, this technique many disadvantages including significant exposure to ionizing radiation, danger of reaction to intravenous iodinated contrast, and

Figure 54-2

Power Doppler ultrasound. Triangular area of cortical ischemia which is well correlated with the results of DMSA Scintigraphy (Pr J. J. Dacher).

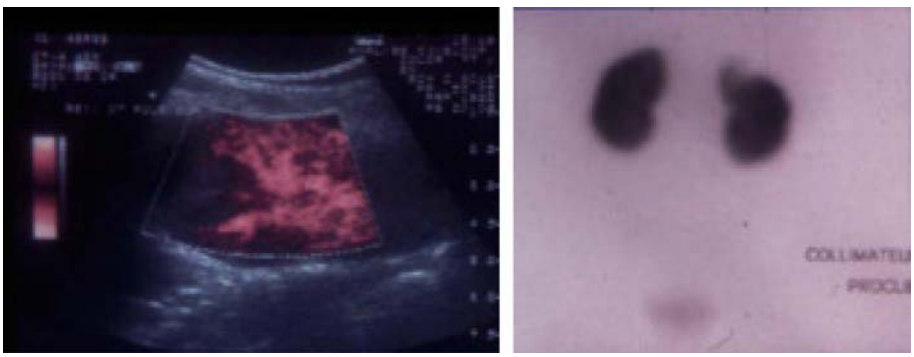


Figure 54-3
Renal ultrasound. Increased echogenicity in a triangular part of the renal parenchyma.

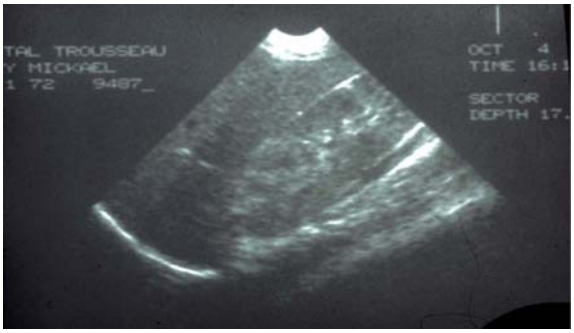
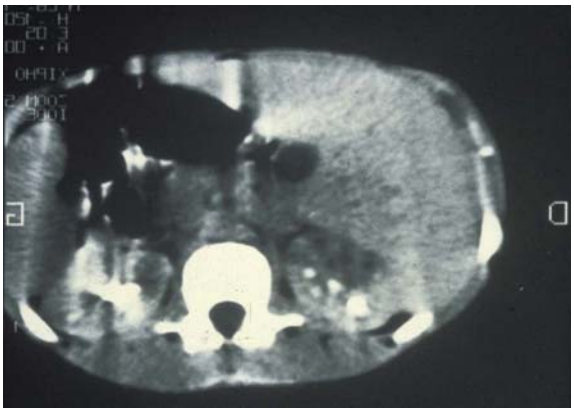


Figure 54-4
Computed tomography. Images of cortical ischemia: decreased contrast enhaucement in infected region, whereas normal renal parenchyma becomes brighter after intravenous contrast.

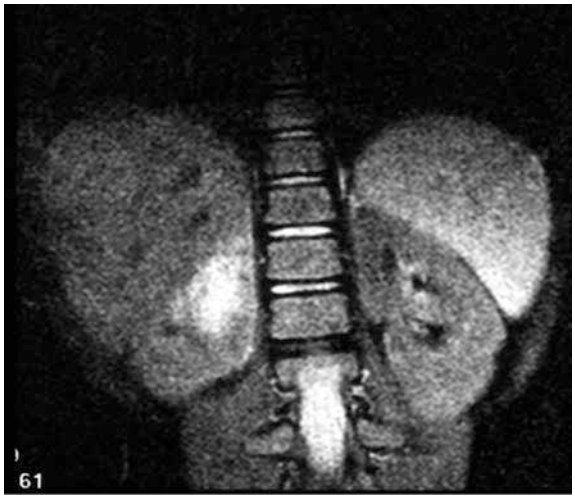


the need for deep sedation in younger children. For these reasons it is reserved for rare situations despite its ready availability and the wide- spread expertise of radiologists in its use.

Magnetic Resonance Imaging

MRI imaging is shown in *Fig. 54-5*. There is a darkening of normal renal parenchyma whereas lesions of pyelonephritis remain bright after intravenous gadolinium contrast (41). The main disadvantages of MRI are the cost and the need for sedation but the lack of ionizing radiation is appealing in the pediatric age group.

Figure 54-5
Magnetic resonance imaging. After IV gadolinium contrast: the lesions of pyelonephritis remain bright and the normal renal parenchyma is dark.



Given the other imaging modalities described, the use of MRI in the diagnosis of pyelonephritis is not necessary. A major disadvantage is the danger of IV gadolinium injection in case of renal insufficiency (42), which has caused strict restriction and in some areas, removal from routine clinical use.

Asymptomatic Bacteriuria (ABU) – Localization of the Infection

In some cases, growth of 100,000 colony forming units is found in freshly voided-urines, but the child is symptom-free (43). Neither symptoms of lower tract infections (dysuria, urgency or frequency) nor symptoms of upper tract infections (fever, shaking, chills and flank pain) are present. In this condition, bacteriuria may be due external contamination leading to a false-positive result. Therefore the diagnosis of ABU requires repeated samples which all demonstrate significant growth of the same organism in a child who has not been ill for at last 2 weeks (44).

There are several lines of evidence that indicate why children with ABU are symptom free and at little risk for clinical problems. The bacteria tend to be of low virulence and do not have significany ability to damage the kidney even if they were to reach the upper tracts (45). ABU is not a significant risk factor for renal scarring. The Escherichia coli isolated from children with ABU are different from those causing symptomatic infections.

In most instances, the organisms cannot be assigned to serogroups, they have an increased sensitivity to the bactericidal effect of serum, and they have poor intrinsic ability to adhere to epithelial surfaces.

The study of Ottolini et al. (46) concluded that ABU was not a significant risk factor for renal scarring in a group of 207 patients with neurogenic bladder treated with clean intermittent catheterization. In this group of patients, ABU was common, persisted for weeks, and was not treated. There was no deterioration of the upper urinary tracts in any patients. The same conclusions were drawn by Shekarriz et al. (47). They studied the morbidity associated with urodynamic evaluation in 69 pediatric patient with ABU. Most of these children had a neurogenic bladder. No patient developed symptomatic UTI. They concluded that routine use of urine cultures or prophylactic antibiotics before urodynamic studies in pediatric patients with a neurogenic bladder is not indicated in the presence of ABU.

Search for the Cause of UTI

The main cause of UTI is the ascending route of the bacteria from the periuretral area. The bacteria originate in the bowel. In boys, the prepuce plays an important role as a reservoir. Several data (5, 6) show a dramatic risk reduction of UTI among circumcised males. There are many published studies that demonstrate that pyelonephritis may be the first indication of an underlying pathological condition such as urinary tract obstruction, nephrolithiasis, vesico-ureteral reflux (VUR), bladder dyssynergy, or incomplete bladder emptying from any cause (48).

The investigation starts with a complete history and physical examination. Recognition of spinal deformaties, history or physical signs of constipation, and/or abnormal bowel or bladder function may suggest a dysfunctional voiding syndrome (49). It may be due to a neurogenic bladder. In most of the cases no underlying neuropathy can be found.

Bladder instability is the most common voiding dysfunction. It frequently is a cause or exacerbating factor in childhood UTIs. It may also be manifested by urge incontinence with and without enuresis, which leads to a characteristic "holding posture" such as squatting. Bladder instability may be due to chronic constipation (50). In this case its treatment may lead to the resolution of urinary incontinence and urinary tract infections.

The Hinman syndrome (51) and the Ochoa syndrome (52) are associated with severe bladder dysfunction and frequent UTIs with no underlying neuropathy.

Imaging Studies

Previous guidelines regarding the utilization of imaging studies in the investigation of childhood UTI recommended that all children undergo renal tract imaging after a first episode of confirmed UTI. The USA guidelines (1999) of the American Academy of Pediatrics (3, 21) recommended a renal ultrasonography and either a voiding cystourethrography (VCUG) or a radionuclide cystography (RNC) in infants and young children less that 2 years of age after a first UTI. The goal of such studies is to identify factors that predispose to recurrent UTI and that increase the risk of renal scarring at the earliest opportunity. More recent studies of Rosenberg (53) and Jodal (54) led to the conclusions that DMSA scintigraphy could replace VCUG. The latter could then be applied selectively, in cases when the DMSA scan or the ultrasound was abnormal. Most of the children with uptake defects on the acute DMSA scan had reflux with dilatation of the upper urinary tract. These guidelines remain open to ongoing discussion among pediatric nephrologists, urologists, and radiologists in many parts of the world. DMSA scintigraphy is very expensive, not often available and requires pediatric experience of diagnostic departments.

A more provocative concern regarding these imaging studies is their actual value in altering management or improving outcomes following a documented case of pyelonephritis in the pediatric population. In a systematic overview of the literature, Dick et al. (55) and Gordon et al. (56) determined that no extant studies provided clear evidence that routine imaging had any effects on the development of renal scars or on improving clinical outcomes in children with their first UTI. They concluded that current recommendations are not evidence-based. Additional studies confirm these findings (57–59).

There is a well-documented association between VUR, renal damage, and pyelonephritis. However, the belief that infection and VUR are the cause of upper tract parenchymal damage is undergoing critical review. There has been increasing knowledge that reflux nephropathy is not always acquired renal, but rather reflects reflux-associated damage related to congenital dysplastic kidneys (60). It may be considered part of the "congenital abnormality of the kidney and the urinary tract" (CAKUT) syndrome (61).

Prior to 2006 most pediatric societies recommended routine voiding cystourethrography for all children with a first febrile UTI. The likelihood of finding a VUR with such a strategy is quite low (20–40%) (62). Most of the VUR diagnosed is low grade. Two recent studies of children with low grade VUR have shown no significant differences in risk for UTI between antibiotic prophylaxis

and no treatment (63, 64). The fact that antibiotic prophylaxis is not necessary in cases of low grade VUR is likely going to affect current guidelines. Regarding the utilization of VCUG in the evaluation of all children with their first febrile UTI. Current practice guidelines focus on performing selected investigations in high-risk children. This group of children have an increased risk of having an abnormal urinary tract that warrants investigations. By targeting investigations to specific children, many unnecessary and invasive investigations will be avoided (65). This is in accordance with the guideline published in August 2007 by the National Institute of Health and Clinical Excellence (NICE) (32, 66) High risk children include:(1) those with an increase in procalcitonin (PCT), which has high sensitivity in identifying children with severe reflux (67); or (2) those with high fever who are younger than 6 months of age, recurrent UTI, clinical signs such as poor urinary stream or palpable kidneys, infection with atypical organisms, bacteriemia or septicemia, prolonged clinical course with failure to respond fully to antibiotic treatment within 48–72 h; unusual clinical presentations such as an older boy, or with a known abnormality on antenatal ultrasound screening of the urinary tract (32, 65, 68). Such high risk children should undergo ultrasonography and VCUG with their first episode of UTI (3, 57, 69). According to the Swedish state of the art conference in children over 2 years of age a DMSA scintigraphy (70) alone with ultrasonography were suggested as methods for identifying risk patients. A voiding cystourethrogram is performed only if the DMSA scintigraphy is abnormal. Therefore, the traditional recommendation to perform the VCUG at 3–6 weeks after the diagnosis of UTI should be reconsidered. Further the recommendation to perform a VCUG, when indicated, early in the course of infection should be reconsidered as the rate of detection of VUR does not increase in relation to the timing of the study (71, 72).

Other Causes of UTI

Malformations of the urinary tract, urolithiasis and bladder dysfuntions are not the only causes of UTI. After an episode of pyelonephritis, none of these causes are found in many children. The urinary tract epithelium plays an important role in UTI through coordination of the innate immune response to infection (73). Urinary tract epithelia express toll-like receptors (TLRs) with the capacity to recognize bacterial components. Engagement of TLRs can lead to activation of inflammatory mediator cascades. The resulting inflammatory infiltration serves to aid bacterial clearance. Dysfunction in this complex innate immune response may lead to recurrent UTI. Host resistance is not the only determinant. The virulence of the infecting strain is also a factor in pathogenesis (74). Specific virulence genes are the focus of ongoing studies. Virulence factors like fimbriae may modify the mucosal chemokine response. This modification may allow the host to adjust the inflammatory response to match the infecting strain (74, 75). There are other poorly understood factors which modify the susceptivility to renal scarring, including the renin-angiotensin system, the plaminogen activating inhibitor, TGFβ, and polymorphisms in collagen (76). Recent findings describe a novel hormonal regulation of innate immune cellular activation. Such studies demonstrate that deamino – 8 – D – arginine vasopressin (dDAVP) is a potent modulator of microbial-induced inflammation in the kidney (77).

Treatment

Treatment is based on the location of the UTI: cystitis and pyelonephritis require different treatment. Most of the time asymptomatic bacteriuria should not be treated with antibiotics. A child with a symptomatic UTI should be given antibiotics without delay. Before the beginning of the treatment a urine sample must be acquired to isolate the bacteria causing the UTI and to define any antimicrobial resistance. A delay in treatment has been identified as a major risk factor in renal scarring with pyelonephritis. Therefore, antibiotic should be initiated on an empiric basis and then modified according to the result of the culture.

Treatment of Acute Cystitis

Oral antibiotics for 3–5 days are recommended (32, 78, 79). The choice of antibiotics should be directed by established guidlines (*Table 54-1*). Cephalosporins should be

Table 54-1
Antibiotics for oral treatment of cystitis in children

Drug	Dose (mg/kg/day)
Trimethoprim-Sulfamethoxazole	30/6 BID
Nitrofurantoin	5 BID
Amoxicillin	25–40 TID
Amoxicillin-clavulanate	40 BID
Cephalosporin – Cefixime	8 BID

avoided if possible and reserved for the treatment of pyelonephritis, given the risk of development of antimicrobial resistance to these agents.

Treatment of Pyelonephritis

A recent Cochrane (80) review of antibiotic treatment for acute pyelonephritis in children identified 18 randomized trials. They compared initial (3–4 days) intravenous treatment followed by oral treatment with completely intravenous treatment (7–14 days). There were no significant differences in the risk of persistant renal damage. These studies suggest that long term parental treatment is not necessary. The recent French guidelines (79) recommend initial treatment with a parenteral antibiotic (2–4 days) followed by oral antibiotics A recent prospective randomized trial (80) confirmed that the incidence of renal scars was similar in patients who received 3 days of intravenous ceftriaxone (1) followed by oral antibiotics compared with treatment utilising 8 days of IV Ceftriaxone.

Hoberman (81), in a randomized controlled trial of 306 children, compared oral treatment (Cefixime) with parenteral treatment. They did not find any significant difference between the two groups in term of renal scarring at six months. The same conclusions were drawn by Montini et al. (82). This multicenter randomized controlled trial demonstrated that oral antibiotics (co-amoxiclav 50 mg/kg/day in three doses for 10 days) was effective as parenteral ceftriaxone (50 mg/kg/day in a single parenteral dose) for three days, followed by oral co-amoxiclav (50 mg/kg/day in three divided doses for 7 days). Despite this new data, caution should be exercised when using only oral antibiotics for the treatment of high-risk children with pyelonephritis (83). Most such children were excluded from the Hoberman and Montini trials noted above. The results of these studies should not be generalized and apply to all children without clear identification of high risk groups (as noted above) who require parenteral therapy to minimize renal scarring. Further, in many countries, antibiotic resistance may limit the empiric use of certain oral antibiotics (84).

The duration of treatment should range between 10 and 14 days according to several guidelines (3, 22, 32) and the review of randomized controlled trials (80, 85). In cases of severe pyelonephritis (acute lobar nephronia, effective treatment can prevent progression to renal abscess. A total of 3 weeks of intravenous and oral antibiotic therapy tailored to the pathogen noted in cultures should be the treatment of choice for pediatric patients with pyelonephritis (22, 23).

The choice of antibiotics should be based on knowledge of local antimicrobial resistance patterns. There is a correlation between pharmacokinetic/pharmacodynamic parameters (*Table 54-2*) and the efficacy of antibiotics in the treatment of UTI (86). A better understanding of these parameters, as well as the rapidly growing field of pharmacogenomics, may increase the efficacy of "individualized" treatments (87). The sensitivity of most urinary pathogens is close to 96% to both Gentamicin and Ceftriaxone. These factors should guide empirical treatment until culture results are known.

Table 54-2

Pharmacokinetic/Pharmacodynamic parameters of the antibiotics studies in acute pyelonephritis (79)

	Amoxillin	Amoxillin clavulanic	Cotrimoxazole	Cefixine	Gentamicin	Cefotaxime	Ceftriaxone	Ciprofloxacin
Dosage	33 mg/kg	33 mg/kg	15 mg/kg	4 mg/kg	3 mg/kg. day	33 mg/kg	50 mg/kg	15 mg/kg
	TID	TID	BID	BID	once a day	TID	once a day	BID
% of sensitive strains	50	70	80	95	97	99	99	98
MIC 50 (mg/l)	4	4	0.5	0.125	0.5	0.06	0.06	0.01
Serum concentration peak	12	10	50/2	2,5	12	30	190	4
Half life (h)	1	1	10	3.3	2	1	8	6
T < MIC (%)	20	20	100	100	100	100	100	100

Antibacterial Prophylaxis

Low-dose, long term prophylaxis has been traditionally used in patients prone to develop recurrent acute pyelonephritis or frequently recurrent lower tract infections. Such therapy has been particularly used in high risk children including those with vesico-ureteral reflux with dilatation of the upper urinary tract, obstructive uropathies, and other high risk conditions. The efficacy of antibacterial prophylaxis has been recently questioned. It is not necessary in case of low grade VUR (63, 64), and may increase the risk of antimicrobial resistance (88). The clinician must decide in any given case whether the risks of scarring justify the use of prophylactic therapy. In some instances, long-term low dose antimicrobial prophylaxis has markedly decreased the recurrence pattern (as well as clinic visits with associated parental/child anxiety) of children with frequent lower tract infections (*Tables 54-3*).

The International VUR Study of Children was specifically designed to study the comparative effectiveness of long term antimicrobial prophylaxis and surgical correction in children with high grades of VUR. Initial reports have described the outcome at 10 years of study (89–92). There was no difference between the two groups of children in terms of renal scarring and/or its complications. These results might lead to the conclusion that antibacterial prophylaxis is efficient in case of high grade reflux. The efficacy of circumcision in decreasing the incidence of UTI in male infants is clear. Data from several groups (4–6) show a dramatic risk reduction of UTI among circumcised males.

In general, there is global agreement that children with totally asymptomatic bacteriuria do not require antimicrobial therapy. Indeed, such therapy may increase antimicrobial resistance and is to be avoided. A child with asymptomatic bacteria is symptom -free because the bacteria tend to be of low virulence, and there is little or no risk of renal scarring.

Table 54-3

Antibiotics used for prophylaxis

Antibiotic	Recommended dosage (mg/kg/day)
Nitrofurantoin	1
Trimethoprim	0.5
Trimethoprim Sulfanamide	0.5 (of trimethoprim component)
Cefadroxil	3–5
Ciprofloxacin	1

Conclusion

Despite the recognition that underlying renal anomalies may be the cause of renal scarring previously attributed to infection, the prevention of renal scarring remains the goal of all therapies for childhood UTI. Although new data has questioned previous "dogmas" re: urinary tract imaging, parenteral therapy of pyelonephritis, and use of antimicrobial prophylaxis, the clinician must be vigilant in recognizing children at risk for complications from UTI. *Such high risk children (those under the age of 6 months with high fever, those with abnormal GU anatomy, and those with a septic presentation at any age) should be treated and investigated aggressively.* The recognition of antenatal urinary abnormalities, improved imaging strategies, better understanding of the molecular and cellular pathophysiology of renal scarring, and the development of new, pharmacogenomically-derived individualized antimicrobial treatment regimens offers the hope of reducing renal scarring and its complications.

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55 Vesicoureteral Reflux and Renal Scarring

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Introduction

Vesicoureteral reflux (VUR) is the retrograde flow of urine from the bladder to the kidneys. This retrograde flow of urine, while the norm in some animals, is not normal in humans. VUR may be an isolated abnormality (primary VUR) or it may occur in association with other congenital anomalies of the kidney and urinary tract (CAKUT), including renal dysplasia, and obstructive uropathy or neurogenic bladder (secondary VUR).

Most cases with VUR are diagnosed after urinary tract infection (UTI). The common belief has been that the presence of VUR increases the risk of UTI and the two together cause renal scarring (reflux nephropathy), which may lead to hypertension, toxemia of pregnancy, chronic renal insufficiency and even end-stage renal disease (ESRD). The traditional management has included a prompt treatment of UTI and long-term antimicrobial prophylaxis until the resolution of VUR. Surgical correction has been advocated in those with high grade VUR, recurrent UTI in spite of antimicrobial prophylaxis, or non-compliance with medical management. Over the years, many studies have debated if one treatment modality is superior to another in preventing renal injury, with most of the studies concluding that the long-term outcome with one intervention is no better than the other. However, the exact role of VUR in the development of renal injury has been difficult to elucidate. Recently, new data have been published regarding the heterogeneity of the secondary effects of reflux, and the role played by voiding dysfunction and/or constipation in the resolution of VUR or the frequency of UTI. In this chapter, we will provide an overview of the important issues concerning primary VUR. This will include: the embryology and anatomy of the ureterovesical junction; the current modalities for diagnosis and management; and a review of current controversies and emerging consensus concerning the role of primary VUR in the pathophysiology of renal scarring.

Prevalence of VUR

The exact incidence of VUR is not known because it is not feasible to do voiding cystourethrograms (VCUG) in a large cohort of healthy children. Its prevalence varies from 1.3% of healthy children (1) to 8–50% of children evaluated after UTI (2–4). In newborns and infants, the incidence of VUR diagnosed after UTI is 36–49% (5–7). Children with VUR detected after UTI are predominantly females (2, 3, 8, 9), though in some studies no gender difference (3, 10, 11) or even male predominance was reported (12). VUR is less common in African American children (13, 14). Only about one-third as many African American as white girls with UTI have VUR and no significant differences in age or mode of presentation exist between the two races (13, 15). The severity of VUR in African American children is less than that in Caucasian children (16).

Embryology and Anatomy

Primary VUR is a congenital anomaly of the structure of the vesicoureteral junction. This is felt to be secondary to a preexisting anatomic abnormality with shortening of the intravesical submucosal length of the ureter. The formation of the ureteral bud from the mesonephric duct signals the beginning of the development of the metanephric kidney – the final stage of renal development. The ureteral bud interacts with the mesenchyme to give rise to the metanephric kidney. As the mesonephric duct is gradually absorbed into the enlarging urogenital sinus (the precursor of the developing bladder) the location of the ureteral bud plays a role in the eventual location of the ureteral meatus within the bladder. If the ureteral bud reaches the urogenital sinus too early, due to the absorption pattern of the mesonephric duct, it is eventually located more laterally and proximally in the bladder.

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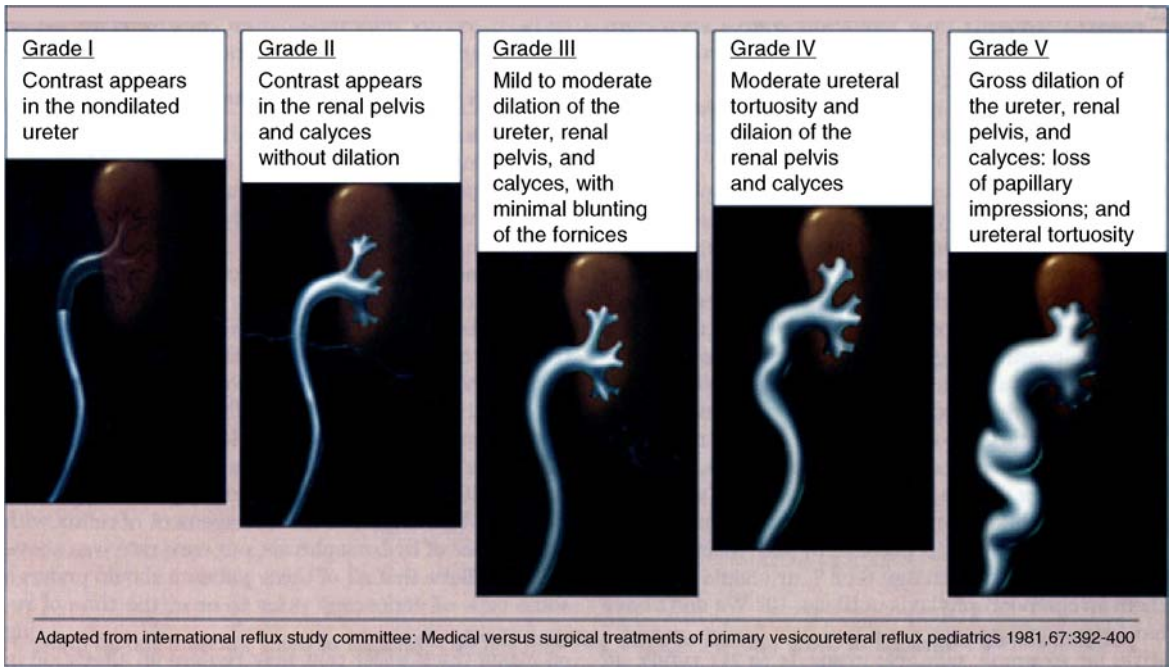
This location is associated with the development of reflux as there is reduction in the intravesical submucosal length of the ureter. Multiple genes have been noted to be involved in the development of VUR. PAX2 (necessary for ureteral budding in mice) and glial-derived neurotrophic factor (GDNF) have been shown to have an impact in the development of VUR in mice, however their role in human VUR remains controversial (17, 18). Comparison of the autonomic innervation and histology of the ureterovesical junction in mutant mice indicated that there was no difference between those associated with VUR as compared to controls (19).

The ureterovesical junction is designed to prevent free reflux of urine from the bladder to the kidney. The ureters pass into the bladder through the detrusor in an oblique path. The distal end of the ureter is located submucosally within the bladder. The length of the submucosal ureter has been noted to be a major component in the prevention of VUR. The muscles of the ureter extend into the trigone of the bladder and enmesh with the fibers from the opposite ureter. This intermingling of fibers allows a degree of fixation of the ureters into the trigone of the bladder. The distal submucosal segment is compressed against the muscular bladder wall with bladder filing acting as an additional mechanism to prevent reflux of urine. Finally, as urine is actively transported down the ureter in an antegrade manner, the tone of the ureter and the meatus in the bladder also work to prevent reflux.

Grading of VUR

Grading has been used in an effort to predict the outcomes of children with VUR. It has long been known that the VUR has the potential to resolve spontaneously, and grading has been used to standardize potential management strategies as well as to compare clinical outcomes. The system that has been most widely accepted classifies VUR into 5 grades based on the appearance of specific features of the voiding cystourethrogram (VCUG) (*Fig. 55-1*). This system was proposed by the International Reflux Study in children (IRSC) (20). Grade I is reflux into the ureter, grade II is reflux into the renal pelvis, without any dilation of the calyces, grade III is reflux to the renal pelvis with mild dilation of the renal pelvis, grade IV is reflux to the renal pelvis with greater dilation of the renal pelvis and papillary blunting. Grade V

Figure 55-1
International classification of vesicoureteral reflux.



reflux includes reflux to the renal pelvis with massive ureteral and pelvic dilation, including ureteric tortuosity. This system, although widely utilized, does have some shortcomings. The differences between grades III and IV are not always obvious. The degree of reflux may be modified by how aggressively the bladder is filled during the VCUG. Also, ureteral dilatation may be present without calyceal dilation leading to further difficulties with grading.

Radionuclide voiding cystourethrogram has also been used to diagnose and follow children with VUR. Unfortunately, exact grading into the five grades as noted above is difficult using radionuclide imaging. Using this modality, VUR can only be graded into mild, moderate and severe. Attempts have been made to correlate this grading system with the IRSC grading as mentioned above (21). Although, good correlation has been noted when combining the radiographic grades into two grades only, this method of evaluation has not been used to predict outcomes of VUR.

Clinical Presentation of Primary VUR

Patients with primary VUR most often present in one of three ways – following diagnostic evaluation of a UTI, during follow-up for antenatal hydronephrosis, and while screening a sibling of a patient with VUR. Some children may present with urinary symptoms only with no fever. Occasionally, an older child with undiagnosed reflux nephropathy may present with symptoms other than UTI, which are noted in *Table 55-1*.

Table 55-1
Presenting Clinical Symptoms of VUR and/or Renal Parenchymal Injury

1. Fever with or without urinary symptoms
2. Urinary symptoms without fever
3. Flank pain
4. Proteinuria
5. Nephrotic syndrome due to focal segmental glomerulosclerosis
6. Hypertension
7. Secondary enuresis due to coexisting voiding dysfunction or diminished urine concentrating ability
8. Renal stone, mostly due to *Proteus mirabilis* UTI
9. Renal failure, including end-stage renal disease

Reflux Identified following a Urinary Tract Infection

The most common clinical setting in which VUR is identified is during a radiographic evaluation following a UTI, particularly in a young child. In a large cohort of children with urinary tract infections, VUR was diagnosed in 46% of infants and 9% of preschool children (22). Patients may present following a febrile or symptomatic UTI. It has long been recognized that the presence of VUR may potentiate clinical symptoms of UTI in children (23). Current recommendations for evaluation of UTI include performance of ultrasonography and VCUG after the first UTI in both boys and girls. A search for other modifiable host factors e.g., voiding dysfunction, constipation – is crucial following documentation of a UTI. The identification of VUR can lead to an increased awareness and early identification and treatment of UTI. Following a diagnosed UTI, DMSA renal scanning has been suggested to determine if scarring is present. The majority of children identified following a documented UTI are girls, however when considering infants <6 months of age no gender difference is noted (24). Additionally, 65% of children diagnosed with VUR following a UTI were less than 7 years of age. Toilet trained children that are diagnosed with VUR following UTI have a 43% incidence of dysfunctional voiding (25) that can potentially lead to recurrent infections and compromise the resolution of VUR.

Reflux Identified Secondary to Antenatal Hydronephrosis

Diagnosis may be suspected in utero with unilateral or bilateral hydronephrosis and confirmed at birth with VCUG. When prenatal hydronephrosis has been noted, post natal evaluation with VCUG will demonstrate the presence of VUR in 10–30% of infants (26). There is a higher incidence of boys in the group of infants diagnosed with VUR following identification of antenatal hydronephrosis (27). Infant boys with VUR that are left uncircumcised have a significant incidence of UTI despite antibiotic prophylaxis (28). Studies have indicated that the natural history of VUR identified following antenatal evaluation of hydronephrosis indicates a high potential for spontaneous resolution (59% by age 4 years) even in those with grades IV – V reflux (29). Analysis of children with resolution of the VUR revealed that that there was higher incidence of resolution in boys with lower grades and unilateral reflux; infection rates were noted to be low in this cohort (30). Further study of this group of infants

revealed that the VUR was of lower grade in female infants and less likely to be associated with renal damage as compared to newborn boys (31). The higher grade of VUR in newborn boys was noted to be secondary to abnormal urodynamic patterns indicating higher bladder pressures associated with dyssynergia of the urethral sphincter which improves with time (32, 33).

Sibling Reflux

Initial studies by Jerkins and Noe (34) indicated that 32% of siblings of an index patient have VUR. Sirota et al. in 1986 studied a cohort of 16 families with VUR in 33 children. These children were noted to have a higher incidence of bilateral VUR than non-familial cases (35). In a study of asymptomatic siblings VUR was identified in 45% using radionuclide cystography (36). A follow-up study by Noe et al. indicated that 75% of children identified with VUR by sibling screening were asymptomatic, and there was a slightly higher incidence of VUR in female siblings of female index patients. The incidence of renal damage was much lower in the siblings diagnosed with VUR as compared to the index patient with VUR (37). Evaluation of children of parents with VUR revealed that 66% of the children had VUR indicating a significant parent to child transmission (38). With the identification of sibling/familial reflux, debate began about the benefit of screening of asymptomatic siblings of index patients (39, 40). Review of the natural history of VUR in siblings, indicated that 52% had resolution of the reflux at follow-up of 18 months, with yearly resolution rates of 28% indicating that most could be managed non-operatively (41). Children over age 7 were found to have less potential to have VUR when screened, although renal injury was noted irrespective of age (42).

UTI with progression of scar was noted in only 5% of siblings with VUR followed for 3–7 years and the vast majority of those with grades I & II VUR had resolution (43). The more "benign" course of sibling reflux compared to reflux identified following a UTI (44) has led many to suggest limitation of those being screened. Analysis of siblings presenting with renal injury indicated that reflux grade, history of UTI and age at diagnosis were the most significant factors (45). Interestingly, Houle et al. identified a higher incidence of scarring in those siblings evaluated after age 2 years than those identified prior to age 2 and therefore recommended early screening (46). Their findings were supported by a later report indicating that in siblings with lower grades of reflux, greater scars were noted when evaluation was performed after age 3 years (47). At this time, most would suggest screening younger siblings (<age 5 years) of index children with VUR and would reserve screening of older siblings to those who present with a UTI or other symptoms.

Voiding Dysfunction and Constipation in VUR

VUR may be associated with voiding dysfunction, which in some cases may present as dysfunctional elimination syndrome (DES). The symptoms of DES include daytime wetness, urgency, frequency, infrequency, constipation or fecal incontinence in "toilet trained" children with no underlying anatomic or neurological abnormality (25, 48, 49). The exact pathogenesis of voiding dysfunction or DES is not known. It seems to be an abnormally learned spectrum of voiding, which evolves from poorly learned voiding technique and attempts to suppress impending or active bladder contractions by inappropriately contracting the pelvic floor muscles and tightening external sphincter (49, 50). This results in increased voiding pressure and/or inefficient voiding (51, 52).

Voiding dysfunction predisposes to recurrent UTI, induces and perpetuates VUR, and may result in permanent renal damage (53, 54). In a study by Koff et al., DES, besides increasing the rate of breakthrough UTI, delayed resolution of reflux and adversely affected the results of ureteric reimplantation (25). In another study that involved use of a questionnaire in 310 children enrolled in the European branch of the International Reflux Study in Children, a strong negative correlation was seen between recurrences of UTI, as well as disappearance of VUR and nonneuropathic bladder/sphincter dysfunction (55).

Voiding dysfunction or DES is a diagnosis of exclusion that mandates a detailed history and physical examination to rule out any underlying neurological or neuromuscular etiology. The diagnosis is usually evident clinically, and urinalysis, bladder ultrasound, and VCUG in selected cases are helpful in making a diagnosis. The role of urodynamic studies is not well-established, partly because study results are inconsistent (49, 56, 57). The procedure is invasive and the study result does not change therapy or influence the final outcome. A thorough history and physical examination lead to the correct diagnosis and treatment in the majority of children (58).

Constipation, besides being a part of dysfunctional elimination syndrome, may be an isolated finding, which, by itself, increases the risk of recurrent UTI or voiding dysfunction in children with VUR. This is believed to result from compression of bladder and

bladder neck that increases bladder storage pressure and post-void residual urine volume. Also, distended colon and/or soiling provide an abundant reservoir of pathogens (59–61). Constipation in children increases the likelihood of urinary incontinence, bladder overactivity, dyscoordinated voiding, a large capacity and poorly emptying bladder, recurrent UTI, and deterioration of VUR (62). In a study that involved 366 children, constipation/encopresis was reported in 30% cases, day-time wetting in 89%, night-wetting in 79%, and recurrent UTI in 60% of the patients. VUR was present in 20% of the patients who underwent VCUG. A multidisciplinary management helped resolution of VUR in 53% of the patients after a mean follow-up period of 22 months (63).

Adults with VUR Diagnosed in Childhood

Studies in adults who were diagnosed with VUR in childhood revealed interesting results. In a study in 21 adults (mean age 23.9 years) with gross VUR diagnosed in infancy, proteinuria and renal insufficiency was diagnosed in 3(23%) of the 13 patients with unilateral reflux nephropathy and 2 (50%) of the 4 patients with bilateral reflux nephropathy (64). In another study of 127 adults (mean age 41 years) with VUR diagnosed during childhood, 44 (35%) had unilateral renal scarring, 30 (24%) had bilateral renal scarring, 30 (24%) had albuminuria, and 14 (11%) had hypertension. Of the 30 patients with bilateral renal scars, 25 (83%) had abnormal GFR (65).

Genetics of VUR

A genetic basis for VUR is supported by an increased incidence of VUR in children of affected parents as well as siblings of an affected child, a low incidence in African Americans, occurrence in some inherited syndromes, and a 100% concordance in identical twins and 50% concordance among dizygotic twins (35, 36, 66). In comparison to the general population, the first-degree relatives of individuals with VUR have a 30–50% increased risk of having VUR (37, 38). The inheritance is variable, with most cases demonstrating a dominant inheritance, which may be related to a single gene (67) or polygenic (68). Recessive (69) as well as X-linked inheritance has also been reported (66, 70).

Numerous studies have reported candidate regions/genes with VUR in association with other renal abnormalities and syndromes (71, 72). Renal-coloboma syndrome which is associated with VUR is caused by PAX2 mutations of chromosome 10q (73). VUR in some patients with CAKUT has been linked to mutations in ROBO2 gene (74). Weak linkage to the HLA locus has been reported (75). While targeted disruption of uroplakin III genes (an integral bladder protein) or angiotensin type II receptor genes in animals results in the phenotype of VUR, other studies in uroplakin III (76) and angiotensin II (77) have excluded linkage to these human loci. Similarly, while some studies suggested an increase in the D allele of the angiotensin converting enzyme (ACE) gene in patients with VUR (78), other studies have failed to support those findings (79).

In the first genome-wide search of VUR and reflux nephropathy, 7 European families with apparently dominant inheritance were studied. The study demonstrated linkage to chromosome 1p13 *(GATA176C01 to D1S1653);* genetic heterogeneity at chromosome 1 locus, and 12 additional loci were identified genome-wide. No significant linkage to chromosome 6p or to PAX2 was detected (80). Another genome-wide search in 31 patients from eight families from a single geographic region in southern Italy with primary VUR identified four genomic areas on chromosome 1, 3, 4, and 22, with the best results corresponding to chromosome 3 (D3S3681 to D3S1569); no common ancestral haplotype was identified., (81). The presence of several loci in both studies once again demonstrates genetic heterogeneity of VUR.

Some of the difficulties with genetic studies in VUR relate to difficulties with sample size, study design differences, the clinical variability of study subjects, difficulties in diagnosing asymptomatic carriers, change in phenotype by spontaneous resolution of VUR by the time genetic study is done, difficulties in explaining why only one side is involved, and possible role for environmental factors leading to phenotypic variability (72, 80, 81).

Natural History of VUR

Primary VUR generally improves with time, and this is attributed to the lengthening of the submucosal segment of the ureter (82). Most important factors identified with resolution of VUR are non-white race, lower grades of reflux, absence of renal damage and lack of voiding dysfunction (83). The reported rate of improvement is not consistent because of differences in patient selection, definition of resolution, duration of follow-up, and the use of one compared to two negative VCUGs to confirm resolution of VUR.

The Birmingham Reflux Study, which used a single negative VCUG, reported about 50% cessation of VUR

after 5-year follow up in medically managed moderate to severe VUR (84). In the International Reflux Study in Children, which used two consecutive negative VCUGs, the corresponding number was 25.0% (85). In a study by Wennerstrom and colleagues, the end point of reflux grade I or less was reached in approximately 75% of children after 10 years of followup (3). The rate of resolution is higher in younger children. In a 5 year followup study in children less than 5-years old, including 60% less than 2-years old, grade I VUR resolved in 82%, grade II in 80% and grade III in 46% of the ureters (5).

The rate of resolution is better in undilated as compared to dilated ureters and low grade VUR as compared to high grade VUR. The International Reflux Study in Children reported that VUR disappeared in more than 80% of undilated and about 40% of dilated ureters (2). Schwab and colleagues reported that grades I-III VUR resolve at a rate of 13% per year for the first 5 years of followup and 3.5% per year during subsequent years; whereas grades IV and V VUR resolve at a rate of 5% per year (86). In the International Reflux Study in Children (IRSC), resolution of VUR was significantly better in grade III versus grade IV VUR (87). In newborn babies with prenatal VUR, 67% of severe VUR and 78% of mild or moderate VUR resolved by the age of 2 years (74).

Many studies have reported that the resolution of VUR occurs slower in children with bilateral VUR (85–88), but no such difference has been reported by others (3). Arant and colleagues reported that in children less than 5-years old with grade I-III VUR, the resolution rate of left unilateral VUR was better than right VUR (5). There are contradictory reports on the effect of age (3, 87), recurrent UTI and bladder dysfunction (86, 89, 90), and gender (3, 87), on the rate of resolution of VUR. VUR resolution occurs earlier in black children. In one study, the mean time until spontaneous resolution in black children was 14.6 months, which was significantly shorter than the mean of 21.4 months in white children (15). A systematic review of published literature on the resolution of VUR, concluded that increasing age at presentation and bilateral VUR decrease the probability of resolution, and bilateral grade IV VUR has a particularly low chance of spontaneous resolution (88).

VUR & Renal Scarring

VUR predisposes to pyelonephritis in patients with UTI, and the two are associated with renal scarring. Hodson and Edwards in 1960 associated VUR with chronic pyelonephritis (91)), which subsequently came to be known as reflux nephropathy (92). Studies have revealed that more than 75% of children under the age of 5 years with febrile UTI have acute pyelonephritis (93–96), and renal scarring occurs in 10% to 64% of all children with febrile UTI (97–100). The two most important factors that are believed to influence the probability of renal injury in patients with VUR are the severity of the VUR and the age at the onset of UTI. The role of VUR, initially proven in piglets (1), has been reported in multiple clinical studies (101–103). Moreover, children with higher grades of VUR have an increased likelihood of developing renal scarring (6, 104–106). Young age at the onset of UTI is a major determinant of renal parenchymal injury in the presence of VUR. Renal injury is more common in infants because of their unique renal papillary morphology which permits intrarenal reflux (107, 108).

The International Reflux Study reported that renal injury is more frequent in children less than 2 years old, particularly in the presence of high grade VUR (109). Ditchfield and colleagues reported that the risk of renal injury is higher in children less than 2 years old, partly because of the greater severity and prevalence of VUR (110). Many studies have reported significant improvement in renal function and growth after antireflux surgery in infants operated for VUR in the first 2 years of life (111–113) or in babies before a UTI develops (114). A comprehensive literature review on febrile UTI in children by the Committee on Quality Improvement (Subcommittee on Urinary Tract Infection) of the American Academy of Pediatrics identified children less than 2 years old as being at highest risk of renal injury with febrile UTI (115). The risk is aggravated by diagnostic challenges, non-specific clinical presentation, difficulties in getting urine specimens, and a higher prevalence and severity of VUR in smaller children. Other factors that affect the probability of renal scarring in children with VUR and UTI include delayed treatment of UTI (116), recurrent UTI (117, 118), and bacterial virulence (119, 120). Finally, there is evidence that genetic factors predispose patients with VUR to scarring, as demonstrated by studies of angiotensin converting enzyme (ACE) gene polymorphisms (78, 121).

Prevalence of cortical defects with febrile UTI in the presence of VUR is about 45% as compared to 24% in those without VUR (122). A prospective study on a population-based cohort of 1221 children aged 0–15 years with symptomatic UTI revealed primary scarring during initial evaluation in 86% of the boys and 30% of the girls. Girls had significantly more febrile UTI recurrences and acquired renal scarring than boys. The authors concluded that most boys had primary, probably congenital, VUR-associated

renal damage, whereas most girls had acquired scarring related to recurrences of febrile UTI (123).

The possibility of congenital rather than acquired renal scarring due to UTI is also supported by the presence of renal parenchymal defects in children (1) with VUR diagnosed during follow-up for antenatal hydronephrosis and no history of UTI (31, 124, 125), (2) presence of dysplastic changes in kidneys believed to be scarred due to VUR and UTI (126), and (3) the presence of VUR and renal scarring without UTI in siblings of an index patients with VUR (37).

Furthermore, of patients that showed renal scars on DMSA renal scans that were done 6 months after the initial episode of pyelonephritis, none of the patients with grade II VUR had an abnormal DMSA scan, whereas 30% and 67% of those with grade III and IV, respectively, had abnormal scans. These percentages are similar to the number of abnormal DMSA scans in children with prenatal VUR and renal dysplasia (127).

These observations raise some doubts about the accuracy of previously published data on reflux nephropathy because it is quite possible that many such patients had preexisting renal injury that had nothing to do with UTI. It is very likely that the inclusion of such patients in the studies in the past may have falsely increased the number of patients reported to have reflux nephropathy.

Mechanism and Complications of Renal Injury

After acute pyelonephritis, renal scarring takes about 1–2 years to develop (128, 129). Shindo et al. reported that the mean time from discovery of VUR to the appearance of a renal scar was 6.1 years (130). Several studies have shown that scarring develops at the same site as previous infection (131, 132). The exact pathogenesis of renal scarring following acute pyelonephritis is not well understood. According to Roberts and colleagues, the acute inflammatory response that is meant to eradicate the invading bacteria is also responsible for early renal parenchymal damage and subsequent scarring (133). The process is an inflammatory response, with chemotaxis and phagocytosis, release of lysosomal enzymes and superoxides, production of peroxide and hydroxyl radicles, tubular ischemia, and reperfusion injury (134–136).The fibrosis that follows is initiated mainly by macrophages (137). Cytokines produced by these cells, which include transforming growth factor-beta 1, platelet-derived growth factor, and fibroblast growth factor, attract and stimulate proliferation of fibroblasts that are ultimately responsible for collagen production and scarring (138). Explanations for progressive parenchymal renal injury even after VUR has ceased include autologous tubular antigens, hyperfiltration of intact nephrons, reaction to Tamm-Horsfall protein, superoxide production, and persistent hypertension (139). Hyperfiltration of intact nephrons causes glomerulosclerosis, activation of rennin-angiotensin system, and a gradual deterioration of renal function (140, 141) Studies have indicated that the mesangial alterations occur early in the course of reflux nephropathy, even before proteinuria is detectable by routine analysis (142).

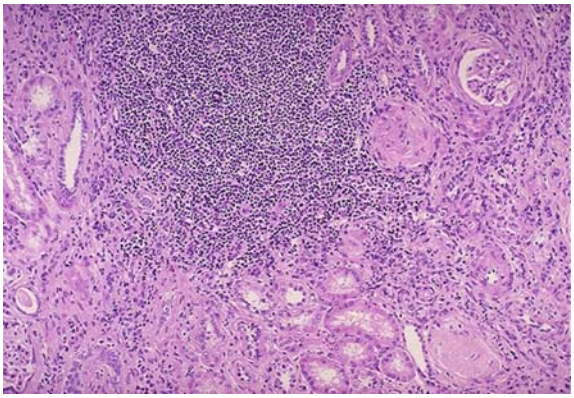
Significant bilateral renal scarring my lead to end-stage renal disease. Reflux nephropathy is responsible for 12–21% of all children with chronic renal failure (143, 144).According to the North American Pediatric Renal Transplant Cooperative Study (NAPRTCS) annual report of 2006, 536 (8.4%) of the 6405 children with chronic renal insufficiency had reflux nephropathy, which, according to the registry, is the fourth most common cause of chronic renal insufficiency after obstructive uropathy, renal aplasia/hypoplasia/dysplasia, and focal segmental glomerulosclerosis (FSGS) (145).

Renal parenchymal injury may cause persistent proteinuria, which may lead to nephrotic syndrome in some cases. The pathogenesis of proteinuria in reflux nephropathy is not well understood and is variably attributed to immunologic injury, macromolecular trapping and mesangial dysfunction, vascular alterations and hypertension, and glomerular hyperfiltration (146). Of these explanations, the one that is most widely accepted is that hyperfiltration in remnant nephrons result in modifications of permselectivity to macromolecules such as albumin and progression of renal disease (147, 148). The histologic hallmark in such patients is hypertrophy of surviving nephrons with focal and segmental glomerulosclerosis (149). Other glomerular changes that may occur are periglomerular fibrosis, sclerosis and retraction of the tuft towards the hilar pole with deposition of collagen within the capsular space (obsolescent glomerulus), solidified glomeruli that are reduced in size and eosinophilic with obliteration of capillaries, and necrotizing changes due to severe hypertension (*Fig. 55-2*).

Hypertension occurs in 10–30% of children and young adults with renal scarring (150, 151) and may take up to 8 years to develop (130). The exact cause for hypertension due to renal scarring is not known, but is believed to be due to segmental ischemia with increased rennin secretion and it does not depend on the severity of the scarring (99), (152, 153). Of the 306 patients entered into IRSC study, 3 had hypertension at study entry. Of the

Figure 55-2

Renal biopsy in reflux nephropathy showing glomerular solidification (obsolescence), tubular loss and substantial number of chronic inflammatory cells in the interstitium.



three, two became normotensive within 5 years of follow-up and the third one required antihypertensive medication at the end of the study. Three more patients became hypertensive during follow-up (154). In another study involving 664 patients diagnosed with VUR between 1970 and 2004, 20 (3%) developed hypertension. The estimated probability of hypertension was 2% (95%CI, 0.5–3%), 6% (95%CI, 2–10%), 15% (95%CI, 11–20%) at 10, 15, and 21 years of age, respectively. The survival analysis revealed that about 50% of children with unilateral and bilateral renal damage develop persistent hypertension at about 30 and 22 years of age, respectively. The presence of hypertension strongly correlated with the renal damage at entry (155).

An increased risk of nephrolithiasis has also been reported in children with VUR (156). Renal scarring may also cause pregnancy-related complications such as recurrent pyelonephritis, hypertension, toxemia or pregnancy, low birth weight babies, and miscarriage (157–161).

Diagnosis of VUR and Renal Injury

Ultrasonography

Ultrasonography is the initial modality for the evaluation of post natal hydronephrosis and UTI in children. Ultrasound has replaced the use of intravenous pyelography for the evaluation of the upper tract (162) and has the benefit of being free from contrast agents and radiation. Ultrasonography has been used to identify other urologic abnormalities, including renal and/or ureteral dilation associated with high grades of VUR (163, 164). Ultrasound is normal in lower grades of VUR and overall, it is poor for identifying VUR (165). Ultrasound has also been utilized for the follow-up of children after surgical correction of VUR (166). Ultrasonography has been used as a screening tool in siblings of children with VUR to determine if high grade reflux is present.

Efforts to use ultrasound for the primary diagnosis of VUR have been studied. The resistive index has been used as a method to identify various degrees of VUR in patients with prior confirmed diagnosis of VUR (167), however this has not had widespread acceptance. Color flow Doppler sonography has been used to identify ureteral jets associated with reflux. Good correlation was noted with VCU (168).

Renal US has very low sensitivity for diagnosing acute pyelonephritis. In one study, US abnormalities compatible with acute pyelonephritis were reported in only 20–69% of patients with acute pyelonephritis as compared to 40–92% by DMSA scintigraphy (169). Nonetheless, it is also useful in detection of renal abscess, pyonephrosis, and abnormalities of the perinephric space. Recently, there have been some encouraging results with power Doppler ultrasonography in the diagnosis of acute pyelonephritis, with a sensitivity of 80–87% and specificity of 81% and 92% (170, 171). Renal ultrasound is not a sensitive method for diagnosing renal scars. In a study involving 159 patients who underwent DMSA renal scan as well as renal US for the evaluation of hypertension of unknown etiology. Thirty-three (21%) patients were found to have renal scars. The sensitivity and specificity of renal US, in comparison with the DMSA renal scan, in diagnosing renal scars were 36% and 94%, respectively (172).

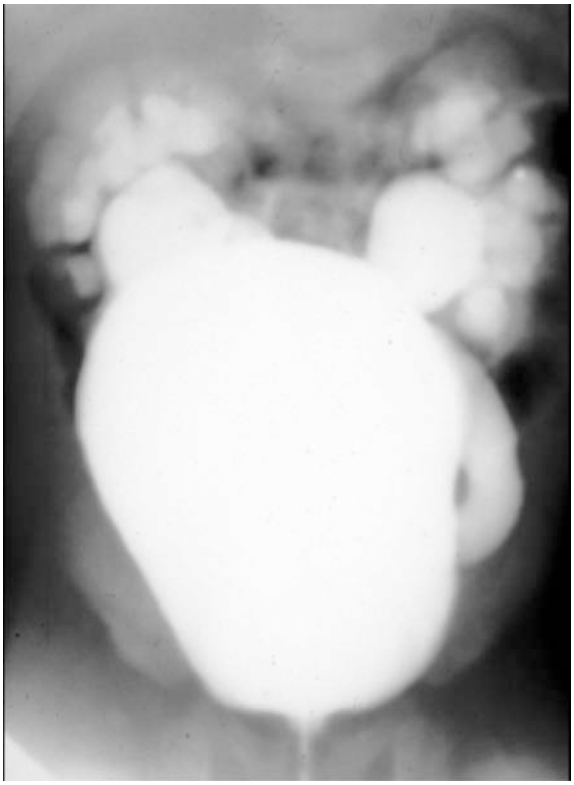
Ultrasound has been used to identify success of dextranomer/hyaluronic acid (DX/HA) injection therapy. The amount of DX/HA was measured to determine persistence of the implant in the immediate postoperative period as a correlate for resolution of VUR (173). Preservation of DX/HA on follow-up ultrasound has been not been noted to correlate with successful reflux correction (174).

Voiding Cystourethrogram

Voiding cystourethrogram is the primary diagnostic modality for the identifying VUR (*Fig. 55-3*). This is an invasive diagnostic procedure that requires catheterization. This requirement for catheterization has led to significant distress among both children and parents. The grading of VUR is based on appearance on radiographic VCUG.

Figure 55-3

Bilateral severe (grade V) vesicoureteral reflux with intrarenal reflux.



Concerns that early performance of VCUG would unmask low grades of insignificant reflux have not been borne out by studies to date (175). The VCUG should be obtained as soon as the child has completed the course of antibiotic therapy of the urinary tract infection. Delay in performance of the VCUG has led to 50% of children not getting the study leaving them at risk of undiagnosed VUR (176). Evaluation with VCU has been suggested after the first febrile infection in children younger than age five. VCUG has also been performed following successful surgical correction of VUR. The high success rates associated with open surgical correction has led to suggestion that VCUG can be avoided following open reimplantation. Avoiding VCU after open reimplantation can lead to significant cost savings (177) as well as reducing the necessity for an invasive procedure.

The results of the VCUG can be affected by size and type and position of the catheter, rate of bladder filling, height of the column of contrast media, state of hydration of the patient, and volume, temperature, and concentration of the contrast medium (178–180). Utilization of sedation to perform VCU is known to compromise the results of the study in identifying VUR, as children may be unable to void to completion when sedated for the study (181). The result of a VCUG is also affected by the number of bladder fillings during the procedure. In a study that involved multiple fillings of the bladder, Jequier reported discrepancy of presence and/or grade of VUR from one filling to the other in 12% of patients with two cycles and in 20% of patients with three cycles. No changes were observed with cyclic voiding in cases of grade IV reflux (182). The intermittent nature of VUR has been confirmed by other studies (183, 184) and was the basis for the International Reflux Study in Children using two successive negative VCUGs for confirmation of VUR resolution (85, 41). However, no prospective study has been done to study the clinical outcome differences between single versus two negative VCUGs for confirmation of VUR resolution. The clinical relevance of repeating this uncomfortable procedure in patients with no recurrence of UTI after the first negative VCUG is questionable, particularly since it is very likely that the missed VUR is most likely to be a low grade.

The timing and pressure of reflux during voiding cystourethrography has been used to attempt to predict spontaneous resolution of VUR. Arsanjani and Alagiri (185) in a recent article showed that reflux associated with filling was less likely to have spontaneous resolution as compared to reflux associated with voiding.

Follow-up of children on antibiotic prophylaxis for the management of VUR with the use of VCU has been studied to determine if an optimal time index could be identified that would balance duration of antibiotic prophylaxis with performance of multiple invasive studies. Thompson et al. have suggested that VCU every 2 years for mild reflux and every 3 years for moderate to severe reflux would lead to reduction in the number of VCUs by 42% with a subsequent reduction in cost of 33% over standard regimens and would only lead to a 19% increase in antibiotic use (186).

Nuclear cystography has been utilized to reduce the radiation exposure for children during follow-up of VUR. Nuclear cystography, while being more sensitive, does not permit specific grading of VUR or reveal anatomical defects such as ureterocele or diverticulum. This is a very useful study in determining resolution of reflux during follow-up or after surgical correction.

The major concern with the VCUG has been the radiation exposure. However, recent introduction of low-dose radiographic equipment, including digital fluoroscopy, has helped reduce radiation exposure significantly (187, 188).

Technetium 99 m Dimercaptosuccinic Acid Renal Scan (DMSA Renal Scan)

DMSA is currently the accepted gold standard for diagnosing acute pyelonephritis (189, 190) and renal scarring (191, 192) (*Fig. 55-4*). Single-photon emission computed tomography (SPECT) DMSA scintigraphy is superior to planer imaging for detection of renal cortical damage (193, 194). DMSA scintigraphy is more sensitive than ultrasound, intravenous urography or CT scan in diagnosing renal scarring (169, 172, 195). The sensitivity of DMSA scintigraphy in experimentally induced acute pyelonephritis in a pig model was reported to be 92%, when compared to histological findings (196). Sensitivity of more than 92% and specificity of more than 98% has been reported in the clinical setting (197, 198). By using standardized criteria for its interpretation, high levels of intra- (95.9% and 90.6% respectively, $p < 0.05$) and inter-observer agreement (84.4%, $p < 0.05$) were reported (199).

An abnormal DMSA scan during a febrile UTI allows the identification of children at risk of developing renal scars (200). For acute pyelonephritis, DMSA scintigraphy can be performed within 2–4 weeks (122, 201) after the onset of UTI symptoms. For renal scarring, DMSA scintigraphy should ideally be performed 6 months after acute infection in order to allow acute reversible lesions to resolve (202).

Dysplasia secondary to congenital reflux will appear similar to renal scarring following post natal infections. A baseline DMSA scan in a child presenting with VUR will allow identification of renal dysplasia and permit subsequent identification of scarring following an infection (203).

Recently, Jodal et al. have suggested the use of DMSA renal scanning as an initial study in the evaluation of urinary tract infections in children. This "top down" approach has been suggested as a way to identify significant VUR in children that have a presenting urinary tract infection. DMSA renal scanning was able to identify 26/27 children that had VUR (204). Since the studied cohort included all children with UTI that would have gone on to have VCU, that authors concluded limiting VCU to those children that had positive DMSA scans would save many children from requiring VCU. The benefit of using DMSA renal scanning as a screening tool in this manner should be balanced with the fact that at many institutions, DMSA scanning is an expensive study.

Figure 55-4
DMSA renal scan showing bilateral reflux nephropathy (R > L).

PIC Cystography

Positional Instillation of Contrast (PIC) cystography has been used to identify children with "occult" VUR that present with recurrent febrile UTI in whom standard and nuclear cystograms fail to reveal VUR (205). Under general anesthesia, the ureteral orifice is identified and contrast is injected directly at the ureteral orifice with fluoroscopic evaluation to identify VUR. Treatment of children with VUR identified on PIC cystography that had resolution of VUR, did not have febrile infections develop during immediate follow-up (205). This method for evaluation and management of VUR remains controversial. Proponents point to reduction in the numbers of febrile infections following identification and resolution of "occult" VUR (206).

Magnetic Resonance Imaging (MRI)

Recently great interest has developed in MRI for the diagnosis of renal scars because it allows discrimination between areas of swelling from scarring, both of which would be interpreted by DMSA scintigraphy as renal scarring. MRI also allows diagnosis of other coexisting conditions such as nephrolithiasis (207, 208), which is not diagnosed by DMSA scintigraphy. Newer imaging methods that show promise in diagnosing renal scarring include dynamic contrast-enhanced MRI (209), and MRI using a gadolinium-enhanced short-tau inversion-recovery (STIR) sequence (210, 224). However, routine use of MRI is less practical because of limited availability, need for prolonged sedation, and high cost.

Biomarkers of Renal Injury

Numerous non-invasive biomarkers have been investigated for their usefulness in early diagnosis and monitoring of the progression of renal scarring. These include proteinuria, renal tubular enzymes such as N-acetyl-beta-d-glucosaminidase (NAG) (211), renal tubular brush-border antigens (BBA) (212), epidermal growth factor (213), cytokines

such as interleukin (IL)-8 (214) and IL-6, soluble tumor necrosis factor (TNF) receptor-1 (215), endothelin-1 (216), and prostaglandin E2 (217). Low molecular weight (LMWP) proteins, such as beta 2-microglobin (B2M), retinol-binding protein (RBP), alfa 1-microglobin (A1M) and lysozyme pass relatively freely through the glomerular basement membrane and are almost completely reabsorbed by the proximal renal tubule (218). Their urinary excretion increases in proximal tubular damage, including that due to reflux nephropathy (219, 220). An increased serum level of B2M has also been reported as being a marker of reflux nephropathy (221).

Proteinuria is a well-known predictor of renal disease progression due to reflux nephropathy (142). Proteinuria is severe when associated with focal segmental glomerular sclerosis (FSGS). A significant correlation exists between albuminuria and GFR (219). In contrast to overt proteinuria, persistent excretion of small amounts of albumin, also called microalbuminuria, is helpful in diagnosing glomerular damage at a very early stage, as has been amply demonstrated in patients with diabetes mellitus (222). In patients with reflux nephropathy, microalbuminuria has been reported at the same time as the appearance of low molecular weight (LMW) proteins in urine (219, 220), and its excretion increases with increasing severity of VUR and renal scarring (223). In a study in children with bilateral VUR with renal scarring and normal creatinine clearance, microalbuminuria was detected in 53.5% of the cases (148). The diagnosis and monitoring of microalbuminuria offers the potential therapeutic possibility of using an angiotensin converting enzyme inhibitor (ACEI) and/or an angiotensin receptor blocker (ARB) to slow the progression of renal damage (224, 225).

Medical Versus Surgical Management of VUR

For many decades, the management of VUR has been driven by the belief that VUR predisposes to recurrent UTI and renal parenchymal damage. Various treatment strategies have been used with the ultimate objective of preventing renal injury. The two main treatment modalities that have been practiced for a long time are long-term antimicrobial prophylaxis and surgical correction. Surgical correction of VUR was common until the concept of antimicrobial prophylaxis for childhood UTI was introduced in 1975 (226). Numerous subsequent studies revealed that medical management of VUR is as good as the surgical treatment and there is no significant outcome difference between the two treatment modalities.

In the International Reflux Study in Children (IRCS), which included 306 patients, no significant difference in outcome was found between medical or surgical management in terms of the development of new renal lesions or the progression of established renal scars (227). Similar results were reported by Birmingham Study (84). In the International Reflux Study Group in Europe, 287 children with severe VUR were randomly allocated to medical (n = 147) or surgical (n = 140) groups. Follow-up with DMSA renal scans for a period of 5 years revealed no difference in outcome between children managed surgically or medically (109).

In its final report, the IRSC compared long-term outcome of medical versus surgical management in 252 children less than 11 years old with non-obstructed grade III/IV VUR, history of UTI, and a GFR of $\geq$ 70 ml/min/1.73 m^2. The follow-up period was 10 years. UTI recurrences and renal growth were similar in the two groups except that medically treated group had more febrile infections. The report concluded that with close supervision and prompt treatment of UTI recurrence, children in medical or surgical group did equally well (154).

In 1997, American Urologic Association (AUA) published its guidelines on the management of VUR in children. The recommendations were based on a systematic review that involved 168 articles on VUR that were published from 1965 to 1994. The seven treatment modalities that were studied included (1) intermittent antibiotic therapy, (2) bladder training, (3) continuous antibiotic prophylaxis, (4) antibiotic prophylaxis and bladder training, (5) antibiotic prophylaxis, anticholinergics and bladder training, (6) open surgical repair, (7) endoscopic repair. The key outcome measures were resolution of VUR, risk of pyelonephritis and scarring, and complications of medical versus surgical management. The study panel recommended antibiotic prophylaxis for all grades of VUR in children less than a year old because of a very high rate of spontaneous resolution. For children 1–5 years old, it recommended antibiotic prophylaxis for all grades of VUR with surgical options in grades III to V if VUR is bilateral or renal scarring is present. For children older than 6 years it recommended antibiotic prophylaxis for grades 1 and II (unilateral or bilateral) and unilateral grades III and IV, with surgical options if renal scarring is present; and surgical repair for bilateral grade III and IV, and unilateral or bilateral grade V VUR with or without scarring as the VUR has the least possibility of spontaneous resolution (228).

The Swedish Medical Research Council published its recommendations in 1999, which suggested no antimicrobial prophylaxis for VUR grade I and II at first examination,

for those with VUR grades III and IV on first examination, it recommended antimicrobial prophylaxis for 1 year at the end of which it should be discontinued if VUR grade decreased to grades 0–II. If there is no change in the VUR grade and the patient is a boy, the prophylaxis can be discontinued whereas it recommended continuation of prophylaxis or surgical intervention in such girls. For grade V VUR at all ages and bilateral grade IV in children less than a year old on the first examination, antimicrobial prophylaxis should be started, the management should be individualized, and surgical intervention should be considered if there is no change in VUR 1 year. The importance of bladder function evaluation and hygiene, and a careful follow-up of patients, including DMSA renal scan emphasized (229).

Medical management seems appropriate for VUR grades I–III unless patient has recurrent UTI, has allergy to the antimicrobial agent, or has compliance issues, For VUR grade IV, medical versus surgical management is controversial. Spontaneous resolution rate is <40% after 5-year follow up. Decision for surgical intervention depends on patient's age, renal function, duration of follow-up, and other factors as for those with VUR grades 1-III. VUR grade V has the lowest rate of spontaneous resolution, particularly in older children, and some advise surgical intervention if there is no improvement within a year, certainly if patient has recurrent infections on antimicrobial prophylaxis (230–232).

Cost Considerations in the Management of VUR

Multiple studies have evaluated the cost efficacy of the various management strategies for VUR. Using a computer based analysis, the cost of medical management was compared to upfront surgery (233). This analysis indicated that medical management was the most cost effective for grades I–IV of VUR. The presence of dysfunctional voiding in children with VUR increased the cost of management by 50% in some children due to the increased incidence of recurrent infections (234). Further extension of this model was used to compare the use of endoscopic management with dextranomer/hyaluronidase as an immediate option for management (235) and then compared to open surgical management for those failing medical management (236). Using these models, the use of dextranomer/hyaluronidase was not shown to be cost effective when compared to standard medical management, however when compared to open surgical management for those failing medical management, there was a cost benefit for those with lower grades of VUR (I–III). Similar cost benefit has been noted by other investigators, however these benefits seem to be best when lower grades of VUR are considered (237). There was no cost benefit for those with higher grades of reflux. There are increased numbers of surgical procedures – specifically endoscopic procedures – for the management of VUR indicating that this management strategy is increasingly being embraced by physicians and patients (238).

Medical Management of VUR

Since the natural tendency of the VUR is to improve with time, the medical management is generally tailored to cover the period during which the VUR is likely to cause the renal damage. The medical management of VUR involves long-term antimicrobial prophylaxis, appropriate management of voiding dysfunction and/or constipation, if present, and follow-up renal imaging to assess the resolution of VUR and the renal injury.

The antimicrobial agents most appropriate for prophylaxis include trimethoprim-sulfamethoxazole (TMP-SMZ), trimethoprim alone, nitrofurantoin, or cephalexin (115, 239). According to one report, nitrofurantoin is more effective than TMP-SMZ as a prophylactic agent (240). In view of an increasing resistance of *E.coli*, ampicillin or amoxicillin are less effective as prophylactic agents (115, 241, 242), and are not used for this purpose beyond the first 2 months of life during which period it is advisable to avoid TMP-SMZ. In toilet trained children, the medication is generally administered at bed time, though this recommendation is not evidence-based.

The prophylactic dose of antimicrobials is one-fourth to one-half of the therapeutic dose for acute infection (115, 239). The dosage for commonly used antimicrobials is shown in *Table 55-2*.

Follow-up of patients with VUR and UTI requires close monitoring for an early detection and prompt treatment of UTI, change of antimicrobial prophylaxis if the patient has recurrent breakthrough UTI, and the monitoring of the VUR by periodic VCUG examinations. The timing for follow-up VCUG is not well-defined though most practitioners do it yearly. In an effort to figure out the optimum timing for follow-up VCUG examinations, Thompson et al. derived a decision-tree model from the published data on VUR and validated the model by using a retrospective cohort of 76 patients. The authors concluded that delaying the VCUG from yearly to every 2 years in children with mild VUR (grades I and II) and every 3 years in children with moderate/severe VUR

Table 55-2
Dosage of Antimicrobial agents for UTI Prophylaxis

TMP-SMZ	TMP 2 mg per kg as a single dose OR
	5 mg of TMP per kg twice per week
Nitrofurantoin	1–2 mg/kg as a single daily dose
Cephalexin	10 mg/kg as a single daily dose
Ampicillin	20 mg/kg as a single daily dose
Amoxicillin	10 mg/kg, all as a single daily dose

(grades III or higher) yields substantial reductions in the average numbers of VCUG examinations and costs, with a modest increase in antimicrobial exposure (186).

The duration of antimicrobial prophylaxis and the potential surgical intervention depends on the age of the patient, the severity of the VUR, frequency of UTI, and the degree of renal scarring, if present. Some recommend cessation of antimicrobial prophylaxis after age 5–7 years even if the low-grade VUR persists (243).

Limitations of Antimicrobial Prophylaxis

Long-term antimicrobial prophylaxis has its limitations. It is not always effective, the breakthrough UTI rates in children with VUR range from 25–38% (84, 230). Antimicrobial resistance is a major concern with long-term antimicrobial prophylaxis. In one study, children who received the medication for more than 4 weeks in the preceding 6 months had more resistant *E.Coli* when compared to those not on such treatment (OR = 13.9; 95% CI, 8.2–23.5) (244). In another study on childhood UTI, a generalized decrease in bacterial susceptibility to common antibiotics was seen in the year 1999 when compared to those previously seen in 1991 (244). Approximately 10% of children on long-term prophylaxis have adverse reactions, the most of which occur within the first 6 months. These include gastrointestinal symptoms, skin rashes, hepatotoxicity, and hematological complications with SMZ-TMP, and mostly gastrointestinal symptoms with nitrofurantoin. More adverse reactions such as marrow suppression, and rarely Stevens-Johnson syndrome may also occur with SMZ-TMP (245, 246). Compliance for daily administration of the medication over a prolonged period of time is questionable. In one study, 97% of the parents reported compliance with low-dose daily antimicrobial prophylaxis and yet the medicine was found in only 31% of the patients' urine (247). Other concerns with long-term antimicrobial prophylaxis are the patient inconvenience with repeated follow-up VCUG examinations to monitor the VUR resolution and the cost of the procedure.

Controversies Over Antimicrobial Prophylaxis

Many studies have raised serious doubts about the relevance of long-term antimicrobial prophylaxis in the prevention of renal injury in patients with VUR. Shindo et al. observed that renal scarring, the injury presumably having been initiated by VUR, can progress despite correction of the reflux and prevention of UTI (130). Arant et al. reported that in spite of good medical management, even mild and moderate VUR can be associated with renal injury (5). In a study on 51 children (mean age 8.6 years) with VUR (grades I–IV), the prophylactic antibiotic was discontinued after a mean period of 4.8 years. After a mean follow-up period of 3.7 years, only 11.8% patients had UTI and no new scars developed in any of the patients (243). Other reports that raise doubts about the usefulness of long-term antimicrobial prophylaxis include the observation that up to half of patents with severe VUR exhibit no evidence of renal damage (9), incidence of renal scars does not always match the severity of VUR (248), and the frequency of pyelonephritis is similar with and without the resolution of VUR (230). In a retrospective study in 611 children aged less than 6 years with first UTI, patient gender and low grade VUR (I–III) were not associated with risk of UTI recurrence. Factors associated with increased risk included white race, age 3–5 years, and grades IV-V VUR. The authors concluded that antimicrobial prophylaxis in the studied patient population was not associated with decreased risk of recurrent UTI, but was associated with increased risk of resistant infections (249).

In a systematic analysis that compared antibiotics with placebo or no treatment for preventing UTI in susceptible children, Williams and colleagues concluded that most published studies to date have been poorly designed with biases known to overestimate the true treatment effect (250). Another systematic analysis, which evaluated the value of identification of VUR after a symptomatic UTI and the effects of various interventions on the occurrence of UTI and subsequent renal parenchymal damage, concluded that it is uncertain whether the identification and treatment of children with VUR confers clinically important benefit and any intervention, including antibiotic prophylaxis or surgery for VUR, is better than no treatment (251). Another systematic analysis, which evaluated the predictability of renal parenchymal damage by

diagnosing VUR in hospitalized children with febrile UTI, revealed that VUR is a weak predictor of renal damage in such children (252).

Voiding Dysfunction and Constipation

Treatment of constipation by dietary measures, behavioral therapy, and laxatives helps reduce UTI recurrence and the resolution of enuresis and uninhibited bladder contractions (60, 253). The treatment of voiding dysfunction or DES may include the use of laxatives and timed frequent voiding every 2–3 hours. Pelvic floor exercises, behavioral modification, and/or anticholinergic medication may be required. A combined conservative medical and computer game assisted pelvic floor muscle retraining decreased the incidence of breakthrough UTI and facilitated VUR resolution in children with voiding dysfunction and VUR (254). Similar results of improved outcome with medical management have been reported by others (63, 255).

Hypertension and/or Proteinuria

Appropriate management of hypertension and/or proteinuria is important to decrease the progression of renal disease. Despite the large number of available antihypertensive drugs, the angiotensin converting enzyme inhibitors (ACEI) or angiotensin II receptor blockers (ARB) are preferred ones because of their renoprotective effect. Studies have revealed that ACEI, besides lowering the blood pressure, reduce proteinuria due to reflux nephropathy (256). In a study by Lama et al. (224), 15.5% of children with VUR and reflux nephropathy had microalbuminuria. Treatment with an ACEI (captopril) for 2 years was associated with decreased microalbuminuria, whereas GFR, serum creatinine, and BP remained stable. Microalbuminuria decreased from 69.5 + 58–12.5 + 9.7 mg/l during the first 6 months of therapy and remained stable during the subsequent 18 months of treatment in most patients. Therapy with ACEI may be particularly beneficial for patients with reflux nephropathy, especially those born with D alleles of the ACE gene (225). A combination of ACEI and ARB significantly improves the renoprotective effects of ACEI (257). However, it is not known whether this antiproteinuric effect slows down the progression of the renal disease. In some patients with very poorly functioning scarred kidney and a healthy other kidney, the removal of a poorly functioning kidney may help cure hypertension (258).

Surgical Management of VUR

Surgical management of VUR has been relegated a second line management strategy for those patients that fail medical management with prophylaxis and follow-up. Current indications for surgical management of vesicoureteral reflux are recurrent infections despite compliance with a prophylactic antibiotic regimen, worsening of renal scars following a urinary tract infection or inability to comply with a prophylactic regimen. The recent introduction of minimally invasive modalities for the management of VUR, have made some clinicians consider surgical correction as a potential first line therapy. Immediate correction could potentially offset the need to keep children on prophylactic antibiotics. Although most surgical techniques have high success rates, most studies indicate that surgical correction of VUR does not prevent urinary tract infections or eventual renal scarring (259).

Endoscopic Management

Endoscopic treatment is performed in an outpatient setting and involves the injection of a bulking agent in a sub trigonal location at the ureterovesical junction in the bladder (STING procedure). With the use of cystoscopy, the ureteral orifice is identified and a special endoscopic needle is utilized to inject material under the ureter. Endoscopic correction of VUR was initially described using a porcine model by Puri and O'Donnell in 1984. This technique with the use of polytetrafluoroethylene paste (PTFE/Teflon) was noted to be very successful in the correction of reflux in children (260).

Unfortunately questions were raised regarding the migration of particles of Teflon to other organs in the body (261). These concerns led to restriction of the use of Teflon in the United States. Use has continued successfully in Europe with very high short and long term successes reported (262–264). Teflon has also been shown to have good efficacy in those children with high grades of reflux and those associated with other congenital anomalies e.g., Ureteroceles (262, 265).

The concerns regarding the use of Teflon in the United States has lead to consideration of other bulking agents. Experimental success with the use of blood was not met with ongoing clinical application (266). Collagen was widely utilized for the correction of reflux in children (267). Success rates were noted to be 69% at 1 year follow-up (267). Long-term follow-up however has demonstrated eventual recurrence of reflux in many children that were initially successful with the use of collagen.

Bovine collagen was utilized for VUR correction and skin testing was required to identify those that were allergic to collagen – therefore not all children were candidates. Endoscopic management with collagen was found to have a cost benefit when compared to open ureteral reimplantation (268). Atala et al. devised a implantable balloon that was used with good success in a porcine model (269). They were also able to demonstrate success with the use of alginate seeded with chondrocytes in both animal models (270, 271) and in human trials (272). This technique requires the use of chondrocytes obtained via prior biopsy of the ear, necessitating two surgical procedures with their attending anesthetic risks. Overall success was noted to be 83% (272).

Polydimethylsiloxane was used as an alternative to Teflon and was found to have no migration in animal studies (273). This agent was not approved for use in the United States but results from European centers have indicated good long-term success rates (86%) for correction of reflux (274). In 1995 Stenberg and Lackgren published the first evidence for the use of dextranomer/hyaluronidase in animals and children (275). The dextranomer microsphere implant promotes the ingrowth of collagen and maintains the increased resistance of the ureteral orifice that prevents VUR (*Figs. 55-5–55-7*). This implantable material marketed as Deflux (Q Med Inc) has become widely accepted as the injectable bulking agent of choice for the management of vesicoureteral reflux. Five year follow-up indicated success rates of 68% (276). There has not been any demonstrable evidence of particle migration with the use of Deflux (277). Significant experience has been gained with the use of dextranomer and success rates of 89% have been noted with improved implantation techniques (278, 279). These improvements in clinical results have led clinicians to utilize dextranomer for reflux associated with increasingly complex urological conditions (280) including ureteroceles, duplicated systems and those associated with prior failed reimplants. Cost analysis has indicated a benefit for the use of dextranomer when compared to open surgery for the management of VUR (236).

The success of dextranomer has led many to consider changing the management paradigm for VUR. Concerns regarding patient compliance with antibiotic prophylaxis and the frequency of urinary tract infections in some children with VUR have led to consideration of dextranomer injection as first line therapy (281, 282). One study has shown a benefit with the use of dextranomer injection in the prevention of urinary tract infections when compared to antibiotic prophylaxis (283). A recent report has also indicated reduction in urinary tract infections in those children managed with dextranomer when compared to those having reflux correction with open surgery although no concrete explanation was offered for this improved outcome (284). This management strategy has not been shown to have a cost benefit over standard medical management with antibiotic prophylaxis (235). The presence of a refluxing megaureter was felt to be a contraindication to the use of dextranomer in children (285).

Meta- analysis of all of the endoscopic modalities used for management of VUR indicate that rates of cure per ureter for grades I and II was 78.5%, grade III was 72%, grade IV was 63% and grade V was 51%. Success rates of 68% were noted for those patients failing first injection and 34% for those failing a second injection. Reduced success rates were noted for those patients with duplicated systems and had neurogenic bladders (286). The success rates and the minimally invasive nature of endoscopic procedures have led to the substantial increase in the number of procedures being performed (238).

Figure 55-5

(a) The endoscopic appearance of a right vesicoureteral reflux (b) Correction of right VUR following injection of dextranomer/hyalurnidase.

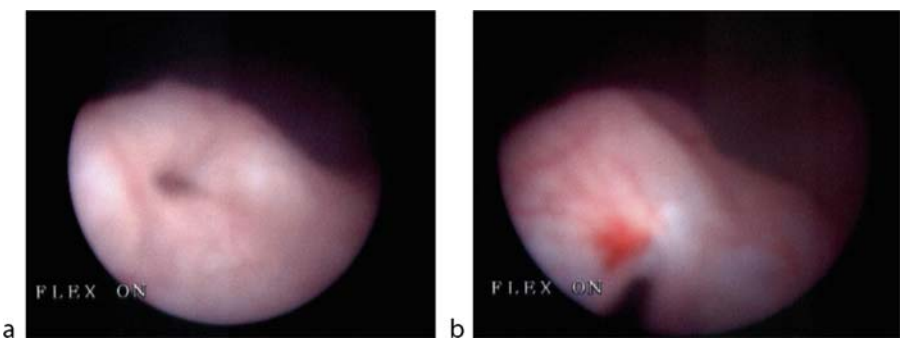


Figure 55-6

Greyscale ultrasound image of a "Deflux mound" seen in a patient after undergoing the Deflux procedure for vesicoureteral reflux.

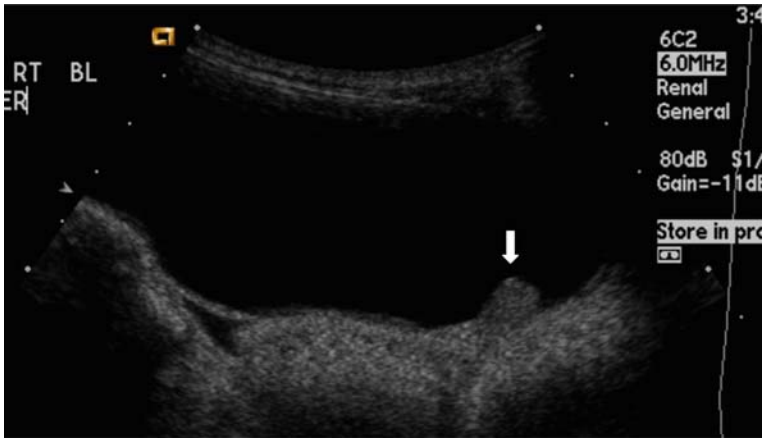
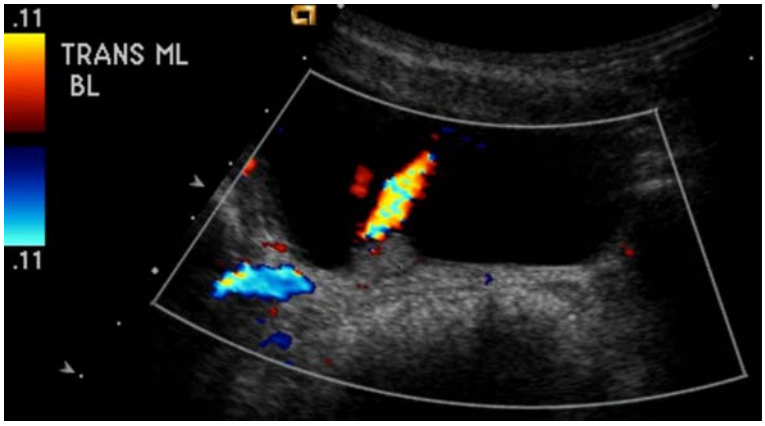


Figure 55-7

Trans Doppler ultrasound with jet ("lava from volcano") after Deflux procedure.



Persistent of VUR is considered a failure of injection therapy. Contralateral VUR is noted in 13% of children having unilateral injection therapy with dextranomer/hyaluronidase (287). There was a higher incidence in girls younger than 5 years of age. Many of these children will require repeat injection treatment or open surgical correction. The low incidence of this entity however does not justify preemptive injection of the contralateral ureter at the time of initial DX/HA injection (288). Open surgical correction for endoscopic failure has similar success rates as when performed as a primary procedure (289). Post operative ureteral obstruction has been noted in patients following injection therapy. This is a rare complication and occurs in 0.7% of patients injected. There is a higher incidence of this complication in children with voiding dysfunction and neurogenic bladder (290). Calcification of the implant of dextranomer/hyaluronic acid has been shown to mimic a distal ureteral calculus (291). Patients have been mistaken as having abdominal pain secondary to this and have had delay in diagnosis of the true cause of the abdominal pain.

Minimally Invasive Procedures

Trigonoplasty was described by Gil Vernet in 1984. This procedure was performed by placing an imbricating

stitch in the trigone and pulling the two ureteral orifices together to the midline (292). This open technique has been performed using an endoscopic technique to make it a minimally invasive procedure. While associated with reasonable initial success, long-term failure rates were high (293), and most have abandoned this approach in favor of endoscopic injection techniques.

Another minimally invasive outpatient procedure has been described with the use of small inguinal incisions to correct reflux. This method utilizes an extravesical surgical approach to the correction of VUR (see below). Although good results have been reported by a single center (294), the successful use of endoscopic bulking agents has overshadowed this and other minimally invasive techniques.

Laparoscopic Ureteral Reimplantation

Laparoscopic ureteral reimplantation has the benefits of providing results that are comparable to open surgery, with the promise of a minimally invasive approach. Initial enthusiasm has been tempered by the long operative times and potential for complications. The procedure is typically performed using a transperitoneal approach and reimplantation is performed using an extravesical technique (295) (see below). This is a technically challenging procedure in the young child and most patients require a 24–48 hour hospitalization. Intravesical laparoscopic ureteral reimplantation has also been described with good success rates. This also is a challenging procedure and requires a reasonable bladder capacity to allow the instruments to be placed successfully (296, 297). The intravesical approaches either utilize the trigonoplasty as described by Gil Vernet, or a standard intravesical cross trigonal approach as described below. Success rates are noted to be high, however, the technical challenge associated with these procedures as well as the widespread acceptance of endoscopic techniques has made these approaches less utilized.

Laparoscopic reimplantation has been associated with long operative times (298) and high cost when disposable equipment is utilized. Additionally, appropriate patient selection is critical for surgical success using laparoscopy. Ureteral injury has been noted with this procedure (295) and is typically felt to be related to the steep learning curve associated with complex laparoscopy. Additional complications that have been noted include ureteral leak in 12.5% and ureteral stricture in 6.3% (299). Overall success with correction of VUR is similar to the open procedures (299).

Open Surgical Techniques

The gold standard for the surgical management of VUR remains the open approaches. The most commonly performed open technique is the Cohen Cross Trigonal reimplantation (300). This procedure has wide applicability and can be performed even in the presence of duplicated systems and other intravesical abnormalities. The procedure involves mobilization of the ureters from their intravesical submucosal tunnels, and placement in a longer submucosal tunnel. The success rates of this procedure for the correction of reflux are 98% (301) and can so consistently be achieved that most clinicians have stopped performing follow-up studies to confirm reflux resolution (177). Improvements in surgical anesthesia and post operative pain management have allowed children to be managed with a 24–48 h hospitalization. Some children have been managed as outpatients.

The Lich Gregoir extravesical reimplantation has also been used successfully for the management of children with reflux. The ureter is dissected outside of the bladder and then placed into a submucosal tunnel by incising the bladder muscle over the ureteral path. While high success rates have been noted for the correction of reflux, extensive mobilization of the bladder when performing bilateral reimplantation has been associated with transient urinary retention, requiring intermittent catheterization in some children (302). To limit this complication, nerve sparing procedures have been developed (303). Many clinicians have restricted this procedure to those children that have isolated unilateral VUR.

Older procedures including the Politano – Leadbetter reimplantation have been successfully utilized for ureteral reimplantation in special circumstances. All of the open techniques have very high success rates for correction of VUR and have been widely accepted as the gold standard for comparison of newer modalities for management.

Following open correction, expected success rates for reflux correction are 97–99% (304). If residual VUR is noted following surgical reimplantation, continued monitoring of the patient and repeat VCU in 3–4 months may show further resolution in many. Most children that have residual VUR following open surgery, have a lower grade of VUR. Contralateral VUR is noted in 19% of patients that had unilateral ureteral reimplantation (305), the majority resolve over the ensuing 2 years and are felt to be secondary to trigonal changes following ureteral reimplantation. Ureteral stricture has also been reported and is secondary to ischemia of the distal ureter. Endoscopic access to the ureter following ureteral reimplantation can be difficult and therefore later management of stones may be difficult.

Intervention Versus Surveillance in VUR

Recently, studies have compared surveillance only with antibiotic prophylaxis in children with primary VUR. In the first such study, Garin et al. randomized study involving 236 with acute pyelonephritis in the age group of 3 months to 18 years. Included in the study were 113 children with grade I-III VUR (age group 3 months to 12 years) and 115 patents without VUR (age group 3 months to 17 years). Patients were randomly assigned to prophylactic antibiotic (SMZ/TMP) or no prophylaxis. DMSA renal scans were done to document renal scarring. At the end of 1 year no difference was noted the incidence of UTI, pyelonephritis, or renal scarring between the two groups. The authors concluded that the study did not support any role for prophylactic antibiotics in preventing the recurrence of infection or the development of renal scars after acute pyelonephritis in children with or without VUR (306).

In another study, Roussey-Kesler et al. randomized 225 children with grade I-III VUR to daily antibiotic prophylaxis (SMZ/TMP) or no prophylaxis. The age of the patients ranged from 1 month to 3 years. After a follow up period of 18 months, there was no significant difference in the occurrence UTI (17% in treatment group and 26%, in untreated control group ($p = 0.2$) between the two groups. No difference based on the grade of VUR was noted. A significant association was found between treatment and patient gender ($p = 0.017$) with significantly reduced UTI in boys, particularly in those with grade III VUR (307).

In the third study, Pennesi et al. recruited 100 children with grade II to IV VUR diagnosed after first episode of acute pyelonephritis. Patients were randomly assigned to receive SMZ/TMP antibiotic prophylaxis or no prophylaxis. The mean ages of patients in the prophylaxis and no prophylaxis groups were 9 months and 8.3 months, respectively. At the end of 2 years, prophylaxis was discontinued and patients were followed for another 2 year period, for a total follow up period of 4 years. DMSA renal scans were done to diagnose renal scars. There was no difference in recurrence of acute pyelonephritis at 2-year (36% versus 30% for prophylaxis and no prophylaxis, respectively) or 4-year period. DMSA renal scans in were abnormal in 0%, 30%, and 67% of patients with grade II, III, and IV VUR, respectively. No significant differences in renal scarring was noted at 2 years and no patients were noted to have new renal scars during the 4 year period (127).

These trials, though important, do not conclusively invalidate the role of antimicrobial prophylaxis in VUR. They were limited by lack of blindness, no use of placebo, a relatively small number of patients, urine collection by sterile bags in non-toilet trained children, exclusion of patients with high grade VUR that are normally associated with a highest risk of renal injury, relatively short duration of follow up, wide age group in one study, and not addressing the issue of interobserver variability in the interpretation of DMSA renal scans.

Conclusion

The emerging knowledge about VUR has generated a lot of interest among clinicians as well as researchers. Clinical and possibly genetic differences between VUR diagnosed during sibling screening, during follow-up for antenatal hydronephrosis or after UTI, as well as the role played by voiding dysfunction and/or chronic constipation in the resolution of VUR or the frequency of UTI, have not been completely elucidated. Prevention of renal scars in children with VUR and the preservation of renal function in those with renal scars remain the most important objectives. While in the midst of a potential paradigm shift in the management of VUR, there lies a risk of not appropriately investigating young children with UTI or not using long-term antimicrobial prophylaxis in those with VUR. It is advisable that until the results of more, appropriately designed studies, particularly placebo-controlled and double blind study such as the RIVUR (Randomized Intervention for Children with Vesicoureteral) study or any other similar study become available, that VUR and UTI continue to be considered major risk factors for renal scarring.

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56 Obstructive Uropathy

Robert L. Chevalier · Craig A. Peters

Introduction

Urinary tract obstruction initiates a complex sequence of events resulting in impaired renal function (1), and obstructive uropathy is a major cause of renal impairment in infants and children. In early development, chronic urinary tract obstruction impairs renal growth and development. In view of its clinical importance, the pathophysiology of urinary tract obstruction has been studied intensively, leading to the development of numerous experimental models, including complete and partial ureteral obstruction, unilateral and bilateral obstruction, and acute and chronic obstruction. Most experiments have been performed in adult animals: therefore comparisons with obstructive uropathy in human infants and children must be made with caution. To address this concern, studies of urinary tract obstruction have been performed in fetal or neonatal animal models. Advantages of surgical models include experimental manipulation of the severity, duration, and timing of obstruction, as well as the study of recovery following release of obstruction. The functional effects of obstruction are directly dependent on the cellular responses, with the resultant effects on renal growth. The latter are of particular importance in the period of rapid somatic growth.

Congenital Urinary Tract Obstruction: Candidate Genes

As with most congenital anomalies, those of the kidneys and urinary tract are likely to be polygenic. A number of transgenic mice have been found with phenotypes including hydronephrosis or evidence of urinary tract obstruction. These include knock-outs for genes involved in formation and patterning of the ureteric bud, ureteral differentiation, and junction formation with the bladder and pelvis (2). Mice lacking a functional gene for a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS)-1 develop fibrotic changes at the ureteropelvic junction, with a renal phenotype of obstructive uropathy (3). Mice lacking lysosomal membrane protein LIMP-2/LGP85 develop unilateral or bilateral ureteropelvic junction obstruction (UPJO) secondary to disturbed uroplakin expression (4). Deletion of most of the components of the renin-angiotensin system or calcineurin results in defective pyeloureteral peristalsis, functional UPJO, and obstructive uropathy (*Fig. 56-1*) (2, 5, 6). A mutant mouse has also been developed which develops megabladder (mgb) due to a primary defect in the development of smooth bladder muscles (*Fig. 56-2*) (7). These animals develop progressive hydroureteronephrosis and renal failure similar to patients with Posterior Ureteral Valves (PUV). Although these mutant animals have not yet revealed the primary morphogenetic mechanisms responsible for clinical congenital obstructive uropathy, they underscore the importance of a functional urinary tract in assuring normal renal development.

Effects of Urinary Tract Obstruction on the Fetal Kidney

The determination of the effect of relief of fetal urinary tract obstruction on functional development of the kidney is of primary clinical importance. Moreover, in view of the association of pulmonary hypoplasia and oligohydramnios, the effect of severe urinary tract obstruction on the development of the fetal lung is of more immediate importance to the neonatal infant. Several laboratories have attempted to develop such models in the fetus or embryo (8, 9). Ureteral obstruction in the chick embryo results in hydronephrosis, but not in dysplastic changes as are present in infants with severe degrees of obstruction (10). Ureteral ligation in the fetal rabbit results in a rapid decrease in the number of glomeruli, whereas relief of obstruction partially restores them (11, 12). The opossum has also been studied because it is a marsupial and most "fetal" development is completed in the maternal pouch rather than while attached to a placenta in utero. Unilateral ureteral obstruction (UUO) in the fetal opossum results in tubular atrophy and interstitial fibrosis, with interstitial fibroblasts appearing similar to undifferentiated mesenchyme (13). In this model, relief of UUO does not affect weight or function of the ipsilateral kidney (14). In fact, relief of obstruction tends to further decrease the

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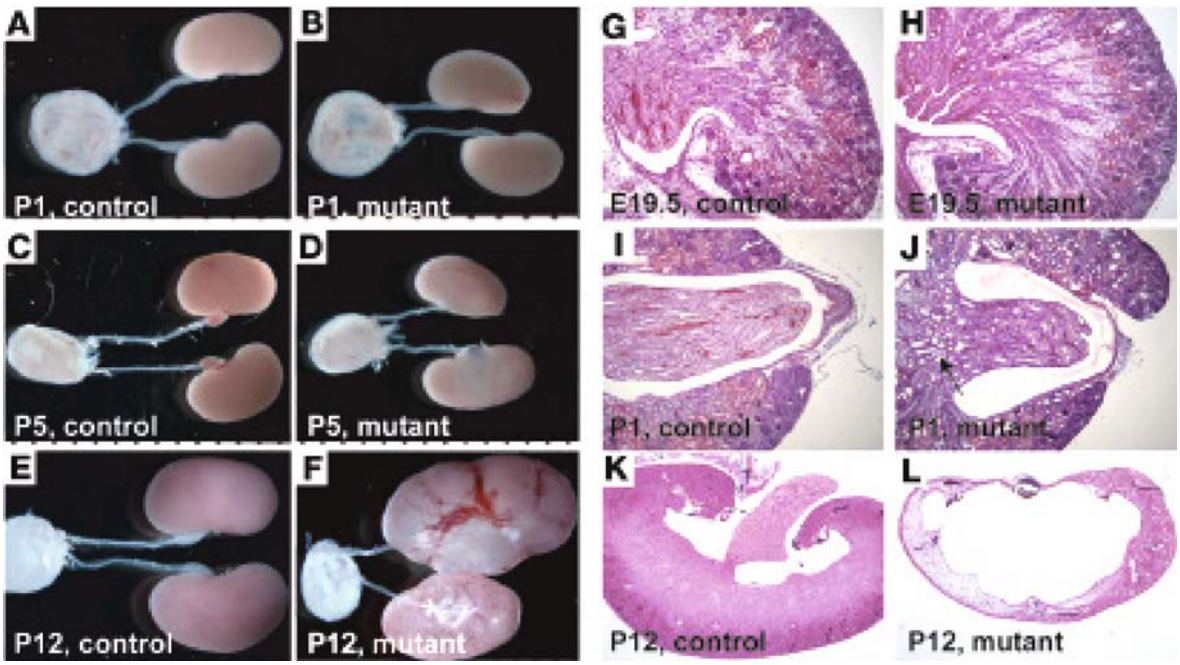
Figure 56-1

Mouse model of congenital ureteropelvic junction obstruction (UPJO) resulting from conditional deletion of *Cnb1*. (A–F) Urinary systems from controls (A, C, and E) and their littermate mutants (B, D, and F). Pictures were taken under different magnifications according to their size. The unit on the ruler is 1 mm. (G–L) H&E-stained paraffin sections from the controls (G, I, and K) and mutants (H, J, and L). Arrow in J points to a mildly dilated collecting tubule. From reference (5) with permission. (See color plate 32)



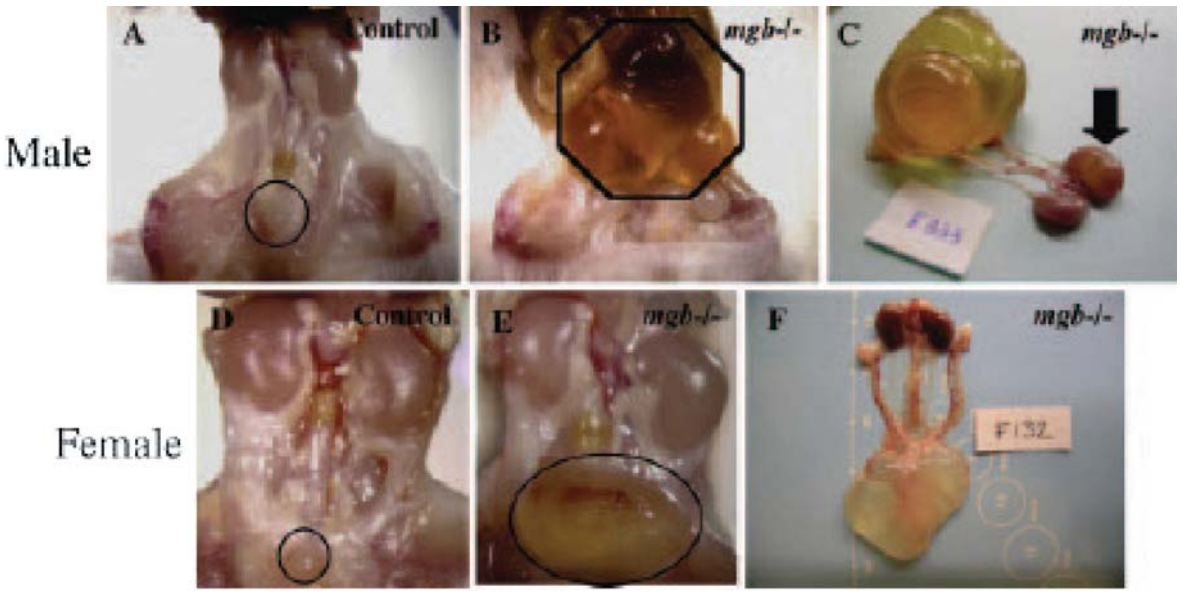
weight and function of the kidney, an effect that is ascribed to a renal inflammatory response (14). In addition, growth of the intact opposite kidney is also greater following relief of obstruction (14), results that are the reverse of those following relief of partial UUO in the neonatal guinea pig (15). As in the neonatal rat (16), the administration of insulin-like growth factor ameliorates the development of interstitial fibrosis in the fetal opossum subjected to complete UUO (17). This supports the role of growth factors in mediating or modulating the renal cellular response to UUO in the fetus; as well as postnatally.

Because of their larger size and presumed greater similarity to the human fetus, a number of investigators have studied urinary tract obstruction in the fetal sheep. Initial studies in this species revealed that urethral obstruction prior to closure of the urachus results in a persistent umbilical fistula that prevents the development of hydronephrosis (18). Ureteral ligation performed during the first half of gestation results in dysplastic changes, whereas delay in obstruction until the last half of gestation results in preservation of renal architecture with little interstitial fibrosis or inflammation (19). It is likely that the dependence of the renal response to the timing of obstruction in the fetus relates to the changing rate of cellular proliferation, which is maximal near the midpoint of gestation (20). Partial bladder outlet obstruction in the fetal sheep increases renal renin expression, and increases renal expression of the AT2 receptor (21). A greater abundance of the AT2 receptor would be expected to enhance the apoptotic response to endogenous angiotensin.

Harrison and his colleagues have described an elegant model of bladder outlet obstruction in the fetal sheep, in which the urachus as well as the urethra are obstructed (22). Relief of obstruction at 112–124 days' gestation after 15–27 days' obstruction in the fetus lessens the degree of hydronephrosis and of pulmonary hypoplasia, resulting in a marked improvement in viability (22). Ureteral obstruction in the first trimester (43–45 days' gestation) or early midtrimester (58–66 days gestation) results in marked interstitial fibrosis, primitive epithelial structures, and parenchymal disorganization typical of dysplasia (23, 24). One animal developed a

Figure 56-2

Mouse model of congenital bladder outlet obstruction. Homozygotic *mgb* mice develop megabladder and hydronephrosis. Postnatal day 17 control male mouse bladder (A, circle) *versus* male *mgb–/–* mouse shows severely enlarged bladder (B, hexagon, and C) and grossly evident hydronephrotic kidney (C, arrow). Postnatal day 12 control female mouse bladder (D, circle) *versus* female *mgb–/–* mouse shows enlarged bladder (E, oval, and F). From reference (7) with permission. (See color plate 33)



wrinkled, distended abdomen, deficient abdominal wall musculature, and undescended testes, comparable to the prune belly syndrome in man. Recovery of renal function following relief of obstruction was found to be directly proportional to the duration of intrauterine decompression, and inversely proportional to the duration of obstruction (25). As in the fetal rabbit model, relief of obstruction prior to the completion of nephrogenesis in the fetal sheep can lead to preservation of nephrons (26).

Chronic partial bladder obstruction in midtrimester in the sheep actually increases RBF and GFR, while renal architecture appears normal despite thinning of the cortex (27). These findings, which are the reverse of those described in postnatal animals, underscore the importance of the degree of obstruction in the determination of the renal response in the fetus. In contrast to bladder outlet obstruction, complete UUO in the fetal sheep decreases RBF, but not as severely as in adults (28). Complete UUO in the fetal sheep reduces ipsilateral renal blood flow (29), with a reduction in the number of glomeruli proportional to the duration of obstruction (30). Peters et al. (31) report that UUO early in gestation results in compensatory growth of the opposite kidney in the fetal lamb. This finding illustrates the operation of counterbalance in the fetus, which indicates that compensatory renal growth is not dependent on functional demand.

Experimental UPJO in the fetal monkey impairs growth of the kidney, which exhibits cystic dysplastic changes, interstitial expansion, and collecting duct apoptosis (32). A novel observation in this model is the prominent glomerular abnormality, with significant podocyte apoptosis (32, 33). Although data are limited, congenital human obstructive uropathy is also associated with glomerular as well as tubular changes (34, 35).

Effects of Urinary Tract Obstruction on Renal Growth and Maturation

Unlike the primate, the sheep, and guinea pig, in which nephrogenesis is completed before parturition, only 10% of nephrons are present at birth in the rat or mouse, with the remainder being formed postnatally (36). Thus, the early postnatal period in these species is analogous to the midgestational period in the human, and serves as a model for urinary tract obstruction during the critical period of rapid nephrogenesis. Surgical models of UUO in the neonatal rat and mouse permit the study of the direct effects of obstruction on the developing kidney,

without intrinsic defects of renal development. The use of genetically altered mice permits the elucidation of molecular mechanisms involved in the response of the developing kidney to obstructive injury (37).

A number of studies indicate that the developing kidney is uniquely susceptible to impaired growth from chronic ipsilateral (UUO). Complete UUO in the neonatal rat impairs the normal maturational increase in renal mass and the DNA content of the ipsilateral kidney (38). In the adult, in contrast, kidney DNA content is significantly increased by UUO (39). Chronic UUO in the neonatal rat results in prolonged renal expression of Wnt-4, and impairs the normal differentiation of mesenchyme to epithelium (40). Three weeks of partial UUO in the neonatal rat increases interstitial collagen deposition (primarily types I, III, and V), with an increase in type IV in the thickened and tortuous tubular basement membrane (41). The quantity of collagen deposited is directly correlated with the degree of pelvic distention (41). Renal growth impairment is dependent on the severity of partial UUO, with greater than 60% stenosis leading to a marked slowing of renal growth (*Fig. 56-3*) (42).

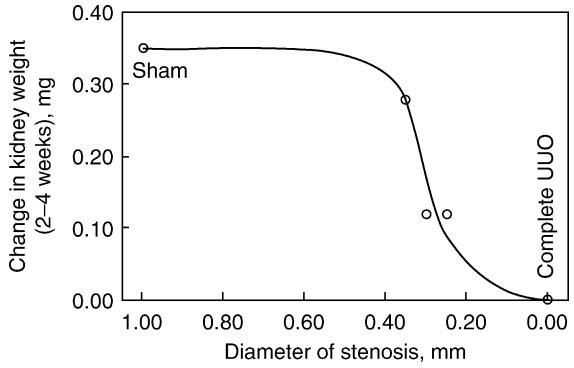
Number of Nephrons

In both human studies and animal models, fetal and neonatal urinary tract obstruction is associated with a significant reduction in the number of nephrons. Two days of UUO in the fetal rabbit reduces the number of nephrons by one third, while UUO in the fetal sheep can decrease the number of nephrons by 50% (11, 31, 43). In the human fetus, urinary tract obstruction also reduces the number of nephrons (44). Following partial UUO in neonatal pigs, the number of glomeruli is reduced by 28% (45). Either partial or complete UUO in the neonatal rat decreases the number of nephrons, with the magnitude of reduction being dependent on the duration and severity of obstruction (42, 46, 47). Interestingly, UUO in the neonatal rat in the period immediately following the completion of nephrogenesis also significantly reduces the number of nephrons, most likely due to massive cystic tubular dilatation that compresses the neighboring nephrons (47). Severe tubular dilatation also contributes to nephron loss in human obstructive uropathy (48).

Nephron loss due to urinary tract obstruction can occur through a number of mechanisms. Following relief of complete UUO in the neonatal rat, glomeruli may undergo sclerosis (49). Severe partial UUO in the neonatal rat leads to phenotypic transition of glomerular cells and delayed disappearance of glomeruli (42). In contrast, severe partial UUO in the neonatal mouse leads to apoptosis of the glomerulotubular junction and formation of atubular glomeruli (*Fig. 56-4*) (50). An improved understanding of the process of nephron loss may lead to new therapies to slow or prevent progression of renal insufficiency in obstructive uropathy.

Figure 56-3

Interval renal growth as a function of unilateral ureteral obstruction (UUO) between 14 and 28 days after surgical partial obstruction of left ureter of neonatal rat. Each point represents mean for group. Complete UUO is represented by luminal diameter = 0, while sham operation is represented by luminal diameter = 1.0 mm. From reference (42) with permission.



Renal Vascular Development

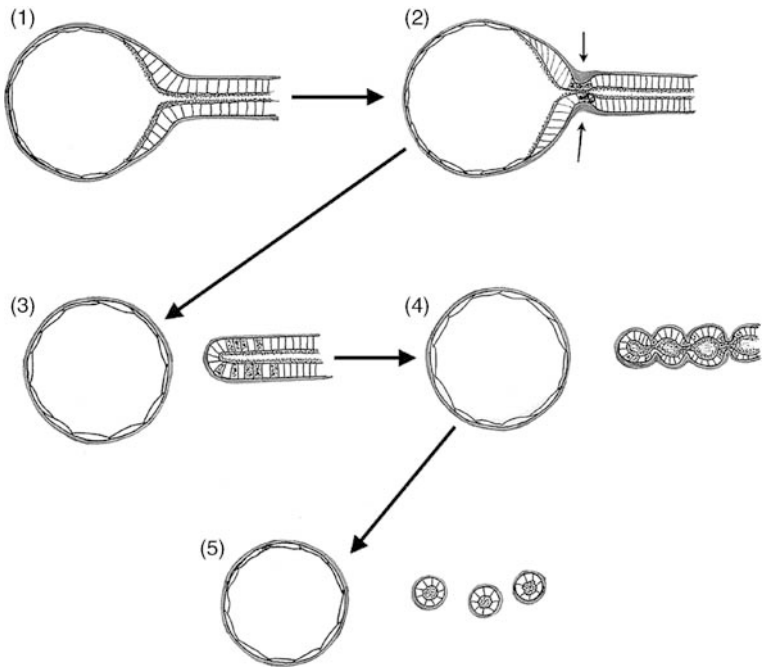
Chronic UUO in the neonatal rat leads to a marked increase in renal renin expression, and persistence of the fetal pattern of renin distribution (38, 51). It is now clear that renin expression by the renal microvasculature is a marker of vascular immaturity, rather than of terminal differentiation (52). As described below, relief of obstruction reduces the extent of renin distribution along afferent arterioles of the neonatal rat (53), and normalizes the renin content of the postobstructed kidney of the neonatal guinea pig (54). Tubular vascular endothelial growth factor (VEGF) decreases with neonatal UUO, while vascular VEGF receptors increase (55). Notably, the administration of exogenous VEGF to neonatal rats with UUO aggravates interstitial lesions (56).

Glomerular Development

Following the induction of glomeruli, glomerular capillaries increase in number, associated with flattening of

Figure 56-4

Steps in the separation of the glomerulus from its proximal tubule in the neonatal mouse subjected to partial UUO. Glomerular tuft is omitted for clarity in this diagram. (1) In the mouse glomerulus tall epithelial cells make up part of Bowman's capsule, blending into the proximal convoluted tubules (PCT). (2) Apoptotic nuclei appear in the neck region where the PCT emerges from the glomerulus, leading there to thickening of the basement membrane (between small arrows) and marked thinning of the tubule diameter. (3) The PCT becomes disconnected from the glomerulus. The severed PCT begins to undergo degeneration through a combination of epithelial cell apoptosis and necrosis. (4) Though the glomerulus remains intact, the disconnected tubule continues to degenerate, assuming an irregular shrunken profile. (5) Eventually the PCT breaks into spherical fragments, while the atubular glomerulus persists for an indeterminate time, though it may undergo ultimate resorption. From reference (50) with permission.



podocytes and increasing surface area for filtration (57). Chronic UUO in the neonatal rat either during nephrogenesis or during the subsequent maturational phase delays glomerular maturation (47, 53), a response that can be prevented by the administration of exogenous epidermal growth factor (58).

Tubular Development

Tubular maturation can be measured by the progressive disappearance of markers normally expressed only in early development, such as Wnt-4 and clusterin (glycoproteins) (40, 59), or KS (a kidney-specific gene with unknown function) (60). Chronic UUO in the neonatal rat leads to persistent tubular expression of clusterin, Wnt-4 (40) and KS (39, 60). Additionally, chronic UUO in the neonatal rat suppresses the normal maturational increase in tubular expression of EGF (38), while administration of exogenous EGF reduces clusterin expression (61).

Renal Interstitial Development

The normal maturation of renal interstitial fibroblasts is reflected by the transformation of myofibroblasts to fibroblasts, such that fibroblasts lose their expression of vimentin and alpha-smooth muscle actin (62). Chronic UUO in the neonatal rat causes prolonged expression of alpha-smooth muscle actin by interstitial fibroblasts (38), and dilated tubules from infants with severe congenital obstructive uropathy are also surrounded by myofibroblasts (63).

Summary

During normal renal development, nephrogenesis proceeds with increasing numbers of glomeruli and progressive glomerular maturation with proliferation of capillary loops. The maturing renal vasculature is characterized by the disappearance of renin from the length of the afferent arteriole, such that its localization is restricted to the juxtaglomerular region. Tubular maturation is reflected by the disappearance of vimentin and the increasing expression of EGF, while interstitial fibroblasts lose their expression of alpha-smooth muscle actin and vimentin. Chronic UUO causes a delayed or arrested maturation of the entire nephron and interstitium. The effects of chronic UUO on maturation of the developing kidney are shown in *Fig. 56-5*.

Mechanisms of Obstructive Renal Cellular Injury

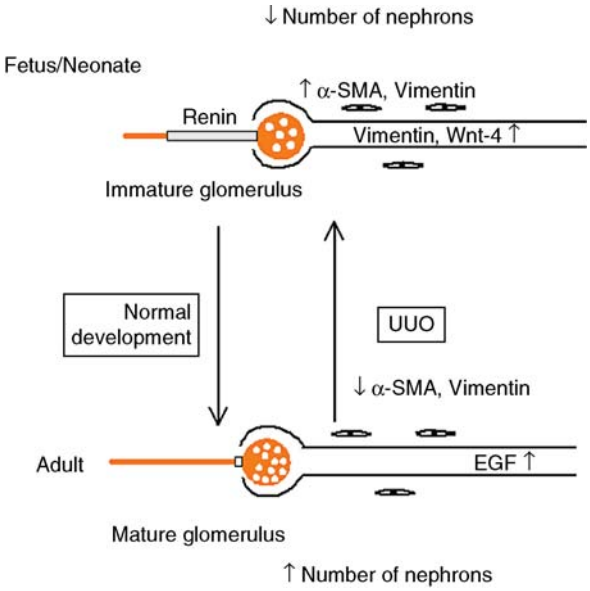
Many of the cellular mechanisms responsible for obstructive uropathy are being elucidated using models of surgically-induced UUO in mutant mice with targeted deletion of key molecules (37). These studies show that obstructive uropathy results from dysregulation of apoptosis, cellular phenotypic transition (epithelial-mesenchymal transition), interstitial inflammation, and interstitial fibrosis (64, 65). As discussed in the following sections, the creation of surgical models of UUO in the neonatal rodent has revealed the unique responses of the developing and maturing kidney to obstructive injury.

The Role of Cell Death

Urinary tract obstruction results in epithelial cell death of proximal tubules and collecting ducts. Cell death in the proximal tubule occurs by necrosis or apoptosis, whereas in the collecting duct, cells primarily die by apoptosis (50, 66). Apoptosis is characterized by condensed chromatin and cytoplasmic blebs (67, 68). Compared to the adult, UUO in the neonatal rat results in greater ipsilateral renal apoptosis (39). This may account at least in part for the fall in renal DNA content in the neonate (39). Apoptosis has also been reported in renal tubules of fetuses and infants with obstructive uropathy (69, 70). Because renal tubular apoptosis leads to tubular atrophy (67), attention has focused on factors regulating apoptosis in the hydronephrotic kidney.

Figure 56-5

Effects of unilateral ureteral obstruction (UUO) on the developing kidney. The fetal/neonatal nephron is shown in the upper panel, and the adult nephron in the lower panel. With normal maturation, the number of nephrons increases, and the glomeruli develop increased numbers of capillary loops. In addition, whereas renin extends along the afferent arteriole in the fetal kidney, renin is localized to the juxtaglomerular region of the mature kidney. During maturation, renal tubular vimentin and Wnt-4 expression disappears, and EGF expression increases. Whereas fetal interstitial fibroblasts express vimentin and α-smooth muscle actin, fibroblasts normally lose these markers with maturation. As a consequence of chronic UUO, the immature pattern of renal development persists. Adapted from reference (58) with permission.



Chronic UUO in the neonatal mouse results in apoptosis and necrosis of renal proximal tubular cells, and apoptosis of distal tubular and collecting duct cells (50). Since proximal tubules undergo significant hypoxia following UUO, it is likely that necrosis of this tubular segment is consequent to decreased oxygen tension and reactive oxygen species, as is the case following ischemic acute renal failure. Whereas the proximal tubule undergoes minimal dilatation following complete UUO in the neonatal mouse, the collecting duct is significantly dilated (66). Moreover, tubular apoptosis is directly related to the magnitude of dilatation. Since apoptosis can be triggered by the mechanical stretch of tubular cells, and is proportional to the magnitude of axial strain, it is likely that the

greater compliance of distal tubules contributes to the greater apoptosis of this tubular segment. However, partial UUO leads to proximal and distal tubular dilatation in the neonatal mouse, as is found also in the primate (32) and in human studies (48, 69).

Growth Factors

Ureteral obstruction in the adult rat decreases the expression of endogenous renal prepro epidermal growth factor (EGF) (71, 72). While EGF does not appear in the kidney until after birth (73), it is likely that activation of the EGF receptor plays a role in perinatal renal development (74). The normal developmental increase in renal EGF expression is prevented by ipsilateral UUO in the neonatal rat (38), while the administration of exogenous EGF to neonatal rats with UUO decreases tubular apoptosis in the obstructed kidney by 80% (61). In addition, EGF treatment reduces clusterin expression by renal tubular cells, and significantly reduces tubular atrophy (61). Moreover, short-term administration of EGF markedly attenuates both tubular and interstitial renal injury one month following the release of UUO in the neonatal rat (75). Although the renal expression of IGF-1 or its receptor is not altered by UUO in the neonatal rat, the administration of exogenous IGF-1, like EGF, significantly reduces renal tubular apoptosis, tubular atrophy, and interstitial fibrosis (16). Chronic UUO induces transient tubular expression of heparin-binding EGF, which can be induced also in tubular cells subjected to stretch (76). Since heparin-binding EGF is also anti-apoptotic, it is likely that expression of this growth factor serves to minimize apoptotic damage in the hydronephrotic kidney.

The mechanism whereby growth factors reduce apoptosis in the hydronephrotic kidney has been localized to the phosphorylation state of BAD, a proapoptotic molecule. When stretched, rat tubular cells undergo apoptosis, which can be reduced by 50% by treatment with either EGF or IGF-1(77). Either UUO in vivo or cell stretch in vitro decreases tubular cell BAD phosphorylation, which is inhibited by growth factors (77). When dephosphorylated, BAD dissociates from cytoplasmic chaperone proteins and complexes with BclX on the mitochondrial membrane, resulting in the release of cytochrome C and the initiation of apoptosis. EGF and IGF-1 activate their respective kinases to maintain BAD phosphorylation and thereby increase cell survival. However, in contrast to the rat and man, EGF *potentiates* tubular cell death in the neonatal mouse, a species-specific response mediated by elevated Src activity in mouse tubular cells (78, 79). These observations underscore the importance of understanding the mechanisms of cell signaling in the species being used to test therapeutic agents for potential implementation in human disorders. Stretching of human renal tubular epithelial cells increases their susceptibility to tumor-necrosis factor-α (TNF-α)-induced apoptosis, which is caspase-dependent (80); inhibition of TNF-α activity in the rat with UUO reduces caspase activity and tubular apoptosis (81).

Although transforming growth factor-β1 is generally considered a fibrogenic cytokine (see below), it also plays a significant role in promoting apoptosis in the hydronephrotic kidney (82). Stretching of tubular epithelial cells stimulates their expression of TGF-β1, and induces their apoptosis, a response that can be inhibited by antibody to TGF-β1 (83). The stretch-induced apoptotic response may therefore result at least in part from increased production of this pro-apoptotic cytokine. The action of TGF-β1 can be attenuated by an endogenous renal proteoglycan, decorin. Mice lacking a functional decorin gene manifest increased tubular apoptosis and tubular atrophy following UUO (84). Another member of the TGF-β superfamily, bone morphogenetic protein-7 (BMP-7) is also produced by the kidney, and acts as a survival factor by suppressing tubular apoptosis. Administration of exogenous BMP-7 attenuates tubular apoptosis and interstitial fibrosis following relief of UUO (85). In addition to its anti-inflammatory role, BMP-7 reverses TGF-β-dependent epithelial-mesenchymal transition (EMT) of renal tubular cells by reinduction of E-cadherin (86).

Other Molecules Modulating Apoptosis

Chronic UUO induces the renal expression of a number of apoptosis-promoting molecules, including Fas, Fas ligand, TNF-R1, TRAIL, TRADD, RIP, caspases, FADD, and FAP (87, 88). The rise and fall of expression of these molecules parallel changes in renal apoptosis in the hydronephrotic kidney. The presence of a nonfunctioning Fas receptor in C57B16/lpr mice reduces distal tubular apoptosis resulting from chronic UUO (89). Similarly, p53-deficient mice exhibit a significant reduction in apoptosis following UUO (90). These findings underscore the importance of apoptosis-regulatory molecules in the hydronephrotic kidney. Complete UUO in the fetal opossum kidney induces tubular apoptosis that is associated with an increase in Bax, a pro-apoptotic molecule, and a decrease in Bcl-2, an anti-apoptotic molecule (91). In the neonatal rat subjected to UUO, renal tubular Bcl-2 expression is reduced in dilated apoptotic tubules,

but not in nondilated tubules (92). This is consistent with endogenous Bcl-2 normally inhibiting tubular apoptosis, which is dysregulated in the obstructed kidney. Gene therapy with hepatocyte growth factor (HGF) increases Bcl-2 expression, and inhibits tubular apoptosis in experimental UUO (93). Whereas osteopontin stimulates the accumulation of interstitial macrophages in the obstructed kidney (thereby contributing to interstitial fibrosis), this phosphoprotein also inhibits tubular apoptosis, and maintains tubular integrity (94). In contrast, death-associated protein kinase (DAPK) mediates tubular apoptosis in mice with UUO, but inhibits interstitial fibrosis (95). These observations underscore the complexity of cell signaling in the obstructed kidney, such that molecules serving a protective role in one renal compartment may aggravate injury in another compartment. While tubular cells themselves generate molecules regulating their own apoptosis, infiltrating cells can do so as well. Chronic UUO in mice deficient in selectins (an adhesion molecule) have reduced interstitial macrophage infiltration, along with decreased tubular apoptosis and tubular atrophy (96).

The sphingolipid ceramide has been shown to induce apoptosis in the kidney (97). The very high prevalence of renal apoptosis in the normal developing rat is associated with elevated levels of intrarenal ceramide, and both ceramide production and endogenous renal apoptosis decrease to adult levels during the first month of life (98). Prolonged UUO in the neonatal rat (but not the adult rat) increases endogenous renal ceramide, and probably contributes to the prolonged renal apoptotic response of the neonatal obstructed kidney (99). Chronic UUO results in a maladaptive downregulation of endogenous renal antioxidant enzymes (100), and tubular catalase is protective against both tubular apoptosis and interstitial fibrosis due to UUO (101). Treatment with antioxidants may therefore have therapeutic benefit in slowing the progression of obstructive uropathy.

Summary

As shown in *Fig. 56-6*, the regulation of tubular apoptosis in the hydronephrotic kidney is extremely complex, and represents a balance between factors promoting apoptosis and countering survival factors. Stimuli for apoptosis include mechanical stretching of tubular cells which results from the initial increase in hydrostatic pressure in a compliant tubular segment. Infiltrating macrophages contribute to apoptosis of tubular cells. Enhanced renal production of ceramide may also contribute to the increased susceptibility of the developing kidney to apoptosis. Apoptosis can be activated by oxidant injury, TGF-β1, caspases, DAPK, Fas, or p53. In contrast, growth factors such as EGF, HB-EGF IGF-1, HGF, and BMP-7 can inhibit apoptosis, and tilt the balance in favor of survival. A better understanding of the renal cell biology of apoptosis should lead to improved means of preserving renal mass in patients with obstructive uropathy.

Interstitial Infiltration and Fibrosis

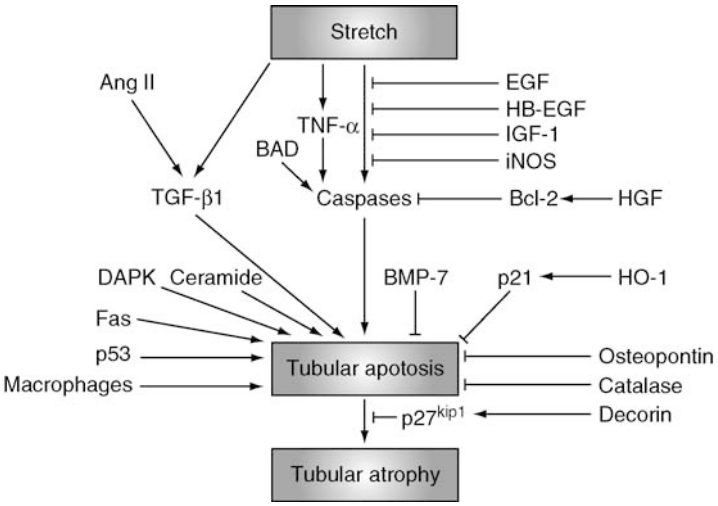
Following UUO in the adult rat, the renal interstitium of the ipsilateral kidney is infiltrated with leukocytes, beginning after 4 h and becoming maximal at 24 h (102). The cells produce a variety of vasoactive compounds, including prostaglandins, thromboxanes, and leukotrienes (103, 104). Inflammation is mediated in part by activation of the intrarenal renin-angiotensin system, with subsequent upregulation of NFκB (105), osteopontin (106), and rho/ROCK (107).

Macrophages

Although lymphocyte infiltration is not required for progressive tubular atrophy and interstitial fibrosis following UUO (108), macrophages appear to play a major role. Adhesion molecules, such as selectins, play a significant role in localizing macrophages to the renal interstitium in the developing kidney subjected to UUO (96). Mac-1 is the predominant leukocyte integrin responsible for leukocyte recruitment to the kidney after UUO (109). The macrophages, in turn, induce apoptosis of adjacent tubular epithelial cells (96), mediated by the release of soluble factors (110). Infiltrating macrophages may produce tissue inhibitors of metalloproteinase (TIMP), which in turn prevent degradation of extracellular matrix (111, 112). Following partial UUO in juvenile rats, renal expression of monocyte chemoattractant protein-1 (MCP-1) is increased (113). Since urinary excretion follows renal expression of MCP-1, and levels parallel the severity of obstruction, this molecule shows promise as a prognostic marker for infants and children with congenital hydronephrosis (114). Moreover, therapeutic inhibition of such chemokines may attenuate interstitial cellular influx and interstitial fibrosis in the hydronephrotic kidney (115, 116). While plasminogen activator inhibitor-1 (PAI-1) contributes to interstitial fibrosis resulting from UUO (117, 118), tissue inhibitor of metalloproteinase-1 (TIMP-1) does not (119).

Figure 56-6

Pathogenesis of renal tubular apoptosis in obstructive nephropathy. Several factors activated during obstructive nephropathy contribute to apoptosis of tubular epithelial cells, a major pathway to tubular atrophy. The fate of cells is determined by a balance between death signals (indicated with arrows on the left side of the figure) and survival signals (indicated by blunt arrows on the right side). Main factors and pathways identified to date are delineated, and described in the text. Axial strain on tubular cells due to stretching of dilated tubules activates caspases, and contributes to tubular apoptosis. Cytokines – such as TGF-β1 and TNF-α – released by tubular cells and macrophages provide additional apoptotic stimuli. Growth factors such as EGF, HB-EGF, IGF-1, HGF, and BMP-7 act as survival factors in most models. Ang II, angiotensin II; BMP-7, bone morphogenetic protein-7; DAPK, death-associated protein kinase; EGF, epidermal growth factor; HB-EGF, heparin binding EGF-like growth factor; HGF, hepatocyte growth factor; HO-1, heme oxygenase-1; IGF-1, insulin-like growth factor-1; iNOS, inducible nitric oxide synthase; TGF-β1, transforming growth factor-β1; TNF-α, tumor necrosis factor-α. From reference (64) with permission.



In contrast to the evidence for the role of renal interstitial inflammation in postnatal obstructive uropathy, complete UUO in the fetal sheep causes renal interstitial fibrosis without apparent inflammatory infiltrate (30). However, renal interstitial inflammation is present in human fetal obstructive dysplasia. Increases in urinary MCP-1 in rodent models as well as infants and children with obstructive uropathy suggests that inflammation is likely to contribute to the postnatal progression of clinically significant obstructive uropathy (113, 114).

Fibroblasts and Transforming Growth Factor-β

The source of interstitial fibroblasts appearing in the obstructed kidney is not clear at this time. Possibilities include proliferation of resident interstitial fibroblasts, pericytes, or transdifferentiation of renal tubular epithelial cells (epithelial-mesenchymal transition, or EMT) (120). Myofibroblast transformation, in which fibroblasts express α-smooth muscle actin, takes place in the interstitium of the obstructed kidney (121). Whereas α-smooth muscle actin normally disappears from the interstitium in the neonatal rat, this process is delayed by ipsilateral UUO (38). Myofibroblasts appear to be involved in extracellular matrix accumulation (122), and may play a role in the enhanced contractility of the renal cortex following UUO (123).

Transforming growth factor-β1 (TGF-β1), a cytokine, stimulates extracellular matrix synthesis, inhibits its degradation, and induces the expression of α-smooth muscle actin in myofibroblasts (124, 125). TGF-β1 has been localized to renal tubular epithelial cells (126) and renal interstitial cells (127, 128). Chronic UUO results in a significant increase in renal TGF-β1 expression (38, 72, 126, 127). It is possible that expression of α-smooth muscle actin by fibroblasts is controlled by the increased generation of TGF-β1 in the obstructed kidney (121, 122, 129). TGF-β1 also inhibits collagen degradation, and UUO results in a ten-fold reduction in interstitial collagenolytic activity (130).

The role of TGF-β1 in mediating renal interstitial fibrosis following UUO has been elegantly demonstrated by the introduction of TGF-β1 antisense oligodeoxynucleotides into interstitial fibroblasts (131). With the recent elucidation of TGF-β signaling, a number of studies have shown that manipulation of the downstream Smad intracellular proteins can markedly alter the severity of interstitial fibrosis resulting from UUO (64). In mice subjected to UUO, renal production of phosphorylated Smad2 and Smad3 (profibrotic Smads) is increased, while Smad7 (inhibitory) is decreased (132). Conversely, either deletion of Smad3 or gene transfer of Smad7 attenuates renal fibrosis in rodents with UUO (133, 134). Administration of exogenous hepatocyte growth factor to mice with UUO markedly reduces endogenous TGF-β1 expression, myofibroblast activation and interstitial fibrosis in the hydronephrotic kidney (135, 136). Bone morphogenetic protein-7 (BMP-7) can also counter the effects of TGF-β1 by preventing EMT (86). While it appears likely that apoptosis of renal tubular epithelial cells contributes to the development of tubular atrophy, apoptosis may also be beneficial in countering fibrosis by clearing myofibroblasts from the interstitium (137, 138). Although interstitial cells play a role in progressive interstitial fibrosis resulting from UUO, tubular cells also contribute to the fibrotic process. Administration of osteogenic protein-1 inhibits tubular apoptosis in rats with UUO, as well as inhibiting interstitial cellular infiltration (139).

Oxidative Stress

As a consequence of UUO, oxidative stress increases in the renal interstitium of the hydronephrotic kidney (140). Inhibition of oxidative stress reduces interstitial fibrosis resulting from UUO (141). Following UUO, endogenous renal antioxidant enzyme activity is reduced (100). While sodium depletion stimulates renal antioxidant enzyme production in the intact kidney, UUO inhibits this normal adaptive response (100). The impaired antioxidant production by the hydronephrotic kidney is a maladaptive response that presumably contributes to the progressive renal injury resulting from urinary tract obstruction.

The Renin-Angiotensin System

Cellular and Molecular Role of Angiotensin in Hydronephrosis

The increased generation of a pressor material in the kidney with ipsilateral UUO has been known for over 50 years. Beckwith reported that extracts of kidneys from rats with UUO induce hypertension when injected into normal animals (142). More recent detailed studies of the intrarenal renin-angiotensin system have been performed in rats subjected to complete UUO during the first 48 h of life. In contrast to kidneys from sham-operated rats, in which 55% of juxtaglomerular apparatuses contain immunoreactive renin, the fraction is increased to approximately 75% of glomeruli in kidneys of 4 week-old rats with chronic ipsilateral UUO (51). Moreover, in contrast to the pattern of distribution in sham-operated rats, in which immunoreactive renin is localized to the juxtaglomerular apparatus; renin protein extends along the length of the afferent arteriole of 4 week-old rats with UUO (51). This pattern is similar to that of the late gestational normal rat fetus (143). While renal renin content is increased in the kidney with ipsilateral UUO, it is suppressed in the intact opposite kidney (51). In contrast to the neonate, UUO in the adult rat does not result in redistribution of immunoreactive renin along the renal microvasculature.

Studies of renin secretion in vivo, or in isolated perfused kidneys, generally rely on measurements of plasma renin activity. The hemolytic plaque assay, however, permits direct measurement of hormone secretion by isolated cells (144). This technique has been used to determine whether renin secretion is increased by cortical cells from the rat kidney with ipsilateral UUO since birth. Rather than there being an increased amount of renin secretion per cell, the obstructed neonatal rat kidney demonstrates a recruitment of cortical cells secreting renin (145). It is likely that the enhanced secretion results from the increased transcription and storage of renin in the microvasculature of the immature kidney.

Following acute UUO in the adult rat, renin expression is increased after only 1 hour (146). Following 24 h UUO in the adult rat, blood vessels of the post-obstructed kidney overexpress angiotensin converting enzyme and angiotensinogen (147). In addition to increased renin production, these changes may contribute to increased intrarenal angiotensin II production following ipsilateral UUO.

Additional studies in the neonatal rat with UUO have addressed the mechanism of activation of the renin-angiotensin system in both ipsilateral and intact opposite kidneys. Renal denervation in developing rats with UUO prevents the expected increased proportion of juxtaglomerular apparatuses containing detectable immunostaining renin, as well as the extension of renin along afferent arterioles (148). In addition, denervation also suppresses the increased renin gene expression of the immature kidney with ipsilateral UUO, indicating that renal nerves modulate

renin gene expression (148). While renal nerves do not mediate the vasoconstriction of the obstructed kidney, they modulate vascular tone of the intact opposite kidney (149). Renal nerves have also been shown to play a role in the enhanced renin gene expression normally observed at the time of birth in the fetal sheep (150). Chronic UUO in the guinea pig increases angiotensin-dependent vasoconstriction in the obstructed kidney independent of renal innervation (151). However, UUO decreases angiotensin-dependent vasoconstriction of the opposite kidney, an effect unmasked by sympathectomy (151).

Modulation of growth by ANG II depends on the type of receptor present on the cell (152). Whereas the angiotensin AT1 receptor appears to mediate many forms of renal vascular and tubular growth (153, 154), activation of the AT2 receptor has been shown to inhibit cell growth (152, 155), and even to induce apoptosis (156, 157). AT1 receptors are present along the afferent and efferent arterioles, proximal tubules, and the luminal surface of the distal nephron (158). Although renal AT1 and AT2 receptors are downregulated following acute UUO in the neonatal rat, the AT1 receptor is upregulated after 28 days of UUO, at a time when intrarenal angiotensin II is increased (159). El Dahr et al. have shown that renal angiotensinogen, angiotensin converting enzyme, and ANG II are also increased in the obstructed kidney (160). It is possible that increased ANG II levels contribute to upregulation of AT1 receptor levels, as has been described in the proximal tubule (161). UUO in the neonatal rat reduces proliferation and stimulates apoptosis at least in part through the activation of AT2 receptors (162). In addition, ANG II stimulation of AT2 receptors promotes clusterin expression in the neonatal obstructed kidney (163).

The quantitative contribution of angiotensin to interstitial fibrosis resulting from neonatal UUO has been demonstrated using mice having zero to four functional copies of the angiotensinogen gene (164). Renal interstitial collagen in neonatal mice with chronic UUO increases linearly with angiotensinogen expression, from a fractional area of 25% in zero-copy mice to 54% in two-copy mice (164). Thus, angiotensin regulates at least 50% of the renal interstitial fibrotic response in obstructive uropathy. The remaining fibrotic response may be due in part to endogenous renal production of tumor necrosis factor-α (TNF-α) (165), whose expression is greater in the neonatal kidney than the adult kidney following UUO (166).

Angiotensin-Induced Molecules

In addition to its direct actions as a growth factor, angiotensin also stimulates other growth-related compounds, including TGF-β1 (167), platelet-derived growth factor (PDGF) (168), osteopontin (169), adhesion molecules (170) and α-smooth muscle actin (168). These have been associated with deposition of collagen, the development of interstitial fibrosis, and tubular atrophy and dilatation (168).

The increase in TGF-β1 in the obstructed adult kidney is regulated by ANG II (126, 128, 171, 172), and intrarenal injection of a transgene expressing angiotensinogen antisense mRNA in rats with UUO reduces renal TGF-β1 production and collagen accumulation (173). In addition, ANG II has been shown to convert TGF-β1 to its active form (174). While the administration of enalapril *reduces* hydronephrosis and proteinuria following 21 days of partial UUO in the weanling rat (175), inhibition of endogenous angiotensin with enalapril or losartan within the first 20 days of life in rats with partial UUO actually *exacerbates* the renal lesions (176, 177). This is because angiotensin is necessary for normal renal development and maturation: caution must therefore be exercised in administering angiotensin converting enzyme inhibitors or angiotensin receptor blockers in the early postnatal period.

Whereas α-smooth muscle actin normally disappears from the interstitium in the neonatal rat, this process is delayed by ipsilateral UUO (38). Since ANG II exerts transcriptional regulation of the human α-smooth muscle actin gene (178), the persistent α-smooth muscle actin expressed by the interstitial vasculature of the neonatal obstructed kidney may be due to greater generation of tissue angiotensin resulting from recruitment of renin-secreting cells (145). It is also possible that expression of α-smooth muscle actin by fibroblasts is controlled by increased generation of TGF-β1 in the obstructed kidney (121, 122, 129).

Angiotensin and the Genesis of Obstructive Uropathy

Although the AT2 receptor mediates apoptosis induced by ANG II, this receptor also appears to be important in limiting renal interstitial myofibroblast transformation in the hydronephrotic kidney (179). Moreover, there is evidence that by interfering with the apoptosis of undifferentiated mesenchymal cells, mutations in the AT2 receptor may underlie a variety of urinary tract malformations, including obstructive uropathy (180, 181). However, such mutations could not be confirmed in all populations (182).

In addition to the importance of ANG II in mediating the renal response to UUO, this peptide may also play a role in the development of the lower urinary tract. Mice

lacking a functional AT1 receptor develop functional urinary tract obstruction similar to wild-type mice subjected to complete UUO (183). The renin-angiotensin system clearly plays a critical role in many of the processes involved in congenital obstructive uropathy, and many of its protean effects remain to be elucidated.

Renal Functional Effects of Ureteral Obstruction

Ureteral ligation initially causes increased ureteral pressure and a transient increase in renal blood flow (RBF), followed 12–24 h later by decreased RBF (184, 185). A decrease in GFR follows the decline in RBF due to ipsilateral ureteral obstruction (186). Ureteral obstruction does not affect all nephrons equally: deeper nephron function is lost at the expense of compensation by cortical nephrons (187). Following 18 h after the release of UUO in the weanling rat, however, superficial nephron GFR is reduced to a greater extent than that of juxtamedullary nephrons (188). Following either UUO or bilateral ureteral obstruction (BUO), the number of filtering nephrons increases steadily during the 1st hours following relief of obstruction (189).

The chromium EDTA technique has been used to measure GFR in adult rats with complete UUO (190). Glomerular filtration rate is reduced more severely than RBF, and following relief of obstruction of 7–14 days, recovery of GFR diminishes with the duration of obstruction (190). Scanning electron microscopic examination of the rat kidney 24 h after the release of 2 weeks of UUO reveals ballooning of tubular epithelial cells, resulting in obliteration of the lumen despite relief of obstruction (191). After 2 weeks of recovery, the epithelial lining normalizes in some tubules, but deteriorates further in others, indicating a marked heterogeneity in nephron injury (191). Tubular fluid flow is present in most nephrons 5–7 weeks after relief of 2–35 days of UUO and subsequent contralateral nephrectomy in the adult rat (192). However, the number of nephrons capable of recovery decreases with the duration of obstruction (192).

Hemodynamic Effects of Urinary Tract Obstruction

In all mammalian species studied to date, complete postnatal ipsilateral UUO results in significant renal vasoconstriction. Complete UUO in the neonatal rat reduces renal blood flow by 77% (149). It is likely that at least some of the renal consequences of UUO are the result of ischemia secondary to afferent arteriolar vasoconstriction, which develops even following single nephron obstruction (193). However, there is no catch-up growth of the ipsilateral kidney despite improved renal blood flow following relief of partial UUO in the neonatal guinea pig (15). The dissociation of renal growth from renal vasoconstriction resulting from neonatal UUO suggests that hemodynamic factors alone cannot account for the impaired renal growth. Alterations in renal growth factors (discussed above) probably contribute to these effects.

Eicosanoids

The severity of reduced RBF resulting from ureteral obstruction has prompted a thorough investigation of potential mediators of renal vasoconstriction. Much attention has focused on eicosanoids as mediators of vascular tone, and the initial increase in RBF resulting from ipsilateral UUO has been shown to be associated with increased renal production of vasodilator prostaglandins (194). The later increase in vasoconstriction, on the other hand, has been ascribed in the past to increased production of the vasoconstrictor prostanoid, thromboxane A2 (195–197). Glomeruli from rats with bilateral ureteral obstruction (BUO) produce increased amounts of PGE2, 6-keto-PGF1a and TxB2, all of which can be suppressed by angiotensin converting enzyme inhibition (198). This suggests that phospholipase A2 or cyclooxygenase is stimulated by enhanced angiotensin II formation by glomeruli from kidneys with BUO. In rats with congenital unilateral hydronephrosis, the reduction in single nephron GFR is due to a reduction in the ultrafiltration coefficient mediated by both angiotensin II and thromboxane A2 (199). Urinary excretion of PGE2 and thromboxane B2 is increased in human neonates and infants with congenital obstructive uropathy (200). A balance between vasoconstrictors and vasodilators may play an important role in determining the vascular tone of kidneys with ureteral obstruction. Increased glomerular phospholipase A2 and cyclooxygenase activity are reduced to normal when animals with BUO are treated with either angiotensin or thromboxane inhibitors (201).

An important finding that is discussed in more detail below is an increased production of vasodilator prostanoids by glomeruli from the intact kidney (as well as the obstructed kidney) of rats with UUO (202). This increased prostaglandin synthesis could be suppressed by angiotensin converting enzyme inhibition, and restored by angiotensin II, suggesting that the prostaglandin

production is angiotensin-dependent (202). The reverse may also be the case: the increased renin release by glomeruli from kidneys with UUO can be abolished by prostaglandin inhibition (194). In this regard, the administration of a cyclooygenase-2 inhibitor reduces TGF-β1 and interstitial fibrosis in the obstructed kidney (203).

Angiotensin

The role of the renin-angiotensin system in mediation of vasoconstriction resulting from ureteral obstruction is of particular interest in the maturing animal, because the activity of this system is markedly enhanced in early development. The involvement of angiotensin in renal vasoconstriction is suggested by a doubling of the renin content of the obstructed kidney during partial UUO and its normalization after relief of obstruction (54). Chronic administration of enalapril (an angiotensin converting enzyme inhibitor) to neonatal guinea pigs with partial UUO prevents the decrease in RBF and glomerular contraction normally resulting from obstruction (204, 205). This confirms the dependence of vasoconstriction on angiotensin.

Although RBF does not return to normal following relief of 5 or 10 days of partial UUO in the neonatal guinea pig, enalapril has no additional salutary effect (54). While enalapril has no effect on RBF of the intact kidney of neonatal guinea pigs with persistent contralateral UUO, following relief of obstruction, enalapril reduces renal vascular resistance of the intact kidney by 40% (54). Moreover, the vasoconstrictor response of the intact kidney to exogenous angiotensin II infusion is increased, suggesting an increased sensitivity of angiotensin II receptors in the intact kidney in response to relief of obstruction in the contralateral kidney. Thus, other factors are presumably responsible for the persistently depressed RBF of the postobstructed neonatal kidney. However, administration of captopril to rats during 1–3 weeks of UUO resulted in better preservation of kidney weight and GFR of the postobstructed kidney 3 months after relief of obstruction (206). As discussed above, angiotensin inhibitors to protect the developing kidney from obstructive injury can aggravate, rather than ameliorate renal lesions if administered during nephrogenesis or subsequent renal maturation.

Arginine Vasopressin

Plasma levels of vasopressin are elevated in rats with 24 h of BUO, but not in those with UUO (207). The increase in vasopressin in rats with BUO is most likely due to hypernatremia and hyperosmolality. Treatment of animals with a specific antagonist of vasopressin V1 receptors increases GFR of the postobstructed kidney by 60% and effective renal plasma flow more than doubles (207). These results suggest that vasopressin contributes to the vasoconstriction and renal functional impairment resulting from BUO.

Kininogen-Kinin System

Earlier studies of the adult rat following relief of 24 h of UUO showed that administration of either carboxypeptidase B (which destroys kinins) or an inhibitor of kinin synthesis does not diminish the salutary effect of captopril on RBF of the postobstructed kidney (195). This suggests that angiotensin converting enzyme inhibition does not act through potentiating of vasodilator kinins in this model. In fact, inhibition of kallikrein by aprotinin further decreases renal vasoconstriction following relief of 24 h of UUO (208). This action appears to be due to stimulation of thromboxane and angiotensin by kinins in the postobstructed kidney (208). However, kallikrein mRNA and protein are significantly reduced by chronic ipsilateral UUO in the rat (209). The role of the kininogen-kinin system in ureteral obstruction therefore remains to be clarified.

Nitric Oxide

After unilateral release of BUO in adult rats, infusion of L-arginine, the substrate for nitric oxide synthase, increases effective renal plasma flow and GFR (210). Treatment of rats subjected to UUO with enalapril prevents many of the sequelae of obstruction in part by an increase in nitric oxide generation (211). Additional effects of UUO include an increase in glomerular soluble guanylyl cyclase activity (through angiotensin II stimulation) while blocking phosphodiesterase activity (212). By increasing the production of cyclic GMP, a vasodilator, these actions would serve to counter angiotensin-dependent vasoconstriction. Endogenous nitric oxide production also counters tubular apoptosis triggered by tubular stretch secondary to UUO (213), and attenuates interstitial fibrosis resulting from UUO (214). Mice lacking functional endothelial nitric oxide synthase (eNOS) develop spontaneous tubular apoptosis and glomerulotubular disconnection similar to lesions resulting from chronic partial UUO (50, 215).

Endothelial cells also produce endothelin, a potent vasoconstrictor. Following relief of temporary UUO in the rat, abnormal responses of the postobstructed kidney to angiotensin II are mediated by endogenous endothelin (216).

Summary

Urinary tract obstruction induces marked changes in the activity of local vasoconstrictors and vasodilators, with a shift in the balance toward vasoconstriction. The role of angiotensin and eicosanoids appears to be clearly established, while that of other compounds, such as kinins and endothelial factors, remains to be elucidated. Interpretation of studies is made more difficult by the complex interactions among the vasoactive compounds, including stimulation of vasoconstrictors by vasodilators and vice-versa. In addition, the protean effects of many vasoactive compounds include the regulation of cell proliferation, apoptosis, and fibrogenesis, all of which are profoundly affected by urinary tract obstruction.

Tubular Responses to Urinary Tract Obstruction

Postobstructive Diuresis

Following the relief of BUO, natriuresis and diuresis are increased; a phenomenon described as postobstructive diuresis. Much of the effect is presumably due to an osmotic diuresis resulting from excretion of accumulated solutes. However, in addition to changes in tubular transport characteristics, altered circulating factors, such as increased circulating atrial natriuretic peptide and enhanced synthesis of prostaglandins may play a role (217, 218). In addition, altered sensitivity of the tubuloglomerular feedback mechanism also contributes to the diuresis (219). It is of interest that in addition to BUO, relief of UUO in neonates can be associated with postobstructive diuresis (220). This may be due to a relative glomerulotubular imbalance.

Sodium

Ureteral obstruction results in decreased reabsorption of sodium and water in juxtamedullary proximal tubules (188). Following relief of obstruction, sodium reabsorption is decreased in the thick ascending limb of Henle, which contributes to a reduction in medullary tonicity. Sodium transport is reduced also in the medullary-collecting duct (221). It appears that the impaired sodium transport can be explained at least in part by alteration in the lipid environment of the basolateral membrane, leading to reduced Na/K ATPase activity (222, 223). Another factor implicated in postobstructive diuresis is a "functional denervation" of the kidney following relief of BUO (224). Acutely, postobstructive natriuresis may be beneficial in providing mobilization of extracellular fluid, and reducing hypertension. However, prolonged salt wasting in some patients can lead to severe volume contraction and circulatory impairment. Chronic sodium depletion can also aggravate renal interstitial fibrosis resulting from UUO (100). Whereas sodium depletion normally stimulates endogenous renal antioxidant enzyme activity, UUO impairs this response, thereby contributing to the fibrogenic stimulus of renal oxidant injury that results from UUO (100, 140). Since infants and children with severe obstructive uropathy frequently have significant salt wasting, adequate sodium supplementation may be beneficial in slowing progression of renal interstitial fibrosis.

Urinary Concentration

As indicated above, reduced removal of solute from the thick ascending limb of Henle leads to decreased solute content of the papillary interstitium, and reduces the urinary concentrating capacity. A relative increase in blood flow to the papilla increases washout of solute, contributing further to the loss of concentrating capacity. A third factor leading to the impairment of the concentrating capacity is a reduced response of the cortical collecting duct to vasopressin. This occurs at a site distal to the generation of cyclic AMP (225). Frokiaer et al. have demonstrated that BUO and release of obstruction in the rat is associated with downregulation of the vasopressin-regulated water channel aquaporin (226, 227). Renal sodium transporters and aquaporins are downregulated in response to partial UUO in the neonatal rat, associated with increased sodium excretion and decreased solute-free water reabsorption (228). The maximal urinary concentration following administration of DDAVP to young rabbits with UUO and contralateral nephrectomy is inversely proportional to the degree of hydronephrosis (229). This may be due at least in part to the distortion of the medullary architecture: loops of Henle are splayed and atortuous 3–4 days follow after UUO in the rabbit (230). Temporary ureteral occlusion in the adult rat results in ischemic tubular necrosis in the inner and outer

medulla, which also presumably contributes to the "concentrating" defect (231). In addition, there is a disproportionate loss of juxtamedullary nephrons. The severity of the renal concentrating defect in children with obstructive uropathy is highly variable, but in some patients it can approach that of nephrogenic diabetes insipidus, leading to episodes of hypernatremia in affected infants (232). Chronic polyuria secondary to obstructive uropathy may accelerate renal injury: in weanling rats with partial UUO, chronic high urine flow rates induced by osmotic diuresis resulted in a significant decrease in GFR (233).

Potassium and Hydrogen Ion Handling

Obstructive uropathy leads frequently to defective distal tubular potassium and hydrogen ion secretion ("type 4" renal tubular acidosis) (234, 235). This can be due to impaired turnover of the sodium/potassium pump, diminished sensitivity of the distal tubule to aldosterone, or the loss of H + -ATPase in the apical surface of intercalated cells. Clinically, these changes lead to hyperkalemia and metabolic acidosis, even with unilateral obstruction, and may not resolve following surgical correction of obstruction (235–237).

Calcium and Phosphorus Handling

Experimental UUO results in reduced urinary calcium and phosphorus excretion due in part to a decreased renal response to PTH. In patients with distal renal tubular acidosis due to obstructive uropathy ("type 1"), urinary calcium excretion can be increased. Release of UUO in thyroparathyroidectomized dogs results in hypophosphaturia, due to reduced filtered load of phosphate and increased tubular phosphate reabsorption (238).

Renal Counterbalance

While the UUO model represents an extreme case of asymmetrical kidney function, the severity of urinary tract obstruction in clinical practice is often unequally distributed between the two sides. Partial UUO in the immature guinea pig results in a compensatory increase in GFR of the intact opposite kidney; the younger the animal at the time of ureteral obstruction, the greater the response (239). The concept of "counterbalance" by the intact kidney in response to the loss of function by the obstructed kidney was developed by Hinman in 1923 (240). The exaggerated adaptive response by the intact kidney of animals undergoing UUO in early development is demonstrated also by the remaining kidney following loss of renal mass in the neonate (241, 242). The dynamic nature of the response is illustrated by the attenuation of compensatory hypertrophy of the intact kidney following relief of UUO in the guinea pig at 10 days of age (15), or in the mouse at 7 days of age (50). Temporary UUO in the neonatal rat inhibits growth of the obstructed kidney, and accelerates growth of the opposite kidney in direct proportion to the duration of obstruction (*Fig. 56-7a*) (243). Similarly, renal growth is impaired (50), and contralateral renal growth increases (244) in relation to the severity of obstruction. Compensatory growth takes place at the single nephron level, as glomerular area in the contralateral kidney is proportional to renal mass (*Fig. 56-7b*). This demonstrates clearly the very fine tuning of the counterbalance mechanism in early development. Also demonstrating the plasticity of the counterbalance phenomenon is the maintenance of normal RBF and glomerular volume in kidneys of neonatal guinea pigs with ipsilateral UUO, if the contralateral kidney is removed at the time of birth (245).

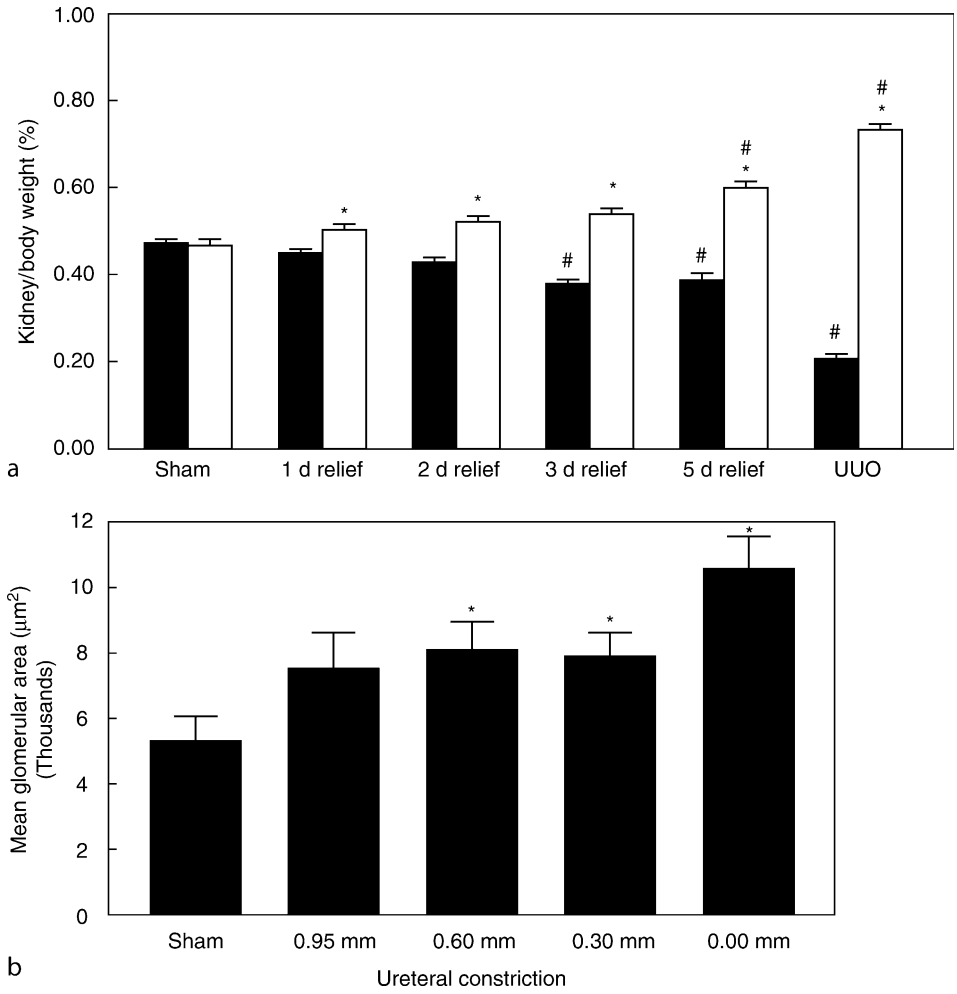
Clinical Implications of Experimental Studies of Urinary Tract Obstruction

Fetal Diagnosis and Intervention

The results of experimental fetal studies have important implications for the management of infants found to have severe obstructive uropathy in utero: prenatal intervention may allow preservation of renal mass prior to completion of nephrogenesis as well as improved pulmonary development. The major problem is the lack of accurate prognostic criteria for the identification of infants who would benefit from intervention. The primary limiting factor at present is the difficulty in visualizing the developing kidneys by ultrasonography prior to 18 weeks gestation (246). In a report of a ten-year experience with prenatal intervention for hydronephrosis due to lower urinary tract obstruction, successful vesicoamniotic shunt placement was found to aid pulmonary development, but often did not prevent renal insufficiency (247). The experience at Children's Hospital of Philadelphia was similar, with one third requiring dialysis and transplantation, although the majority reported a satisfactory quality of life (248). Significant technical advances will be required to allow the required anatomic definition, as well as the development of reliable functional parameters.

Figure 56-7

A. Kidney weight (factored for body weight) of obstructed (black) and contralateral (white) kidneys of neonatal rats 28 days following sham-operation, following relief of 1–5 days ureteral obstruction, or with persistent ureteral obstruction (UUO). *p < 0.05 vs. opposite kidney; #p < 0.05 vs. sham. There is a fine balance between the growth of the obstructed and opposite kidneys, such that combined renal mass remains constant. From reference (243), with permission. B. Mean (± SE) glomerular area vs. ureteral constriction of intact kidneys from 45 day-old rats subjected to neonatal contralateral partial UUO. *p < 0.05 vs. sham. From reference (244) with permission.



Use of Biomarkers in the Evaluation and Management of Obstructive Uropathy

The lack of reliable markers of significant urinary tract obstruction is a critical issue. A biomarker has been defined as, "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" (249). Currently utilized markers of congenital obstructive uropathy include sonographic renal pelvic diameter, quantitative diuretic renography, and markers of glomerular and tubular function (250). Numerous experimental studies have demonstrated renal activation of TGF-β1 in the hydronephrotic kidney, and TGF-β1 may provide an index of obstruction. Urinary TGF-β is increased in children with severe urinary tract obstruction, and the correlation provides 80–100% sensitivity (251–253). The urinary concentration of TGF-β1 in

the pelvis of the obstructed kidney is 4-fold than that of bladder urine, which in turn is 3-fold higher than in controls (252). Upregulation of renal MCP-1 and suppression of EGF are also a consequence of experimental UUO, and urinary MCP-1 is increased, while EGF concentration is markedly reduced in children with severe UPJ obstruction (114). While attempts to correlate renal histological changes with differential renal function have been disappointing (34, 254), in situ hybridization and immunohistochemical analysis may improve specificity. Renal TGF-β1 expression is increased and EGF decreased in patients with UPJ obstruction (255).

Isolation of urinary polypeptides by capillary electrophoresis and mass spectrometry (CE-MS) was shown to predict the outcome of infants with UPJO (256). Comparison of urinary polypeptide patterns with currently available renal imaging studies allowed assignment of infants to a group likely to resolve and a group likely to require surgical intervention (*Fig. 56-8*). A combination of proteomics (to discover new biomarkers) and testing of candidate molecules (identified from mechanistic studies of animal models) should be complementary (257). Ultimately, a broad approach will be necessary to develop the most effective matrix of biomarkers to predict which patients will require surgical or medical intervention, and to track the evolution of the renal lesions over time. In addition to identification of molecules of interest in urine, similar identification in blood or amniotic fluid may be useful in evaluation of the fetus (258). Moreover, imaging or analysis of biopsy tissue (including immunohistochemistry or laser-capture microscopy) may provide significant advantages. Marked changes in renal structure and function follow normal development from fetus to neonate to adulthood, and biomarkers must be established for each developmental stage. In the case of unilateral obstruction, urine from the contralateral kidney may complicate detection of markers originating from the obstructed kidney, such that it may be necessary to obtain renal pelvic urine samples.

Recovery and Long-Term Outcomes

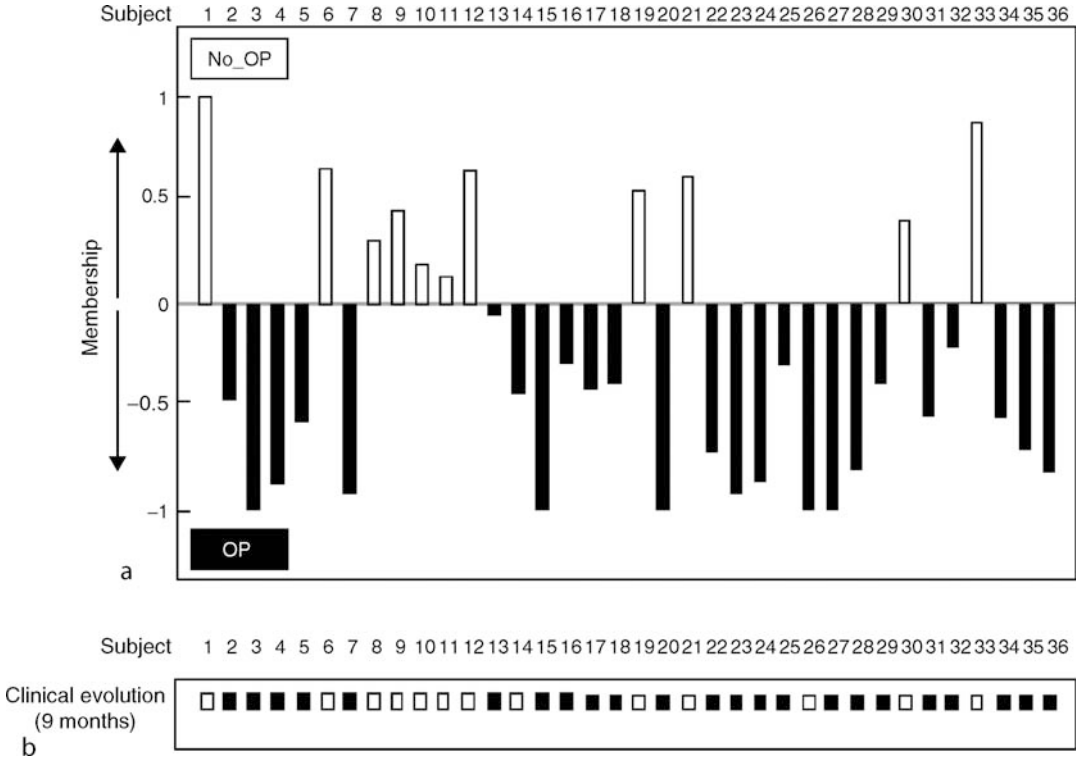
One of the primary concerns in the management of infants with obstructive uropathy is the expected recoverability of renal mass and renal function following relief of the obstruction. Temporary UUO in the neonatal rat impairs renal growth proportional to the duration and severity of obstruction prior to relief (*Fig. 56-7*) (243, 244). Although relief of complete UUO attenuates the renal cellular injury, and GFR of the postobstructed kidney is normal one month following relief (53), one year later, GFR is decreased by 80%, and proteinuria has developed (49). Importantly, glomerular sclerosis, tubular atrophy, macrophage infiltration, and interstitial fibrosis are increased not only in the postobstructed kidney, but also in the opposite kidney (49). These findings suggest that despite normal early postoperative GFR in infancy, children undergoing pyeloplasty for UPJO should be followed into adulthood. Following relief of 5 days complete UUO immediately after the completion of nephrogenesis, renal damage and loss of renal function are even more severe than those following relief of UUO during nephrogenesis (47). This suggests that if significant urinary tract obstruction is diagnosed either in the prenatal or neonatal period, its relief should not be delayed.

Timing the relief of obstruction in the postnatal period is complicated by differences in available experimental models. As described above, severe partial UUO in the neonatal guinea pig leads to arrested growth of the ipsilateral kidney, which is not improved by relief of obstruction after 10 days (15). This suggests that any delay in relief of severe neonatal obstruction can result in irreversible damage. Measurement of renal function by nuclear scintigraphy in rats studied up to one year after neonatal UUO reveals a 40% reduction in function, and a similar reduction in kidney weight (259). Early relief of neonatal partial UUO in the rat prevents the development of hydronephrosis, and reduction in renal function (260). If weaning rats are subjected to partial UUO, the morphologic effects are less severe than those resulting from UUO within the first two days of life (261). If partial UUO is delayed even longer (performed at 6 weeks of age), the impairment in GFR is minimal and does not progress over 15 weeks despite the presence of hydronephrosis (262). Thus in the rat, as in the guinea pig, susceptibility of the kidney to injury from ipsilateral ureteral obstruction decreases with age, while recoverability depends on the duration and severity of obstruction.

In the neonatal mouse subjected to moderate partial UUO, proximal tubular apoptosis leads to glomerulotubular disconnection, a process that can be arrested by relief of obstruction (50) (*Fig. 56-4*). Relief of severe partial UUO does not result in recovery. Atubular glomeruli are also found in the kidneys of children with obstructive uropathy, as well as in a variety of renal disorders, including pyelonephritis and cystinosis (263). Since glomerulotubular disconnection is presumably irreversible, a better understanding of the mechanisms of nephron loss is of critical importance in the development of improved interventions and therapies.

Figure 56-8

Membership of urinary proteomic patterns of 36 newborns with intermediate ureteropelvic junction obstruction (UPJO) and the clinical outcome of these individuals 9 months after group membership prediction. (a) Urinary protein profiles of individuals from the OP_Poss group (n = 36, grade 3/4 hydronephrosis and/or Pd > 15 mm and mild functional obstruction (isotopic MAG3 renogram)) were classified using a hierarchic disease model based on the polypeptides used to discriminate between healthy newborns, No_OP and OP groups. Each OP_Poss individual was scored with this model using support vector machines. This scoring results in membership values between -1 and 1. A negative value suggests evolution toward the OP profile and a positive value suggests evolution toward the No_OP profile. White bars indicate membership to the No_OP profile and black bars indicate membership to the OP profile. (b) Clinical outcome of the OP_Poss individuals 9 months after sample analysis. White squares indicate that the individual had evolved toward the No_OP group (spontaneous resolution of the obstruction) and black squares indicate that these individuals underwent surgery (OP). This resulted in 34 out of 36 correct predictions (94%). From reference (256) with permission.



The solution to this dilemma will depend on improved methods for imaging the developing kidney, as well as more reliable means of monitoring renal function. In view of the exaggeration of renal "counterbalance" in early development, the greater adaptation of intact nephrons may mask the deterioration of obstructed ones, necessitating the accurate measurement of differential renal function. Such advances, along with improved understanding of the mechanisms underlying the renal response to urinary tract obstruction during maturation, should allow intervention prior to the development of irreversible changes in remaining nephrons.

Congenital Obstructive Uropathy: Clinical Evaluation and Management

The clinical management of COU continues to be a challenge to the pediatric nephrologist and urologist due to the wide spectrum of severity, the variable clinical progression, the inability to predict long-term functional consequences, and the inability to alter established renal damage incurred during development. Nonetheless, COU is a common clinical entity and represents the largest cause of end-stage renal failure in young boys. The advent of prenatal ultrasonographic imaging has revealed a large

number of children with asymptomatic hydronephrosis, some of whom will have no long-term ill effects, yet others are at risk of severe renal damage. This section will review the common clinical entities causing COU, the basics of clinical evaluation and fundamental management principles.

Clinical Definition of Obstruction

At the heart of much of the controversy surrounding COU is a lack of agreement as to the definition of urinary obstruction in children. Obstruction present during the development of the kidney and urinary collecting system is fundamentally distinct from that acquired later in life. The effect on kidney development is something that cannot occur once the kidney has fully formed. In this way, COU must be seen and defined separately from later acquired obstruction. A common definition of obstruction is "a condition of impaired urine flow that if untreated will result in loss of renal function" (264, 265). Yet, if a child is born with an obstructive condition in which one or both kidneys are not functioning normally, it would make no sense to wait for deterioration of function to consider intervention. Impaired development, just as with pediatric growth retardation, represents a pathological condition, and if due to obstruction, intervention should be considered. Intervention may not always normalize or even improve function, but it may prevent further deterioration. This question becomes very important and controversial when approaching children with asymptomatic hydronephrosis where the uncertainty regarding the necessity of intervention is significant. Choosing between an observational or interventional approach has become the focus of current debate.

Operationally, it is the authors' view that congenital obstruction is a condition of impaired urine flow or transport that has impaired or can impair normal renal functional development (266). It would seem as this were a cautious and protective view with regard to the child's developing renal functional potential.

Ureteropelvic Junction Obstruction (UPJO)

The most common cause of COU is ureteropelvic junction obstruction (UPJO) (267). More severe versions are less common. The process is more common on the left (2:1 – left to right) and more common in boys (2:1 – male to female) (268). Bilateral obstruction is uncommon, but present in 10–40% (269), depending upon the diagnostic threshold. UPJO represents an obstructive process at the proximal portion of the ureter where the pelvis narrows into the ureter. It may be either intrinsic or extrinsic, or both. Intrinsic obstruction is considered to be an abnormality of the development of the ureteral wall and histologically is seen as disordered bundles of smooth muscle with a preponderance of extracellular matrix material (270). It may appear visibly as a simple narrowing of the proximal ureter with a dilated pelvis above, or as a tortuous segment of the ureter, often fixed to the inferior aspect of the pelvis with fibrous bands (*Fig. 56-9*). Nearly always the lumen is patent, albeit narrow and irregular. Studies have examined the possible underlying causes, including abnormal innervation, expression of various cytokines, and disordered regulatory proteins of the smooth muscle and matrix (34, 271, 272). All of these studies are interesting, yet fundamentally limited due to there being no way to determine cause and effect; they are simply associational. Similar studies have examined the distal aspect of the ureter in the context of ureterovesical junction obstruction, with similar limitations. How these

Figure 56-9

Operative view of the ureteropelvic junction (UPJ) in a child with significant obstruction. The obstruction appears to be related to several fibrous bands (arrow) and not a typical crossing vessel. The pelvis (P) is dilated but ureter (U) itself does not appear markedly abnormal. The UPJ is usually resected and re-anastomosed. (See color plate 34)

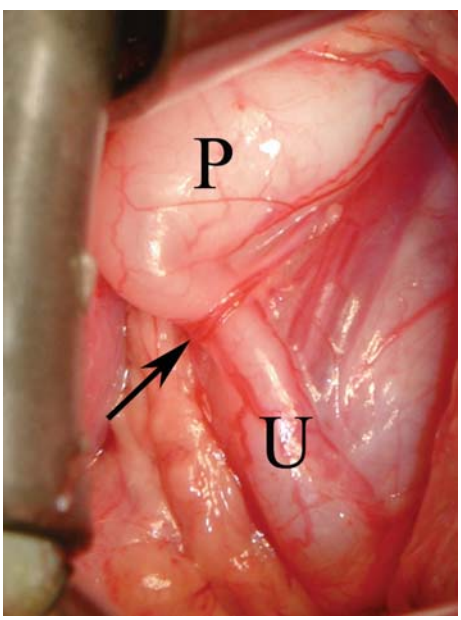
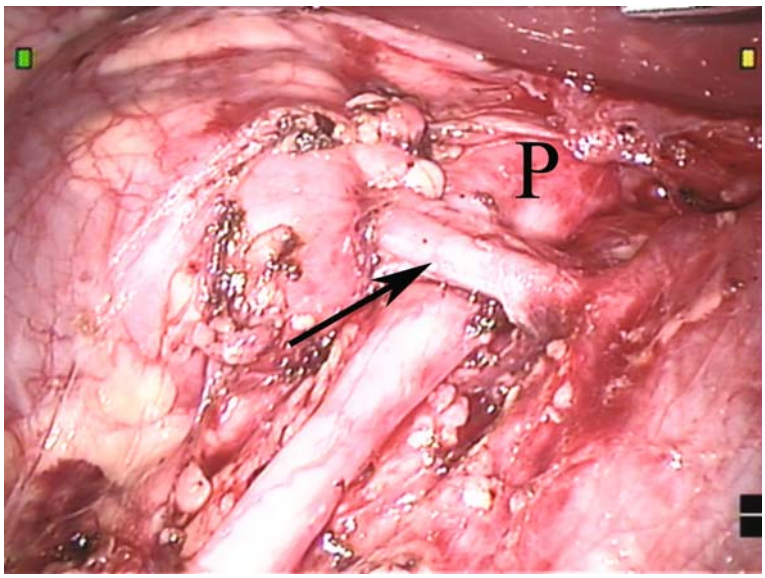


Figure 56-10

Operative (laparoscopic) picture of a lower pole crossing vessel (black arrow) associated with UPJ obstruction. At this point the pelvis (P) is not markedly dilated, but the patient experienced episodic severe pain with associated hydronephrosis. Pain resolved after surgical pyeloplasty with transposition of the ureter anterior to the vessel. (See color plate 35)



observations relate to the genetic models of UPJO remains to be determined (273).

In some instances, a clear intrinsic cause is seen in the form of a ureteral polyp, usually based just at the UPJ (274). These are fibroepithelial polyps that are entirely benign, although on occasion, pathological interpretation may indicate low-grade papilloma. In children the risk of malignant transformation is exceptionally low.

Extrinsic obstruction is somewhat more apparent, and usually represents a mechanical effect of a lower pole renal vessel, either artery, vein or both (*Fig. 56-10*) (275, 276). This fixes the ureter in position and with pelvic dilation, abrupt kinking may occur. Not all so-called "crossing vessels" are obstructive and it may be difficult to discern at times. It is also unclear as to how often the ureter is abnormal in addition to there being a "crossing vessel". This is emerging clinically in the form of surgical attempts to correct obstruction by simply moving the vessel up onto the pelvis and leaving the ureter alone (see below).

Clinical Presentation

Most UJPO is detected prenatally today, but some may still emerge with symptoms of abrupt flank pain, nausea, vomiting and dehydration. In the extreme form, this pattern, termed Dietl's crisis when bilateral (277), is characteristic, yet continues to be clinically unrecognized. Many of these children are thought to have gastrointestinal symptoms and a few have been through extensive negative workups. The pattern of abrupt onset with severe abdominal or flank pain associated with nausea and vomiting, often in the late evening is typical. Some of the children note that leaning over a chair or curling up may help. They will dehydrate and the pain subsides completely, usually within 2–4 h. The frequency and intensity of this pattern can evolve, yet may be unpredictable. If suspected, imaging with ultrasound during an episode will usually reveal the cause. When not symptomatic, the affected kidney may be normal, but usually has some degree of hydronephrosis.

Infection is an uncommon presentation for UPJO, and should prompt an evaluation for vesicoureteral reflux or bladder abnormalities. Infection in the setting of UPJO can be quite severe due to urinary stasis, and may require urgent intervention and drainage.

Prenatal detection of hydronephrosis has revealed a large population of children with an even wider spectrum of effect and usually without any symptoms (278). While mild dilation with no ureteral dilation is technically a

form of UPJO, some chose to consider this as "physiological hydronephrosis", "non-specific hydronephrosis", or a normal variant. These may be appropriate, but finding the line of demarcation is challenging. It also requires some sense of natural history of the condition, which is not always apparent. Guidelines for evaluating and managing these children remain in evolution. At present, there is a movement away from intervention, which may produce delays in treatment (279–282).

Natural History

One of the remarkable aspects of UPJO, as with ureterovesical junction obstruction (UVJO), is the potential for spontaneous improvement of the apparent obstructive effect (283–286). This is manifest by the resolution of the hydronephrotic changes and is most often seen in children with prenatally detected dilation. The mechanism of resolution, as with the mechanism of obstruction, remains undefined. The fact of resolution should not be interpreted to mean there was no obstruction, only that it was a transient effect. It may produce some negative renal consequence, although that is very difficult to determine. The possible causes include maturation of the pelvi-ureteric function as peristaltic pumps, or changes in down-stream urinary dynamics, particularly the bladder. It is known that apparent UPJO is more common in boys, who are also known to void at high pressure early in life, presumably due to sphincteric immaturity. With maturation of this system, up-stream dilation may slowly resolve. This has been indirectly shown in the fetal sheep (286), yet the correlation with humans is speculative. It is unquestioned, however, that significant hydronephrosis can resolve with an apparently normal kidney remaining. All management of asymptomatic UPJO must consider this aspect.

Consequences

The spectrum of UPJO ranges from minimal renal effects to a non-functioning kidney. The mechanisms of effect have been discussed, but it is clear that UPJO can produce severe renal impairment, both pre and postnatally. The ability to detect this effect clinically is sometimes limited and our current methods are not ideal. Biopsy studies have shown a wide range of changes and a lack of agreement between conventional clinical imaging and pathological changes (34, 35, 254, 287). Global renal functional impairment in the setting of unilateral UPJO with a normal contralateral kidney is rare, yet bilateral obstruction poses the risk of global functional impairment. Even so, some have advocated observational management, as the severity of one side may often be minimal (288). Later development of symptoms is a potential long-term consequence, and while this can be prevented with intervention, a wait and see approach is often chosen. Late onset hypertension is documented to occur, although uncommonly (289, 290). Certainly any child with hydronephrosis or surgically treated obstruction should be monitored for hypertension. On occasion, early onset hypertension will respond to surgical cure.

Ureterovesical Junction Obstruction (UVJO)

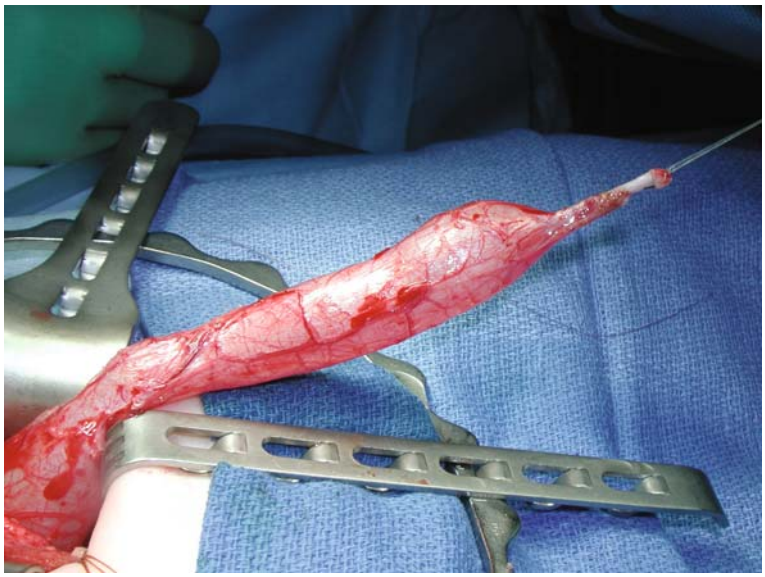
Ureterovesical junction obstruction (UVJO) represents a similar clinical process to UPJO at the distal end of the ureter. The term obstructive megaureter, or primary obstructive megaureter (POM), is often applied to this entity, and should be distinguished from a refluxing megaureter. The term non-refluxing, non-obstructive megaureter is often used, but again begs the question as to the definition of obstruction. The same controversy applies to UVJO as to UPJO. Histological studies have demonstrated changes of disordered smooth muscle and excessive, disordered extracellular matrix in resected UVJO, but again, the underlying cause remains indeterminate (*Fig. 56-11*). UVJO is less common than UPJO, representing 23% of cases of prenatally detected hydronephrosis compared with the 41% being UPJ obstruction (267). As with UPJO, UVJO is seen more often in boys and left sided involvement is more common. Bilateral involvement occurs and may pose major challenges to reconstruction and maintenance of normal renal function.

Clinical Presentation

UVJO rarely presents with pain and while it is now mostly detected through prenatal ultrasound, infection is clearly a more frequent clinical presentation. In all cases, evaluation for VUR is important as obstruction and reflux may occur concomitantly. This combination is particularly a matter of concern for severe infection. In the setting of presentation with infection, it should be borne in mind that during acute infection the magnitude of the ureteral dilation may appear far greater than when uninfected (291). This is presumably due to the effect of bacterial toxins on the ureteral smooth muscle tone. Further evaluation after the acute episode is important to clarify the pathophysiology.

Figure 56-11

Operative view of primary obstructive megaureter with narrow distal segment and abrupt transition to the dilated proximal ureter. The narrow segment will be resected and dilated ureter tapered for reimplantation. (See color plate 36)



Natural History

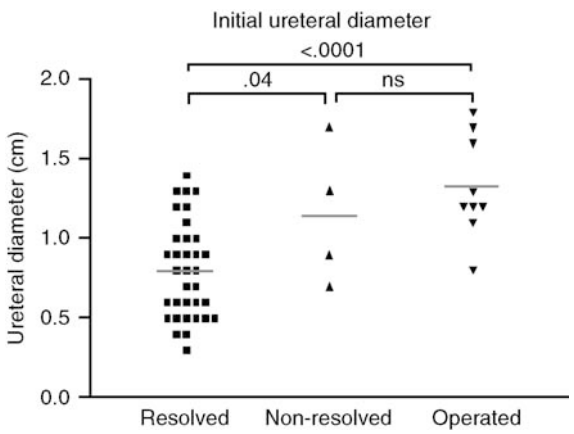
As with UPJO, UVJO can resolve spontaneously, perhaps even more readily (292–294). Similarly, however, it is difficult to predict with any precision just when and in whom it may resolve. The more severe the dilation, the longer it will likely be before resolution (*Fig. 56-12*). The pattern of resolution is clearly one of cranial to caudal, and often the distal ureteral dilation is the last to disappear. Indeed, isolated distal ureteral dilation with a normal kidney may be seen in older children and adults. It is unlikely to pose any risk. Reports of spontaneous resolution demonstrate the high degree of variability and the potential for late deterioration. The author (CAP) has seen functional decline in the affected kidney as late as 8 years postnatal, and similar reports are in the literature (295).

Implications

There are no data as to the renal pathological effects specifically of UVJO, since it is difficult to biopsy these kidneys, even at the time of surgery. It may be presumed that they would follow a similar pattern as seen with UPJO, and the limited correlation of histopathological changes with conventional clinical imaging must be considered.

Figure 56-12

Outcome of children with non-refluxing primary megaureters in relation to initial ureteral diameter. Although it cannot be stated strictly that ureters larger than 1 cm all need surgical intervention, follow-up should be more cautious. From reference (294), with permission.



Posterior Urethral Valves (PUV)

Posterior Urethral Valves (PUV) represents a very different element of COU, largely due to the usually greater severity and because it affects the entire urinary system.

The long-term implications are profound in boys and it remains a leading cause of end-stage renal failure. The ability to intervene in utero is technically present, yet the selection of appropriate cases and the long-term outcomes remain highly problematic. Unlike UPJO and UVJO, there is little controversy as to the need for intervention, and in the context of PUV, the challenge rests in maximizing renal functional development and maintaining the best level of bladder function possible. It is the impact of PUV on bladder functional development that can pose the most significant impediment to maintaining maximal renal function and can impact on functioning of a renal transplant in the setting of renal replacement. The details of these management strategies are beyond the scope of this chapter, but it is essential that any child with PUV be recognized as having potentially serious bladder dysfunction (296–298).

Description

PUV has been recognized as a pathological entity since the mid-1800s, although the clinical implications were best described by HH Young in 1919 (299). PUV occurs exclusively in boys, and any bladder outlet obstruction in a girl would be a distinct entity and very rare. The name is probably not appropriate as the obstructive process is not a valve and the term COPUM (congenital obstructive posterior urethral membrane) has been proposed (300, 301). It is unlikely to catch on and PUV continues to be the best understood term. PUV are seen as a web of abnormal tissue emanating from the veru-montantum in the posterior urethra with an opening inferiorly and fusing anteriorly to look somewhat like a spinnaker sail (*Figs. 56-13* and *56-14*). It is presumed to be an abnormal remnant of the site of ureteral budding, although this is speculative. The typical PUV or Type I of Young's classification, is located at the distal aspect of the veru, but a second type (Type III) can be seen distal to the sphincter and often is a concentric web with a tiny opening, or even a "wind-sock" appearing structure. Young described a Type II valve from the bladder neck to the veru, yet these are likely just folds of non-obstructing prostatic tissue made apparent by the dilation of the prostate from Type I valves or from a sphincteric abnormality.

The upstream effects of developmental obstruction are associated with PUV and these may be profound. In the most severe cases, renal development is very abnormal (302), there is massive hydronephrosis and ureteral dilation, and the bladder is markedly thickened from smooth muscle hypertrophy and matrix deposition (303). The bladder neck may appear markedly thickened and some have considered it to be obstructive in the past, even to the point of resecting it. In fact, it is simply made more evident by the dilation of the bladder above the posterior urethra below, and is not likely to be functionally obstructive. The clinical evidence of ongoing bladder dysfunction has come to be recognized as a manifestation of secondary bladder dysfunction from the PUV and not from bladder neck obstruction (298, 304).

There is a mortality associated with PUV, although it has decreased markedly in recent decades. In the 1950s, 50% of children with the diagnosis of PUV would die in childhood. Today that number is likely to be close to 1%, and those probably represent children with pulmonary insufficiency due to the most severe degree of obstruction associated with oligohydramnios and pulmonary hypoplasia (305).

This aspect of PUV has challenged the perinatal caregiver and prompted ongoing attempts at fetal decompression to permit enhanced lung and kidney development (247, 248, 306–308). These efforts are still evolving,

Figure 56-13

Posterior urethral valves as evidenced on a voiding cystourethrogram with massive left vesicoureteral reflux. The bladder is not markedly trabeculated but the posterior urethra is dilated. The valve leaflets are indicated by the white arrow. The very thin caliber of the urethra is indicative of the severity of obstruction and limited flow, but is of normal caliber.

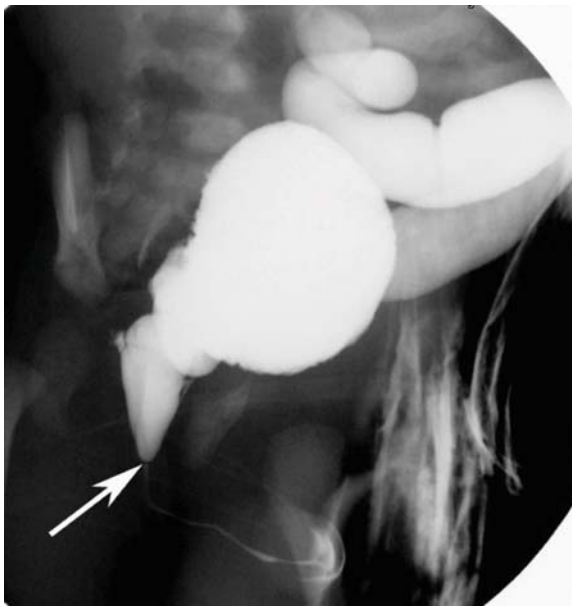
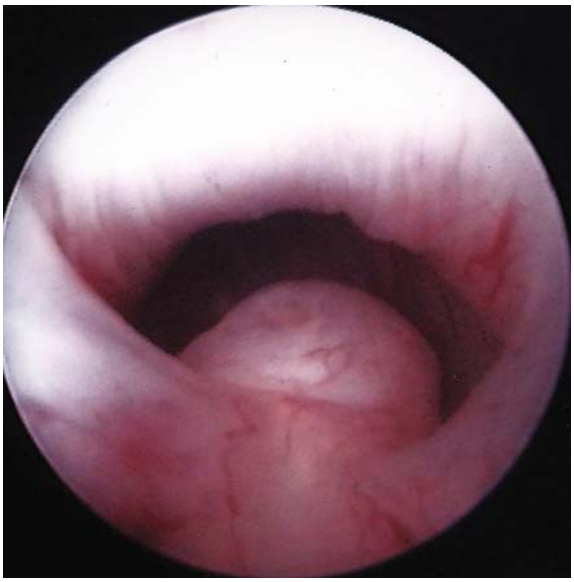


Figure 56-14

Endoscopic image of posterior urethral valves with the veru montanum in the center and the posterior valve leaflets emanating from it laterally and distally. The anterior aspect of the valve is not visible in this image. (See color plate 37)



particularly in the area of patient selection for salvageable renal function. The pathophysiological mechanisms of oligohydramnios-associated pulmonary hypoplasia remain incompletely defined (309–311), yet the association is clear, and these children with PUV are most at risk. Even without pulmonary hypoplasia, renal functional impairment is a significant risk, and it may be slow to emerge.

Clinical Presentation

PUV is often detected prenatally, yet even today many cases are not identified until later in childhood. The severity of the obstruction is a major determinant of the likelihood of prenatal detection and the subsequent delay in clinical diagnosis. The wide spectrum of severity makes this challenge even more difficult. Prenatally, the identification of a male fetus with bilateral hydronephrosis, particularly if the ureters are dilated, and with bladder dilation or thickening, should trigger consideration for PUV (312–315). The status of the amniotic fluid is important as it is related to lung development. The character of the renal parenchyma can predict renal functional outcomes to some degree. Sonographically "bright" kidneys in the setting of PUV may be associated with early renal insufficiency, although the precision of these estimates is poor (313). The pattern of change during gestation may offer some insight as to the outcomes, particularly with rapidly enlarging renal units. The definitive diagnosis of PUV cannot be made with certainty prenatally, but it should be considered probable until proven otherwise. Unless there is consideration for fetal intervention, there is little change in obstetric management that would be undertaken except the site of delivery. Early delivery has not been shown to offer benefits and exposes the child to the risks of prematurity. Evaluation at birth will usually reveal the underlying cause.

In older children, the clinical presentation may be widely variable, ranging from urosepsis and renal insufficiency, to incontinence or UTI. Milder degrees of PUV may not present until adolescence and while it had been common wisdom that late presentation was less severe, it has been clear that these boys can be severely affected (316). Suspicion for PUV should be raised in a boy with a weak stream, failure to thrive, urosepsis or UTI, or even new onset renal failure with no predisposing factors. In most cases, there will be evidence of an abnormality on renal ultrasound and confirmation of PUV by cystography can then be made.

Natural History

The natural history of PUV reflects its wide spectrum. While there are no formal studies of the outcomes of untreated valves, the clinical presentation of older boys, often with severe renal and bladder dysfunction, provides that information. The long-term implications of even acutely treated PUV highlight their natural history.

Long-Term Implications

PUV is a life-long condition (317). While a few boys will have no long-term sequelae, this should not be assumed until they have been followed for several years. For the majority, renal impairment and bladder dysfunction will be a part of their early years and have consequences into adulthood. Recognition of the sometime slow progression of both renal and bladder dysfunction in these boys is critical to any treatment program and it is essential to permit optimizing renal function and bladder control. The dynamic and interdependent nature of the renal and bladder functional impairment continues for years and must be recognized and anticipated. For example, impaired renal concentrating ability exposes the bladder

to higher urine volumes per unit time, which will further aggravate a small capacity, stiff, poorly compliant bladder, thereby raising intravesical and subsequently intra-renal pressures (318). This will only serve to further exacerbate the concentrating defect, creating a vicious cycle of dysfunction. Recognition and interruption of the cycle is essential. Infection is always a threat to both renal and bladder function, and may require a variety of interventions to protect the urinary tract and the child.

As with any condition affecting childhood renal function, PUV can have profound systemic effects on growth, development, and with the attendant medical interventions, socialization. Caring for these children is a lifelong involvement.

Basics of Clinical Diagnosis of COU

The clinical management of COU is defined by imaging. The aim of any evaluation approach is to define the anatomy and functional state of the entire urinary tract. Failure to recognize the potential for other areas of abnormality or dysfunction is a common cause for misdirected therapy. No one imaging technique is ideal in representing the state of the urinary tract or kidney in obstructive conditions, and we are constrained by their limitations. Developing a means by which to integrate these studies into our clinical management is essential to deal with the wide spectrum of severity of COU and provide reasonable treatment recommendations to patients and their families.

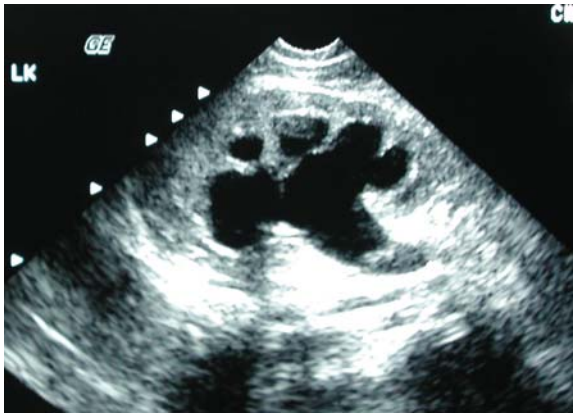
Modalities

Ultrasonography

The most common and perhaps the most versatile imaging tool today is the ultrasound. It offers a non-invasive means to obtain detailed structural information about the urinary tract with ready inference of functional data. While ultrasound is a static image and is often referred to as non-functional, appropriate inference can afford functional information. The state of hydronephrosis relative to bladder filing is important. If the kidney is dilated with a full bladder and yet drains with bladder emptying, all visible on ultrasound, the degree of hydronephrosis must be interpreted differently than if it is unchanging. The degree of hydronephrosis is an excellent basic marker of the severity of obstruction. In the absence of calyceal dilation, it is fairly clear that the degree of obstruction is limited. Indeed in these patients we would not recommend functional imaging such as a renal scan. In contrast, dilation of all of the renal calyces, even with less renal pelvic dilation (*Fig. 56-15*), suggests a greater degree of obstructive change than when these are non-dilated. Pelvic dilation is often measured as an indicator of severity, yet may be exaggerated with an extra-renal pelvis that makes this number high and worrisome, yet may be relatively insignificant.

Figure 56-15

Ultrasound image of hydronephrosis consistent with a ureteropelvic junction obstruction (UPJO) based on the absence of any ureteral dilation. All calyces are dilated. The renal parenchyma is distended but not markedly stretched.



The degree of hydronephrosis and the state of the associated renal parenchyma are key factors in describing any hydronephrotic state, along with laterality, the state of the bladder, and whether there is ureteral dilation as well. While there is no universal ultrasound grading system for the kidney, the Society of Fetal Urology (SFU) system is as close as available (319). This provides a level of grading that is useful and facilitates communication. Simply stating the presence of hydronephrosis is completely inadequate in a radiological or clinical communication and an indication of the relative severity, distribution and associated findings is essential.

The severity of hydronephrosis is a fairly good representation of the severity of obstruction in most cases (320). This correlation will fail in previously operated cases, in the co-existence of reflux, and in some anatomic conditions such as megacalycosis (321). In the transplant population, hydronephrosis is not always present in the setting of an obstructive effect. In general, however, all obstruction produces hydronephrosis, but not all hydronephrosis represents obstruction. From a practical

standpoint, simple pelvic dilation is rarely associated with a functionally significant obstruction. As relative severity increases, progressive dilation of the calyces and distention of the cortical tissues will be evident. The change in the shape of the kidney does not always indicate loss of function or tissue. The use of terms such as cortical atrophy or cortical thinning suggests loss of function, yet this may simply be stretching of the tissue with dilation.

In the child with asymptomatic hydronephrosis, usually with prenatal detection, the degree of dilation has correlated with the risk of functional loss on longitudinal studies (322). There is no clear cutoff at which it is safe to ignore the finding, and it is unclear what effect longer times of observation might reveal with lesser degrees of dilation. Despite modern imaging, this is one of the few clear findings that predicts functional outcome statistically. However, it is difficult to translate that into clinical practice in many cases. Further imaging is usually used to better define the status of the kidney and the risk of deterioration.

Nuclear Renal Imaging

The most commonly used means to assess the severity and functional effects of obstruction is the MAG-3 diuretic renogram. MAG-3 (MercaptoAcetyl tri-Glycine tagged with ^{99m}Technetium) is principally secreted by the tubules with 10–20% filtration. The measure of relative uptake is comparable to the relative function by GFR, and is measured by the relative amount of tracer uptake by each kidney 2 min after injection. It is always important to recognize that this does not assess global function unless the total fraction extracted by the kidneys is estimated and these estimates can be difficult and imperfect. When a kidney is markedly dilated, the assessment of relative function may be less precise due to a greater impact of background uptake, particularly on the right with the overlying liver. On occasion the affected kidney will demonstrate greater than the expected 50% uptake and it remains controversial as to whether this is a real effect of obstruction or a technical artifact (323–325).

The washout phase is used to assess the severity of obstruction and is conventionally performed by administering a dose of furosemide when the affected renal pelvis is full and monitoring the time required to clear one-half of this amount (t1/2) in minutes. The conventional interpretation is that if the half-time is less than 10 min then drainage is normal, while 10–20 min is indeterminate, and over 20 min is indicative of obstruction (*Fig. 56-16*). It is necessary to interpret these numbers in the context of the individual and to examine the patterns of washout to permit a clinical interpretation. Simply going by the number is unlikely to be satisfactory. Some washout curves are biphasic with a rapid decline to partial drainage than a flat curve. Depending upon the means used to calculate the half-time, these curves may give falsely high or low numbers (326). Other factors that may cause differing interpretations of a diuretic renogram include posture, the fullness of the bladder and whether a catheter is used, and the timing of the diuretic administration. More recently interest has developed with diuretic administration concurrent with or 15 min prior to the tracer, the so-called F-0 and F-15 renal scan (327–330). It is critical to recognize that none of these studies or their thresholds for obstruction has been validated in any useful way in the infant population. In a study of the histopathology of pediatric UPJO, diuretic renal scan findings correlated poorly with histological changes (35).

The timing of the MAG-3 is subjective and some advocate not using this study in the very young child, but it is clear that reasonable results may be obtained in children at one month. The other issue is the need for repeat studies. This is a complicated and invasive study by most parents' perceptions, and accordingly, we try to limit its use. In the typical patient with significant hydronephrosis and generalized caliectasis, the MAG-3 would be performed at 2–3 months of age, then again at 1 year. If the relative function is decreased initially, a study at 6–9 months may be obtained, but this is rarely different from the initial study. Patterns of change over time are probably the most useful parameters to indicate the status of the affected kidney.

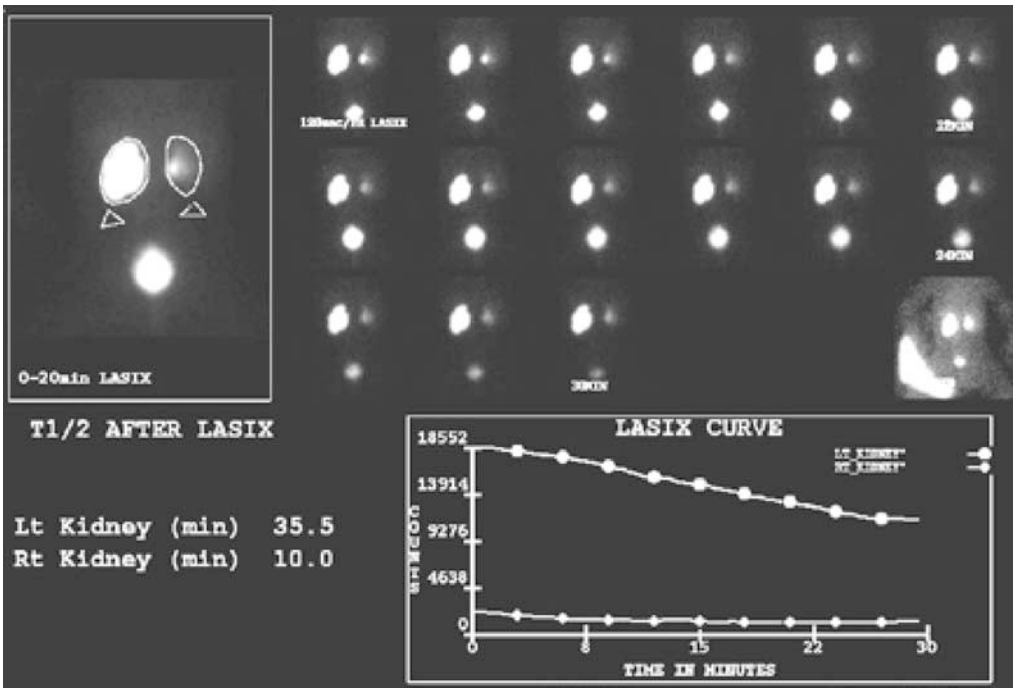
Nuclear studies remain the "gold-standard" for the functional assessment of the hydronephrotic kidney, but better modalities are clearly needed.

Magnetic Resonance Imaging (MRI)

The most promising new modality for functional imaging of the urinary tract is magnetic resonance imaging (MRI) with the ability to provide exquisite detail of the anatomy as well as providing functional assessment (*Fig. 56-17*) (332). The anatomic value is best seen in complex cases where the ultrasound images are difficult to interpret. This is most often in cases of duplication and where the ureter is obstructed near the bladder and may be ectopic. With fine resolution, the presence of a crossing blood vessel causing ureteral obstruction may be detected. These cases are the minority of pediatric hydronephrosis where the anatomy is well defined by ultrasound, but the functional implications are uncertain. In this regard there is much potential with MRI, but it has not yet moved to that level of widespread use. Several centers have focused on using this study, but the functional assessment of obstruction

Figure 56-16

MAG3 diuretic renogram in prenatally detected ureteropelvic junction obstruction (UPJO), demonstrating delayed washout of the tracer on the left side (posterior view). The $t_{1/2}$ of 35 min would be strongly suspicious for obstruction. The slightly elevated $t_{1/2}$ for the right side only reflects the fact that by the time the diuretic was given and counting began, the pelvis has washed out nearly completely.



remains at a level similar to that of the MAG-3. The correlation with functional outcomes has yet to be established. Until that time, it will be difficult to justify the extra expense of these studies in the routine UPJO in whom the functional impact of the obstruction is uncertain.

Excretory Urography

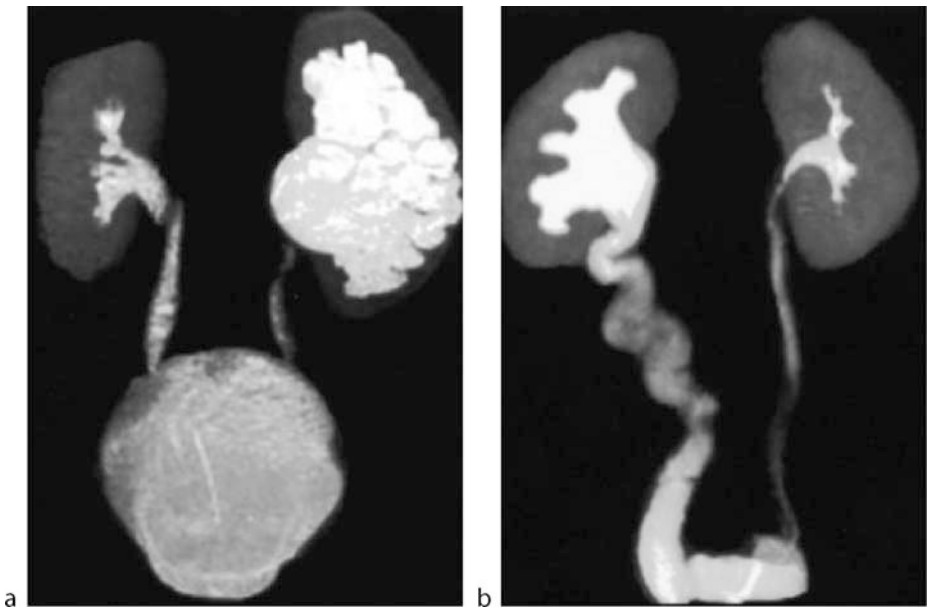
The IVP (intravenous pyelogram) remains a tried and true standard to provide anatomic and functional information regarding the urinary tract. While uncommonly performed today, it remains a useful tool in select situations. As with the MRI, these are cases in which the anatomy is not fully defined by ultrasound. The functional interpretation of the IVP rests on the fact that the contrast agent is handled similarly as with most other tracers and the rate and symmetry of passage through the kidney is a clear indicator of the level of obstruction. However, because it cannot be quantitated, and there are no numbers attached to this assessment, it has been considered subjective. This is true and for longitudinal comparisons, the diuretic renogram is preferable, but it must be recognized that the actual significance of any of the numbers on a renal scan is to some degree subjective. The radiation exposure of a well performed IVP, and the reduced cost are attractive, yet it is being used increasingly less in most centers.

Retrograde and Antegrade Pyelography and Pressure Perfusion Studies

It is rare that direct contrast instillation into the affected kidney provides information that is necessary to define clinical management. However, when this is needed, either retrograde pyelography or antegrade pyelography can be a useful tool. Often they can be performed in conjunction with an operative procedure or after a temporary nephrostomy has been placed for drainage. Both are invasive and require general anesthesia in most cases in children. Retrograde pyelography requires cystoscopy to place the ureteral catheter and it is important to recognize that this may trigger a significant infection if no other drainage procedure is performed. It is very risky to use a

Figure 56-17

Magnetic resonance images obtained by maximum intensity projection reconstruction after Gd-DTPA infusion. a. Left ureteropelvic junction obstruction (UPJO). b. Right ureterovesical junction obstruction (UVJO). From reference (331) with permission.



simple diagnostic retrograde pyelogram to assess a UPJO if a stent or operation is not performed.

Antegrade pressure perfusion studies have had some interest in the past, but their utility remains limited. Several versions of the classic Whitaker test (333, 334) have been introduced using different patterns of flow into the renal pelvis to stress the drainage system and to determine pressure differentials between the kidney and ureter or bladder (335). Most are non-physiologic, and none have been correlated with functional outcomes in the population most of interest. They have some utility in the previously operated kidney with persisting dilation that may simply represent ectasia of a dilated system or ongoing obstruction. Static renal pelvic pressure measurements have been explored as possible indicators of the severity of obstruction, but are not clinically used to date (336). Interpretation of these studies remains more of an art than science and they are rarely used.

Cystography

The voiding cystourethrogram (VCUG) is an essential part of most basic evaluations of the child with hydronephrosis, even when the presentation was asymptomatic. Since reflux can both cause and co-exist with hydronephrosis, and contribute to separate obstruction due to ureteral dilation, its assessment is important. In addition, the VCUG provides a thorough evaluation of bladder function in terms of the structure of the bladder, the patency of the urethra, bladder emptying and if there is reflux, an anatomic definition of the upper tracts. In the setting of reflux and hydronephrosis, grading of reflux is difficult if some element of obstruction is present. This is most evident when there is a mismatch between the ureter and renal pelvic dilation; it is also evident if there is dilution of the refluxed contrast due to renal stasis. In such a setting, it is imperative that the radiologist perform delayed images to look at washout of the contrast agent. Prompt washout of a dilated system eliminates the need to obtain a diuretic study and confirms non-obstructive dilation. There are some situations, however, when discriminating between ureteral dilation from reflux and a possible co-existing UPJO is very difficult. At times a somewhat arbitrary choice to correct one is needed followed by close observation to determine the response of the other (337).

Clinical Interpretation

Creating a synthesis of the imaging information with the clinical scenario remains a challenge and must reflect the

apparent severity of obstruction, the health of the child, the preferences of the parents, the ability to maintain follow-up, and the availability of surgical resources. There is no single formula for managing the child with asymptomatic hydronephrosis. In contrast, the child who presents with symptoms or who develops them on observation can be moved to surgical correction promptly. While it may at times be uncertain whether the symptoms are due to the obstruction, most often this is evident. There is very little reason to delay therapy.

General Management Principles

As a practical approach for the infant with asymptomatic hydronephrosis, usually after prenatal detection, segregation by severity can be based on the initial ultrasound. Those children with moderately severe to severe hydronephrosis, with caliectasis, will require ongoing monitoring and functional assessment. This will be with a MAG-3 diuretic renogram and the differential function and washout times will be the most closely followed. If the initial function is lower than 45%, follow-up MAG-3 may be appropriate in 6–9 months rather than waiting for 1 year. Ultrasound imaging should be obtained in the short term, 4 months, to ensure that this is not increasing, a rare but obviously important observation. We have found that assessment at one year to be most useful if no significant improvement has occurred, and function is stable, then it is less likely that spontaneous improvement will occur within the first 5 years of life. It appears as if the first year is the most dynamic, either for better or worse. These are not hard-and-fast rules and require adaptation to the clinical context.

For patients with less severe hydronephrosis, functional imaging is not usually useful and regular ultrasound imaging can provide useful data and can detect worsening hydronephrosis that would warrant a functional assessment. Imaging intervals are usually increased over time. It is our practice to monitor until normal, and while there are no data to indicate that this is essential, it seems cautious.

The child with posterior urethral valves or an anatomic abnormality that will not resolve spontaneously, such as an ectopic ureter or ureterocele, is best managed with definitive intervention and decompression. While there are some reports of expectant management of ureteroceles (338), this seems to be inappropriately risky with little real advantage. We have seen several cases of initial presentation of ureteroceles, and even ectopic ureters that are more complex than if they had been treated early in life. There seems little rationale for planned delayed management of obvious structural anomalies in children.

Specific Management

The management of each specific form of urinary obstruction is well defined and there is relatively little controversy in this regard. New modalities that are intended to be less morbid are in the process of replacing open surgical methods, although they are still not in the realm of being the gold standard. Their introduction has raised the question of changing the threshold for intervention if that intervention results in less morbidity.

UPJO Management

UPJO is best treated with a dismembered pyeloplasty, either open or laparoscopic. This is a well-established and predictable operation that can be performed with minimal invasiveness and children can be sent home on the same or the first post-operative day. Laparoscopic pyeloplasty is not yet widespread and even with open surgery; children do very well with this operation. Alternatives such as endopyelotomy or cutting of the stenosis and stenting to permit healing in an open state, is attractive, but has not had adequate outcomes (339–342). Even for reoperative procedures, this modality has not lived up to its initial enthusiasm.

Laparoscopic pyeloplasty in children has been used for over a decade (343), but still remains of limited availability. This is largely due to the difficulty of learning to perform accurate and efficient suturing for this procedure. Success rates are good and morbidity seems to be decreased. With the advent of robotic surgical technology, the learning curve for this type of laparoscopic surgery is markedly shortened (344–347). The DaVinci® (Intuitive Surgical Corp., Sunnyvale, CA) surgical system permits precise, well visualized and delicate surgery for this type of reconstruction in children as young as 3 months with good outcomes. While not yet widely available or used in children, this technology offers significant potential for reducing morbidity for complex reconstructive surgeries. The procedure is performed through three laparoscopic ports, guided through a master-slave "robotic" system with the surgeon controlling the movements at a console. Visualization is in three dimensions and the precision is excellent. While the system was not designed for children, it has been used successfully. Older children will demonstrate a more apparent improvement in

operative morbidity over open surgery, but it can be seen subjectively in younger children as well.

Open surgery is still the gold standard for correction of UPJO through the dismembered pyeloplasty. This can be performed in the flank position or through a dorsal lumbar incision. Both are cosmetically reasonable and do not require muscle cutting. The procedure consists of resection of the stenotic segment, some reduction in the size of the dilated renal pelvis, and re-anastomosis of the ureter to pelvis. This is often performed with an internal ureteral stent to facilitate early healing and drainage; some surgeons use a wound drain or a nephrostomy. Success rates should be in the 95–97% range based on reduction in hydronephrosis and on functional parameters on renal scan (348).

Recovery is quick in infants but slower in older children, with 1–4 day hospital stays. The exposure is excellent. There has been a movement toward smaller and smaller incisions, largely in response to laparoscopy, and this has raised concerns regarding exposure and the ability to manipulate delicate tissues precisely. Exposure is a critical principle of surgery and the current laparoscopic images offer exceptional views of the kidney. This should facilatate surgical reconstruction. Where minimally invasive technology will evolve for pediatric renal surgery remains uncertain, but there has clearly been significant progress in reducing perioperative morbidity due to a variety of means, and this is likely to continue.

Management of Ureterovesical Junction Obstruction (UVJO)

Obstruction of the distal ureter is more difficult to repair than to just resect the obstructed segment as with a UPJO. The stenotic segment is at the end of the ureter, which must then be detached and re-anastomosed into the bladder, and in such a way as to prevent urinary reflux. The standard procedure today is a tapered ureteral reimplantation. This author (CAP) favors an excisional tapering to provide adequate reduction in ureteral caliber to permit effective reimplantation. The key elements of this procedure are to preserve ureteral vasculature and provide an effective anti-reflux reimplantation. Ureteral stenting is necessary but can be done with internal stents and extraction strings, limiting the hospital stay to 2 or 3 days. Ureteral plication has been used widely as well, but is not satisfactory for larger ureters. Success rates in general are good, approaching 90% with no residual obstruction or reflux (349).

Less invasive methods have been described but have few data to support their widespread use to date (350). These include prolonged stenting of the obstructed megaureter to permit dilation (351). This method has been used in infants, but is reported to require an open incision. Laparoscopic methods have been used to a limited degree, with success, but this is a challenging operation with a subjective loss in manipulative ability due to the working area. In time, this is likely to develop as methods are evolved to facilitate surgical manipulation.

In general, megaureter repair is a less frequently performed operation and as a result, many surgeons are less comfortable with it and will often avoid it. When it is deemed appropriate, an experienced surgeon should be involved, as the consequences of failure are significant.

Management of Posterior Urethral Valves (PUV)

Surgical management of PUV begins with endoscopic valve resection in most children, but frequently requires multiple further surgeries to address the secondary consequences of bladder obstruction, including ureteral reimplantation, often with tapering of the ureters, bladder reconstruction with augmentation or continent catheterizable stomas. The details of these procedures are beyond this chapter and are readily available in basic surgical texts. Managing the child with valves requires experience and a willingness to employ these procedures.

Endoscopic resection of valves is a brief procedure using a cystoscope, usually passed through the urethra. It requires significant skill and experience (352). Even after years of experience, this author (CAP) finds this procedure a significant challenge and it should never be viewed as simply snipping some tissue. The potential for injury to the urethra and creation of strictures is significant. Parents are usually counseled that a lesser resection is safer, even if it requires a second operation to achieve complete valve resection. None-the-less, endoscopic valve resection is a well-established and successful procedure with limited morbidity. It can be done as an outpatient in the healthy child and may or may not require a catheter. Follow-up cystography is needed to confirm patency of the urethra and to reassess any reflux.

Subsequent clinical follow-up is dependent upon the initial status of the urinary tract to address the response to decompression, both functional and anatomic. Long-term monitoring of the renal and bladder function is essential in most cases.

In the rare case of very severe valves, temporary proximal diversion may be appropriate to permit growth and development of the infant with maximal renal drainage. This can be in the form of a vesicostomy or ureterostomies. The latter are rarely performed today, but may

be useful in cases where the bladder is profoundly abnormal and there is ineffective drainage from the ureters into the bladder. In general, these diversions are only used when the child is too small to undergo endoscopic resection or if catheter drainage of the bladder does not produce satisfactory improvement in renal functional parameters or hydronephrosis (353, 354). Controversy persists as to the optimal approach in these patients, and in the impact on bladder and renal function (304, 355). Diversion is always performed with a plan for re-functionalization after a period of decompression, often 6–12 months, depending on the renal function and particularly the bladder function.

Ectopic Ureter and Ureterocele

Management of the obstructed elements associated with an ectopic ureter or ureterocele are dependent upon the anatomy, functional status of the affected renal segment, and associated conditions such as reflux. The basic approaches include removal by way of a partial nephrectomy if there is a poor function or a drainage procedure. Drainage can be accomplished by joining the affected ureter to the unaffected segment or by ureteral reimplantation of the affected ureter, usually in combination with the unaffected ureter (a so-called double-barrel or common-sheath reimplantation). Similar strategies are applicable with ureteroceles, except that it is possible to decompress a ureterocele through a simple endoscopic incision, with the risk of developing new reflux that may need later surgical management (356, 357). This less invasive approach is particularly useful in the infant.

As noted above, some have advocated for an observational approach to ectopic ureters and ureteroceles, and indeed a rare few might never cause a problem, but this is a condition that will not resolve on its own and due to stasis of urine, is at risk for infection. Late presentations of previously asymptomatic ureteroceles and ectopic ureters would argue to definitively correct the obstruction at presentation.

Summary

Congenital obstructive uropathy remains a challenging and important clinical condition affecting large numbers of children to widely varying degrees. In parallel with most pediatric conditions, the overall goal in managing COU is that of maximizing the functional potential of the urinary tract. The unique aspect of COU being ongoing during the development of the kidney and urinary tract is a key aspect of this condition that distinguishes it completely from acquired obstruction and demands a distinct perspective. The most appropriate management must be determined individually, although broad guidelines are clear. It remains an essential element of management to perform a thorough functional and anatomic assessment of the entire urinary tract and to avoid focusing exclusively on the affected segment. Clearly some of these conditions will resolve spontaneously without apparent harm, but others will inevitably progress and produce irreversible renal injury or developmental retardation. Distinguishing those patients is the crux of the current clinical challenge and requires a clinical synthesis that recognizes the inherent uncertainties. It is clear that current clinical indices are not fully reflective of the developmental renal pathology present. An approach that may allow some over-treatment is seen as preferable to one that may permit under-treatment.

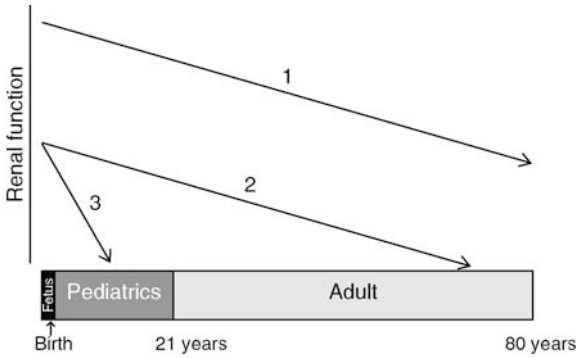
Long-Term Follow Up

The long-term prognosis for infants and children with congenital obstructive uropathy is poorly defined. Unfortunately, many children undergoing pyeloplasty for UPJO, and found to have a satisfactory immediate postoperative course, are lost to follow up after several years. In light of the experimental evidence, as well as the limited clinical data available, it is likely that some of these patients will eventually develop proteinuria, hypertension, or chronic renal insufficiency. Although a large proportion of infants with equivocal hydronephrosis identified prenatally appear to have normal renal functional development without intervention (358), others may demonstrate progressive impairment (359, 360). Failure of the patient's family to comply with follow up imaging may contribute to renal functional deterioration if surgical correction is deferred (361). Obstruction has thus been defined as "a condition of impaired urinary drainage which, if uncorrected, will limit the ultimate functional potential of a developing kidney" (266).

The prognosis for children with PUV is more grim: a majority of patients diagnosed in fetal or neonatal life develop renal insufficiency by 10 years (307, 362). The rate of deterioration of renal insufficiency for an individual patient with congenital obstructive uropathy depends on both the severity of fetal renal maldevelopment and ongoing injury due to obstruction or infection. Mild fetal obstruction is likely to have a minimal impact on renal growth or function, although changes may become apparent in late adulthood. Moderate obstruction may lead to significant renal deterioration in early adulthood, while severe obstruction results in renal insufficiency in

Figure 56-18

Scheme showing long-term impact of congenital obstructive uropathy. The urinary tract abnormality develops in embryonic and fetal life, but if mild (1), the consequences of the condition may become apparent only later in adulthood, if at all. If moderate (2), progression of renal insufficiency may develop earlier in adulthood. If severe (3), renal failure develops in infancy or childhood. Fetal intervention should take into account not only the health and welfare of fetus and mother, but the long-term implications of perinatal management. From reference (258) with permission.



infancy or childhood (*Fig. 56-18*). At a minimum, all children with obstructive uropathy should have regular measurement of blood pressure, plasma creatinine, urinalysis, and periodic renal ultrasound. Every attempt must be made to maintain normotension, and to minimize proteinuria. Nonsteroidal anti-inflammatory drugs should be avoided. In the transition of care from pediatrician to internist, this follow up should be maintained throughout adulthood.

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57 Bladder Dysfunction in Children

Michael E. Mitchell, MD · Anthony H. Balcom, MD

Normal Bladder Development

In Utero Development of the Bladder

Although the molecular biology of bladder development is still the subject of research, some basic developmental phases of bladder embryology are known. The bladder starts to develop in the fourth week of gestation and probably represents the fusion of two different structures. The first is the urogenital sinus, the anterior portion of the cloaca, which is contiguous with the allantois derived from endoderm. The second is the caudal end of the mesonephric duct, the common excretory duct derived from intermediate mesoderm, which joins with the urogenital sinus on the 24th day of gestation and proceeds to differentiate into the trigone, posterior bladder neck and posterior proximal urethra.

The result is that the urogenital sinus becomes the body of the bladder, or the detrusor, and the mesonephric duct differentiates into the bladder base and proximal urethra. Because of this fusion of different embryonic tissues it is not surprising that innervation and vascular supply may also be unique relating to tissues of origin. Furthermore, the need for coordination in function in the "developed" organ may be complex.

By the 10th week of gestation the bladder is tubular in shape, lined by a single layer of cuboidal epithelial cells and connected with the allantois via the urachus. By 12th week the urachus is closed and the bladder is now lined with two layers of cuboidal epithelium. At 12 weeks the bladder wall has thickened with the development of connective and muscle tissues. This process starts at the bladder dome and progresses to the bladder base. By the 21st week the epithelium is similar to mature urothelium (4–5 cell layers thick).

Bladder compliance is low initially but increases throughout in utero development. This process seems to correlate with production of urine and bladder filling and empting, which is intuitively logical, and a very important concept that can be expanded and applied to repair of an abnormal bladder. The mechanism of the bladder wall change seems to be that of decrease and change in the collagen structure of the developing bladder wall and the increase in smooth muscle fibers (1). Beuboeuf, et al. (2) used a mouse model to demonstrate that mechanical forces influence bladder wall structure and organization.

On the other hand continence mechanisms in utero have not been the focus of study so far. However, it is known that by 15 weeks gestation there is condensation of smooth muscle fibers at the caudal extent of the developing bladder that ultimately forms the bladder neck and proximal urethra. The distal portion of this smooth muscle is wrapped anteriorly with striated muscle. The result is the formation of the voluntary urethral sphincter (3). In boys this is connected laterally to the prostate, but in girls it is attached to the lateral aspect of the distal vagina as both of these structures are developing at this point.

The development of the bladder and sphincteric mechanisms has been tightly linked with the production of urine in utero determined by ultrasound (4) (*Fig. 57-1*). However these fetal bladder volume measurements may actually correlate more accurately with the development of functional bladder storage. Clearly, bladder storage and urine production are closely related. Support of this concept comes from the well-known observation that in the fetus with renal agenesis the lower urinary tract does not progress in its development; the bladder and ureters are present but hypoplastic. Thus, not only is there apparent bladder storage in utero, but also bladder empting. This would imply that the developmental process is also associated with development of a complex neurologic control of the lower urinary tract. Furthermore, because of the convergence of two different embryonic tissues one might predict that the neurogenic coordination of the mature organ may be complex. This may help to explain the complexity of the spectrum of dysfunction of the bladder.

The First Year of Life

The bladder of the newborn child is not like that of an older child in terms of structure and function. During growth and development, before and after birth, the bladder is in a state of continuous change. (1, 5) The impetus

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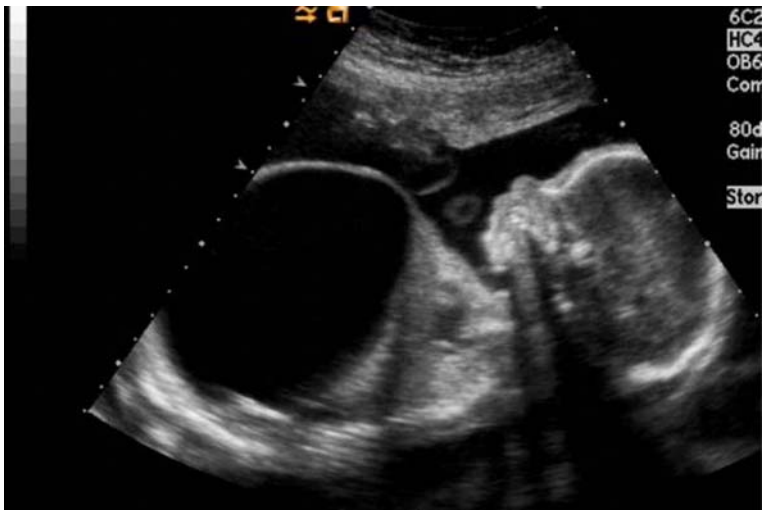
Figure 57-1

In Utero ultrasound image of the Fetus. This image demonstrates a pathologically enlarged urinary bladder at about 26 weeks of gestation, consistent with either Prune-Belly Syndrome (which was the diagnosis established after delivery of this fetus), or Posterior Urethral Valves.



for this change seems to relate to the physical forces exerted, both filling and emptying, on the developing bladder wall (6). In utero the bladder is small with low compliance. After birth there is continuous bladder change with increasing capacity and compliance which reflects bladder wall elasticity. Truly coordinate voiding, however, does not occur until after the first few months of life, which may partially explain the increased potential for infections in this period. As far as urine control is concerned, however, it is evident that voiding remains automatic; coordinate but not socially appropriate, until the end of the first year of life. Thereafter, potential for volitional voiding function develops with the ability to inhibit automatic voiding. However socially appropriate voiding; potty training, does not usually occur until ages 2–5 depending on numerous factors related in part to the maturation of the spinal tracts and social norms and pressure. What we are coming to realize is that, just as there is change with normal growth and development, pathologic conditions in the bladder originating prior to birth do have potential to reverse in the first years of life given the appropriate physical stimuli, specifically filling and empting (7).

Physiology of the "Normal Bladder" Defined by Anatomy and Function

The physiology of normal bladder function is a complex 'moving target' in young children. Bladder function is usually defined by the measurable characteristics of storage and empting.

Bladder storage function is defined and dependent on four parameters. These are:

1. *Bladder Volume or capacity* (V in ml.) which is usually measured at the point of maximal filling, the point of desire to void, or overflow leaking.
2. *Bladder Compliance*, or elasticity, measured by the ratio of detrusor pressure at a given volume, V/P, ml/cm H_2O, or the slope of filling phase curve. This is the Pressure in the bladder plotted against the Volume instilled into the bladder.
3. *Bladder Sensation* measured as both the first sensation of fullness, and point of desire to void.
4. *Sphincteric function* measured by the ability of the bladder neck and proximal urethra to remain closed during bladder filling. This observation is usually made with fluoroscopy, or by simply observing leakage from the urethral meatus.

Each of these parameters changes with normal development and depends on multiple parameters such as patient age, size and development.

Bladder empting function is both defined and dependent on two parameters. These are: 1. The ability of the bladder detrusor to contract and generate pressure to facilitate flow; and 2. the ability of the sphincteric mechanism to relax in a consistently coordinate manner, usually measured by Electromyographic (EMG) activity of the

pelvic floor musculature. However, functionally, bladder empting is measured by urine flow rate during voiding and ability to empty the bladder completely.

Urodynamics is the mechanism by which the parameters of filling and empting are evaluated. In this study the bladder is filled with either H_2O, or CO_2 while the pressure is monitored in the bladder via bladder catheter and in the abdomen via rectal balloon while monitoring the EMG activity of the pelvic floor, measured by needle or surface electrodes in the perineum. The study is often performed with fluoroscopy, "Video Urodynamics", so that the appearance of the bladder wall, presence or absence of vesico ureteral reflux, efficiency of empting, and most importantly, the appearance of the bladder neck and urethra, and their respective pressures can be simultaneously evaluated and recorded. With a carefully performed study and with proper patient cooperation, which is the real limiting factor in using this evaluation in children, the anatomy and physiology of the lower urinary tract can be defined.

The idealized data of a normal urodynamic study is shown in *Fig. 57-2*. The tracing shows the relationship between the true detrusor pressure (corrected by subtracting abdominal pressure from measured bladder pressure: P_{ves}- P_{abd} = P_{det}) and the volume in the bladder. Bladder pressure and vesical pressure are both terms for the same parameter, while detrusor pressure is a subtracted pressure. The rate of filling in ml/min is usually 0.1x anticipated bladder capacity, e.g., a 200 ml bladder would be filled at 20 cc/min.

Bladder capacity is defined as the volume at which the patient either voids spontaneously, or leaks, or requests the filling stop because of discomfort or the strong urge to void. Compliance is defined by the slope of the filling phase (tonus limb) curve prior to the initiation of voiding. Sphincteric, striated or external sphincter EMG activity will increase with bladder filling ("recruitment") but stop abruptly with initiation of void. Bladder neck competence and opening with voiding is best seen with fluoroscopy. Efficiency of voiding is measured by measuring urinary flow rate and completeness of bladder empting. Abnormal bladder function represents deviation from anticipated normal values.

In reality, however, a good history of normal bladder function can be elicited from parents and older pediatric patients, which obviates the need for, or can accurately predict the findings of a urodynamic study. Prior to potty training wetting should be periodic without dribble between or undue straining with voiding. Most parents will have observed a child's voiding "stream" and will be able to characterize it as full, thick or thin, continuous or interrupted, so called "staccato voiding". Toilet training in years 2–4 is normal, persistent daytime wetting accidents after age five may indicate a problem. Nighttime accidents are not uncommon through age seven years. Most parents can usually accurately describe the various forms of incontinence (stress, urgency, dribble, and insensate) in their child but will usually not volunteer the information unless specifically questioned.

The Abnormal Bladder

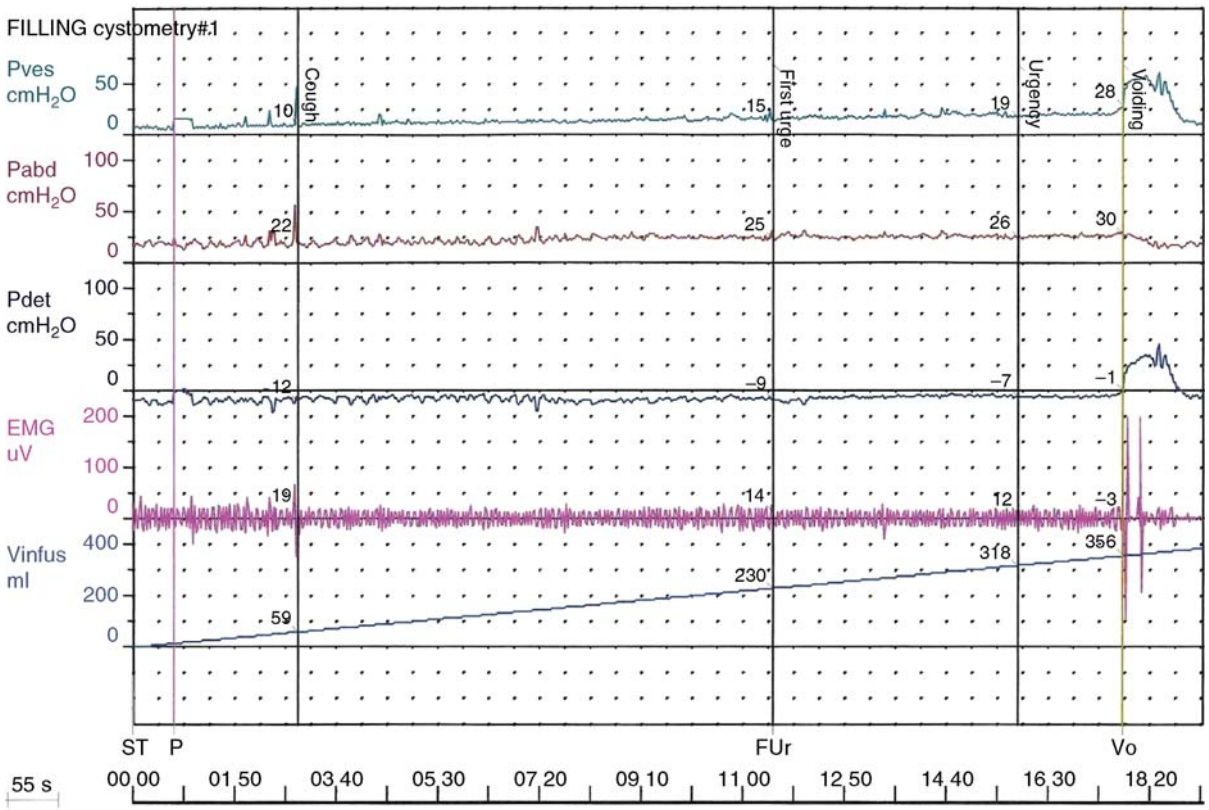
Pathophysiology of Bladder Dysfunction

The pathophysiology of the "abnormal" or "dysfunctional" bladder represents a breakdown of any one, or a combination of some or all of the physiologies of the normal bladder, and can represent a broad spectrum of potential pathologies and abnormal physiologies resulting in the symptoms of incontinence or infection. For example, urinary incontinence related to abnormal storage is characterized on urodynamic studies by uninhibited bladder contractions, e.g., overactive bladder, especially in the first third of filling or, the middle part of filling, rather than at near capacity. Urinary incontinence may also relate to small bladder capacity, e.g., congenitally small bladder, or normal potential capacity with poor bladder compliance, e.g., bladder scarring from infection or surgery, posterior valves, myelodysplasia, or sacral agenesis. Abnormal storage of urine can also be characterized by poor compliance; that is, bladder pressure rises too quickly with bladder filling, this can be observed in patients with bladder out flow obstruction cause by urethral valves, myelodysplasia or after bladder neck and urethral surgery. *Figure 57-3* shows a urodynamic study of a child with these findings.

Bladder neck dysfunction is also discernable with a video urodynamic study as prematurely opening during filling, associated with stress incontinence, or remaining closed during attempted voiding; resulting in overflow incontinence, urge incontinence and large post void residuals. Normally, the bladder neck is closed until voiding begins, at which point it opens completely. However, some children demonstrate a fixed, open bladder neck: myelomeningocele, neurogenic, and bladder exstrophy patients after surgical repair. An alteration of the normal sacral spinal cord nucleus (Onuf's nucleus) including the afferent and efferent nerves can cause a spectrum of bladder neck dysfunction. For example, lower motor neuron injuries will typically contribute to an open, patulous

Figure 57-2

Normal Urodynamic study. This figure depicts normal bladder storage and emptying characteristics during urodynamic evaluation. The patient is a 9-year-old female found to have a thickened filum terminale by Magnetic Resonance Imaging (MRI), but normal voiding. The bladder pressure curve, Pves, the top line, demonstrates a normal filling phase with the first sense of urgency at volume of 230ml. Bladder pressure stays low, which is normal, up to volume of about 356ml, at which point patient begins voiding with true detrusor contraction. The second line from the top is Pabd, a reflection of "abdominal pressure" by rectal catheter. The third line is Pdet, "detrusor pressure", which is a subtracted pressure: Pves minus Pabd. The fourth line from the top is EMG activity, demonstrating normal baseline activity of the striated pelvic floor musculature, and then quieting with voiding. The bottom line, Vinfus, is "volume infused in milliliters", representing bladder capacity at each point. On this urodynamic study one can see a frequent clinical situation we encounter, which is "the computer" subtracting abdominal pressure from measured intravesical pressure, which results in a "negative" pressure in the bladder, which is artifactual.

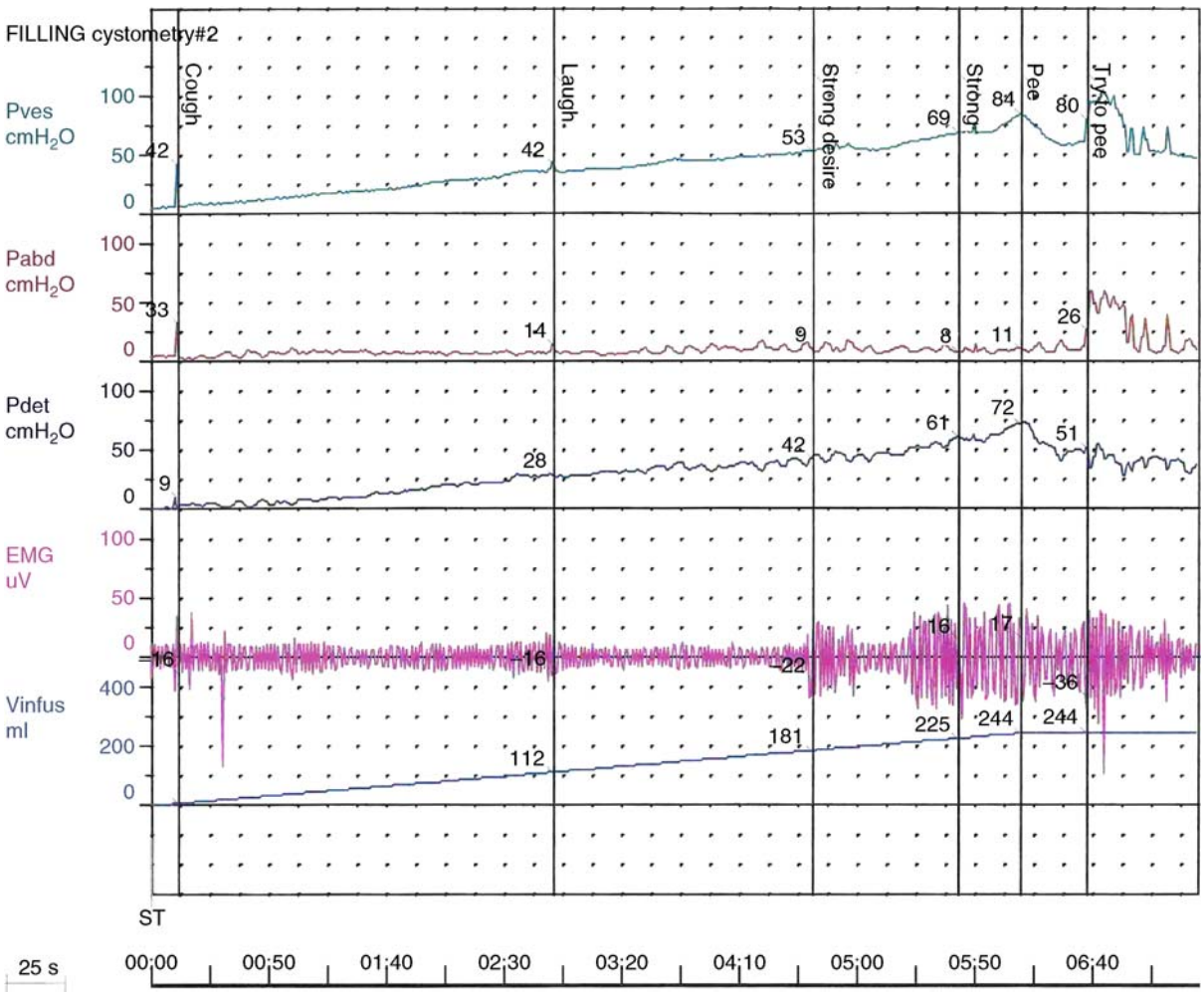


bladder neck. However, in some patients a "tightening" of the bladder neck with filling can be observed (this may also be observed in patient with posterior urethral valves).

Urethral sphincteric dysfunction can also represent a broad spectrum of pathologies including spinal cord injury patients, bladder exstrophy, or myelodysplasia, and is associated with dysfunctional voiding, which is the lack of consistently coordinate sphincteric relaxation with detrusor contraction. However this problem can also be seen in otherwise neurologically intact children who demonstrate by voiding cystourethrogram (VCUG) a "spinning top" urethra configuration, or by videourodynamics showing bladder neck hypertrophy with failure to completely relax the pelvic floor, with increased EMG activity, with voiding. These patients will also demonstrate significant post void residuals and abnormal flow rate (either reduced flow rates or interrupted flow during voiding attempts) and recurrent infection. All three would be demonstrated by non-invasive urodynamic studies: uroflow (urine flow rate) and post void residual urine volume by ultrasound measurement easily performed in the clinic setting, as shown in *Figs. 57-4* and *57-5*.

Figure 57-3

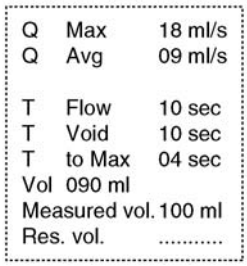
Example of urodynamic study demonstrating 1) very poor bladder compliance, and 2) uninhibited contractions near capacity. The patient is a 14-year-old male with spina bifida and subsequent neurogenic bladder dysfunction who, without treatment (intermittent catheterization and anticholinergics) is at risk to develop hydronephrosis. This study also demonstrates 3) detrusor-sphincter dyssynergia, EMG activity increases inappropriately with detrusor contractions.



Bladder detrusor abnormality and urethral dysfunction are often intertwined and interdependent. Abnormal urethral function often results in abnormal bladder detrusor function and vice-versa. Not surprisingly, the result may be wetting or infection because of inappropriate bladder emptying and/or poor bladder storage. Detrusor inactivity/hypoactivity, a large poorly contractile bladder, can be determined by a combination of video urodynamics and uroflow studies. Poor detrusor contraction is seen in the bladder pressure tracing curve; flat or sloped tonus limb without evidence of contractions, and an inconsistent interrupted pattern of voiding demonstrated on uroflow study. However, detrusor inactivity can still result in elevated bladder pressures and even progressive hydronephrosis if there is a problem with bladder empting, e.g., voiding dysfunction in poorly managed myelodysplasia or mechanical obstruction from valves or stricture. This condition typically arises because of dyscoordinate-type voiding and collagen deposition in the bladder wall muscle fibers. The bladder neck vs. the external sphincter

Figure 57-4

Uroflow study. This non-invasive urodynamic evaluation determines the voided volume and the flow pattern. Depicted here is the apparatus for measuring uroflow, and a normal uroflow pattern for an 8 year old boy. The voided volume, 100 ml, is low for age; a normal 8 year old boy would be expected to have a maximum bladder capacity of 300 ml; 8 + 2 = 10, 10 × 30 = 300 ml.



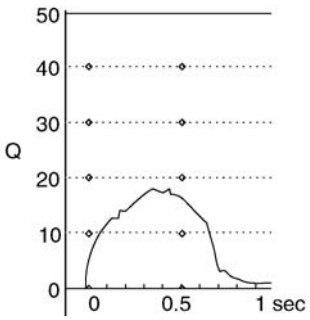
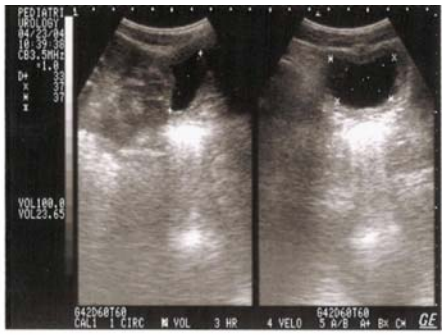
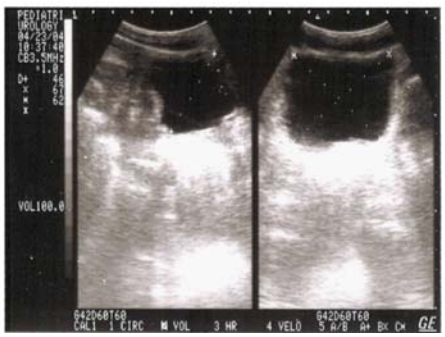


Figure 57-5

Post void residual urine (PVR) volume determination by ultrasound of the bladder in the clinic. This non-invasive technique measures the volume of urine in the bladder after a uroflow study. Combining the uroflow and the PVR non-invasively provides (1) functional bladder volume, (2) uroflow pattern, and (3) voiding efficiency.





can also be dyssynergic. A commonly misunderstood physiology is that hydronephrosis related to lower urinary tract dysfunction results primarily from high urethral resistance not from the bladder *per se*. Even in a small bladder poor compliance alone cannot cause hydronephrosis without associated elevated outflow resistance (usually > 40 cm H_2O). The proximal urethra may also show dyssynergy best seen on video urodynamic studies.

Definition and Spectrum of Bladder Dysfunction

The definition and spectrum of bladder dysfunction has been characterized according to the International Children's Continence Society (8). Recent advances in enuresis and incontinence research require clarification and modification of terminology for bladder dysfunction. There are now well-standardized definitions of urinary incontinence in children as shown in *Table 57-1*. Functionally however, the basis of lower urinary dysfunction can be grouped into three major categories, relating to (1) abnormal anatomy, (2) abnormal innervation, and (3) not associated with any known abnormality.

1. Anatomic causes that would include such diagnoses as: bladder exstrophy/epispadias, urogenital sinus malformations cloaca, ectopic ureter, ureterocele etc. Patients with anatomic abnormalities may also have a neurogenic basis for bladder dysfunction, as in the exstrophy patients and cloacal malformation patients.
2. Abnormal innervation or neurogenic causes would include such diagnoses as: spinal cord injury, myelodysplasia, sacral dysgenesis/agenesis, tethering of the spinal cord, tumors of the spinal cord, transverse myelitis etc.
3. Dysfunctional causes are probably the most common and yet most poorly defined category of voiding dysfunction as it occurs in otherwise normal patients, therefore treatment of these patients can represent a major challenge.

Signs and Symptoms of Bladder Dysfunction in Children

Often a urinary tract infection is the first sign of bladder dysfunction in children. The tight linkage between bladder dysfunction by any mechanism and infection cannot be overemphasized. Therefore, clinically, the initial symptoms are those of a urinary tract infection, dysuria, frequency, suprapubic pain, gross hematuria, chronic urinary incontinence, and even new onset of urinary incontinence. The cause of the infection may not be uncovered until after treatment of the infection. Most commonly, after treatment of a urinary tract infection in a child, either new or recurrent, consideration of potential for poor emptying due to bladder dysfunction is very important even in the apparently "normal" child. Unfortunately this consideration is often forgotten. Perhaps infection of the urinary tract should be considered more the inability to clear the

Table 57-1

Terminology Related to Lower Urinary Tract Dysfunction

Older, Previous Terminology:	International Children's Continence Society Preferred Terminology
Wetting	*Incontinence;* *A. Continuous Incontinence* (Implies Day-time) 1. Primary 2. Secondary, i.e., after a dry interval of 6 months B. Intermittent Incontinence 1. Day-Time incontinence a. Primary b. Secondary, i.e., after a dry interval of 6 months C. Nocturnal Incontinence = Enuresis (Implies Night time only)
Bladder Instability	*Overactive Bladder;* Usually *Idiopathic Detrusor Overactivity,* a urodynamic diagnosis, implies lack of identifiable neurologic lesion.
Detrusor Hyperreflexia	*Neurogenic Detrusor Overactivity;* implies known/identifiable neurologic lesion.
Discoordinated voiding	*Detrusor Sphincter Dyssynergia;* requires urodynamic diagnosis, implies neurologic lesion.
Voiding Dysfunction	*Dysfunctional Voiding;* Inability to relax sphincter with voiding. Diagnosis is by Uroflow measurement. Implies lack of identifiable neurologic lesion
Stress Urinary Incontinence	*Urodynamic Stress Urinary Incontinence;* requires urodynamic diagnosis.
Lazy Bladder	*Underactive Bladder;* requires urodynamic diagnosis.

inevitable ascension of bacteria from the perineum. The primary cause of this failure is inability to consistently empty the bladder, commonly caused by increased pelvic floor activity, with high bladder pressure. Other host defense mechanisms may be altered, i.e., GAG layer disruption, bladder wall ischemia from high intravesical pressure, poor lymphatic flow from high intravesical pressure, and the child has difficulty clearing bacteria.

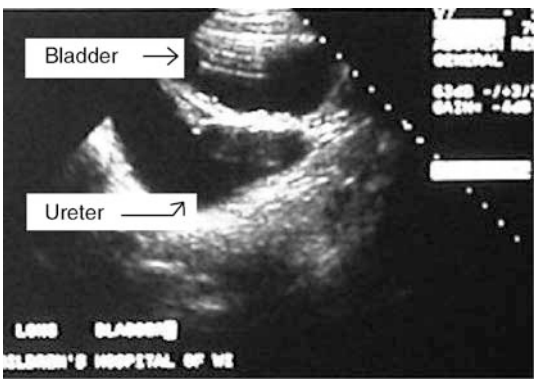
Symptoms of voiding dysfunction can also mimic those of infection. For example, new urinary incontinence can be the symptom of overflow incontinence that has developed from increasing post void residuals and bladder decompensation. Or, more commonly, urinary incontinence in children may relate to detrusor overactivity, the "overactive bladder" causing leakage of relatively small amounts of urine. Complete uninhibited detrusor contractions can also occur for a few years after potty training, causing a flood-type of urinary incontinence. Therefore, patients symptomatic but with negative urine culture may be manifesting a true voiding dysfunction and should be evaluated by a careful history, physical exam and ultrasound study of kidneys and bladder.

Hydronephrosis, particularly bilateral hydronephrosis, can on rare occasions be the presenting sign of significant bladder dysfunction in children. This typically is associated with a major degree of bladder outflow obstruction; however, other anatomic causes must be ruled out these would include bilateral ureteral pelvic junction obstruction or bilateral distal ureteral obstruction, known as obstructive megaureters (*Fig. 57-6*).

The failure to achieve potty training is often the presenting symptom of bladder dysfunction in children, particularly in those children with anatomic cause of the incontinence. Because of the wide variation in timing of potty training a true problem is often delayed in diagnosis until the onset of formal education when diapers no longer are acceptable.

Figure 57-6

Megaureter: Ultrasound study of the pelvis showing a dilated distal ureteral adynamic segment, also known as megaureter, behind a normal bladder.



Anatomic Causes of Bladder Dysfunction in Children

Classic anatomic conditions that can contribute to bladder dysfunction and urinary incontinence routinely misdiagnosed would include female epispadias, ectopic ureter, urogenital sinus, occult neurogenic bladder, tethering of the cord and sacral deformities. Even though labeled anatomic, each condition may be associated with a degree of bladder dysfunction.

Undiscovered female epispadias is a classic but uncommon reason for "failure to potty train" in little girls. A thorough history will usually reveal a stress component to the leakage. A careful inspection of the perineum will demonstrate a bifid clitoris and short urethra (*Fig. 57-7*).

Although the anatomic malformation in bladder exstrophy results in abnormal development of the bladder neck and proximal urethra (sphincteric incompetence), bladder dysfunction, specifically a small bladder with poor compliance, may result from poor urethral resistance. The bladder develops abnormally because of low urethral resistance. Most of these changes can be corrected by a single operation performed shortly after birth to reconstruct the bladder neck and proximal urethra. These maneuvers facilitate normal bladder cycling and normalization of bladder function just like in classic bladder exstrophy in males. The problem is that most of these females are not diagnosed soon enough to take advantage of the window of opportunity that seems to close sometime during the first few months of life.

Epispadias is also often associated with incontinence in the boy and is easier to diagnose. As in the girl it should be treated as early as possible to salvage bladder function. Unfortunately, this is has not been the general practice and many boys with epispadias have delay in bladder neck repair and resultant poor bladder function.

Fundamentally, the lower urinary tract anatomy and physiology reflect the embryology of the urinary tract. The ureteric bud, arising from the mesonephric duct, classically was felt to have one-way induction, that is, be responsible for the induction of the metanephric blastema to become the functioning kidney. However, it is becoming more and more clear that there is also induction of the mesonephric duct by the developing ureter, i.e., there is induction of adjacent mesenchyme in both directions. In fact recent evidence shows that the ureter may develop

Figure 57-7

Female Epispadias: This condition was not recognized at birth, and the child was referred at 3 years of age for continuous urinary incontinence. Note the short urethra, the bifid clitoris, and the widely separated pubis. Ideally correction at birth allows proper bladder cycling, and therefore definite bladder growth.

from the center outward to initiate the formation of the kidney, and also in the other direction to initiate development of the trigone, bladder neck, and proximal urethra; to the urethral meatus in the female and just distal to the prostatic utricle in the male. This important role would include the stimulus for neurologic development of these structures. Therefore, abnormalities detected in the ureter must lead one to anticipate potential abnormalities not only in the kidney but also in the bladder base, bladder neck and proximal urethra (*Fig. 57-8*).

Vesico-Ureteral Reflux and Bladder Function

High grade vesicoureteral reflux may indicate significant abnormality in development of the trigone and the bladder base. This is not dissimilar to the effect of abnormal positioning or timing of appearance of the ureteric bud during early embryology on the metanephric blastema resulting in renal dysplasia (9). Children with high grade vesicoureteral reflux, particularly those with bilateral reflux, should signal significant abnormality in development of very delicate and highly tuned structures which affect bladder and bladder neck coordination and function. It is not surprising, and rather is to be anticipated, that children with high-grade bilateral reflux have higher potential for renal dysmorphism and low potential for spontaneous resolution, in large part because of high potential for dysfunction of both the bladder neck and sphincter. Treatment with antibiotics and reimplantation surgery without recognizing the high potential for voiding dysfunction can lead to very unacceptable results.

Ureterocele

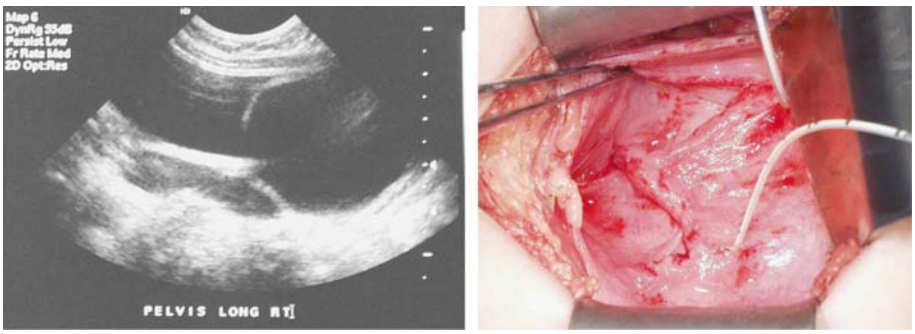
Ureterocele is an abnormality of the distal end of the ureter, an obstructive cystic dilatation of the caudal end of the ureter. A ureterocele is frequently associated with the upper pole in a duplex kidney, and this associated renal segment frequently shows cystic dysplasia. It should be viewed as a cystic induction of the trigone, but may also involve the bladder base, neck and proximal urethra. Bilateral large ureteroceles that extend down the urethra (ceco-ureterocele) must be considered to be associated with high potential for bladder dysfunction. Fortunately, because most ureteroceles are associated with less severe dysplasia of the trigone and bladder neck, surgical excision results in quite good potential for coordinate voiding and continence. The classic treatment is resection of the involved renal moiety and ureter, however, transurethral puncture of smaller ureteroceles may be considered as potentially curative. Puncture of a larger ureterocele which involves the bladder neck or proximal urethra may result in high grade reflux or "valve like" mechanical obstruction of the proximal urethra from a flap from the wall of the intraurethral ureterocele. Urinary tract infection or incontinence can be a consequence in cases of severe, bilateral ureteroceles (*Fig. 57-8*).

Ectopic Ureter in Girls

An ectopic ureter, with ureteral insertion distal to the sphincteric mechanism in a young girl will cause persistent wetness day and night in the face of normal

Figure 57-8

An example of abnormal, dysplastic development of the trigone and bladder neck from the presence of bilateral ureteroceles. The ultrasound image shows abnormal laxity of the posterior bladder wall/trigone form the ureterocele. The photograph of the opened bladder demonstrates severe thinning of the trigone after the ureteroceles have been resected.



voiding and bowel control post potty training. A unilateral ectopic ureter ("single ectopic ureter") is not usually associated with bladder dysfunction per se, therefore the usual presentation of uteral ectopia in a girl is that of normal voiding and potty training with voiding in the face of chronic low grade wetness, unresponsive to all efforts at therapy, in many cases including psychotherapy.

An ectopic ureter in a girl is frequently the upper pole ureter of a duplex renal collecting system. These ectopic ureters frequently "bypass" the sphincteric mechanism and kidney and may be either unilateral or bilateral. Continuous urinary incontinence develops. The child will typically void normally every 2–4 hours, but mothers notice that their child is wet within 15 min of voiding and report that the girl is "always" wet. Ectopic ureters can go to the bladder neck, mid-urethra, fourchette, vagina, cervix uterus, uterus, or Fallopian tube. The upper tracts in patients whose ureter enters the bladder neck typically are poorly functioning, dysplastic. Diagnosis can be difficult, but usually a Renal Ultrasound and VCUG suggest the anomaly. Other than the constant wetting, bladder function in these patients will be normal.

Ectopic ureters are never a continence problem in boys because a ureter can only link up with mesonephric duct derivatives, and the external sphincter in the male is distal to the mesonephric duct derived tissues.

Diagnosis can be difficult, but usually renal ultrasound suggests a duplication anomaly. VCUF typically show VHCR Computed Tomography can be helpful in elucidating the exact anatomy, and Magnetic Resonance Imaging with contrast is emerging as a useful technology in this regard.

Treatment of ectopic ureter in the female is guided by the amount of associated function in the renal parenchyma, with upper pole partial nephrectomy being a good option when poor renal parenchymal function exists, compared to uretero-neocystostomy or ipsilateral uretero-ureterostomy when good renal function is present in the associated renal moiety.

Bilateral Single Ectopic Ureter in Boys and Girls

Bilateral single ectopic ureters, with uppertract single systems bilaterally are often associated with bladder hypoplasia and dysfunction; again, because of the absence of bladder filling and empting in utero. The very rare situation of bilateral single system ureters ectopic to the bladder neck presents with urinary incontinence or urinary tract infection in females and UTF in males. Girls tend to have very poor outcomes when subjected to uretero-neocystostomy without recognition of the abnormal bladder neck. They frequently require institution of clean intermittent catheterization (CIC), and if they fail anticholinergic medication, progress to bladder augmentation. Boys with single system ureteral ectopia to the bladder neck are exceedingly rare, and may succumb in utero to renal failure. If they survive, they invariably have renal compromise, and despite efforts like CIC, anticholinergic medication, and bladder augmentation, usually progress to renal transplantation.

Cloaca and Urogenital Sinus

In girls, a cloaca represents the persistence of a common cavity composed of the (1) lower urinary tract, (2) genital

tract and (3) hind gut. A urogenital sinus anomaly represents the confluence of the urinary tract and genital tract. Each is part of a broad spectrum of pathologies that may involve the bladder, bladder neck and urethra, as well as spinal cord development. The cloaca, which is by definition a junction of the urinary tract and the GI tract, represents severe embryologic perturbation of early development, week 3–4 of gestation. The "urorectal partitioning" by the urorectal septum is usually quite altered. There may also be an abnormality of the tail bud during embryogenesis contributing to this anomaly.

Urogenital (UG) sinus malformations can result in poor formation of the posterior bladder wall and urethra. The spectrum of this malformation is broad, and is associated with the entire range of poor bladder formation and sphincteric incompetence. There are three basic categories: (1) the low confluence of the urethra and the vagina, (2) the mid-level confluence, and (3) the high confluence.

The imperforate anus in a cloacal anomaly is frequently located in the vaginal portion of the urogenital sinus. Urogenital sinus secondary to excessive androgen stimulation, the adreno-genital patients with congenital adrenal hyperplasia, is not generally associated with bladder and urethral dysfunction, except in the most severe cases in which the proximal urethra is severely foreshortened.

The spectrum of malformation in the cloaca patients is even broader than that of the UG sinus group. The rectum frequently connects with the vaginal portion of the urogenital sinus. There is often also a pelvic floor dysmorphism that is associated with failure of development of appropriate nerve supply to the pelvic diaphragm. The pelvic floor tends to be fibrotic and somewhat "non-relaxing" in these patients. The bladder may be poorly developed, and the complete spectrum of ureteral abnormalities, i.e., reflux, ectopia of the ureters, poor trigonal development and bladder neck malformations is present.

Early treatment of the cloaca in the neonatal period entails fecal stream diversion with double barreled colostomy. This limits fecal soiling of the common chamber and maximizes the potential to maintain sterile urine. Surgical reconstruction is usually done at a later date through a posterior sagittal approach. A perineogram, whereby contrast is injected into the single perineal opening, is extremely helpful in terms of guiding surgical intervention. The perineogram is usually done with a "Christmas tree" adapter on a syringe of contrast in the operating room by the pediatric urologist at time of reconstruction, and is frequently followed immediately by endoscopy to gain further insight into the anatomy. Then, typically a posterior sagittal approach for surgical correction is undertaken. Anterior repair of the urogenital sinus can be performed at the same time. On rare occasions, this may often involve complete mobilization of the urogenital sinus. Depending on the anatomy in terms of urethral length, the urethra and bladder base frequently require reconstruction. The native vagina may also need to be repositioned.

The spinal cord develops at the same time as the lower urinary tract; therefore, the patients with cloaca malformations have significant potential for abnormalities of the spinal cord. These would include tethering of the spinal cord, a tight filum terminale, intradural lipomas, diastematomyelia, and syrinx of the spinal canal.

In the case of patients with cloaca or high imperforate anus, but in almost all girls with cloacal malformations, bladder dysfunction must be considered to be the rule rather than the exception. Approximately 33% of children with cloacal or urogenital sinus anomalies will have demonstrable abnormality of the central nervous system, especially the spinal cord. Bladder dysfunction is to be found in 80% of these patients (10, 11). Most of these children have difficulty with coordinate voiding and often are dependent on intermittent catheterization. Because of the associated anatomic abnormalities of the urethra many require construction of alternative channels to the bladder such as an appendico-vesicostomy. Some of these children also have neurogenic bladder dysfunction resulting in small or poorly compliant bladders and require anticholinergics or bladder augmentation. Some benefit from temporary urinary diversion.

Technically boys do not have cloaca or urogenital sinus malformations. However, boys with imperforate anus, particularly high imperforate anus, are subject to the same potential bladder dysfunction as girls with cloaca. Any child with high imperforate anus should be assumed to have high potential for bladder and urethral dysfunction and minimally should be evaluated initially with ultrasound to evaluate the kidneys and a VCUG to evaluate for vesicoureteral reflux and bladder configuration.

Exstrophy/Epispadias Cloacal Exstrophy Complex

Exstrophy represents a spectrum of pathology that impacts bladder, bladder neck and urethral anatomy and function. It is unique in that it seems to be the result of premature senescence of the infraumbilical abdominal wall creating a defect (a hole) through which developing

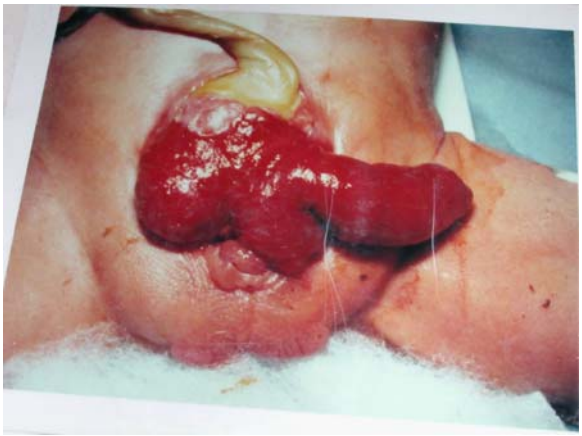
structures herniate. The timing of this herniation dictates the degree of severity of the condition. If the event occurs at 3–4 weeks gestation, the period of the cloaca, cloacal exstrophy develops which involves bladder, kidneys, hindgut, genitalia, urethra and spine. Often, the lower extremities and upper abdominal wall are involved as well (*Fig. 57-9*).

Classic Bladder Exstrophy represents an event that occurs at approximately weeks 4–7, and involves only the bladder, urethra and genitalia. Epispadias represents a problem after 7–8 weeks gestation and can involve the urethra, bladder neck and genitalia. All forms of the spectrum of exstrophy are associated with abnormalities of urine storage and emptying, except for boys with minimal form of epispadias, because of the structures involved: bladder, bladder neck, and proximal urethra (*Fig. 57-10*).

The cause of the defect is not known but may represent a problem with epigenetics. The important consideration with exstrophy is that the classic and epispadias forms are rarely associated with other significant problems. If the bladder, bladder neck and urethra are repaired early, i.e., first month of life, there is reasonable potential to achieve normal function in terms of voiding with continence. However, delaying repair or not achieving bladder cycling early after birth results in reduced potential for normal function. The condition is rare, 1/30,000 births, but provides a window into the potential for surgical repair and physiology of the lower urinary tract. For example, the bladder histology of the patient with classic exstrophy at birth is similar to that of a pre-20-week fetus, before significant urine production and bladder cycling have occurred. With careful repair at birth however, normal function of the bladder can be achieved. Restoring the anatomy therefore can potentially achieve normal function in many patients with classic exstrophy and epispadias. With early repair of classic and epispadias patients it should be possible to achieve voiding with continence in more than 80% (12). In contrast, the significant potential for spinal involvement in the cloacal exstrophy group severely limits the potential for normal bladder and urethral function and the same may be true for the earlier forms of classic exstrophy. The spectrum does point to the tight linkage between ectodermal (central nervous system) and mesodermal organ systems. An example of a urodynamic study in a girl with classic bladder exstrophy is shown in *Fig. 57-11*.

Figure 57-9

Cloacal Exstrophy. This newborn male has the anatomical conditions of cloacal exstrophy, including two separate bladder halves separated by the posterior wall of the cecum, prolapsed ileum, and a small omphalocele.



Posterior Urethral Valves

Boys with posterior urethral valves (PUV) can represent the complete spectrum of bladder dysfunction. In fact, it might be said that if one completely understood the potential pathologies of a patient with valves one would

Figure 57-10

Classic Bladder Exstrophy in a newborn boy. Note the exposed bladder plate, the epispadiac penis, and the widened pubic symphisis.

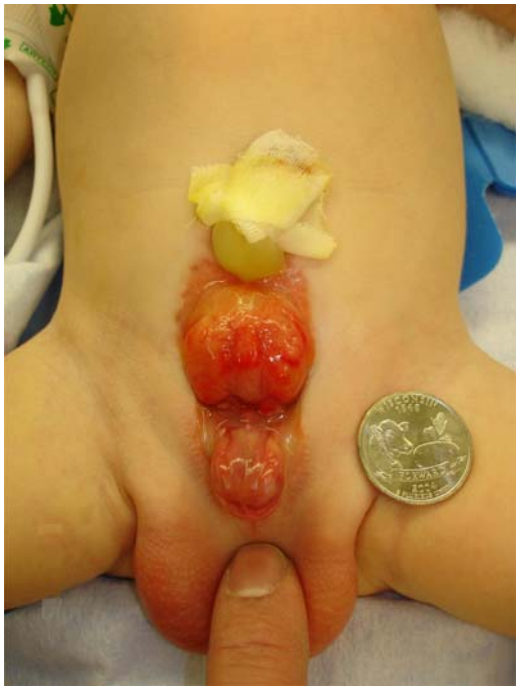
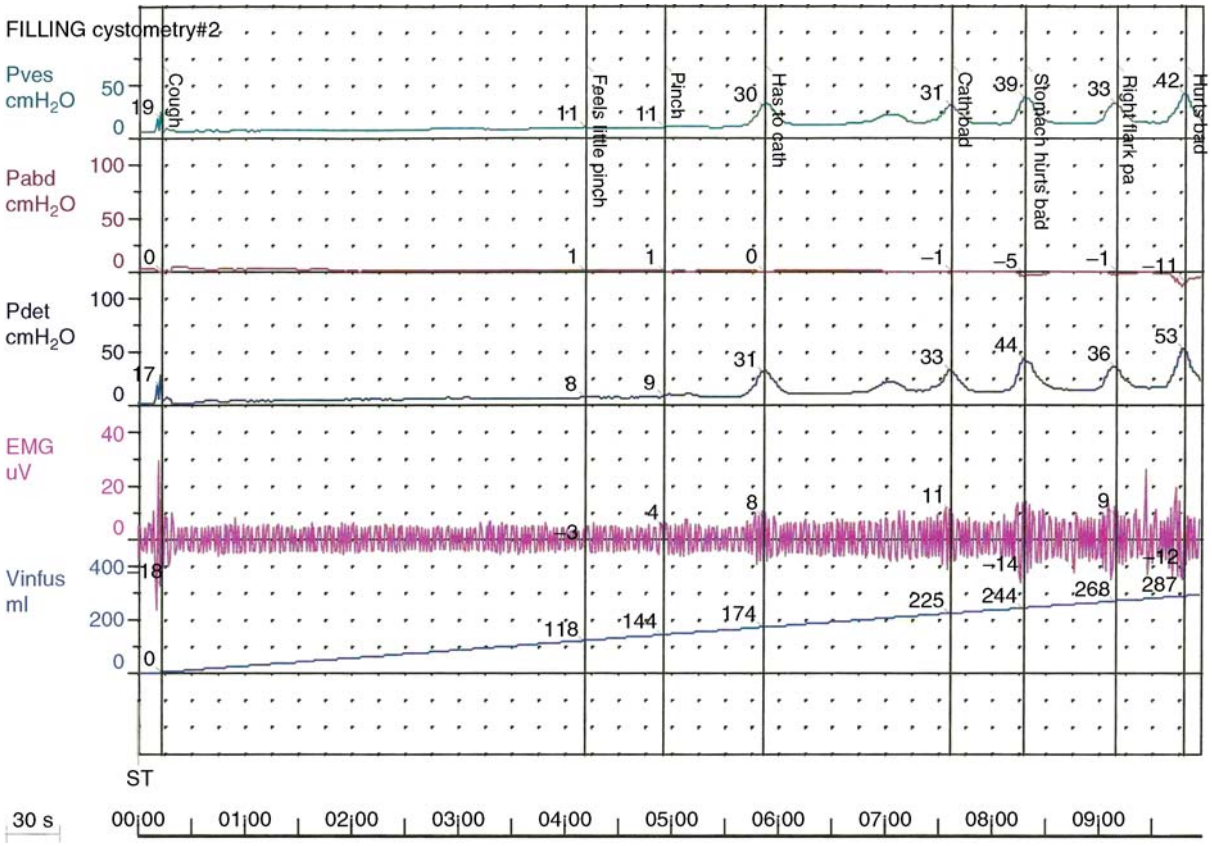


Figure 57-11

Example of urodynamic study demonstrating detrusor overactivity in a 12-year-old female with classic bladder exstrophy. Vesical pressure, Pves, demonstrates beginning of abnormal, uninhibited bladder contractions at a volume of about 174ml. These contractions become more frequent until capacity of about 287ml.



have the complete grasp of bladder dysfunction in children. The protean nature of this pathology relates to physics of pathologic stress during development in utero. Obstruction at the distal end of the prostatic urethra, perhaps a pathologic junction of the proximal and distal urethra, occurring at weeks 10–12, just prior to the production of urine by the developing kidneys (13) seems to be the cause of PUV. Development in the face of high-grade bladder outlet obstruction in utero impacts all aspects of the urinary tract.

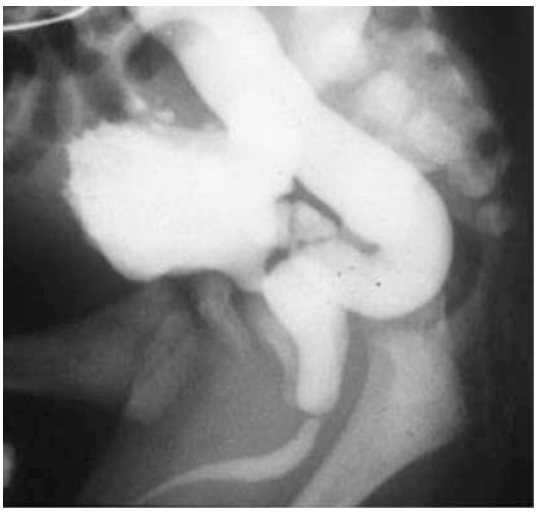
The kidneys become hydronephrotic, and in severe cases dysplastic, but most importantly often lose distal tubular function and cannot produce concentrated urine, hence the patient has obligatory high urine volumes. The ureters develop in an environment of obstruction and pressure work and are dysmorphic and are ineffective in peristalsis. The bladder develops in an environment of high pressure work and becomes dysmorphic with cellules, diverticuli, hypertrophy of muscle and encasement of muscle with collagen (14). The dynamics of the bladder are altered and demonstrate loss of compliance and capacity (*Fig. 57-12*).

At potty training the child tries to hold against abnormal pressures and develops a learned dyssynergia. The symptoms of daytime and nighttime wetting, polyuria, urgency/frequency, and constipation because of chronic state of dehydration predominate. Clinically the patient becomes more hydronephrotic and may go into renal failure. Urodynamics demonstrate increasing bladder pressures with filling, poor compliance; decreased sensation to extremely high bladder pressures, relatively poor capacity; and, to make matters worse, inability to completely empty the bladder, dysfunctional bladder wall kinetics, and sphincteric dysfunction (*Fig. 57-13*).

This constellation of pathologies is called the "Valve Bladder Syndrome" (VBS) (15, 16). Interestingly, if pati-

Figure 57-12

Voiding Cystourethrogram of a newborn boy with Posterior Urethral Valves. Note the dilated posterior urethra, the valve leaflets in the membranous urethra, the bladder trabeculation at the dome, and the massive vesico-ureteral reflux.



ents are treated by early valve ablation in the first weeks of life, and with subsequent normal filling and empting of the bladder many of the pathologic functions of the lower urinary tract can be reversed (17). Unfortunately, the abnormal renal function is not affected by early ablation.

Treatment of the patient with VBS includes: improvement of bladder volume and compliance with anticholinergics or bladder augmentation, or rarely diversion; improvement of bladder empting with biofeedback and/or intermittent catheterization, often through a Mitrofanoff channel and possible drainage at night; and, careful management of the renal failure.

Neurogenic Causes of Bladder Dysfunction in Children

Spinal Dysraphism

Myelomeningocele: Children with myelomeningocele had abnormal neural tube development between 2 and 5 weeks of gestational age. The neurologic dysfunction is multifactorial and has definite contributions from dysplasia of the neural elements from failure of the neural tube to completely form, and amniotic fluid "irritation" of the lower spinal and nerve roots (18).

Urodynamics should be done after spinal "shock" from newborn closure of the myelomeningocele resolves, typically at 6 weeks. Urodynamics will typically show that 2/3 of these children will have bladder contractions; about 1/3 will have detrusor areflexia. They frequently will have bladder sphincter dyssynergy characterized by pelvic floor/striated sphincter increased EMG activity concomitant with detrusor contraction (19). This combination potentially places the upper tracts at risk of hydronephrosis because of the increased bladder pressures. Therefore, clean intermittent catheterization would be initiated if the detrusor pressure at leak point is greater than 40 cm of water; and/or if the detrusor sphincter dyssynergia is clearly demonstrated with bladder filling (20). Oral anticholinergics (Ditropan, Detrol or Vesicare) are frequently initiated. Recently, placement of nighttime catheter gravity drainage has been advocated to keep the bladder completely decompressed for at least 8–10 h/day (21). Such early and aggressive treatment of these patients has resulted in a reduction in the need for bladder augmentation in many of these patients by protecting the development of the bladder in the first year of life (*Fig. 57-14*). In some cases urinary diversion with vesicostomy has been used as an alternative to early CIC (22).

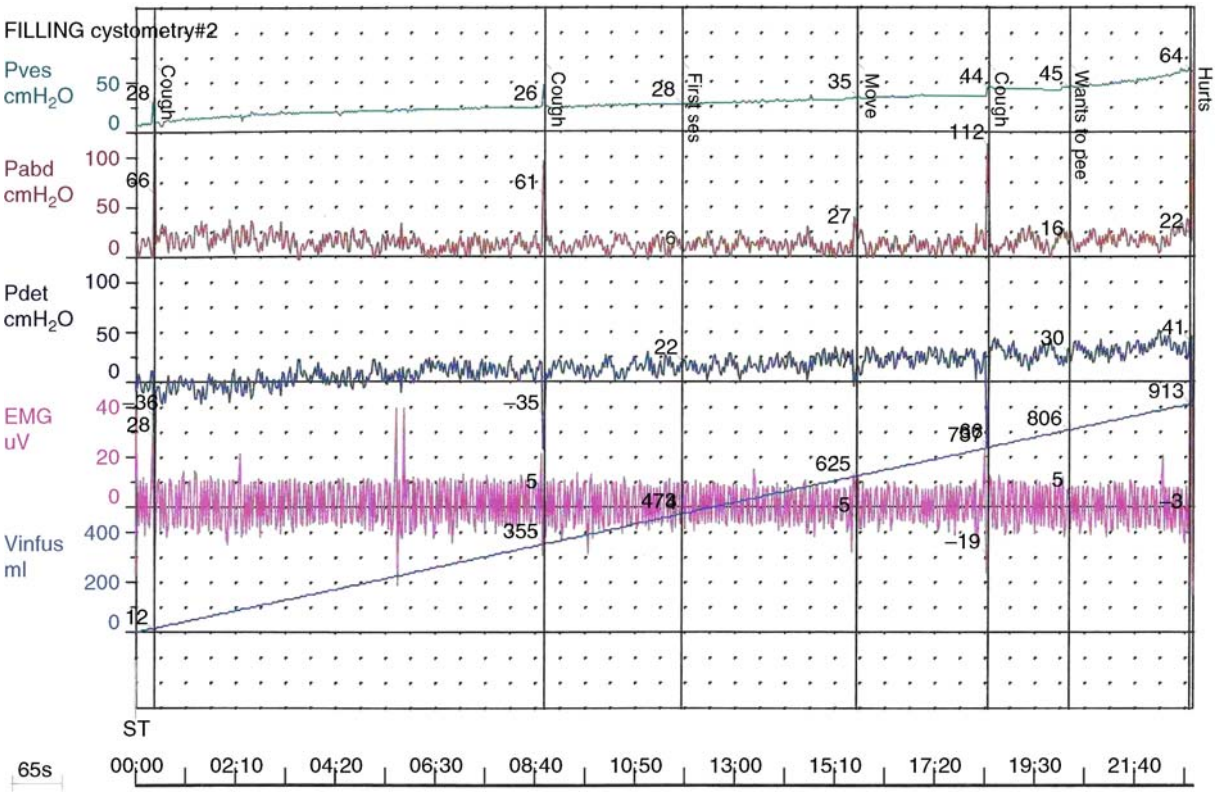
Lipomeningocele represents a challenging disorder to treat. Children with this disorder are somewhat similar to those with sacral agenesis in that they frequently do not have motor abnormalities of their lower extremities, therefore, socially, they are in diapers, but they ambulate normally. This increases the propensity for teasing and such by peers. Their bladder dysfunction is typically characterized by incontinence from uninhibited detrusor contractions, poor bladder wall compliance, and, particularly, an open bladder neck. They usually need to undertake intermittent self-catheterization in conjunction with anti-cholinergic medication to achieve social continence (23).

Sacral Malformations

Lower spinal canal lesions such as sacral agenesis, both partial and complete, are technically anatomic abnormalities which can affect bladder function. Most commonly these conditions result in detrusor-sphincter dyscoordination, but because they represent lower spinal cord lesions, they can be associated with the complete spectrum of obstruction and to low outflow resistance problems. Children have bladder dysfunction that is in some ways similar to myelomeningocele, but in some ways different.

Figure 57-13

Example of urodynamic evaluation on 15-year-old male with history of posterior urethral valves (PUV). This study demonstrates poor compliance of the bladder as seen on the top (Pves, or intravesical pressure) curve. Bladder pressure steadily rises to about 35cm/water at 625cc. This is abnormal. From this point on the filling curve pressure rises even further to a pressure of 64cm/water at a volume of about 913ml. This is definitely an abnormal cystometrogram demonstrating "valve bladder;" poorly compliant, but with typically preserved capacity.

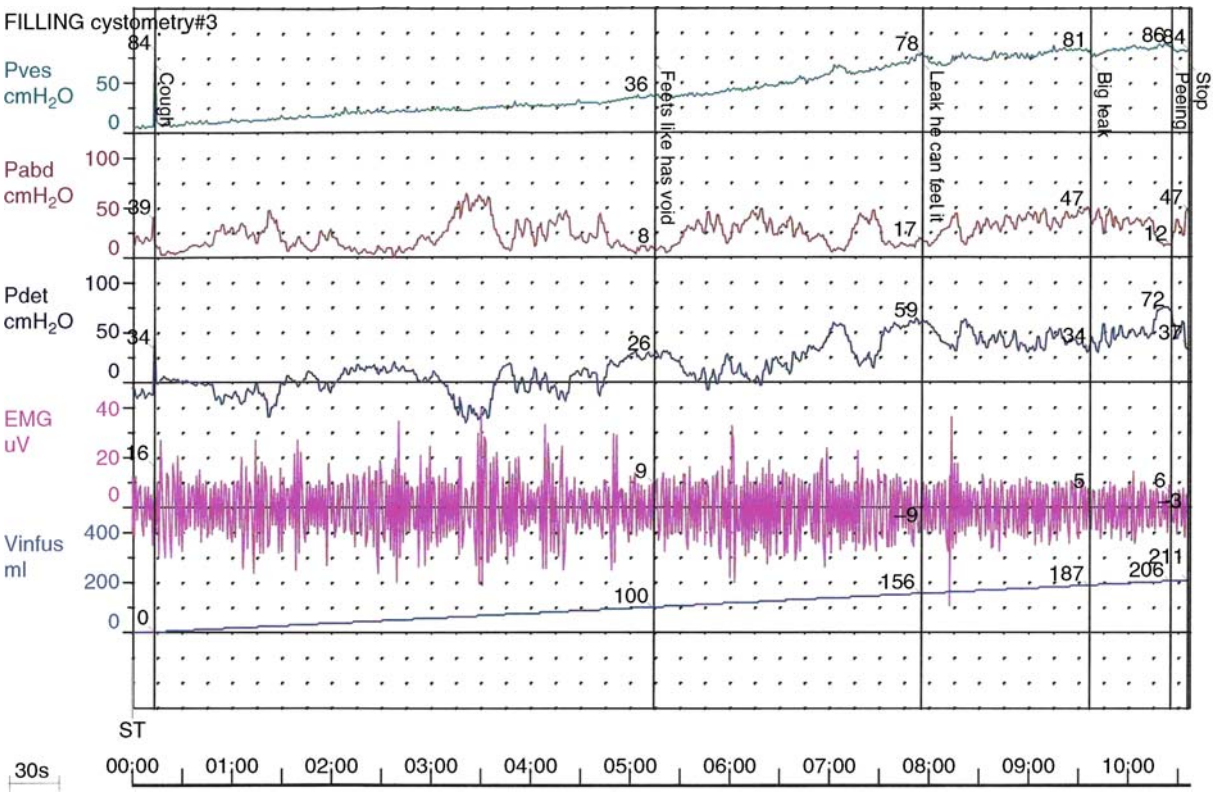


The difficulty with sacral malformations relates to the broad spectrum of potential anatomical defects from hemi sacrum with essentially no neurologic deficit to sacral regression with severe corporal deformity and neurologic bladder deficit resulting in total incontinence, with these patients usually requiring construction of a continent urinary reservoir. However, even in the less severe sacral deformity patient with normal lower extremity function severe urinary incontinence, from low urethral resistance and small bladder capacity, is very possible. Approximately 25% of children with sacral agenesis have no sign of denervation of the bladder, while 35% have upper motor neuron lesions and demonstrate overactive detrusor activity and detrusor-sphincter dyssynergia. The 40% of children with lower motor neuron lesions from sacral agenesis have detrusor areflexia and an open bladder neck, and usually present with urinary incontinence (24, 25).

Children with sacral malformations frequently have high-pressure bladders that do not empty well and can have acquired renal damage unless diagnosed and treated early. Many of these patients require major bladder surgery including bladder augmentation and a bladder neck procedure, and intermittent catheterization to achieve continence, even though by all other clinical measures they seem to be normal. They frequently have fecal incontinence that can be controlled with oral regimens and enemas, but they often will need both intermittent catheterization of their bladder to maintain continence and protect their upper tracts, and construction of antegrade continent enema stoma to achieve bowel continence.

Figure 57-14

Example of urodynamic study demonstrating poorly compliant, atonic neurogenic urinary bladder from myelomeningocele. Patient is 13-year-old male with spina bifida. Note how Pves curve shows steady rise in pressure without detrusor activity. Patient was not on anticholinergics at time of study.



Sacro Coccygeal Teratoma and Spinal Cord Tumors

Neurologic abnormalities of bladder dysfunction can occur from tumors, both sacro coccygeal teratomas and spinal tumors such as vascular or central nervous system tumors. A rare child will have a spinal cord injury in childhood. The evaluation and treatment are very similar to that of the bladder dysfunction from myelomeningocele.

Tethering of the Spinal Cord

Children with tethering of the spinal cord can present primarily with bladder dysfunction, as opposed to the children who have tethered spinal cord associated with spina bifida aperta (cystica), and the children with tethered spinal cord that present with musculoskeletal conditions. The urological problems frequently are initially diagnosed as "behavioral", and frequently the diagnosis of the tethered spinal cord is prolonged and difficult. The presence of secondary onset of urinary incontinence, or prolonged primary incontinence is the tip-off to looking for a tethered spinal cord as the source of the bladder dysfunction. Only half of the patients presenting with urinary complaints who were found to have a tethered spinal cord had other clinical signs thereof, such as back or leg pain, scoliosis, or club foot. The idea of early diagnosis of tethered spinal cord to obviate permanent damage to the urinary tract has been difficult to substantiate. Evaluation is essentially the same as for any child with dysfunctional voiding, with the emphasis on MRI evaluation of the spinal cord if suspicion of spinal cord tethering by history and/or physical exam exists. Treatment is similar if not identical to that discussed in the neurogenic causes of bladder dysfunction section (26).

Evaluation and Treatment of the Child with Anatomic and/or Neurogenic Bladder Dysfunction

Evaluation of the Dysfunctional Bladder

The fundamental approach to evaluating children with neurogenic and/or anatomic bladder dysfunction includes kidney and bladder ultrasonography, VCUG and urodynamic evaluation. VCUG in particular evaluates for vesicoureteral reflux and bladder neck function. Lasix renal scan is employed if there is hydronephrosis to evaluate for uretero-pelvic or uretero-vesical obstruction.

Medical Management of the Dysfunctional Bladder

The management of the patient with dysfunctional bladder is as varied as the spectrum of causes and dysfunctions. Basically, the common denominators are functional bladder size and urethral resistance, impacting effective bladder storage, and securing dependable bladder empting; the mainstay of this is Clean Intermittent Catheterization, (CIC). The basis of all management of abnormal bladder function is clearly CIC. If satisfactory bladder capacity cannot be achieved with anticholinergics or Botox injection, then bladder augmentation will be used. Because of the long-term consequences of bladder augmentation with bowel; infections, mucus, acidosis, stones, potential rupture, and cancer risk, alternatives are contemplated. These would include use of other tissue such as ureter, engineered bladder using autologous tissue culture techniques, or even growing a new bladder with stem cells. However, the problem of bladder expansion has not yet been solved. Malone antegrade continent cecostomy is frequently beneficial to the child with concomitant neurogenic bowel incontinence. There are a few techniques presently employed to make bladders larger and more compliant. These techniques will hopefully evolve in the future. *Table 57-2* lists medical and surgical options in regard to management of bladder problems in childhood.

Bladder Augmentation

Bladder augmentation, also known as Enterocystoplasty, is accomplished by reconfiguring the patient's bladder, using their own tissues. These techniques include the use of segments of large and small bowel and stomach, flaps made from dilated ureters, and tissue produced in the laboratory. Regeneration of new bladder tissue has been attempted; autoaugmentation and implantation of material that forms a framework for new bladder growth, all with some success but at significant cost as well.

Classic bladder augmentation with intestine was described in the 1950s by Gilchrist, using the intact cecum, separated from the GI tract on its vascular pedicle, reconstituting the GI tract, and anastamosing the inverted cecum to the dome of the bladder. Gilchrist also described complete replacement of the bladder: a continent urinary reservoir. Clinical experience, however, taught us that one needs to detubularize the colon segment to obviate peristaltic contractions, so the more "modern" era of bladder augmentation began in the 1980s, where large series of intestinal augmentation of the urinary bladder in children were reported (27). There are different intestinal segments available for use; ileum has been used most frequently, followed by the sigmoid colon. The use of a segment of

Table 57-2

Medical and Surgical Treatment of Anatomic and Neurogenic Bladder Problems in Childhood

Problem	Small Bladder Capacity	Poor Bladder Compliance	Bladder Neck and/or Sphincter Incompetence
Surgical Treatment:	Bladder Augmentation with Intestine or Ureter. Myomectomy/ Autoaugmentation, Neuromodulation.	Bladder Augmentation with Intestine or Ureter. Myomectomy/ Autoaugmentation, Neuromodulation.	Bladder Neck Plasty. Bladder Neck Sling, Bladder Neck Wrap, Artificial Urinary Sphincter, Injection of Bulking Agent at Bladder Neck, Bladder Neck Closure with Continent Catheterizable Channel
Medical Treatment:	Anticholinergic Medications, Combined alpha blockers/ anticholinergics, Botox Injection into dome of bladder	Anticholinergic Medications, Combined alpha blockers/ anticholinergics, Botox Injection into dome of bladder	Alpha agonist Medication

stomach to augment the bladder has a very special niche; that is, in the patient with renal failure. Gastrocystoplasty, however, is now being largely abandoned because of the concern of malignancy at a rate of about 10% in the gastric portion, which is unfortunate because gastrocystoplasties tended to work well. When ileum is used for bladder augmentation, typically a 20–25 centimeter segment is detubularized along the antimesenteric border and retubularized into an upside-down "cup," which is then anastomosed to the spatulated bladder. The ileum, however, does absorb urine and "recirculates" it, which can lead to metabolic problems; specifically hyperchloremic metabolic acidosis. Some people favor the use of the sigmoid colon for augmentation of the bladder. This is frequently a sigmoid loop that is mobilized on its vascular pedicle, detubularized along its antimesenteric border, retubularized into an upside-down "cup," and then anastomosed to the dome of the bladder. The sigmoid colon does not seem to be quite as compliant as the ileum, so typically 25 cm of sigmoid is used. The sigmoid colon is also frequently used for re-do augmentation when the patient has had an ileal augmentation but has insufficient capacity or compliance.

When undertaking ileal augmentation enterocystoplasty one needs to avoid the distal 20 cm of ileum as this is where vitamin B_{12} absorption occurs. Also, from a technical standpoint the front wall of the ileal augmentation frequently is extended anteriorly; almost to the bladder neck. This helps prevent the "hourglass" configuration that can develop after a bladder augmentation. The "hourglass" configuration causes the patient to catheterize only the "lower chamber," which is the native bladder, resulting in incomplete emptying, leading to complications such as stones, infections and potentially perforation (28). Patients augmented with ileum need lifelong monitoring of their serum bicarbonate levels, and sometimes venous pH. If hyperchloremic metabolic acidosis is diagnosed, which is rather frequent in this patient population, they are typically placed on either sodium bicarbonate or potassium citrate supplements orally to try to prevent osteopenia from the systemic acidosis. The incidence of bladder calculi in children with intestinal augmentation using ileum and sigmoid approaches 15% (29). This is felt to be from epitaxic calcification on mucus, so these patients are instructed to irrigate their bladders with large volumes (200–300 cc) of sterile water at least once a day to minimize stone formation.

Augmented bladders can become chronically overdistended, and then perforate, causing peritonitis, which can be life-threatening. For this reason, a great deal of effort goes into evaluating the patient preoperatively in terms of their compliance with an intermittent catheterization schedule. These children are basically almost never able to safely empty on their own, even with the Valsalva voiding. To achieve dryness they frequently need some type of bladder outlet procedure, via injection of a bulking agent, periurethral sling procedure, bladder neck plasty, artificial urinary sphincter or bladder neck closure in combination with appendicovesicostomy, Mitrofanoff's principle. However, the tendency to close the bladder neck frequently, which existed in the 1980s and 1990s, has diminished, and most clinicians are accepting a bit of urinary incontinence near capacity rather than subject the patient to the risks of perforation of the intestinal augmentation.

The potential for development of a malignant tumor in an intestinally augmented bladder seems to approximate 3% at 20 years (30). For this reason some advocate annual screening via cystourethroscopy of the patient who has an augmented bladder. However, malignancies can develop in between annual cystoscopies and become quite clinically significant (31). Efforts are underway to evaluate laboratory methods, such as NNP53 and FISH types of urine evaluations, to enhance the success of screening programs.

If bladder calculi develop in augmented bladders the smaller stones can be dealt with endoscopically via the existing urethra or continent catheterizable channel, with larger stones amenable to treatment by percutaneous access of the augmented bladder. The rare very large stone requires open excision.

In summary, intestinal segments in the urinary tract can be associated with metabolic acidosis and poor bone growth, mucus production with increased potential for infections and stone formation, and potential for cancer to develop with time. Stomach segments are associated with potential for metabolic alkalosis, hematuria/dysuria syndrome and potential for cancer to develop with time. Data for bladder regeneration alternatives is new, and at this point preliminary, as is the potential for engineering artificial bladders for implantation.

Voiding Dysfunction in the Apparently Normal Child

Voiding dysfunction, that is non-anatomic, non neurogenic bladder dysfunction, is by far the most common cause of urinary tract infection and urinary incontinence in children. The typical presenting symptoms of bladder dysfunction in a child are daytime incontinence and urinary tract infection. The history is extremely important in assessing the "normal" child with voiding dysfunction. The age at which they potty trained

is of significance. Typically this would be from 18 months to 3½ years of age. The ease of which they potty train can have some significance; difficult potty training generally leads to many more years of daytime incontinence. The dry interval by history, which is typically 90–240 min in the normal child, needs to be assessed. This interval may be shorter in a child with a neurological abnormality, so this is a useful screening question.

The evaluation typically includes a careful history, a careful physical exam looking for palpable kidneys and/or bladder, examination of the introitus to evaluate for bifid clitoris with epispadias, for example, or labial fusion, which can contribute to a dribbling type of incontinence and recurrent cystitis (*Table 57-3*). The most important part of the physical exam is assessing the lumbosacral spine. Sacral dimples, hairy tufts, hemangiomas and lipomas are all associated with tethered spinal cord, so children with these types of findings need to be evaluated with MRI.

One needs to be cognizant of normal, coccygeal dimples; these are only associated with neurological abnormalities on an extremely rare basis.

Table 57-3
Physical Clues to Neurogenic Bladder

• Sacral dimple, especially with a fixed base (not coccygeal dimples)
• Sacral/lumbar hemangioma
• Sacral/Lumbar hairy tufts
• Sacral/lumbar cutaneous sinus tracts
• Sacral/lumbar lipoma
• Flattening of the intragluteal cleft
• Abnormally-shaped buttock area consistent with sacral agenesis
• Non-palpable sacrum consistent with sacral agenesis
• Imperforate anus
• History of sacral-coccygeal teratoma resection

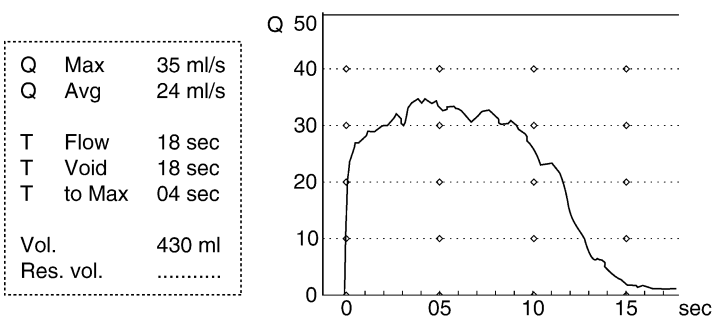
Radiographic Evaluation

The cornerstone of evaluation of the child with dysfunctional voiding is ultrasound of the kidneys and bladder. This study is usually reassuringly normal, but if hydronephrosis or bladder wall thickening are present, further investigation is warranted. If febrile urinary tract infections are occurring, or if upper tract involvement is seen by ultrasound, one needs to consider evaluating the child for vesicoureteral reflux with a voiding cystourethrogram.

Non-Invasive Urodynamic Evaluation

Uroflow is accomplished easily and painlessly in the clinic setting, providing a wealth of information about the lower urinary tract dynamics, without the need for catheterization of the urethra. Use of ultrasound to measure the post-void residual urine volume in the bladder after uroflow completes the investigation of the child's functional bladder capacity and voiding efficiency (*Figs. 57-4* and *57-5*). There are many types of uroflow patterns in children with dysfunctional voiding. A "Normal" uroflow pattern has a relatively rapid rise to a gentle peak, then a gradual decline to no flow (*Fig. 57-15*).

Figure 57-15
Example of normal uroflow study in 14-year-old male. Note rapid rise to peak flow and sustained peak flow phase of curve, followed by a relatively rapid decline to no flow. This is the so-called "Ayres Rock" configuration for normal uroflow in pediatric patient.



A "start and stop" pattern usually signifies some degree of straining with voiding, and is rare in children suggesting detrusor hypotonia. A "staccato" pattern, whereby the flow rate rapidly increases and decreases, but does not stop in between, is very suggestive of incomplete relaxation of the pelvic floor with voiding, the classic "dysfunctional voiding" situation (Fig. 57-16).

A "low flow" pattern is suggestive of urethral stricture in boys, and is almost never seen in girls (Fig. 57-17).

Uroflow with simultaneous evaluation of pelvic floor EMG activity is frequently of value in evaluating the child with refractory voiding dysfunction. Normally, the pelvic floor EMG activity abates with voiding (Fig. 57-18).

Figure 57-16

Staccato uroflow pattern, consistent with inability to relax the pelvic floor with voiding. This is the classic uroflow finding in a girl with dysfunctional voiding.

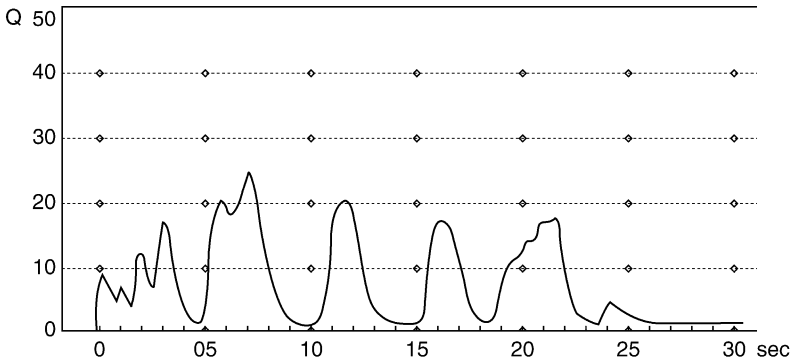


Figure 57-17

Abnormal low uroflow rate from known urethral stricture disease, with elevated post-void residual urine volume by ultrasound of the bladder in the clinic setting. This 15 year old boy with urinary incontinence and trisomy 21 should have a uroflow rate exceeding 30 ml/ sec, and the tracing above shows a maximum flow rate of only 4 ml/ sec. His PVR is clearly elevated at 493 cc.

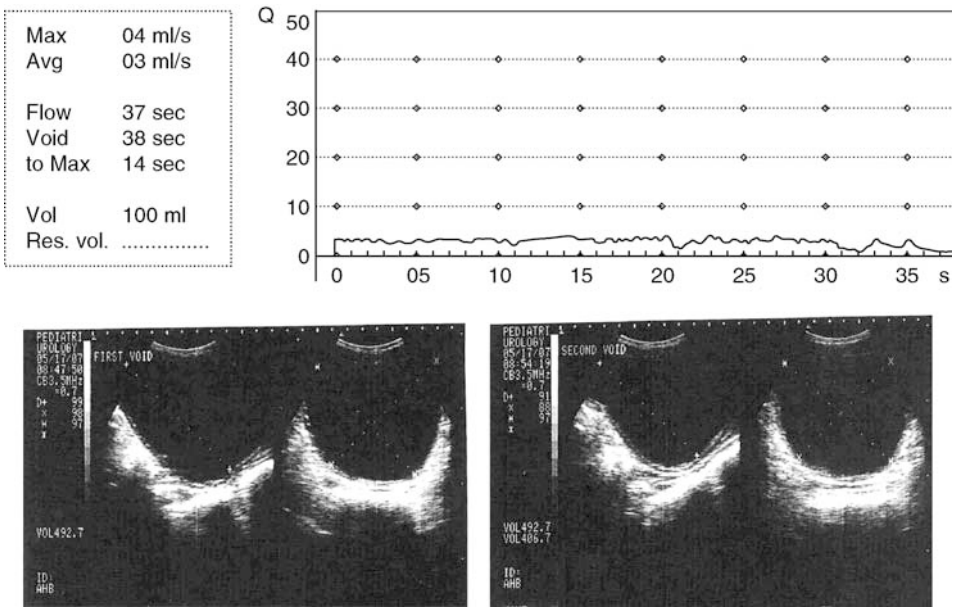
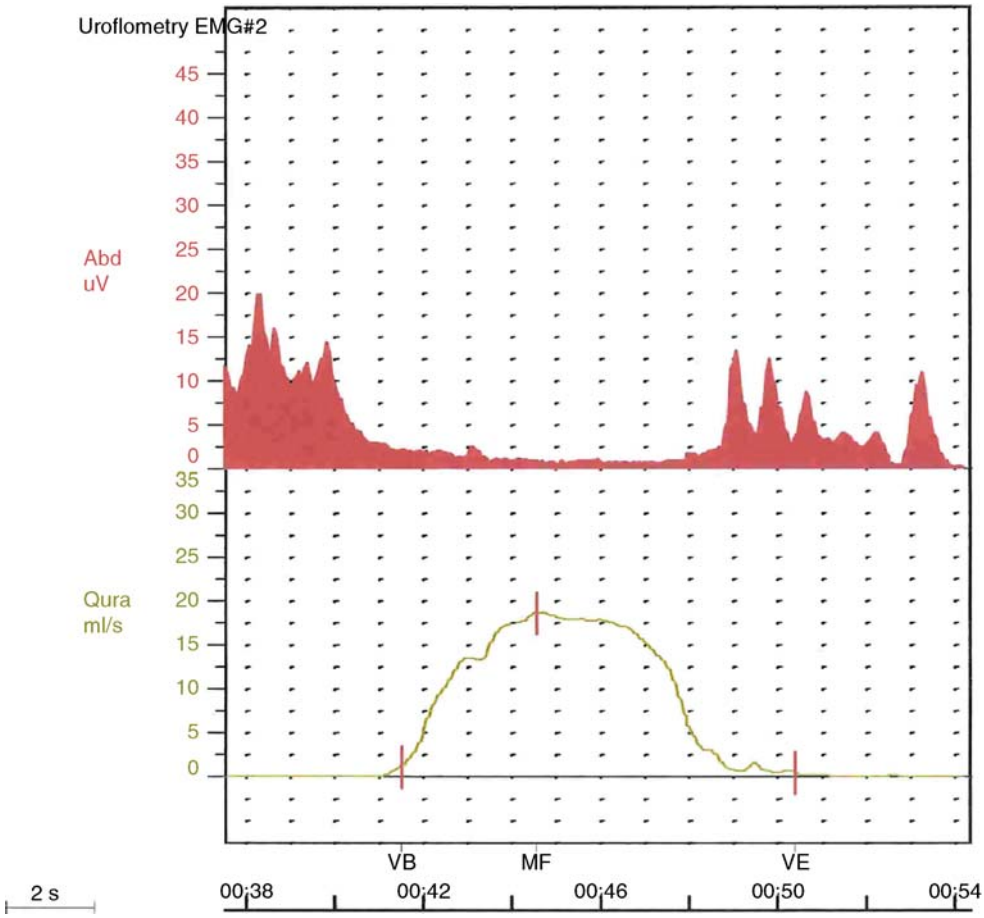


Figure 57-18

Example of normal uroflow/ EMG tracing, note how the upper curve, representing EMG activity of the pelvic floor, disappears completely with voiding, represented by urine flow rate in the lower tracing.



Some dysfunctional voiders are unable to relax their pelvic floor with voiding, frequently as a mal-adaptive learned behavior from intentionally delaying voiding, or from chronic constipation (*Figs. 57-19* and *57-20*).

Full Urodynamic Evaluation

Full urodynamic evaluation is only rarely necessary in the "Dysfunctional Voiding" patients, and should be used only for the most difficult patients who do not respond to therapy, or who present with other signs and symptoms which would implicate a possible anatomic or neurologic cause of the bladder dysfunction.

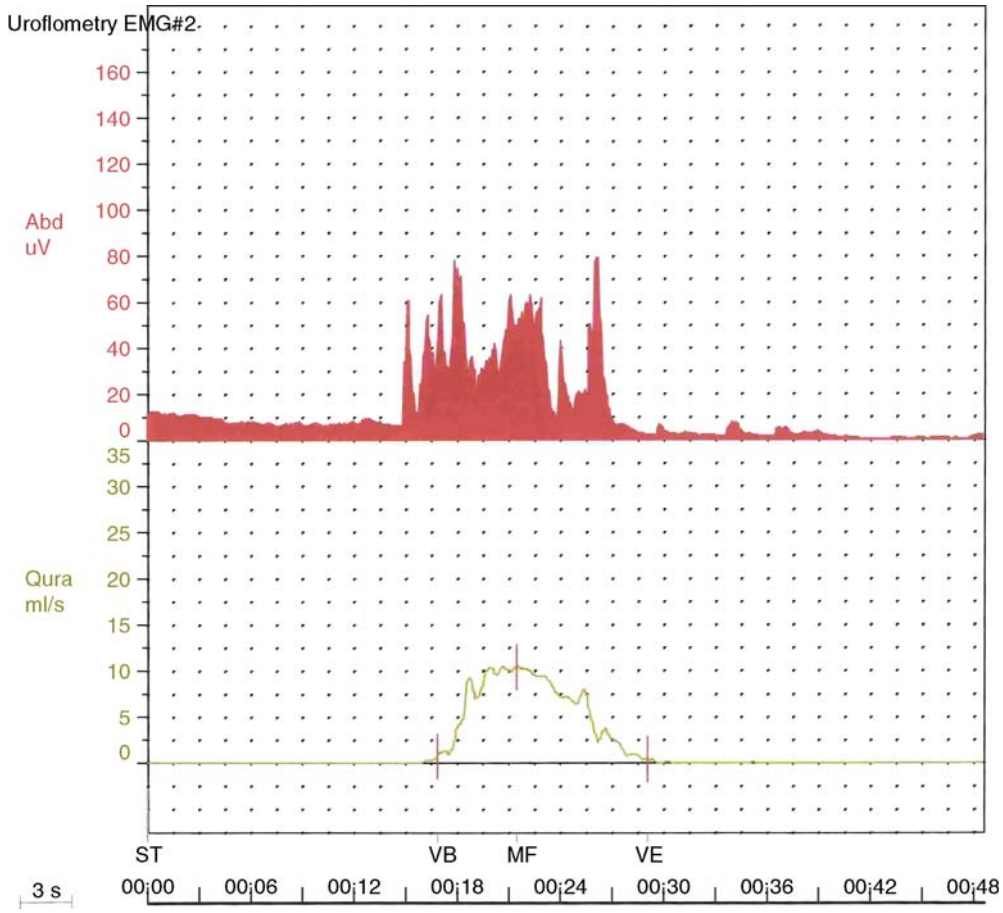
Management of the Child with Voiding Dysfunction

The management of the "normal" child with non-anatomic, non-neurogenic voiding dysfunction begins with behavioral modification. The cornerstone of this is timed voiding, and "star charts" providing visual reward for the child are helpful. Sometimes oral anticholinergic therapy has a role while the child continues to become more aware of how to void properly. Treating constipation if present, which it frequently is, early on with oral regimens is extremely critical to success of achieving continence.

The "normal" child with non-anatomic, non-neurogenic bladder dysfunction frequently has significant constipation that is unrecognized by the child or the parents.

Figure 57-19

Abnormal uroflow/ EMG. This 12 year old girl presented with urinary incontinence and recurrent afebrile urinary tract infections, cystitis. Her study shows a very abnormal amount of activity of the pelvic floor by EMG, as depicted in the upper tracing. The lower tracing shows abnormal low flow rate, from functional bladder outlet obstruction. This patient underwent pelvic floor biofeedback therapy and the results can be seen in *Fig. 57-20*.

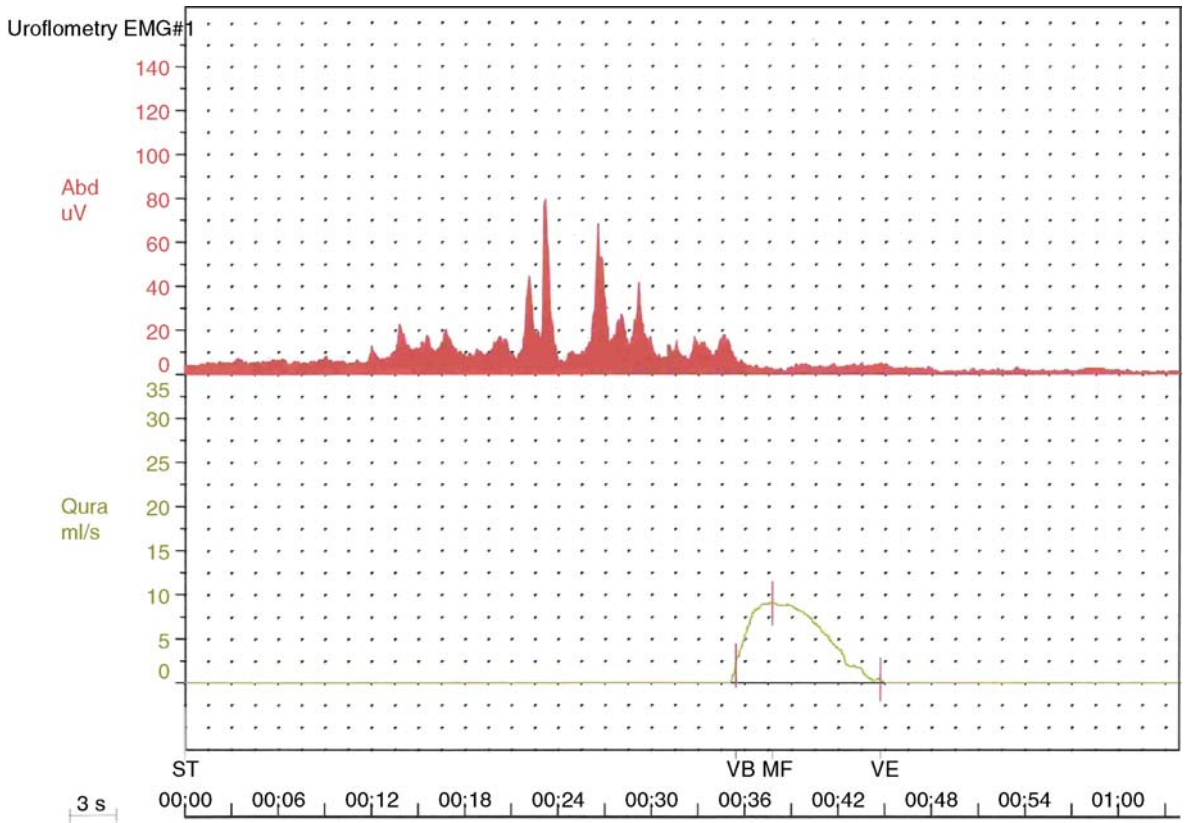


Chronic constipation is usually dietary in origin, and also there is a strong component of behavioral factors such as constantly "being in a hurry." As the "external sphincter" becomes the only way the child can control their bowel movements, they develop the inability to relax the external sphincter/pelvic floor apparatus with voiding, then carry higher residual urine volumes leading to urinary tract infection and incontinence. In order to help these children properly evacuate their rectum a number of recommendations need to be observed by the child and the family. Oral dietary fiber can succeed, but it is important to rotate different fiber sources because any child will typically tire of mandarin oranges, for example, within about 3 days. It is also very important to "make time to go to the potty," and not be in a hurry in the bathroom. Advising the child to get up early enough before school so they can eat breakfast and then have another ½ hour or so at home to take advantage of the "gastrocolic reflex" is important. Many children have resolution of their urinary incontinence with just this maneuver alone, primarily because many children are hesitant to go to the bathroom at school, citing concerns of: it is "dirty" or "not private enough."

For the "normal" child with non-anatomic, non-neurogenic bladder dysfunction who fails conservative measures such as behavioral modification and/or treatment of constipation, the next step is typically empiric anticholinergics. A number of different medications are available, such as oral anticholinergics, alpha blockers, antipsychotics, and occasionally medications like Ritalin will have

Figure 57-20

This shows normal uroflow/EMG curves after pelvic floor biofeedback in this 12 year old girl (see *Fig. 57-19*). Initially, patient has increased pelvic floor EMG activity with initiation of voiding, but then "quiets" her pelvic floor and voids satisfactorily.



value. There is some emerging evidence that a significant proportion of children that are "normal" with non-anatomic, non-neurogenic bladder dysfunction have psychological disturbances (32, 33).

Children who fail anticholinergic treatment are often referred for pelvic floor biofeedback. Non-invasive electrodes are applied to the skin overlying the rectus abdominus muscles and the perineum. Each of these channels is reflected visually on a computer screen for the child. They are initially taught to "contract" the pelvic floor muscles, and then they are taught how to properly identify the pelvic floor muscle group. Only then are they capable of learning how to relax their pelvic floor with voiding. This typically requires sessions twice a week for 2–6 weeks to properly identify and isolate the pelvic floor muscle group from the rectus abdominus muscle group. The motivated child is then expected to undertake these exercises at home, without which pelvic floor biofeedback is doomed to fail.

Non-Neurogenic Neurogenic Bladder, or "Hinman's Syndrome"

The worst end of the spectrum of the "normal" child with voiding dysfunction, even including urinary retention, is the so-called "Hinman's syndrome." This goes by the rather confusing name of "Non-Neurogenic Neurogenic Bladder" dysfunction. Fundamentally, this means that no identifiable neurological lesion is present by MRI. This likely represents an extreme end of the spectrum of voiding dysfunction whereby the child has learned to avoid urinary and fecal incontinence by overusing the striated sphincter/pelvic floor complex. They frequently have vesicoureteral reflux, urinary tract infections and hydronephrosis, and can proceed to renal failure. One needs to screen for a history of for psychological trauma and/or child abuse.

Management of these children is very difficult because these behaviors are very difficult to reverse. Timed voiding, bowel programs, pelvic floor biofeedback,

anticholinergics and alpha blockers are the initial treatment, but many of these children go on to intermittent catheterization. Some of them actually benefit from urinary diversion, at least temporarily, until they are motivated and compliant with intermittent catheterization. Botox injection into the dome of the bladder is beneficial in rare occasions. Also, Botox can be injected into the pelvic floor/striated sphincter apparatus in some children with hypertrophy of the striated pelvic floor musculature, but this is not being done commonly in children at present.

Conclusions

Bladder dysfunction in children represents a very broad spectrum of diagnoses that stretches from a "normal" child with retentive behavior who requires pelvic floor biofeedback and encouragement, to children with severe neurologic and anatomic problems. In most difficult cases our ability to treat lags far behind our ability to diagnose. In a few cases we are able to treat early and coax an abnormal bladder into normal physiology, i.e., newborns with posterior urethral valves and children with exstrophy/epispadias. But for most children with severe abnormalities that result in poorly functioning bladders, we have not yet developed the therapies that result in normalcy.

The critical phase of bladder growth is almost certainly in the first six months of life. When bladder outlet obstruction, such as posterior urethral valves, exists in utero, the bladder does not develop properly and the classic thinking was it is the luck of the draw whether or not the child would have good bladder function. However, there is accumulating evidence that normal bladder cycling, that is complete emptying of the bladder on a regular basis, is a major determinant in terms of beneficial healthy bladder growth. This complete emptying of the bladder also helps determine whether or not the patient can clear infection. Urinary infections are linked with bladder function. Vesicoureteral reflux is much more tightly linked, but not necessarily by cause and effect, to dysfunctional voiding than has been appreciated, almost certainly from an embryological basis of abnormal induction of the trigone from an abnormal ureteric bud.

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58 Urolithiasis

Dawn S. Milliner

Introduction

Urolithiasis is increasingly recognized in pediatric patients and is encountered in a variety of clinical settings: the 8-year-old boy who presents with hematuria, the 14-year old with cystinuria experiencing her fourth episode of renal colic in the past 6 years, the 10-year old with inflammatory bowel disease whose two renal stones were recognized incidentally during imaging for evaluation of abdominal pain, the premature infant with asymptomatic nephrocalcinosis and a stone, the 4-year old in the emergency room with gross hematuria and abdominal pain after recently starting indinavir medication. All have in common particulate material of mineral origin within the urinary tract. An understanding of how and why stones form, along with knowledge of the pathophysiologic states that promote urinary tract calculi, provide the basis for effective clinical management.

Epidemiology of Urolithiasis in Children and Adolescents

Urinary tract calculi account for between 1 in 1000 and 1 in 7600 hospital admissions in children and adolescents in the United States (1–3), a rate that is approximately 1/50th–1/75th that reported in adults (4). However, hospitalization is becoming more frequent. From 1999 through 2001, Pearle noted an increase of 34% in the number of pediatric and adolescent patients hospitalized in the U.S. with a primary diagnosis of urolithiasis (5) Since most patients with urolithiasis do not require hospitalization and there is a continuing shift to outpatient care for stone episodes, such data underestimate the frequency of this clinical problem.

With recent appreciation that presenting symptoms of urolithiasis may be different in children than in adults and improved radiographic techniques used more liberally than in the past, the diagnosis is now being made more often in pediatric patients. The frequency of occurrence has also been influenced in recent years by factors predisposing premature infants to nephrocalcinosis and urinary tract stones and advances in medical care that have resulted in survival through childhood and adolescence of increasing numbers of patients with medical conditions, such as cystic fibrosis, that are associated with urolithiasis. Finally, there is an increasing incidence of urolithiasis overall in recent decades in population based studies in industrialized countries (6).

An explanation for the lower incidence and prevalence of urolithiasis observed in children than in adults is incomplete, but may be related to higher concentrations of urinary inhibitors of crystal formation and crystal cell attachment such as citrate, magnesium, and certain macromolecules in children when compared with adults (7–9). Miyake et al. (8) noted that nucleation of calcium oxalate crystals was more strongly inhibited in urine from children compared with adults. Another study demonstrated greater inhibition of calcium oxalate crystal growth by the urine of stone formers and control subjects less than 20 years of age when compared with older individuals (10). Urinary macromolecules from pediatric patients more strongly inhibit the adhesion of calcium oxalate crystals to renal tubular epithelial cells In vitro than do those from adults (7). Robertson defined a risk index for calcium oxalate lithiasis and also found this index to be lower in children (11).

Among children and adolescents, males show a mild preponderance for stone disease overall (12–15). Male to female ratios of 1.4–2.1:1 were reported in four pediatric series from the United States, United Kingdom, Brazil, and Armenia (16–19). This contrasts with symptomatic adult stone formers, among whom males predominate by a ratio of 2–3:1, reaching a maximum of 4.8:1 from ages 40–60 years (6). There are exceptions among certain groups of stone formers, such as young children with developmental anomalies of the urinary tract and associated infection, who tend to be male and younger in age (12, 17).

Ethnic differences in stone prevalence have been observed. Caucasians appear to have the highest incidence followed by Hispanics, with individuals of African American descent less likely to form stones (20–22). Regional differences in diet, fluid intake, and climate may also influence stone prevalence, for example, a “stone belt” is recognized in southeast U.S.

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Clinical Features

Symptoms of renal colic and gross hematuria, pathognomonic of urolithiasis in adults, are seen less reliably in children (12, 23). Flank or abdominal pain or hematuria accounted for initial presenting features in 94% of adolescents, 72% in school age children, and accounted for presenting features in just 56% of those from birth to 5 years of age in one series (*Fig. 58-1*). In younger children, most report or are observed to have nonspecific abdominal pain, rather than typical renal colic. Indeed, among children up to 5 years of age the diagnosis of urolithiasis was made following a urinary tract infection or as an incidental radiographic finding during evaluation of other problems in nearly half of the patients (12).

In North American series, 60–78% of urinary tract calculi are present in the kidney at the time of diagnosis (12, 16, 24). The majority of those found in the ureters, bladder, or urethra have also originated in the kidneys. The bladder appears to be the site of stone formation in less than 10% of North American children, though in other areas of the world bladder calculi may be more often seen and are often referred to as endemic bladder stones (19, 25). Primary bladder stones tend to occur in younger children (11, 19). Such stones appear related to dietary factors (26, 27). A diet with a high content of whole-grain cereals and/or oxalate-rich vegetables together with a low content of calcium, animal protein, and phosphate is believed to be responsible (11, 25). The resulting urinary biochemical profile favors precipitation of ammonium acid urate and calcium oxalate, the most frequently occurring constituents of endemic bladder stones (25). Endemic bladder stones of childhood are common in several areas of the world including Thailand, India, Turkey, Syria, Iran, Iraq, Tunisia, Pakistan, Indonesia and the Sudan (11). There has been a striking decline in such stones in Europe and other developed countries over the last century (11, 27). Calculi that form in the bladder in North American children are nearly always associated with bladder malformations or prior surgery, such as augmentation cystoplasty, and are characterized by infection.

Stones from the upper urinary tract are comprised of calcium oxalate in 40–60%, calcium phosphate in 15–25%, mixed (usually calcium oxalate and calcium phosphate) in 10–25%, magnesium ammonium phosphate (struvite) in 17–30%, cystine in 6–10%, and uric acid in 2–10% as reported in several pediatric series (1, 12–14, 16, 19, 28).

Diagnostic Evaluation

Recommended initial evaluation of children or adolescents with urolithiasis (*Table 58-1*) includes complete medical and diet histories; family history of stones, hematuria, osteoporosis, or renal failure; and urinalysis with determination of pH, osmolality, and presence or absence of

Figure 58-1

Clinical features at time of initial presentation of 221 children and adolescents with urolithiasis. (Reproduced with permission.)

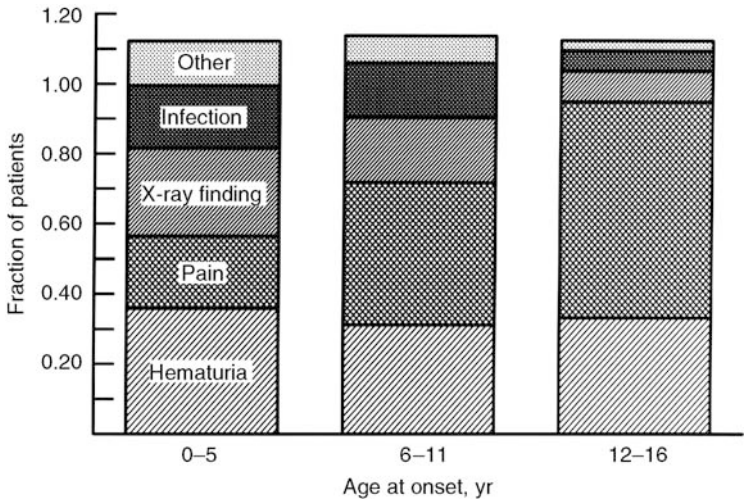


Table 58-1

Initial evaluation of the pediatric or adolescent patient with urolithiasis

Medical history
Diet, fluid intake
Medications, including vitamins, minerals, herbs
Family history
Physical examination
Radiologic appearance of stones and urinary tract
Urine analysis, including pH and microscopy
Urine culture
Urine calcium, oxalate, citrate, sodium, uric acid, phosphorus, cystine[a], creatinine, and volume and/or osmolality
Serum calcium, phosphorus, sodium, potassium, bicarbonate, uric acid, magnesium, creatinine, alkaline phosphatase
Stone analysis[b]

[a]Where indicated
[b]If stone available

crystals. Serum chemistries including calcium, phosphorus, uric acid, bicarbonate, sodium, potassium, magnesium, creatinine and alkaline phosphatase should be obtained. An estimate of daily fluid intake is an important aspect of the dietary history, as is an estimate of the daily intake of calcium, sodium, oxalate, protein, and fluid. The medical and diet histories should also include inquiry regarding use of over the counter food supplements, vitamins, minerals, or herbal preparations. Analysis of stone composition is very helpful in directing metabolic evaluation (*Table 58-2*) and should always be performed when stone material is available.

Urine concentrations, and where possible excretion rates, of solutes should be measured. In infants or young children, older children with developmental delay, or when a reliable timed urine collection cannot be obtained, random urine specimens with solute to creatinine ratio can be very useful. However, care is advised in interpretation since normal values often vary by age, diurnal status, and prandial state. Whenever a child is able to cooperate with a timed 24-h urine collection, this will provide more complete information including daily urine volume. Completeness of each collection should be verified with measurement of urine creatinine. Urine should be analyzed for calcium, phosphorus, oxalate, citrate, uric acid, sodium, creatinine, and total volume. Cystine measurements should be performed if there is any suggestion of cystinuria (stones unknown or of intermediate density, cystine crystals in urine, family history of cystinuria). Metabolic evaluation should be performed while the patient is on his or her usual diet, usual fluid intake, and normal activity. The studies should not be performed shortly after ESWL or a stone removal procedure and should not be obtained when the child is receiving intravenous fluids. If abnormalities are found, further metabolic evaluation such as a full supersaturation profile may be indicated.

Normal values for urine analytes are shown in *Table 58-3*. Excretion rates are higher in infancy. After 2 years of age, urine uric acid/creatinine and oxalate/creatinine ratios vary, decreasing by age, but excretion rates are consistent throughout childhood when determined in a timed collection and expressed as mg/1.73 m^2/24 h (29–32). Uric acid can also be expressed as mg/dL GFR, measured in either a random or timed urine collection (33). Reported normal values for some analytes vary from study to study and for oxalate vary by the measurement method. Ethnic and regional differences can also influence normal values (34). As urine solute measurements are used to guide treatment strategy, it is helpful to keep in mind that the excretion rate of each of these analytes is a continuous variable and should be regarded as a modifiable risk factor rather than focusing on a strictly defined threshold for normal.

Evaluation should include imaging studies of the urinary tract. Renal imaging can provide important clues to the nature of the urolithiasis (*Fig. 58-2* and *58-3*). Stones comprised of calcium oxalate or calcium phosphate are radiodense and readily seen by both ultrasound and CT. Struvite and cystine stones are of intermediate radiodensity. Uric acid, xanthine, 2,8 dihydroxyadenine, and orotic acid stones are radiolucent by conventional radiography but visible by ultrasonography or unenhanced computerized tomography (CT). Indinavir stones and rare matrix stones are radiolucent on conventional radiographs, of low density on CT, and may be difficult to distinguish from surrounding soft tissue by both ultrasound and unenhanced CT. Ultrasonography will be sufficient in most circumstances (35), while CT or intravenous pyelography will be required at times, as will voiding cystourethrography. Small stones and ureteral stones, in particular can be difficult to see by ultrasound. Nimkin compared imaging modalities for detection of stones in the pediatric population, and reported a sensitivity of 57% for plain films, and 77% for ultrasonography when compared with stone detection by CT (3). Others have reported better overall sensitivity for ultrasonography (36), but it may miss 30% of small papillary or calyceal stones (37) and may miss ureteral calculi (36).

Table 58-2
Stone type and associated conditions

Stone type	Associated conditions
Calcium oxalate	Idiopathic stone disease
	Hypercalciuria, idiopathic or of any cause
	Hyperoxaluria, idiopathic or secondary
	Enteric hyperoxaluria
	Primary hyperoxaluria
	ADPKD
	Cystic fibrosis
Calcium phosphate	Idiopathic stone disease
	Hypercalciuria, idiopathic or of any cause
	RTA
	Dent's disease
	Lowe syndrome
Magnesium ammonium phosphate (struvite)	Infection related stones
Carbonate apatite	
Uric acid	Hyperuricosuria
	Metabolic syndrome
	Ketogenic diet
	HPRT deficiency, partial or complete
	Tumor lysis syndrome
	Glycogen storage disease, type 1
	ADPKD
Cystine	Cystinuria

For acute renal colic, noncontrast helical CT scanning is more sensitive and specific for ureteral calculi than all other imaging modalities (38). Computerized tomography also has the advantage of providing detailed anatomic information (usually without the need for intravenous contrast administration), and improved sensitivity for very small stones or those that are poorly radiopaque (35). However, recent information suggesting an increase in malignancies following computerized tomography in childhood has raised concern regarding the degree of radiation exposure with this imaging modality (39). With careful attention to radiographic technique, the radiation exposure with CT can be significantly reduced while maintaining good imaging quality (38, 40) and may be less than that with other renal imaging techniques such as an IVP (41). Nonetheless, ultrasonography is the mainstay for imaging of urinary tract stones in children and adolescents with other modalities used only when specifically needed. For visualization of ureteral stones large enough to be seen by conventional radiography, KUB can be used to supplement an ultrasound.

Urine culture should be obtained in every patient with stones. Colony counts of bacteria in the urine in patients with infected stones may not reach the threshold of 10^5 cfu/mL, such that even lower colony counts should be further evaluated. In selected circumstances, such as suspected Ureaplasma, Corynebacterium urealyticum, or anaerobic infection, special culture techniques may be needed.

Management of the Acute Episode

Stones causing obstruction, acute renal colic, stones with a high potential for acute obstruction (e.g., a large stone in the renal pelvis), and infected stones should be evaluated jointly with a urologist. The majority of stones less than 5 mm in diameter will pass spontaneously, even in young children (15). The use of alpha adrenergic antagonists or calcium channel blockers to facilitate passage of ureteral stones has recently been introduced.

Table 58-3

Urine chemistry: Normal values

	Age	Random, mg/mg creat	Timed	Comments
Calcium[c](34, 143, 262) (52, 230, Butani 2004)	0–6 months 7–12 months ≥ 2 years	<0.8 mg/mg <0.6 mg/mg <0.21 mg/mg	<4 mg/kg/24 h	Prandial variation Sodium dependent
Oxalate[a](32, 116, 263) (204, 231)	< 1 year 1 to < 5 years 5–12 years >12 years	0.15–0.26 0.11–0.12 0.006–0.15 0.002–0.083	≥ 2 y.o.; < 0.5 mmol/1.73 m^2/24 h	Random urine mmol/mmol highly age dependent Excretion rate/1.73 m^2 constant through childhood and adulthood.
Uric acid (29, 33)	Term infant >3 y.o.	3.3 mg/dL GFR[b] <0.53 mg/dL GFR	<815 mg/1.73 m^2/24 h	 Excretion rate/1.73 m^2 from >1 year age constant through childhood.
Magnesium (4, 262)	>2 years	<0.12 mg/mg	<88 mg/1.73 m^2/24 h	Excretion rate/1.73 m^2 constant through childhood.
Citrate		>180 mg/g creat (264) >400 mg/g creat (4)		Limited data available in children
Cystine (4, 23)		<75 mg/g creat	<60 mg/1.73 m^2/24 h	Cystine > 250 mg/g creat suggests homozygous cystinuria

[a]Oxalate oxidase assay

[b](mg/dL U.A.) (S_{creat}/U_{creat})

[c]Regional and ethnic variations have been described (Butani Ped Neph 2004)

The proposed mechanism is relaxation of ureteral smooth muscle, with subsequent inhibition of ureteral spasms and dilatation of the ureter. Tamsulosin has been the most frequently used agent. Meta-analysis of available studies in adult patients suggests benefit when either of these agents are added to standard therapy, with a mean time to stone expulsion of less than 14 days in those receiving alpha-adrenergic antagonists (42, 43). This approach has been most successful with distal ureteral stones. To date, little information is available in pediatric stone patients. Use of corticosteroids or non-steroidal anti-inflammatory agents to reduce ureteral edema, either alone or in combination with alpha adrenergic antagonists, has shown mixed results (42).

Dissolution of cystine, uric acid, or struvite stones can sometimes be accomplished but is challenging, and best reserved for selected situations (44). Calcium stones are not amenable to dissolution.

Larger symptomatic stones are likely to require surgical intervention. The range of effective surgical interventions continues to increase, and now even very young patients can often undergo extracorporeal shock wave therapy (ESWL), ureteroscopic lithotripsy or removal, or percutaneous stone removal procedures (PCNL). Open lithotomy is rarely required. In recent years, improvements and miniaturization of endoscopic instruments have led to an increase in the proportion of stone procedures in children that are accomplished by ureteroscopic approach (45, 46). Outcomes have been good (45, 47, 48). Nonetheless, ureteroscopic procedures may be associated with ureteral or urethral injury, particularly in boys, or vesicoureteral reflux, and require a greater degree of technical proficiency in children than in adults (47).

Due to the potential for high energy shock waves to permanently damage renal tissue, there has been concern regarding possible long-term adverse effects of ESWL on the developing kidney. Similar concerns have been raised regarding percutaneous nephrolithotomy (PCNL) which requires insertion of a nephrostomy tube or other catheter through the renal tissue. A careful study of 16 children

Figure 58-2

Multiple calcium oxalate stones in both kidneys of a 5-year-old boy with primary hyperoxaluria, type I.

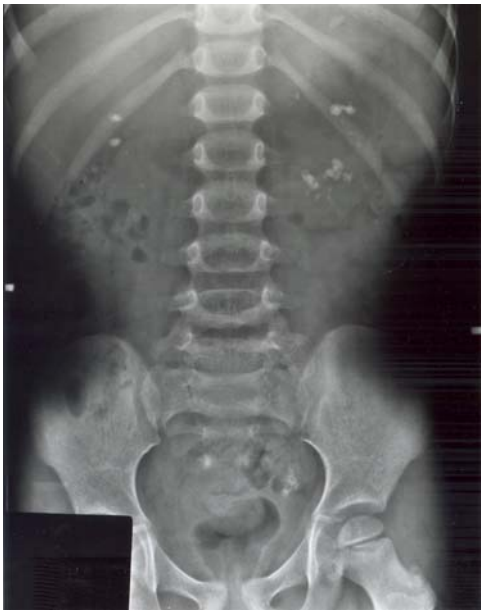
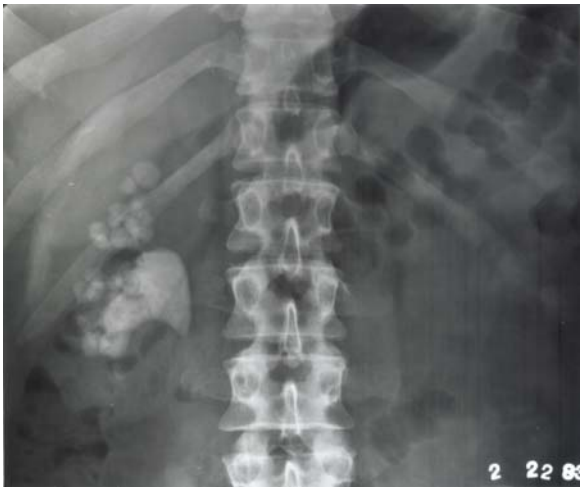


Figure 58-3

Staghorn calculus in right kidney of a patient with cystinuria



treated for a single stone found no morphologic changes in the kidneys by ultrasound following ESWL, and no significant changes in serum creatinine, but did document significant posttreatment increases in urinary beta 2 microglobulin and enzymuria (49). These changes were considered evidence of proximal renal tubule dysfunction, and resolved by 14 days post procedure. One study suggested impaired growth of kidneys in children following ESWL (50), though a more recent study found no difference in observed versus expected growth of treated kidneys during a mean of 6.2 years following ESWL, ureteroscopic stone removal procedures, nor PCNL (46). The number of PCNL patients was small, however. Most information with second or later generation lithotriptors does not suggest clinically significant renal parenchymal scarring, impaired renal growth, permanent renal function abnormalities, nor hypertension in children following ESWL or PCNL (46, 51–53). Information to date is still limited, particularly in children under the age of 6 years at the time of treatment.

The best treatment for infection related stones involves eradication of the infectious agent. Since stone material is often not well penetrated by antibiotics, removal of infected stone material is often required. Urease inhibitors such as acetohydroxamic acid (AHA) can be helpful but have a high incidence of adverse effects (54). Patients with infected stones requiring ESWL, percutaneous ultrasonic lithotripsy, or an open procedure require attentive antibiotic management as bacteria may be released rapidly from stone material on fragmentation during lithotripsy or instrumentation. Struvite stones may be soft and friable, with particular vulnerability to stone fragment retention following ESWL. Any stone fragments remaining may harbor bacteria in the interstices of the fragments, making eradication of the infection very difficult. In addition, stone fragments provide a nidus for new struvite stone formation, which can occur rapidly. It is for this reason that percutaneous nephrolithotomy is still sometimes required to assure complete removal of stone material.

Stones that are not infected and not causing symptoms or associated with impending or established obstruction may be managed medically. Growth in size of existing stones and formation of new stones are common. Treatment is directed to minimize the likelihood of this occurring. For all forms of urolithiasis, increased oral fluid intake to maintain a urine volume of greater than 750 mL/day in infants, 1000 mL/day in children under the age of 5 years, 1500 mL/day to the age of 10 years, and greater than 2 L/day in older children and adolescents is recommended. Avoidance of dietary excess of calcium, oxalate, and sodium are important. However, reduction to less than the RDA may be problematic with respect to nutritional adequacy, normal growth, and development. Reduction to the appropriate RDA for age for calcium, and not lower, is recommended even in children with known hypercalciuria. Indeed, recent epidemiologic studies in adults suggest that dietary calcium reduction is

associated with an *increased* likelihood of urolithiasis (55), perhaps due to greater absorption of dietary oxalate when less calcium is present in the intestinal tract. Dietary sodium restriction is of particular importance in patients with hypercalciuria and those with cystinuria, as it can reduce the urinary excretion of calcium (56, 57) and cystine (58–61), respectively.

Patients with idiopathic urolithiasis who have normal urine chemistries or mild hypercalciuria and a single stone may be managed initially with increased fluid intake and dietary modification alone. If metabolic stone forming activity is not adequately controlled by this approach, addition of potassium citrate therapy, or for those with hypercalciuria a thiazide, is the next step. For patients with predisposing metabolic or other factors, treatment should be directed specifically to the abnormality(ies) found. Suggested initial therapy, and second line therapy if problems persist despite initial intervention, are shown in *Table 58-4*. For optimal management of patients with significant predisposing factors and those with complex and recurring stone disease, an understanding of the mechanisms of stone formation as well as causative factors and pertinent disease states is necessary.

Mechanisms of Stone Formation and Nephrocalcinosis

Several steps are required for stone formation, beginning with crystal formation. Growth of crystals, their aggregation, and adherence of crystals to epithelial cells of the urinary tract follow. Although the concentration of urinary solutes is the principle determinant of supersaturation, and thus of crystal formation, other factors play a role as well, including ionic strength, pH, and naturally occurring promotors and inhibitors present in the urine. Urine in normal individuals is often supersaturated with calcium oxalate, calcium phosphate, or sodium urate, yet most individuals do not develop stones. That is because concentrations of mineral solutes, ionic strength, pH, promotors, and inhibitors in the urine vary throughout the day as influenced by fluid intake, dietary constituents, and normal metabolism. A dynamic process of crystal formation, growth, aggregation, disaggregation, and dissolution of crystals accompanies the diurnal variations in the physicochemical state of the urine.

Promoters of crystallization are not well characterized but may include urinary macromolecules and lipids (62). Lipids are integral to the organic matrices of mineralized tissues as well as to pathologic calcifications (62). Urinary tract stones are no exception. Another type of promotion that has received attention is the role of crystals of one salt in inducing crystallization of another. For example, sodium urate (63, 64) or calcium phosphate (65) acts as a nidus for calcium oxalate crystal formation. These principles help to explain the observation that some patients with calcium stones are hyperuricosuric yet do not demonstrate hypercalciuria (66).

Inhibitors of crystallization act at the crystal surface, most often interfering at active crystal growth sites (67) and by forming soluble complexes with potential crystal ions. Naturally occurring inhibitors of calcium oxalate and

Table 58-4

Treatment for metabolic stone disease

Metabolic abnormality	Initial Rx	Second line Rx
Hypercalciuria	Reduce dietary Na^+ Dietary calcium at RDA Thiazides[a]	Potassium citrate Thiazides Neutral phosphate
Hyperoxaluria	Reduce dietary oxalate Potassium citrate	Neutral phosphate[b] Magnesium Pyridoxine[b]
Hypocitric aciduria	Potassium citrate	Bicarbonate
Hyperuricosuria	Alkalinization	Allopurinol
Cystinuria	Alkalinization Reduce dietary Na^+	Thiola D-penicillamine Captopril

[a]If hypercalciuria is severe or there is osteopenia

[b]Initial therapy in primary hyperoxaluria

calcium phosphate crystal formation in the urine include citrate, pyrophosphate, magnesium, and urinary macromolecules such as glycosaminoglycans, and nephrocalcin. These properties can be used to therapeutic advantage in patients with calcium oxalate and calcium phosphate stones (68, 69).

From the time filtrate is formed at the glomerulus until the urine reaches the bladder there is an environment of constant flow, such that crystals formed are generally washed out of the urinary tract. In order for stones to form, the crystals must either aggregate to a size sufficient to obstruct a renal tubule and impair flow (free particle theory) or the crystal must adhere to the renal epithelial cell, the papillary tip, the wall of a calyx, or other location in the urinary tract (fixed particle theory). Adherence is favored by injury to the renal tubular epithelium or the urothelium. Injury may be induced by toxins, by infection, or by crystals themselves. Once crystals are relatively stationary in the urinary tract, permitting growth and aggregation, a stone may develop. Stasis of the urinary tract, with or without frank obstruction, is an important contributor. Among children who do form stones, predisposing metabolic factors, infection, and/or urinary stasis are identified in the majority (4). Predisposing factors should be systematically sought in every pediatric or adolescent patient with stones, since such factors form the basis of effective treatment interventions.

Nephrocalcinosis is caused by microscopic mineral deposits in renal parenchyma, usually in the medullary regions (70). It is often seen concomitant with urolithiasis and appears to share many of the same metabolic risk factors and pathophysiologic mechanisms. When it occurs independent of urolithiasis, metabolic changes such as marked hypercalciuria or hyperoxaluria, or renal immaturity are typically present (*Table 58-5*). Nephrocalcinosis occurs more often in infants and young children and is more often associated with loss of renal function than is urolithiasis. In one study of 152 children and adolescents with nephrocalcinosis, various hereditary renal tubular disorders were identified in 32% (71). A family history of nephrocalcinosis or urolithiasis was noted in 7.2% and 19.1%, respectively (71).

Table 58-5
Causes of nephrocalcinosis

Prematurity
Hypercalcemia
Williams syndrome
Primary neonatal hyperparathyroidism
Paracellin 1 disorders
Bartter's syndrome
Dent's disease
Lowe syndrome
Cystinosis
Distal RTA
Calcium sensing receptor disorders
Primary hyperoxaluria
Cystic fibrosis

Causative Factors in Urolithiasis

Seventy-five percent of children and adolescents with urolithiasis have identifiable predisposing causes for stone formation (12–14, 28). From compilation of studies that included 492 North American pediatric patients with urolithiasis Polinsky noted that metabolic causes accounted for approximately 33 %, structural urinary tract abnormalities for 32 %, and infection related stones for 4 %, although differences in classification complicated combining individual studies. In most published series, complete metabolic evaluations were not performed in all patients, likely underestimating the contribution of metabolic risk factors. This impression is supported by a study in which all patients were screened for metabolic abnormalities in which a metabolic cause for stone formation was identified in 25/47 patients (53%) (72). In some pediatric reports, especially from Europe, a higher proportion of patients have infection cited as the primary cause of urolithiasis (4, 17, 19, 73), though this pattern may be changing (74). In most series, infants and young children more frequently have infection related stones than do older children or adolescents (12, 17), while adolescents account for the majority of those with idiopathic stone disease. No contributing cause for stone formation was identified (idiopathic stone disease) in 28 % of North American patients and 14% of the European patients compiled by Polinsky (4).

It is common to find more than one predisposing factor in a given patient (12). In one study of 221 pediatric patients with urolithiasis, 36% of those with structurally abnormal urinary tracts were also found to have metabolic abnormalities predisposing to stone formation and 39% had chronic infection (12). Among the patients with infection related stones, 29% also had metabolic abnormalities. Thus infected, obstructed, or structurally anomalous urinary tracts do not obviate the need for careful metabolic assessment. Because of the high likelihood of

identifying causes of stone formation in children and adolescents and their many future years at risk for further stone disease, complete assessment for stone forming factors is indicated in all children.

Metabolic Factors Predisposing to Stone Formation

High urinary concentration of calcium, oxalate, uric acid, or cystine resulting from either increased renal excretion or low urine volume results in supersaturation of the urine, thus favoring crystal and stone formation. Among naturally occurring inhibitors of stone formation in the urine, citrate, magnesium, and pyrophosphate are the best defined. A number of urinary macromolecules including glycosaminoglycans, osteopontin, and urinary prothrombin fragment 1 appear to play a role but as yet are not well studied in children and adolescents (75). Identification of the specific predisposing metabolic factors (*Table 58-6*) in each patient is of importance in developing the most effective therapeutic regimen.

Solute Excess

Hypercalciuria

Hypercalciuria as currently defined (>4 mg/kg/day or > 0.21 urinary calcium/creatinine ratio) is common, occurring in from 5–10 % of healthy children (34, 76). Regional and ethnic variations in urine calcium excretion have been observed (34). Hypercalciuria has been associated with hematuria, dysuria, urinary urgency, and perhaps recurrent urinary tract infections (77–80) as well as to urolithiasis. It is the most common metabolic factor in children and adolescents with urolithiasis, accounting for approximately half of such abnormalities identified. Urine calcium is influenced by a number of dietary constituents. Physiologic hypercalciuria related to dietary excess of sodium, calcium, or protein or to deficiencies of potassium or phosphorus should be identified and is readily addressed. Most hypercalciuria is idiopathic. Inherited forms have been well described, and some appear to follow an autosomal dominant or codominant pattern (81–85). However, when one considers the respective roles of the gastrointestinal tract, bone, and the kidney in calcium economy, it is not surprising that hypercalciuria more often appears to be a polygenic trait. A number of rare, specific monogenic causes of hypercalciuria have also been identified involving the proximal renal tubule (Dent's disease, hereditary hypophosphatemic rickets with hypercalciuria due PHEX gene mutations, Lowe syndrome), thick ascending loop of Henle (Bartter syndrome, activating mutations of the calcium sensing receptor gene, familial hypomagnesemia with hypercalciuria and nephrocalcinosis due to mutations of the claudin 16 gene /paracellin 1) and the distal tubule (pseudohypoaldosteronism type II, primary renal distal RTA), Detailed discussion of each is beyond the scope of this chapter, but the reader is referred to recent reviews (76, 85). Not all are associated with urolithiasis. A family history of stones, isolated hematuria, or early osteoporosis can be informative. Among the monogenic causes of hypercalciuria, a few deserve further mention. They include the x-linked disorders related to mutations of the CLCN5 chloride channel, the gene for which is located on chromosome Xp11.22. This chloride channel is expressed in the cortical proximal tubule, medullary thick ascending limb of the loop of Henle, and α-intercalated cells of the human nephron (91). Mutations of this channel have now been demonstrated in four conditions, previously described as separate clinical entities: X-linked nephrolithiasis with renal failure, Dent's disease, x-linked recessive hypophosphatemic rickets, and low molecular weight proteinuria with hypercalciuria and nephrocalcinosis. These conditions have in common hypercalciuria, nephrolithiasis, nephrocalcinosis, renal tubule dysfunction characterized by low molecular weight proteinuria and impaired absorption of phosphorus, progressive renal insufficiency, and in some cases rickets (92). It has recently been suggested that all be referred to collectively as Dent's disease (93).

Hypercalciuria in patients with Dont's disease is typically of moderate degree, although may be as high as 10 mg/kg/day in some children. The reason for the hypercalciuria is not well understood, but it appears to be responsive to treatment with thiazide diuretics (94). Amiloride was not found to be helpful (92, 94). Proteinuria of up to 1 gram per day in children is a diagnostic feature of males affected with Dent's disease (92) and is frequently present in lesser amounts in carrier females (95, 96). Urinary retinol binding protein is distinctly elevated and of value in diagnosis (95).

Other mechanisms have been implicated in idiopathic hypercalciuria and include renal tubular phosphate leak, increased 1,25 dihydroxyvitamin D synthesis, increased renal prostaglandin E2 production, and enhanced bone resorption, among others (76, 85). A complex interplay of environmental and genetic factors involving the intestinal tract, bone, and kidney appears responsible for hypercalciuria in most patients (86).

Table 58-6

Metabolic abnormalities associated with urolithiasis

Metabolic abnormality	Idiopathic	Physiologic or secondary	Renal tubule transport disorder or inborn error of metabolism
Hypercalciuria	Polygenic trait	Dietary salt excess	Dent's disease (CLCN5 mutations)
		Dietary calcium excess	Lowe Syndrome (OCRL1 mutations)
		Vitamin D excess	Hereditary hypophosphatemia with rickets and hypercalciuria (PHEX mutations/NPT2a)
		Ketogenic diet	
		Loop diuretics	
		Immobilization	RTA (ATP6B1, AE1 mutations, carbonic anhydrase deficiency)
		Metabolic acidosis	
		Phosphate depletion	
		Prematurity	Familial hypomagnesemia – Hypercalciuria (Claudin 16 mutations)
		Prostaglandin E_2	
		Hypercalcemia	Calcium sensing receptor
		Hyperparathyroidism	Mutations
		Hyper- or hypothyroidism	
Hyperoxaluria	Mild, associated with idiopathic stone disease	Dietary oxalate excess	Primary hyperoxaluria I and II
		Enteric hyperoxaluria	
		Gastric bypass surgery	
		Parenteral nutrition in premature infants	
		Orlistat	
		Ethylene glycol	
Cystinuria	–	Renal tubule immaturity in infants	Cystinuria
Hyperuricosuria	Mild, associated with idiopathic stone disease	High protein diet	HPRT deficiency, complete (Lesch Nyhan) or partial
	Familial forms	Ketogenic diet	
		High dose pancreatic enzyme therapy	
		Diabetes	
		Tumor lysis	Glycogen storage disease 1
		Myeloproliferative, lymphoproliferative disorders	
		Sulfinpyrazone	
		Phenylbutazone	
Hypocitric aciduria	Mild, associated with idiopathic urolithiasis	Metabolic acidosis	Distal RTA
		Ketogenic diet	
		Hypokalemia	
		Bacteriuria	
Xanthinuria		Allopurinol therapy	Hereditary xanthinuria

Among children with idiopathic hypercalciuria who did not have urolithiasis at initial diagnosis, the likelihood of developing calculi was 4–17% during 3–11 years of follow-up in three studies involving a total of 121 patients (56, 78–80). The risk of urolithiasis appears to increase with age (79). In addition to the development of urolithiasis and urinary tract symptoms, idiopathic hypercalciuria is associated with reduced bone mass in as many as half of affected patients (87, 88). Fractures have been observed. Treatment with thiazide diuretics is effective in normalizing urine calcium in most patients with idiopathic hypercalciuria, though thiazide failure has been observed in some (89).

Secondary forms of hypercalciuria are common and may occur in response to administration of medications such as loop diuretics or carbonic anhydrase inhibitors. Prolonged immobilization in children or adolescents, excess intake of calcium or vitamin D, and high concentrations of circulating parathyroid hormone all predispose to hypercalciuria. Phosphate depletion causes a hypercalciuria mediated by an increase in 1,25 dihydroxyvitamin D synthesis (90). Chronic metabolic acidosis from any cause has the potential to result in hypercalciuria due to bone resorption. In distal renal tubular acidosis, the deficiency in acid secretion results in significant hypercalciuria. Once the metabolic acidosis is corrected with exogenous administration of base, the hypercalciuria resolves. Hypercalcemia of any cause is likely to result in secondary hypercalciuria.

Hyperoxaluria

Hyperoxaluria accounts for 2–20 % of metabolic factors identified in children and adolescents with urolithiasis (12, 97, 98). Mild idiopathic hyperoxaluria (ranging from 0.45 to 0.6 mmol/1.73 m^2/24 h) is not infrequently observed in conjunction with hypercalciuria in patients with idiopathic stone disease (98, 99). The etiology of the hyperoxaluria is unknown, but has variably been ascribed to metabolic variations resulting in increased oxalate production or to enhanced gastrointestinal absorption (99–101). Approximately 10–20% of the oxalate in the urine has traditionally been regarded as derived from dietary sources (102), although some have argued that the contribution of dietary oxalate is higher (103). Dietary oxalate excess (particularly if there is concomitant low dietary calcium) (99, 103) can lead to hyperoxaluria. Ingestion of starfruit (carambola) or large amounts of sorrel have been associated with acute oxalate nephropathy (104). Ethylene glycol, found in antifreeze, is metabolized to oxalate. Acute ingestion may result in acute renal failure due to oxalate nephropathy.

Under normal conditions, only a small fraction (less than 10%) of oxalate ingested from the diet is absorbed in the gastrointestinal tract (101, 105, 106). Other dietary constituents influence absorption of oxalate, particularly calcium containing foods which reduce absorption by binding to available oxalate. In recent years there has been interest in degradation of fecal oxalate by certain anaerobic bacteria that populate the colon in a significant proportion of healthy individuals. It has been suggested that loss of colonization with such organisms may result in increased GI absorption of oxalate leading to hyperoxaluria and stone formation (98, 107, 108). Of interest, in one carefully done epidemiologic study in adults, the presence of Oxalobacter in the stool correlated inversely with stones across all strata of age, gender, race/ethnicity, and region. Yet median urine oxalate excretion was the same with or without O. formigenes colonization (109), raising the possibility of other mechanisms. Oral administration of oxalate degrading bacteria or the responsible enzyme is currently under investigation as a potential treatment strategy (110).

Increased absorption of oxalate resulting from gastrointestinal disease is termed enteric hyperoxaluria. Any abnormality of the intestinal tract that is associated with malabsorption of fat can result in enteric hyperabsorption of oxalate. Mechanisms include binding of calcium by fatty acids. This leaves less calcium in the lumen to combine with oxalate, and oxalate is thus absorbed more avidly. In addition, bile salts cause injury to the colonic epithelium promoting enhanced oxalate absorption. The degree of hyperoxaluria caused by enteric disease is highly diet dependent and quite variable, from mild to severe. Consequences can include not only recurrent urolithiasis but also oxalate nephropathy leading to renal failure. Treatment includes reduction in dietary oxalate, addition of calcium supplements to bind oxalate in the intestinal tract, increased oral fluid intake, a low fat diet, and in some patients administration of sequestrants of bile acids. If hypocitric aciduria or hypomagnesuria are present, oral supplementation may be helpful to enhance urinary inhibition of calcium oxalate crystal formation.

The primary hyperoxalurias are autosomal recessive inborn errors of metabolism due to deficiency of hepatic alanine:glyoxylate aminotransferase (type I) or glyoxylate reductase/hydroxypyruvate reductase (type II) and are associated with marked elevation of urine oxalate excretion (111, 112). These enzymes are important in the metabolic disposition of glyoxylate, a precursor of oxalate found in hepatic cells. In type I disease, a large number of

mutations of the AGXT gene have been described, all of which lead to a reduction in AGT enzyme activity and/or to mistargeting of the enzyme from the peroxisome to the mitochondria where it is ineffective in disposition of glyoxylate. A marked increase in the hepatic production of oxalate and variable increases in glycolate result. Since excess oxalate must be excreted by the kidney, resulting high urinary concentrations lead to supersaturation of the urine for calcium oxalate. In type II disease deficiency of GRHPR enzyme activity leads to hyperoxaluria and hyperglyceric aciduria. Studies from France suggest a prevalence of type I disease of 1.05 per million population (113). Type II is rare, with fewer than 50 cases to date reported in the literature. Molecular diagnosis can now confirm the diagnosis of type I or II PH in most patients (115, 116). Liver biopsy with measurement of enzyme activity is required in a minority of patients. A small number of children have been reported with marked hyperoxaluria and clinical features similar to those with type I and II, but who have normal hepatic AGT and GRHPR activity. Whether these children have an as yet undefined error of hepatic metabolism or whether another mechanism is responsible remains to be established (98, 106, 114).

Due to the degree of hyperoxaluria and its presence from birth, primary hyperoxaluria is characterized by particularly aggressive stone formation and renal failure. The majority of patients demonstrate urolithiasis and/or nephrocalcinosis during infancy or childhood (*Fig. 58-2*). Over time, due to repeated stone episodes which may involve obstruction or infection and due to nephrocalcinosis and other effects of oxalate on the renal tubules and interstitium, renal failure often results. Type I disease is more severe than that of type II (117), with a higher proportion of type I patients progressing to end stage renal disease at an earlier age. However, there is wide variability of clinical expression in both types, with some patients progressing to renal failure in infancy and others retaining satisfactory renal function through the fourth or fifth decade of life (118, 119). Once the glomerular filtration rate falls below 30 mL/min/1.73 m^2, the plasma oxalate concentration rises rapidly, with resulting deposition of calcium oxalate in multiple organ systems (oxalosis). If oxalosis cannot be prevented or reversed by intensive dialysis and early transplantation, severe morbidity and death are the consequence.

Approximately a third of the patients with type I PH respond to pharmacologic doses of pyridoxine with a marked reduction in, and in some cases normalization of, urine oxalate excretion (118, 120). PH I patients should receive a trial of pyridoxine to assess their response. In all patients with PH intensive efforts to minimize calcium oxalate crystallization and stone formation in the kidneys appear to be helpful in preservation of renal function (118). Neutral phosphate therapy has been shown to reduce both urinary supersaturation for calcium oxalate and to increase urinary inhibitory activity for calcium oxalate crystal formation (121). Citrate therapy has also been advocated (122). There has been recent interest in oral administration of oxalate degrading bacteria, such as Oxalobacter formigenes and in oral administration of oxalate degrading enzymes for treatment of PH (123). These agents appear to not only degrade oxalate within the lumen of the gastrointestinal tract, but to also stimulate enteric oxalate secretion (124, 125).

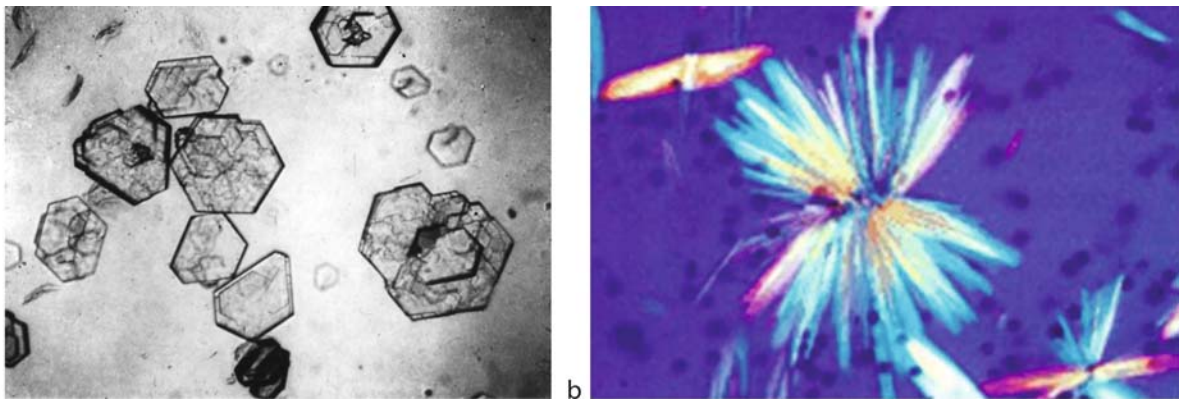
Cystinuria

Cystinuria accounts for 2–8 % of children with metabolic stones (4, 12). An inherited defect in renal tubular reabsorptive transport of cystine and the dibasic amino acids (ornithine, arginine, and lysine) accounts for the high concentrations of cystine in the urine of patients with this disorder. Cystine stones form due to cystine excretion rates that exceed solubility at usual urine volumes. At physiologic pH of less than 7, solubility is limited to 1000 µmol (240 mgm)/L (126). Characteristic hexagonal crystals can sometimes be seen in the urine of patients with cystinuria (*Fig. 58-4*). Cystine solubility is enhanced at an alkaline urine pH, such that alkalinization of the urine is an important component of treatment regimens. However, even at an optimal urine pH of 7, cystine solubility in the urine is limited to approximately 1250 µmol (300 mg)/L of urine (126). The prevalence of cystinuria in the general population in the United States and Europe is estimated at 1 in 7000, but varies widely in various parts of the world ranging from 1/2500 in Jews of Libyan origin to 1/100,000 in Sweden (127, 128). The mutant gene appears to be common, with a frequency approaching 0.01 (127–129).

Historically, three clinical subtypes have been described, based on the level of urinary cystine and dibasic amino acids in obligate heterozygotes (130). Type I accounts for approximately 70% of patients with clinically evident cystinuria and is completely recessive. Patients with type I/I show cystine excretion rates that are generally greater than 1000 µmol/gm creatinine by 1 year of age, with a mean excretion rate of 4500 µmol/gm creat (130). Half or more of such patients develop stones within the first decade of life (130). Mutations of the SLC3A1 gene on chromosome 2p encoding the rBAT protein important

Figure 58-4

Crystals with a distinctive morphology in urine sediment can be of diagnostic importance: A. Cystine crystals in a patient with cystinuria B. Indinavir crystals in the urine after treatment with indinavir medication. (Reproduced with permission.)



in dibasic amino acid transport have been shown to be responsible for type I cystinuria (131, 132). Types II and III are incompletely recessive, identified by elevated cystine excretion in obligate heterozygotes (133). Heterozygotes with type II/N have cystine excretion from 400–2400 µmol/gm creatinine and may form stones. Those with III/N have lower cystine excretion and typically do not develop urolithiasis. Patients with types II/II and III/III have cystine excretion rates similar to I/I. Compound heterozygotes are common. Mutations of the SLC7A9 gene on chromosome 19 are responsible for types II and III (132, 134).

With increasing molecular genetic information, some have challenged the traditional classification system, specifying type A disease as due to two mutations of SLC3A1, and type B disease as due to two mutations of SLC7A9 (135). Type AB patients have been recognized. However, there is not a strict genotype- phenotype correlation with types A and B. It is also recognized that defects in these two genes do not explain all cases of cystinuria (135, 136). Due to immaturity of renal tubule function during infancy, and the complexity of compound heterozygosity, it is difficult to definitively establish the diagnosis of cystinuria prior to 1 year of age (137).

Patients with homozygous forms of cystinuria frequently experience their first stone episode during childhood or adolescence and have recurring, life-long stone formation (*Fig. 58-3*). Medical therapy can be highly beneficial. First line therapy consists of increased oral fluid intake and a low salt diet to reduce urine cystine concentration to less than 300 mg/L, combined with urinary alkalinization to a pH of 7.0. If formation of cystine stones is not well controlled with these measures, addition of alpha-mercaptopropionyl glycine or D-penicillamine is recommended. Though both of these agents have been associated with significant side effects, they can be effective in stone dissolution as well as prevention of new stone formation (135, 138, 139). Titration of dose to maintain free urine cystine below 100umol/mmol creatinine appears helpful (139). Alpha- mercaptoprotionylglycine appears better tolerated than D-pencillamine (140). Captopril has been advocated but results have been inconclusive (141). However, even with optimal medical management urologic procedures are periodically required. With advancing age, elevated serum creatinine concentrations can be encountered in 17–50% of patients, with a small proportion (approximately 3%) eventually requiring dialysis (135, 141, 142).

Hyperuricosuria

Hyperuricosuria is found in 2–10% of children and adolescents with metabolic stones. Mild idiopathic hyperuricosuria may be a cause of hematuria (143) and is often found in conjunction with hypercalciuria (12, 144). A defect in renal tubular transport of uric acid, either due to reduced proximal tubular reabsorption or to increased secretion may be found (143, 145, 146). Idiopathic renal hyperuricosuria is often familial and asymptomatic.

Secondary hyperuricosuria may result from diets high in protein, or to ketogenic diets (147), and from medications including dicumarol, ascorbic acid, probenicid, phenylbutazone, salicylates, and citrate as well as pancreatic extract therapy in patients with cystic fibrosis (143). It can be seen in association with diabetes (148) and the syndrome of inappropriate secretion of antidiuretic hormone (149).

Although uric acid crystals in the urine may contribute to urolithiasis by forming a nidus for calcium oxalate crystallization, uric acid stones are uncommon in childhood. When they occur in children, uric acid stones are generally due to marked overproduction of uric acid such as occurs in tumor lysis syndrome, lymphoproliferative or myeloproliferative disorders, or rare inborn errors of metabolism such as complete (Lesch Nyhan syndrome) or partial deficiencies of hypoxanthine phosphoribosyl transferase (HPRT) enzyme activity. Urolithiasis occurs in a high proportion of patients with Lesch Nyhan syndrome, and not infrequently develops during the first year of life. Of those with partial HPRT deficiency, up to 75% develop uric acid urolithiasis (4). High fluid intake and restriction of dietary purines are recommended. Treatment with alkalinization to a pH of 7.0 will increase solubility and a trial of allopurinol may be beneficial.

Other Solute Excess

Xanthine calculi may occasionally be seen in patients treated with allopurinol for hyperuricemia related to Lesch Nyhan syndrome or as part of tumor lysis syndrome during chemotherapy (150). Markedly increased urine concentrations of xanthine and hypoxanthine are also seen in patients with a rare autosomal recessive deficiency of xanthine oxidase activity. Xanthine calculi develop in approximately a third of patients with hereditary xanthinuria (103, 151). Urinary tract stone disease in childhood may result from urinary solute excess due to other rare inborn errors of metabolism including orotic aciduria, alkaptonuria (152), and adenine phosphoribosyltransferase (APRT) deficiency leading to 2,8 dihydroxyadeninuria (153, 154). Treatment with allopurinol is effective in ARPT deficiency as it blocks production of 2,8 dihydroxyadenine.

Inhibitor Deficiencies

Citrate is a naturally occurring inhibitor of calcium oxalate and calcium phosphate crystallization. Hypocitric aciduria is a frequent finding in a subset of adult patients with idiopathic urolithiasis (155). In a study by Tekin and colleagues, urinary citrate excretion was significantly lower in their population of pediatric calcium stone formers than in a control group of healthy children and was felt to be the most important risk factor identified (156). Baggio found lower urinary concentration and excretion rates of citrate in stone forming children than in controls, but the differences did not reach statistical significance (157). Whether or not citrate is a primary factor in stone formation in a particular patient, increasing a lower citrate concentration to a higher one can be beneficial in minimizing calcium stone forming activity.

Deficiency of urinary citrate occurs predictably as a result of hypokalemia, and systemic or intracellular acidosis. However, in most such situations, the hypocitric aciduria is transient. In distal renal tubular acidosis, hypocitric aciduria appears to be a major contributor to stone formation and persists until the metabolic acidosis is corrected. Citrate measured in the urine may be artifactually low in the presence of a urinary tract infection, and should be repeated after antibiotic treatment. Interpretation of urinary citrate concentrations is complicated by relatively few studies of urinary citrate in healthy children, and the fact that citrate is influenced by prandial state, dietary composition, and age. Pyrophosphate and magnesium in the urine are also known to act as inhibitors of calcium oxalate and calcium phosphate crystal formation. Although both are used for therapeutic benefit, deficiencies of pyrophosphate or magnesium have not been described as a primary cause of urolithiasis.

A variety of urinary macromolecules appear to inhibit calcium oxalate crystal formation. Those studied include glycosaminoglycans, osteopontin, nephrocalcin, and urinary prothrombin fragment 1, among others. Some investigators have observed relatively lower concentrations of some of these inhibitors in the urine of children who form stones when compared with healthy children (158). However, considerable controversy exists regarding the role of urinary macromolecules in clinical disorders of stone formation (159).

Clinical Conditions Associated with Urolithiasis

A number of clinical conditions are associated with metabolic factors that predispose to stone formation. Impaired acidification of the urine favors calcium stones, due to reduced solubility of calcium salts in an alkaline environment, as well as metabolic acidosis (160). Distal renal

tubular acidosis results in hypocitric aciduria, hypercalciuria, low titratable acidity, and high urine pH. Multiple small calcifications in the papillary tips of the kidneys, comprised of calcium phosphate and/or calcium oxalate, are typical and are seen with both incomplete and complete forms of distal RTA (*Fig. 58-5*). In long term follow-up of distal RTA in childhood, Caldas, et al. (161) found nephrolithiasis and/or nephrocalcinosis in 14/28 patients. Nephrocalcinosis tends to occur with increasing age (161). Experience suggests that early initiation of alkali therapy may obviate the development of nephrocalcinosis by correcting the hypocitric aciduria and hypercalciuria (162, 163). A few patients have been reported among kindreds with RTA that appeared to develop nephrolithiasis and/or nephrocalcinosis due to hypercalciuria without systemic acidosis (164). This and other observations in hereditary RTA suggest that the relationships among hypercalciuria, the renal tubule acidification defect, and nephrocalcinosis/urolithiasis are complex (164).

Several autosomal recessive mutations resulting in carbonic anhydrase II deficiency have been described with variable degrees of distal and/or proximal RTA and associated urolithiasis and nephrocalcinosis (165–167). Mutations in the red cell anion exchanger (Band 3, AE1) gene have been confirmed in several families (168), are associated with distal RTA, and usually show autosomal dominant transmission (169). Urolithiasis is a frequent manifestation (168). Secondary distal RTA such as occurs in Wilson's disease and Sjogren's syndrome has also been associated with urolithiasis (170, 171). In type 1 glycogen storage disease hypercalciuria and hypocitric aciduria, which increase with age, and distal RTA have been implicated in the development of nephrolithiasis (172–175). Incomplete distal RTA and urolithiasis have also been reported in cerebrotendinous xanthomatosis, an autosomal recessive lipid metabolic disorder (176).

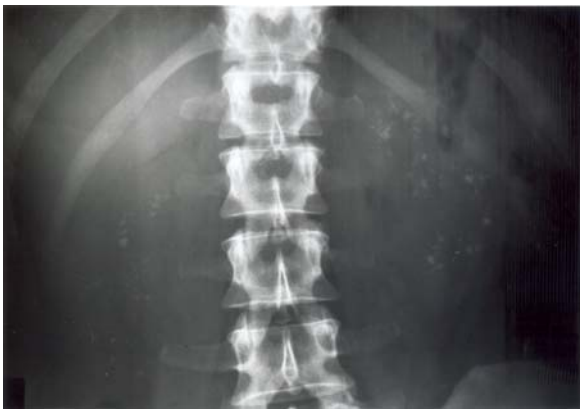
Urolithiasis is not usually associated with proximal RTA (177, 178) nor with type IV RTA (179). Despite hypercalciuria in proximal RTA, the high associated urinary citrate concentrations may protect against stone formation. In type IV RTA, reduced excretion of calcium appears sufficient to compensate for the reduced urine citrate, such that urinary saturation of calcium oxalate remains within normal limits (179). Lower phosphorus and uric acid excretion in patients with type IV RTA may also mitigate against stone formation (179).

Obesity and the associated metabolic syndrome is a risk factor for stone formation. Insulin resistance results in impaired renal ammonia production (180). Uric acid stone formation follows, related primarily to the low urine pH. Well-studied in adult patients, this complication of obesity has now been recognized in early adolescence (181). In addition to metabolic effects of obesity, bariatric surgical procedures, which are increasingly performed in adolescents and even younger children, are associated with hyperoxaluria, low urine volumes, and nephrolithiasis (182). Enteric hyperabsorption of oxalate related to malabsorption is the postulated cause (183). The hyperoxaluria can be marked and in some adult patients has led to renal failure due to oxalate nephropathy (184). The emerging epidemic of obesity in children and adolescents will likely lead to an increased prevalence of renal stone disease.

Medullary sponge kidney is frequently associated with recurrent calcium urolithiasis. Hypercalciuria, hypocitric aciduria, incomplete RTA, and increased urine pH have been variably observed in such patients (185). Nephrolithiasis is seen in 20–36% of patients with autosomal dominant polycystic kidney disease, with approximately half of the stones comprised of calcium oxalate and the other half uric acid (186). The most consistent metabolic abnormality is hypocitric aciduria, with a minority of the patients having hyperuricosuria or hypercalciuria (186, 187). Thin basement membrane nephropathy has been associated with hypercalciuria, hyperuricosuria, and resulting nephrolithiasis (188). An increased frequency of urolithiasis and nephrocalcinosis has also been reported in cystinosis (189–192). Children and adults with Wilson's disease may develop urolithiasis. Distal renal tubular acidosis is the most commonly observed factor (170). However, some of the patients demonstrate hypercalciuria and urolithiasis without evidence of renal

Figure 58-5

Small radiopaque stones scattered throughout both kidneys in a patient with distal RTA. Pattern is characteristic for RTA.



tubular acidosis (193). Since urolithiasis may be diagnosed before Wilson's disease is clinically evident, any patient with renal stones and unexplained neurologic, bony, or hepatic abnormalities should be screened for Wilson's disease (170).

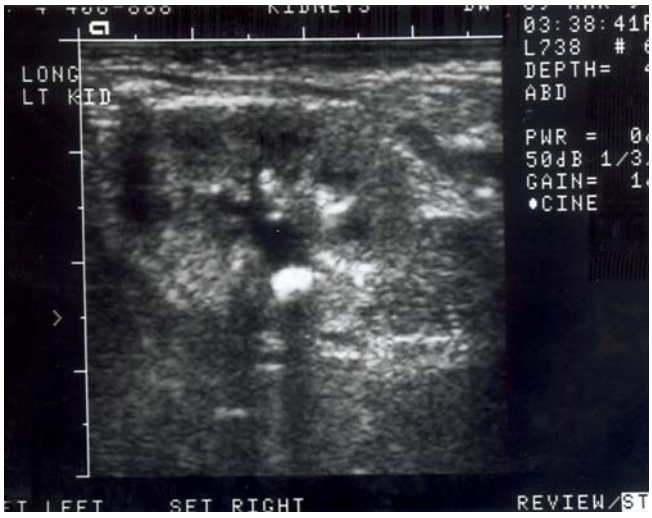
Inflammatory bowel disease and other diseases of the gastrointestinal tract associated with malabsorption can cause hypocitric aciduria and hypomagnesuria due to loss of bicarbonate and magnesium in the stool, hyperoxaluria from enhanced enteric oxalate absorption, hyperuricosuria due to increased cell turnover, and low urine volume because of diarrhea, which together result in reduced inhibitor activity, low pH, and high solute concentrations in the urine (194, 195).

Calcium oxalate urolithiasis has been reported in 5.2–10.4% of young patients with cystic fibrosis (105, 196, 197) and nephrocalcinosis has been observed in another 6.3% (105). Katz found microscopic nephrocalcinosis at autopsy in 35/38 (92%) of patients with cystic fibrosis, including in six patients who were less than a year of age at the time of death (198). Hyperoxaluria appears to be the primary factor (105, 196, 197, 199) although hypocitric aciduria (105, 197) and hyperuricosuria (105) have also been observed. Urine calcium has been variably reported as reduced (199), normal (105), or increased (105, 197). Crystalluria is commonly seen (196, 197). Pancreatic insufficiency with fat malabsorption and resulting enteric hyperoxaluria are suggested by the clinical setting although no correlation could be established between the degree of fat malabsorption and the urine oxalate excretion in two studies (197, 199). Deficiency of enteric Oxalobacter formigenes due to repeated courses of antibiotics has also been suggested as a risk factor for hyperoxaluria in this patient population (107). Pancreatic enzyme replacement and high protein diets may contribute to hyperuricosuria. Damage to renal tubules by antibiotic treatment has been implicated by some authors as a contributing cause with hypercalciuria following a renal phosphate leak (197).

Premature infants have an increased incidence of nephrocalcinosis and nephrolithiasis when compared with healthy term infants (*Fig. 58-6*). In three prospective studies of infants born at less than 1500 gm or less than 32 weeks gestation, renal calcifications occurred in 64, 16, and 28% of infants (200–202). The risk of renal calcifications appears greatest in smaller infants, those receiving furosemide, postnatal corticosteroids, a longer duration of mechanical ventilation and parenteral nutrition, white race, and in those with a family history of urolithiasis (202). Metabolic disturbances including hypercalciuria (200), hypophosphatemia and hypercalcemia (both of which can induce hypercalciuria) (203), and hyperoxaluria related to parenteral nutrition (204) have been implicated. Abnormalities of urine composition related to renal tubule immaturity may play a role. Nephrocalcinosis resolves within a few years time in approximately half of the patients and new stone formation appears to abate in the majority of patients. However, longer term studies performed at 4–12 years of age in very low birth weight children who had a history of renal calcifications demonstrated hypercalciuria, hypocitric aciduria, and reduced ammonium excretion in

Figure 58-6

Nephrocalcinosis and a shadowing stone in the pelvis of the kidney of a 3-month-old infant born prematurely.



response to furosemide when compared with a control group of children born at term (205). At the time of study 18% of the children still had renal cortical hyperechogenicity and 9% had urolithiasis. Whether the renal tubule abnormalities are the long-term result of renal tubular immaturity at birth, secondary to renal damage from nephrocalcinosis, or due to other factors remains to be established.

Pharmacologic Agents Implicated in Urolithiasis

Urolithiasis can result from use of a variety of medications. Renal excretion of the medication or a metabolite may exceed its solubility in the urine, or stones may form due to metabolic effects induced by the medication. The protease inhibitor indinavir, used widely in the treatment of human immunodeficiency virus (HIV), can cause ureteral obstruction and colic, observed in 2–28% of patients treated with this agent (206–208). Indinavir is filtered by the kidney, poorly soluble in the urine at a pH of greater than 5, and can precipitate as radiolucent stones. Distinctive birefringent crystals with plate and starburst structures are observed in 20–25% of patients receiving this antiretroviral agent (*Fig. 58-4*) (207, 209). The indinavir crystals or small stones may also act as a nidus for calcium oxalate or calcium phosphate, and thus can also be associated with partially radiopaque stones of mixed composition. There are a number of such reports in children (206, 210, 211). Due to the radiolucent nature of pure indinavir stones, they are not visible on noncontrast renal tomography and may be difficult to distinguish from soft tissue on CT scanning (210). Ultrasonography, IVP, or contrast-enhanced computerized tomography may be needed to demonstrate indinavir stones and associated ureteral obstruction. Dissolution of stone material may be observed with discontinuation of indinavir, increased fluid intake, and urine acidification (211). Other medications that can precipitate to form urinary tract stones are ceftriaxone (212, 213) sulfonamides, ampicillin, amoxacillin, triamterene, guafenesin (214), phenazopyridine, and oxypurinol (215). Urinary tract stones are rarely observed with these agents, however.

Carbonic anhydrase inhibitors, used for management of epilepsy and glaucoma among other uses, result in alkaline urine, reduced urine citrate, and hypercalciuria (215, 216). Agents of this class, including zonisamide, topiramate, and dorzolamide have been reported to be associated with calcium phosphate and calcium oxalate urolithiasis in children and adolescents (217–219). Commonly used agents such as calcium supplements, vitamin D, and its analogues can be associated with hypercalciuria and can predispose to stone formation. Cyclosporine A has been associated with hypercalciuria. Aminophylline results in hypercalciuria, alkaline urine, and phosphaturia countered, in part, by its diuretic effects (220). Lithium can induce hypercalcemia and hypercalciuria. Orlistat, a lipase inhibitor, can lead to enteric hyperoxaluria and has been associated with oxalate nephropathy (221).

Dietary Considerations

Diets low in animal protein, but high in cereals contribute to formation of endemic bladder stones in children (27, 222). High intake of animal protein predisposes to higher urine uric acid, calcium, and oxalate excretion and reduced urinary citrate and pH, all of which favor calcium oxalate stone formation (6). Frassetto noted that contemporary net acid-producing diets (rich in animal protein and deficient in plant protein and potassium alkali salts) produce a low-grade systemic acidosis in otherwise healthy adults (223). He and others have postulated that the ensuing metabolic changes may, in part, explain the increasing prevalence of urolithiasis in industrialized countries in recent decades (6, 223). Large dietary intake of sodium or calcium may induce hypercalciuria. High dietary oxalate, particularly if diet calcium is low, can result in hyperoxaluria. Fructose intake has been identified as a risk factor for stone disease, based on large epidemiological studies (224). The cause is unclear though fructose intake can lead to hyperuricosuria, insulin resistance, low urine pH, and can be associated with hyperoxaluria (225). Non-fructose carbohydrates do not appear to be a risk factor for stone disease (224). Contemporary weight loss diets low in carbohydrates but high in animal protein, such as the Atkins diet, lead to higher calcium, reduced citrate, and low pH in the urine, increasing the likelihood of urolithiasis (226).

Ketogenic diets used for the management of seizures are associated with nephrolithiasis (147, 227, 228). Stone formation has been noted as early as 1 month after initiation of the diet, but with a median occurrence of 2 years in a 6 year study (147, 227–229). The risk of stones in children may be as high as 25% after 6 years on a ketogenic diet (229). Stone composition includes uric acid, calcium phosphate, mixed calcium and uric acid, and ammonium urate (147, 227) Urolithiasis appears to be related to ensuing hypercalciuria, hypocitric aciduria, low urine pH, and reduced oral fluid intake (147, 227, 230).

Urine uric acid excretion is normal in most, but not all patients (147, 227), such that uric acid stone formation appears related primarily to the low volume and acidic pH of the urine. Based on early clinical experience, treatment with potassium citrate may reduce the formation of stones (229).

Infection as a Lithogenic Factor

Infection of the urinary tract with bacteria producing the enzyme urease leads to hydrolysis of urea with resulting production of ammonium and bicarbonate ions in the urine. In the presence of the increased pH, dissociation of phosphate occurs, with supersaturation of the urine for magnesium ammonium phosphate (struvite) and calcium phosphate apatites, including carbonate apatite (231). Precipitation of stone material follows. A variety of bacterial species produce urease, including Proteus, Staphylococcus, Klebsiella, Providentia, Pseudomonas, Enterobacter, Ureaplasma urealyticum, Corynebacterium urealyticum, and some anaerobes. Struvite tends to form staghorn calculi that can grow rapidly and are challenging to treat. It is difficult to eradicate infection while stone material remains, as antibiotics do not penetrate well within the interstices of the stone where bacteria reside. Just under half of the patients with infected staghorn calculi can be rendered stone free by ESWL (232), such that combined procedures including percutaneous nephrolithotomy are often needed (233). Retained stone fragments can serve as a nidus for early recurrence.

Xanthogranulomatous pyelonephritis is a rare but serious infectious process leading to nonfunction of the infected kidney. Obstruction caused by renal calculi has been found in 68% of affected children (234), with approximately half of such calculi being of the staghorn variety (234). Another author reported nephrolithiasis and/or nephrocalcinosis in 71% of such children (235). Proteus, Escherichia coli, Klebsiella, Pseudomonas, and Enterobacter bacteria (several species of which produce urease) are most often responsible (236). In xanthogranulomatous pyelonephritis, renal stones, obstruction and infection exacerbate each other. Nephrectomy is required in most circumstances (234–236).

Encrusted pyelitis is another unusual manifestation of infection and mineralization (237). Infection of the kidney with Corynebacterium urealyticum, a urea splitting organism, leads to encrustation of the wall of the renal pelvis with struvite and associated apatite crystals and proteins (238). Infection with this slow growing aerobic organism is usually acquired after urologic manipulations of the urinary tract and is most often observed in immune suppressed patients. Most reports in the literature are in renal allograft recipients (237, 239, 240). Obstructive uropathy and loss of renal function are common (239, 240). The encrustations are tightly adherent to the mucosa and are difficult to dislodge. In addition to prolonged antibiotic therapy, placement of percutaneous nephrostomy tubes for irrigation with acidic Thomas solution (237) or use of acetohydroxamic acid (237, 240, 241) have been advocated.

Although infection with urease producing organisms can itself produce urolithiasis, often the infection exacerbates underlying metabolic factors. With careful evaluation, 20–61% of patients with infection stones can be also be demonstrated to have metabolic factors predisposing to stone formation (12, 242). Stasis due to urinary tract abnormalities also predisposes to infection, further demonstrating the interaction among infection, metabolic factors, and structural abnormalities of the urinary tract in the genesis of calculi.

Structural Abnormalities of the Urinary Tract

Stasis of urine often accompanies structural abnormalities of the urinary tract, whether congenital or acquired. By compromising the normal continuous flow of urine from the upper urinary tract or interfering with regular and complete emptying of urine from the bladder, stasis promotes crystal retention and stone formation as well as infection. A wide range of structural abnormalities has been associated with urolithiasis including medullary sponge kidney (185), autosomal dominant polycystic kidney disease, calyceal diverticuli (243), ureteropelvic junction obstruction (156, 244), horseshoe kidney (245), ureteroceles, primary megaureter (24), posterior urethral valves (24), and the bladder extrophy-epispadius complex (246). Medullary sponge kidney is frequently associated with recurrent calcium urolithiasis. In medullary sponge kidney and autosomal dominant polycystic kidney disease, tubular ectasia and cyst formation, respectively, are felt to contribute to stasis of the urine that exacerbates metabolic abnormalities. Patients with myelodysplasia or spinal cord injury often have impaired bladder emptying, recurrent urinary tract infections, and hypercalciuria from relative immobilization. Partial obstruction from any cause can result in defects in renal acidification with accompanying hypocitric aciduria. Enterocystoplasty and urinary diversion employing intestinal mucosa are particularly prone to local formation of stones, with 16–50% of

patients with enterocystoplasty reported to form bladder stones (247–249). The difficulty in eradicating bacteria from the enteral mucosa, stasis, an alkaline urine pH due to infection or to exchange of chloride for bicarbonate across the mucosa, and the presence of mucous in the bladder all favor stone formation in this setting (248). The length and location of intestine resected may also influence the metabolic stone environment if either chronic diarrhea or malabsorption ensues. Foreign bodies such as sutures or staples, or indwelling stents also can act as a nidus for stone formation.

However, with the exception of enterocystoplasty, the overall incidence of stones in children with structurally anomalous, obstructed, or infected urinary tracts is low, on the order of 1–5% (250). This suggests that while these factors are permissive, children who form stones may also have underlying metabolic abnormalities. Hypercalciuria, hyperoxaluria, or hypocitric aciduria have been identified in 66–80% of patients with structural abnormalities of the kidneys or ureters who also had urolithiasis and underwent metabolic evaluation (244, 251). Tekin and colleagues demonstrated a similar degree of hypocitric aciduria and hyperoxaluria in patients with ureteropelvic junction obstruction and urolithiasis as in those with calcium stone formation but without urinary tract abnormalities. Both were significantly different from the control group of normal children (156). The importance of metabolic abnormalities in patients with stones and urinary tract abnormalities is further suggested by long term follow-up of a group of pediatric patients with ureteropelvic junction obstruction and renal calculi. Recurrent urolithiasis developed in 68% of the patients despite successful UPJ repair. Further, 10/19 (53%) of recurrent stones formed in the contralateral, uninvolved kidney (244). Accordingly, complete metabolic evaluation is just as important in children with structural abnormalities of the urinary tract or infected stones as it is in those without.

Long-term Management of the Stone Patient

Urolithiasis in children and adolescents, like that in adults, frequently recurs. In one study of 221 children followed for a mean of 59 months (median 36 months), 67 % developed two or more stones during initial evaluation and follow-up (12). Other authors have reported recurrence rates of 20–40 % with variable follow-up periods (15). Recurrence rates are higher in children with demonstrable metabolic abnormalities (15). Accordingly, long-term follow-up with periodic reassessments is indicated. Evaluation of metabolic stone forming activity, as determined by growth in size of existing stones or formation of new stones over time, is important in monitoring the effectiveness of treatment. The frequency of renal imaging required will depend upon the type and number of stones and the severity of the metabolic abnormalities detected. In most circumstances and in the absence of symptoms or infection, once yearly or every other year imaging is sufficient. Patients with significant metabolic problems such as primary hyperoxaluria, cystinuria, marked hypercalciuria, and those with infected stones (which can develop and grow quickly) may require more frequent evaluations. Acute symptoms at any time should prompt reevaluation.

Ultrasonography has the advantage of visualization of radiolucent as well as radiopaque stones, detection of hydronephrosis, and the absence of radiation exposure and is preferred for most routine follow-up assessments. However, lack of sensitivity for small stones, difficulty in comparing size of individual stones over time, lower sensitivity for visualization of ureteral stones, and the possibility of obstruction in the absence of hydronephrosis will, at times, dictate other imaging modalities.

If existing stones increase in size, or new stone formation occurs despite treatment, a more intensive regimen should be implemented. In patients with active stone formation, additional studies can be helpful in assessing the degree of metabolic stone formation and providing guidance as to the most effective therapies. These may include determination of crystalluria by phase contrast microscopy, supersaturation of the urine (252), measurement of urinary inhibitor activity, or measurement of enteral absorption of oxalate, among others. Though tests intended to differentiate between absorptive and renal leak hypercalciuria have limited utility in pediatric stone patients, they can be helpful in selected circumstances.

In children of all ages, if compliance with recommendations can be secured, response to treatment is typically excellent, with reduced frequency or elimination of active stone formation. However, many metabolic abnormalities, such as idiopathic hypercalciuria, are life long. Subtle degrees of damage to the renal tubule are suggested by studies showing increased urinary excretion of renal N-acetyl-beta-glucosaminidase in children with hypercalciuria (253) and in children with urolithiasis (254), a deficiency of distal acidification in children with hypercalciuria and urolithiasis that is not seen in children with hypercalciuria alone (255), and families in which hypercalciuria appears to be the primary event, with distal RTA developing over the years as an apparent complication of hypercalciuria (164, 256). Others have argued that the

renal tubule acidification defect is primary and that the hypercalciuria and urolithiasis occurs secondarily (257). Jaeger and colleagues studied renal tubular function in patients with urolithiasis related to primary hyperparathyroidism, medullary sponge kidneys, hyperuricemia, cystinuria, struvite stone disease, idiopathic hypercalciuria, and normocalciuric idiopathic urolithiasis (58). They found that a significant number of stone formers have dysfunction of the proximal renal tubule, that the abnormalities were found in patients of all of the diagnostic groups, and that the occurrence was related to the presence of stones in the collecting system at the time of study (58). The authors concluded that tubulopathy in nephrolithiasis is the consequence, rather than the cause, of stone disease.

The risk of renal insufficiency has been estimated at 1.7% in idiopathic calcium oxalate urolithiasis (258). In a group of 40 patients with a mean duration of cystinuric stone disease of 26 years, renal insufficiency was found in 30% of patients, though none had reached end stage renal disease (259, 260). In cystinuria patients, stone-preventive treatment appeared effective in preserving renal function (259, 261). Infection staghorn stones eventually are associated with end stage renal disease in 20–30% of adult patients with unilateral involvement and a higher percentage of those with bilateral stones (258). Several forms of more severe urolithiasis, such as primary hyperoxaluria, Dent's disease, and 2,8 dihydroxy adeninuria are frequently associated with renal insufficiency or end stage renal disease.

For these reasons, and despite the excellent response to treatment noted in most children with urolithiasis, long-term nephrologic care is indicated particularly for those with more complex forms of renal stone disease.

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59 Pediatric Renal Tumors

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Overview

Renal cancers in pediatric patients are relatively common, with an incidence of almost 8 per 1,000,000 representing approximately 7% of all childhood cancers (1). The vast majority (95%) of these are Wilms Tumors (WT) but several other histologic types of renal tumors also occur in children (*Table 59-1*). The incidence of each type of renal tumor is tightly correlated to the age of the patient. WT, most common in children under age five, is very rarely seen in adolescents and young adults. An adolescent with a renal tumor is more likely to have renal cell carcinoma. Rhabdoid tumor of the kidney (RTK) and congenital mesoblastic nephroma (CMN) are seen almost exclusively in infants less than a year, and clear cell sarcoma almost always occurs in children less than four years old.

This chapter will touch on several pediatric renal tumors, but, given the overwhelming prevalence, will focus largely on WTs. The principles of the diagnostic evaluation, surgical management, and the use of appropriate chemotherapy and radiotherapy used in WT can be generalized to some of the other less common renal tumors.

In 1899, Max Wilms made a critical contribution to the field of pediatric renal tumors, describing seven children suffering from renal "mixed tumors" – later identified as nephroblastoma, or Wilms Tumor (*Fig. 59-1*) (2). He provided a meticulous description of the triphasic morphology of this tumor, comprised of three defining components: epithelium, blastema, and stroma (*Fig. 59-1*). It is now recognized that WTs can have great histologic diversity. Cell types seen in normal developing kidney can be present, as well as diverse elements such as adipose tissue, cartilage, skeletal muscle, and neuroglial tissue. These elements appear to arise from stromal differentiation. Epithelial differentiation can be seen with the presence of renal tubules, glomeruli, or comma and S-bodies. Tumors can be triphasic, with all three components; monophasic, with epithelial differentiation only; or biphasic, containing exclusively blastemal and stromal cells. Max Wilms also offered the important insight that all of these tumor components developed from a common undifferentiated germ cell. Along with the observation that the morphology of WT correlates with phases of normal renal development, this has helped build an understanding into the link between organogenesis and tumorogenesis in the kidney (3). Study of this relationship has fostered understanding of the correlation between WT and a variety of renal abnormalities. It has also helped elucidate the connection between the persistence of embryonic renal tissue (termed nephrogenic rests) and risk for the development of WT. The expanding knowledge of the genetics of WT has added both answers and new questions into this link.

Genetics of Wilms Tumor and Associated Syndromes

Approximately 90% of WTs are unilateral and considered to be sporadic. The most often present in children 5 years old or younger, without a male or female preference (4, 5). A smaller portion, ranging in studies from 5 to 5% of cases, is caused by sporadic mutations in the Wilms tumor-1 tumor suppressor gene (*WT1*). The low percentage of WTs in which mutations of the *WT1* gene are found suggests that other tumor suppressor genes may be involved in the pathogenesis of WTs. Recently, one such additional tumor suppressor gene for WT, *WTX*, located on the X chromosome, has been identified (6). Much less commonly, in around 1–2 percent of cases, WTs are caused by inherited mutations in genes that in some cases have yet to be identified (7). Finally, there is recent evidence that mutations in *CTNNB1* (encoding β-catenin), a gene important in several major signal transduction pathways and in cell-cell adhesion, may have an important role in WTs (8–11). A summary of molecular pathways leading to various types of WTs is shown in *Fig. 59-2*.

The "Two Hit" Hypothesis

WTs can either be unilateral or bilateral. The median age of onset of bilateral tumors is earlier, at age of 32 months compared with 44 months for unilateral disease (4). The earlier onset of bilateral WTs is similar to what is observed for retinoblastoma, another childhood tumor that can present either unilaterally or bilaterally. These observations led Knudsen and Strong to propose a "two-hit"

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Table 59-1

Pediatric renal tumors

Nephroblastomic tumors	Nephroblastoma (Wilms Tumor)
	• Favorable histology
	• Anaplasia (diffuse, focal)
	Nephrogenic rests and Nephroblastomatosis
	Cystic nephroma and Cystic partially differentiated Nephroblastoma
	Metanephic tumors
	Metanephic adenoma
	• Metanephic adenofibroma
	• Metanephic stromal tumor
	• Favorable histology
Mesoblastic nephroma	Cellular, classic, mixed
Clear cell sarcoma	
Rhabdoid tumor	
Renal epithelioid Tumors of childhood	Papillary renal cell carcinoma
	Renal medullary carcinoma
	Renal tumors associated with Xp11.2 translocations
	Oncocytic renal neoplasms following neuroblastoma
Angiolipoma	
Ossifying renal tumor of infancy	

A variety of histologic types of renal tumors occur in children, the overwhelming majority however (95%) are Wilms Tumors

hypothesis for these tumors (12, 13). This model suggests that genetic predisposition to a tumor is conferred by an inherited or de novo germline mutation in one allele of a tumor suppressor gene, such that only one additional "second hit" during somatic development is sufficient to cause somatic homozygosity by inactivating the second allele. In contrast, individuals without this genetic predisposition would require two distinct genetic events, resulting in the deletion or inactivation of both copies of a tumor suppressor gene, and would therefore present at a later age (14).

Loss of Heterozygosity in Wilms Tumors

Loss of heterozygosity (LOH) is the hallmark of a tumor suppressor gene, and is the molecular correlate of the "second hit" discussed in the preceding section. For a tumor to result from loss of a tumor suppressor gene (assuming it is encoded on an autosomal chromosome), both copies of the gene must be lost in a cell that initiates the tumor. Thus, a first event, or "first hit" must be the loss of one allele of a tumor suppressor gene. This loss may be inherited in the germ line or may occur in somatic tissue. At a molecular level, LOH is an event in which an individual loses function of the remaining second allele. This is referred to as LOH because the cells involved are no longer heterozygous for the tumor suppressor gene, but rather, harbor mutations in both copies of the gene. LOH may occur through point mutation or deletion of the second allele, either through a local event, or loss

Figure 59-1

(a) and (b) (Gross and microscopic views) favorable histology. Gross pathologic specimen of favorable histology Wilms Tumor. Typically, these tumors are described as friable, pink or variegated in color, often with cystic changes, areas of hemorrhage and necrosis. Classic triphasic histology of Wilms Tumor, containing blastemal, stromal and epithelial elements.

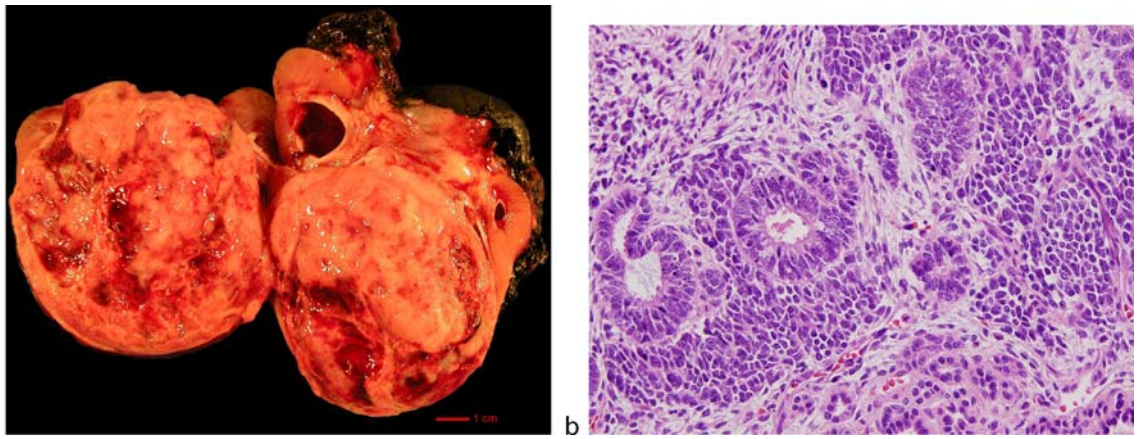
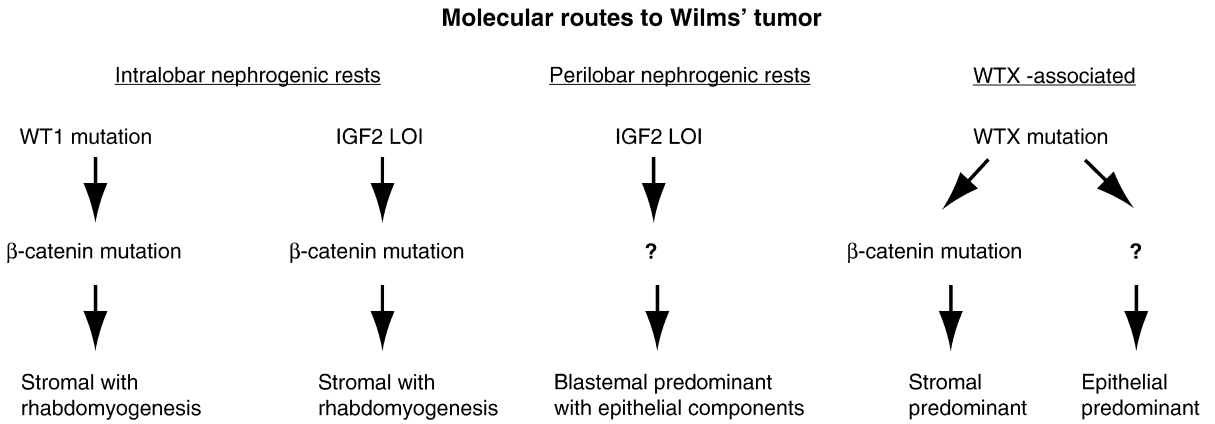


Figure 59-2

A summary of the presently identified molecular pathways leading to various types of Wilms' Tumors. The references are cited in the text. It is not known yet whether WTX mutations will be found to be mainly associated with perilobar or intralobar nephrogenic rests. Since the presently identified mutations and epigenetic changes are not found in all tumors, this figure must be considered incomplete. Additionally, it is likely that additional mutations and chromosomal aberrations are involved in tumorigenesis, that remain to be identified. Additionally, exceptions to these generalizations are described in the literature. Abbreviation: LOI, loss of imprinting.



of the entire chromosome or a gene conversion event that copies a mutation from the originally mutated allele to the second copy.

LOH at chromosome 11 occurs in 30–40% of WTs, encompassing allelic loss of both the *WT1* locus at chromosome 11p13 and the locus telomeric to it at 11p15 (15–18). Other chromosomes at which LOH is evident in WTs are chromosome 16, for which LOH is observed in 20% of sporadic WTs (19), and at chromosome 1p with a frequency of 11% (20, 21). Notably, patients with tumor-specific LOH at these locations have significantly worse outcomes (22), indicating that additional tumor suppressor genes may exist on these chromosomes. A further 5% of cases exhibit LOH at 4p, 7p, 8p, and 17, 17q and 18q (22, 23).

Loss of Imprinting in WTs

WTs are often associated with loss of genomic imprinting (LOI). Imprinting refers to situation in which only one inherited allele, either the maternal or the paternal, is transcribed. LOI is common to several embryonal cancers and many other tumor types (24). The 11p15 locus, containing the *IGF2* and *H19* genes is a well-known site of genomic imprinting. LOI at 11p15 is observed in 30% of sporadic WTs (25–27), and involves activation of the normally silent maternal allele of *Insulin-like growth factor 2* (*IGF2*) resulting in biallelic *IGF2* expression (26), silencing of the normally active maternal allele of *H19* (28, 29), and hypermethylation of the differentially methylated region upstream of the maternal copy of *H19* (30). It is likely that increased expression of *IGF2* associated with LOI at 11p15 may have a role in the pathogenesis of some WTs (31–34). 11p15 may also harbor as yet unproven WT suppressor genes (35, 36).

Known Tumor Suppressor Genes and Oncogenes Involved in WTs

To date, *WT1* (16, 37, 38) and *WTX* (6) are the two identified WT suppressor genes. Loss-of-function mutations in *WT1* account for 10–15% cases of sporadic WT (39). *WT1* mutations include both nonsense and missense changes, as well as the generation of aberrant splice forms, and are distributed throughout the coding sequence (40–42). These same tumors may also exhibit constitutive activation of β-catenin, the central effector of the *Wnt* signaling axis (3, 11, 43), implicating another signaling pathway critical for normal kidney development in WTigenesis (16), and suggesting potential genetic cross-talk between the *WT1* and *Wnt* signaling axes. However, most sporadic tumors express wild-type *WT1,* often at high levels (44), suggesting that downstream *WT1* signaling is affected in these tumors, or that genetic disruption of other cellular pathways is responsible for tumorigenesis.

WTX is a recently identified tumor suppressor gene involved in Wilms Tumors (6). *WTX* is encoded on the X chromosome, such that males only have a single copy, but females have two, though only one allele should be expressed due to X-inactivation. X-inactivation complicates the understanding of the significance of mutations in *WTX*, since some mutations have been found in the unexpressed allele and are therefore of questionable significance for the tumor (45). *WTX* was originally suggested to be mutated in as many as a third of WTs but more recent studies suggest a frequency closer to 15% (6, 45, 46).

Syndromes Associated with WTs

Ten to fifteen percent of WTs occur in children with recognized malformations, including hemihypertrophy, cryptorchidism, hypospadias, or in association with a recognizable genetic syndrome (7).

WAGR Syndrome (WT, Aniridia, Genitourinary Malformation, Mental Retardation Syndrome (47–49))

WAGR syndrome is caused by a microdeletion at 11p13 that deletes both *WT1* and *Pax6* (47, 49). WAGR is associated with aniridia in all cases resulting from hemizygosity for *Pax6* (50–52), but is variably associated with WT (50% risk of WT) (53) and genitourinary malformations due to hemizygosity for *WT1*. WAGR is also variably associated with mental retardation and congenital heart disease; however the gene(s) on 11p13 responsible for these defects have not been identified (54).

Denys-Drash Syndrome (DDS)

DDS includes the triad of WT (90% risk of WT development) (55, 56), genitourinary malformations and nephropathy (mesangial sclerosis), but various combinations of theses features have been reported (57–59). DDS is caused by intragenic *WT1* point mutations that either eliminate or alter the structure of the zinc finger region. The most common mutation is an arginine-to-tryptophan transition in exon 9 (Arg 394), or other missense alterations in the zinc-finger domains encoded by exons 8 and 9 (3, 16). The increased severity of kidney disease associated with DDS, as compared with WAGR, raises the possibility of a dominant-negative effect that is mediated by dimerization of mutant and wild-type proteins through their N terminal domains (60, 61).

Frasier syndrome bears similarity to DDS, and is characterized by gonadal dysgenesis, often resulting in XY sex reversal in males (3), progressive glomerular nephropathy (focal segmental glomerulosclerosis) (62), gonadoblastoma. Interestingly Frasier Syndrome (FS) (63, 64) much less commonly (less than 5%) includes WTs among its features. FS is caused by mutations in intron 9 of *WT1* that affect splicing and prevent expression of the +KTS isoforms of WT1 (discussed below) (63, 64).

*B*eckwith-*W*iedemann *S*yndrome (BWS) is the most common WT-associated condition, affecting 1 in 13, 000 children, and is probably related to a large degree to LOI at 11p15 (65–67), resulting in hyper-expression of *IGF2*. BWS is characterized by prenatal overgrowth and increased incidence of embryonal tumors of liver (hepatoblastoma), muscle (rhabdomyosarcoma), and kidney. This syndrome carries a 10% risk of WT (68). While the genetics of WT formation in BWS is not completely understood, at least 20% of BWS patients exhibit paternal uniparental disomy for 11p15 that contains the *IGF2* and *H19* imprinting locus strongly associated with sporadic WT (65), and these children have a high risk (64%) of developing embryonal tumors (67). In addition, familial BWS is linked to chromosome 11p15 (69). A third, unidentified tumor suppressor gene on 11p15 has been linked to rhabodomyosarcoma (70), suggesting that at least three genes on 11p15 may predispose to growth abnormalities and WT as well as other embryonal tumors.

Other genetic syndromes associated with increased incidence of WT (71, 72), include Simpson-Golabi-Behmel Syndrome (linked to Xp26) (73–76), Perlman Syndrome (77–80), Sotos Syndrome (linked to 5q35) (81, 82), and Bloom's Syndrome (linked to 15q26) (83, 84).

Familial Wilms Tumor

True familial WT is extremely rare, accounting for only 1–2% of all cases (13), suggesting that de novo germline mutations, rather than familial transmission of a mutant allele underlie the genetic predisposition (3). In addition, reduced fertility may be associated with germline mutations in genes that regulate urogenital development (68). The low number of familial cases may reflect the historic lethality of this cancer in before individuals with tumors reached reproductive age. With the advent of effective therapy in the past few decades, such that most individuals with WTs survive to adulthood, it will be important to observe in the future whether familial cases become

more common in the population. Two familial WT genes have been mapped – *FWT1* (*Familial WT 1 locus;* also known as *WT4*) at 17q12–21 (85) and *FWT2* at 19q13.4 (86), but neither gene has been identified. In addition, familial cases unlinked to any previously identified loci have been reported, indicating that other familial WT genes may exist (87).

The *WT1* Gene, mRNAs and Proteins

Gene Identification

A major step towards the discovery of the *WT1* locus was the identification of cytogenetically visible, germline interstitial deletions in 11p13 as the locus for WAGR Syndrome (48). Together with the discovery that some individuals with sporadic, non-syndromic WT also showed LOH in 11p13 (88, 89), a minimal 30 kb region on 11p13 was identified as an critical locus in the development of WT, and was given the designation of the "*WT1*" locus (90, 91). Two groups independently identified the *WT1* gene by mapping the smallest region of overlap among chromosomal deletions in germline and sporadic tumor specimens (92, 93), and *WT1* was shown to be mutated in several patients with sporadic WT (94).

Structure of WT1

The *WT1* gene is comprised of 10 exons, and encodes a 55-kDa zinc-finger transcription factor with both DNA- and RNA-binding activity (*Fig. 59-1*) (95). Consistent with a probable role as a transcription factor, the carboxyl (C) terminus of WT1 contains four Krüppel-type (Cys_2His_2) zinc-finger domains and two nuclear localization domains that mediate DNA binding. The zinc-finger region and its alternative KTS splice site are critical for WT1 function, as mutations in the zinc-finger domain are associated with WTigenesis as well as the severe urogenital abnormalities observed in DDS (42). The amino (N) terminus of *WT1* contains a glutamine/proline-rich transactivation domain, a dimerization domain (96, 97) and putative RNA-binding domain (92, 98).

Multiple Isoforms of the WT1 mRNA

The *WT1* transcript is alternatively spliced at two major sites, that result in the expression of four major *WT1* transcripts (99), which are expressed at relatively constant ratios in all tissues throughout development and across species (40, 100). The first alternative splice inserts exon 5, this exon is unique to the *WT1* gene of placental mammals. Exon 5 encodes 17 amino acids (99). However, the biological function of the WT1 isoform containing exon 5 remains obscure as mice lacking this exon develop normally and are fertile (101). Nevertheless, *WT1(+exon5)* is often over-expressed in WT and other malignancies (102), and in vitro studies have suggested a role in the regulation of cell division and cell survival (103).

An alternative splice donor site at the end of exon 9 inserts the amino acids lysine, threonine and serine (KTS) at the end of the third zinc fingers, serving to alter the DNA-binding affinity of WT1 (98, 100). The +KTS/−KTS ratio of WT1 protein is tightly regulated and highly conserved from zebrafish to humans (99), and the relative expression levels of the +KTS versus –KTS isoforms is critical for normal urogenital (104) development. Additionally, alternative translation initiation sites both upstream and downstream of the major translational start site (105, 106), along with alternative pre-mRNA splicing (100) and RNA editing (107) can theoretically result in the generation of 24 *WT1* isoforms.

WT1(–KTS) As a Transcriptional Regulator

An emerging body of evidence suggests that the +KTS and –KTS serve distinct functions in the nucleus, with (–KTS) isoforms playing a role in transcriptional regulation, and (+KTS) proteins implicated in posttranscriptional modification of target genes via RNA editing (104, 108). WT1(–KTS) exhibits characteristic properties of a DNA-binding transcription factor (109) and the best predictor of endogenous gene transactivation is a high-affinity 10-bp EGR1-like (WTE) motif (5′GCGTGGGAGT3′) identified from genomic DNA (3, 110).

Early studies demonstrating over-expression of *IGF2* and *Platelet-Derived Growth Factor (PDGF)* in WTs, led to the hypothesis that WT1 functions as a transcriptional repressor of growth-promoting genes (111–113). Subsequently, WT1 was shown to bind and repress promoters of numerous other growth-promoting genes expressed in the kidney including *Pax2* (114), *c-myc* (115), *N-myc* (116) (111), *B-Cell CLL/lymphoma 2 (Bcl-2)* (117, 118), *EGR* (119) *epidermal growth factor receptor (EGFR)* (96) and *colony stimulating factor-1 (CSF-1)* (120, 121), as well as *WT1* itself (122).

Several genes implicated in the control of kidney development appear to be upregulated either directly or indirectly by WT1 in vivo, including *Sprouty1 (Spry1)*

(119, 123) *Amphiregulin (AREG)* (124, 125) and the podocyte-specific genes *Nephrin (NRHS1)* (126–131) and *Podocalyxin (PODXL)* (132–134). Consistent with its role as a tumor suppressor, WT1 activates *p21* (135), leading to G1 cell cycle arrest. WT1 has also been shown to activate the anti-apoptotic gene *Bcl2* (117, 118, 136), previously shown to be repressed by WT1(115). Many other genes have also been suggested to be regulatory targets of WT1, but their possible roles in urogenital development or tumorigenesis is less clear (126, 137, 138).

WT1 as a Regulator of RNA Processing

The KTS insertion disrupts the linker region between zinc fingers 3 and 4, thereby decreasing the DNA binding affinity of WT1 to known target sequences (109). Several results are consistent with the possibility that WT1 (+KTS) functions to regulate RNA processing. Whereas WT1(–KTS) isoforms exhibit diffuse nuclear expression typical of transcription factors, punctate or "speckled" sub-nuclear WT1(+KTS) protein is detected within RNA spliceosome domains (139) and colocalizes with that of RNA splicing factors such as U2AF65 (140) and WTAP (141) as well as small nuclear ribonucleoproteins (snRNPs) (142, 143). An interaction of WT1(+KTS) with *Igf2* mRNA has been demonstrated in vitro (139), and WT1(+KTS) interacts with RNA-export machinery to stimulate translation of unspliced RNA (108).

Developmental Expression Pattern of WT1

WT1 mRNA and protein expression is first detected in the intermediate mesoderm lateral to the coelomic cavity, and is crucial for development of both the kidneys and gonads (144). As WTs derive from the condensing nephrogenic mesenchyme, it was expected that a WT suppressor gene would be expressed in the condensed mesenchyme and not in the reciprocally-induced ureteric bud (68). Indeed, *WT1* expression in the developing kidney is limited to the metanephric mesenchyme and its derivatives. *WT1* is expressed at low levels in uninduced metanephric mesenchyme and expression increases dramatically in the condensed nephrogenic mesenchyme as induction proceeds. During epithelial reorganization, *WT1* expression becomes restricted to the posterior portion of induced nephrogenic structures (145), the region that will form the epithelial podocyte population of the glomerulus by the S-shape stage of nephron development (114). Once kidney development is completed, *WT1* expression declines, except in podocytes, which express *WT1* throughout adulthood (146).

WT1 in Wilms Tumors

Since most WTs do not seem to arise through inactivation of the *WT1* gene, it is equally important to consider the role of *WT1* in these tumors, as well as the molecular consequences of the loss of *WT1*. This consideration is complicated by the many genes identified as putative regulatory targets of the WT1 gene (discussed above), such that there is no single potential target gene, the over or under-expression of which, could likely account for tumorigenesis Indeed, the role of WT1 in WT is likely to be complex and relate to the abnormal transcriptional and post-transcriptional regulation of many genes. Emerging evidence, that finds *CTNNB1* and *WT1* mutations to often occur in the same tumor, and WT1 mutation to be common in stromal or mesenchymal-type tumors, may provide hints to the role of *WT1* in these tumors (9, 11, 147). *WT1* mutations are also associated with a myogenic phenotype in WTs (148–150). In examining mutations within intralobar nephrogenic rests, that are considered to be "precursor lesions" for WTs, it was found that *WT1* mutations were present, but mutations in *CTNNB1* were not, suggesting that mutation of *WT1* is the earlier, *CTNNB1* the later event (10). In a related study by the same authors, LOI for *IGF2* was examined, with regard to its relationship with *WT1* and *CTNNB1* (151). Whereas *WT1* mutations are more often associated with intralobar nephrogenic rests (152–155), tumors associated with loss of imprinting of *IGF2* often arise from perilobar nephrogenic rests (156, 157). Two distinct morphologies were observed among *IGF2* LOI tumors, a blastemal morphology associated with perilobar rests and a myogenic phenotype associated with intralobar rests (151). Several of the intralobar tumors also had *CTNNB1* mutations, despite not having *WT1* mutations, demonstrating that *CTNNB1* mutations may be a hallmark of intralobar rests regardless of their *WT1* genotype (151).

WTX, CTNNB1, and Wnt signaling in Wilms Tumors

β-catenin is a multifunctional protein, acting both as a component of the cadherin cell-cell adhesion apparatus, and as a major protein involved in the "canonical" Wnt signaling pathway. Wnt proteins are secreted signaling molecules that serve as some of the most important signals

regulating developmental processes. Among the several signal transduction pathways regulated by Wnt proteins, the best characterized is the so-called "canonical" pathway, the major purpose of which seems to be to prevent the degradation of β-catenin, which allows it to translocate to the nucleus, form a complex with Tcf/Lef family transcription factors, and regulate genes involved in cell proliferation and differentiation (158). A role for β-catenin in BMP and TGF-β signal pathways, through the interaction with Smad proteins has also been demonstrated (159–161), such that β-catenin appears to be one of the most central proteins in cell growth and differentiation. Mutations in the *CTNNB1* gene have been found in about 15% of WTs, often in the same tumors that carry mutations in *WT1* (9, 11, 43), suggesting that mutation of *CTNNB1*, rendering it constitutively active, may constitute an additional "hit" beyond loss of WT1, in the pathogenesis of WT.

The identification of the *WTX* gene as a second tumor suppressor gene has strengthened the case for a role for Wnt signaling and β-catenin in WT. WTX has been shown to be a negative regulator of the Wnt pathway through its ability to form a complex with β-catenin that promotes it degradation (162). This suggests a mechanism whereby loss of WTX may serve to abnormally up-regulate signaling through β-catenin and contribute to tumorigenesis (162). Indeed, WT1 itself has also been suggested to negatively regulate signaling through β-catenin (163) and some WTs have been found to contain mutations in *WT1, WTX* and *CTNNB1* (9), suggesting that hyperactive canonical Wnt signaling may be a centerpiece of WTs (8, 164, 165). In a particularly intriguing recent report, it was shown that mesenchymal-type WTs appeared to be Wnt-dependent, characterized by mutation and nuclear accumulation of β-catenin and expression of known Wnt target genes, whereas more epithelial-like tumors appeared to be Wnt-independent by these criteria, i.e., no nuclear accumulation of β-catenin or activation of target genes (166). Interestingly, however, *WTX* mutations were found among both groups of tumors, both those that seemed to be Wnt-dependent and independent, indicating that much is still to be learned about the molecular mechanisms involved in the pathogenesis of WTs.

Treatment

The treatment of WT is a paradigm of multimodality management, as well as an example of the importance of collaborative studies in pediatric cancer. Acquisition of knowledge of the appropriate usage of active chemotherapy agents, as well as appreciation of the radiosensitivity of WT, along with advances in surgical techniques and post-operative care, have led to remarkable improvement in the outcomes of children with WT. A universally lethal disease at the turn of the nineteenth century, survival increased to about 25% with surgery only in the early 1900s; the use of routine post-operative radiation therapy resulted in an almost 50% survival rate in the 1950s; the discovery of the effectiveness of chemotherapy drugs (initially vincristine and actinomycin) increased survival to the 70–80% range in the 1970s (167). Further improvements in both the overall outcome of patients with renal tumors, and a decrease in overall toxicity through limiting exposure to unnecessary therapy for low risk patients have come about through the work of large collaborative groups. Although many groups have made important contributions, the two largest are the International Society of Pediatric Oncology (SIOP) and the National Wilms Tumor Study Group (NWTS.) NWTS no longer exists, but the work of this group on the study of renal tumors in North America has now been taken on through the Children's Oncology Group (COG). Although these two groups (SIOP and NWTS/COG) have fundamentally different approaches to the treatment of WT, both have strategies which have resulted in overall survival (OS) rates approaching 90%. SIOP therapies have been based on pre-nephrectomy chemotherapy, and NWTS/COG approach has advocated up-front nephrectomy in almost all cases. Although outcome from each approach has been excellent, it is difficult to extrapolate improvements in therapy from one group to the other, given the confounding variable of the surgical timing.

Risk Stratification

Staging

The treatment of WT is stratified according to the risk of relapse of the tumor. Known prognostic factors, such as age of the patient, size of the tumor, stage, histology, and most recently, genetic factors, are used to risk stratify the patients. NWTS/COG staging is based on anatomical staging at presentation; SIOP staging is based on post-chemotherapy findings. A comparison of the two systems is presented in *Table 59-2*.

Histology

The most powerful prognostic factor for outcome in WT is the histology of the tumor. NWTS and COG define WT as Favorable Histology (FH) if anaplasia is not identified

Table 59-2

Staging systems for pediatric renal tumors

Stage	COG (before chemotherapy)	SIOP (after chemotherapy)
I	(a) Tumor is limited to the kidney and completely excited (b) The tumor was not ruptured before or during removal (c) The vessels of the renal sinus are not involved beyond 2 mm (d) There is no residual tumor apparent beyond the margins of excision	(a) The tumor is limited to kidney, or surrounded with fibrous pseudocapsule if outside of the normal contours of the kidney. The renal capsule or pseudocapsule may be infiltrated with the tumor but it does not reach the outer surface, and it is completely resected (resection margins "clear") (b) The tumor may be protruding into the pelvic system and "dipping" into the ureter (but it is not infiltrating their walls) (c) The vessels of the renal sinus are not involved (d) Intrarenal vessel involvement may be present
II	(a) Tumor extends beyond the kidney but is completely excised (b) No residual tumor is apparent at or beyond the margins of excision (c) Tumor thrombus in vessels outside the kidney is stage II if the thrombus is removed en bloc with the tumor *Although tumor biopsy or local spillage confined to the flank were considered stage II by NWTSG in the past, such events will be considered stage III in COG studies*	(a) The tumor extends beyond kidney or penetrates through the renal capsule and/or fibrous pseudocapsule into perirenal fat but is completely resected (resection margins "clear") (b) The tumor infiltrates the renal sinus and/or invades blood and lymphatic vessels outside the renal parenchyma but it is completely resected (c) The tumor infiltrates adjacent organs or vena cava but is completely resected
III	Residual tumor confined to the abdomen (a) Lymph nodes in the renal hilum, the periaortic chains, or beyond are found to contain tumor (b) Diffuse peritoneal contamination by the tumor (c) Implants are found on the peritoneal surfaces (d) Tumor extends beyond the surgical margins either microscopically or grossly (e) Tumor is not completely respectable because of local infiltration into vital structures	(a) Incomplete excision of the tumor which extends beyond resection margins (gross or microscopic tumor remains post-operatively) (b) Any abdominal lymph nodes are involved (c) Tumor rupture before or intraoperatively (irrespective of other criteria for staging) (d) The tumor has penetrated though the peritoneal surface (e) Tumor implants are found on the peritoneal surface (f) Tumor thrombi present at resection margins of vessels or ureter, transected or removed piecemeal by surgeon (g) The tumor has been surgically biopsied (wedge biopsy) prior to preoperative chemotherapy or surgery
IV	Presence of hematogenous metastases or metastases to distant lymph nodes	Hematogenous metastases (lung, liver, bone, brain, etc.) or lymph node metastases outside the abdominal-pelvic region
V	Bilateral renal involvement at the time of initial diagnosis	Bilateral renal tumors at diagnosis. Each side should be substaged according to the above criteria

NWTS/COG and SIOP staging systems differ mostly in timing of staging, prior to chemotherapy versus after chemotherapy

(*Fig. 59-1*). Anaplasia may be focal or diffuse, and is defined by the presence of large mitotic figures, large and bizarre nuclei, and hyperchromasia (*Fig. 59-3*). Anaplasia is associated with worse outcome (168, 169), and merits intensification of therapy. Patients with low stage focal anaplasia do better than those with diffuse anaplasia (170, 171).

Rhabdoid tumor of the kidney (*Fig. 59-4*) and clear cell sarcoma were initially believed to be subsets of unfavorable histology of WT, but are now understood to be completely distinct tumor types (170, 172).

SIOP bases its histologic classification of WT largely on response to therapy. A revised working classification of all renal tumors was developed by SIOP in 2001 (173)

Figure 59-3

(a) and (b) (Microscopic view) Anaplasia in Wilms Tumor. There is diffuse nuclear enlargement, at least 3 times normal size (compare with tumor cells in top center), with marked pleomorphism. Abnormal, enlarged mitotic figures reflect an increased DNA content.

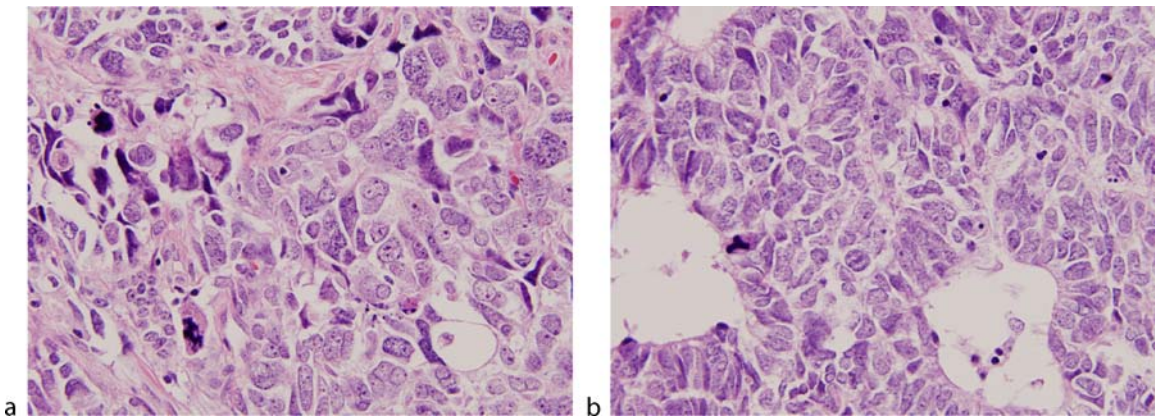
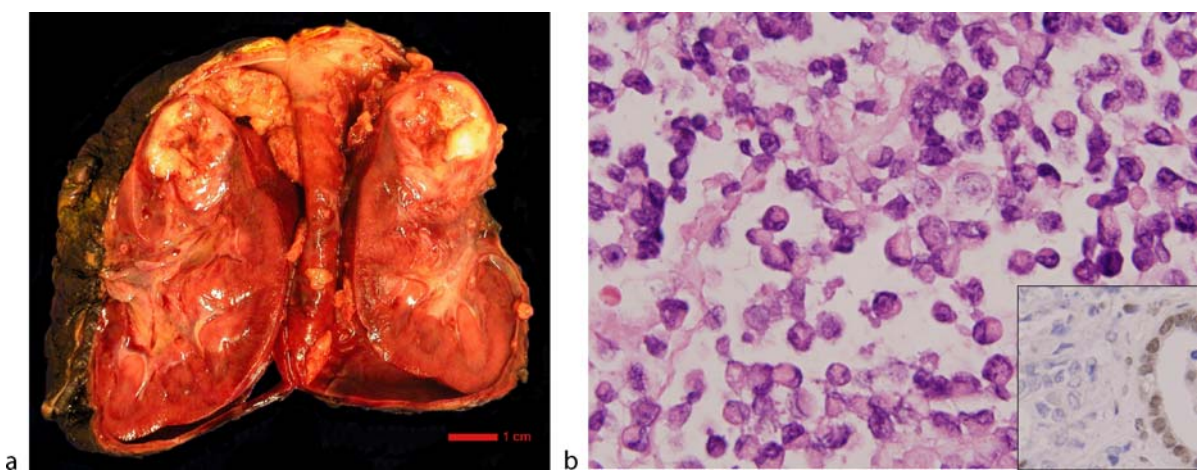


Figure 59-4

Rhabdoid Gross and Microscopic Images. (a) Rhabdoid tumor of the superior pole with extrarenal extension extending to Gerota's fascia. The tumor ruptured the renal capsule and formed a desmoplastic neo-capsule seen at the superior portion of the specimen. There was extensive microscopic extension into the renal sinus vein. (b) Microscopic appearance of a rhabdoid tumor. The tumor cells are frequently discohesive with vesicular nuclei and prominent nucleoli. The nuclei are often indented by an eosinophilic cytoplasmic inclusion composed of whorled aggregates of intermediate filaments. Rhabdoid tumors are characterized by a loss of chromosome 22q11.2 in an area involving the *INI1* gene. The inset is an immunohistochemical stain for *INI1* demonstrating retention in normal renal tubular cells (right) and loss in the tumor cells (left).



(*Table 59-3*). WTs are classified into three risk groups: high risk (blastemal or diffuse anaplasia), intermediate risk (regressive, stromal, mixed, epithelial, or focal anaplasia), and low risk (completely necrotic, or cystic partially differentiated).

Age

Increasing age has been shown to be correlated with decreased prognosis by both cooperative groups (174, 175). Age is used by COG as a prognostic factor,

Table 59-3
Revised S.I.O.P. working classification of pediatric renal tumors

A. For pretreated cases
I. Low risk tumors
Mesoblastic nephroma
Cystic partially differentiated neproblastoma
Completely necrotic nephroblastoma
II. Intermediate risk tumors
Nephroblastoma – epithelial type
Nephroblastoma – stromal type
Nephroblastoma – mixed type
Nephroblastoma – regressive type
Nephroblastoma – focal anaplasia
III. High risk tumors
Nephroblastoma – blastemal type
Nephroblastoma – diffuse anaplasia
Clear Cell sarcoma of the kidney
Rhabdoid tumor of the kidney
B. For Primary nephrectomy cases
I. Low risk tumors
Mesoblastic nephroma
Cystic partially differentiated nephroblastoma
II. Intermediate risk tumors
Non-anaplastic nephroblastoma and its variants
Nephroblastoma – focal anaplasia
III. High risk tumors
Nephroblastoma – diffuse anaplasia
Clear cell sarcoma of the kidney
Rhabdoid tumor of the kidney

along with tumor size. A subset of patients less than 2 years of age, with tumors less than 550 g have been shown to do well with nephrectomy only (176, 177). Adult event-free survival rates of WT have been shown to be lower than WT in pediatric patients, but may be confounded by greater toxicity of treatment seen in adults (178–180).

Biologic Factors

As with other tumors, there is much interest in identifying biologic factors that correlate with prognosis. LOH of both 1p and 16q was found to be a significant adverse prognostic factor in the fifth NWTS trial (181), and is being used prospectively to risk stratify patients on the first COG renal protocols.

Surgical Considerations in Renal Tumors

Surgery was the first modality used in the treatment of renal tumors, and it continues to be of critical importance today. Regardless of tumor type or multimodality treatment protocol, surgery serves as the mainstay to achieve local control, histopathologically assess, and anatomically stage children with renal tumors.

History and Physical Exam

Children with renal masses frequently present asymptomatically, and are often diagnosed either by the caregiver during a routine activity (bathing) or by the healthcare professional on a well-child visit. Seldom do these patients appear sick, and the diagnosis is often surprising to the parents and healthcare professionals who first encounter the child. Patients can also present with symptoms of hematuria or with gastrointestinal complaints, most often constipation. Findings of an abnormal hemogram or urinalysis testing or unexplained hypertension are not uncommon in both symptomatic and asymptomatic patients. Any of these findings should prompt a through abdominal exam and consideration of abdominal imaging.

Surgical consultation is warranted from the time of diagnosis. Once the presence of a mass is confirmed, additional history of associated medical problems that might compromise therapy or predispose the child to risks of peritreatment morbidity should be established. History should include any evidence of developmental delay or unusual growth patterns that may be consistent with predisposing genetic syndromes, as well as any possible history of bleeding disorders in the patient or the family. The accurate assessment of vital signs cannot be overstated. The degree of hypertension in these children can be significant, and this may change the perioperative anesthetic management. The degree of thoracic and abdominal compromise from the mass should also be investigated thoroughly to determine the anesthetic and operative risk for the patient as well. Despite very large masses, most children do not have significant respiratory embarrassment at presentation unless there is considerable metastatic pulmonary disease. Documenting the resting respiratory rate, decreased or absent breath sounds, and presence of effusions or consolidative processes are important. The abdominal exam should focus on the site of the mass and any evidence for involvement of the contralateral side. The presence of ascites should also be considered. A genitourinary exam is also important to investigate the presence of

"new" hernias, hydroceles, or varicolceles that may give indications about the size, location and vascular structures affected by the mass. Tenderness on exam should also alert the physician to the presence of tumor hemorrhage or rupture with subsequent peritoneal irritation.

Laboratory Evaluation

Baseline laboratory studies include urinalysis, complete blood count with differential, chemistry profile, liver function tests, coagulation profile, and blood typing for possible transfusion during surgical intervention. An association of WT and Von Willebrands disease is well established, and should be investigated preoperatively (182, 183).

Radiographic Evaluation

Radiographic studies consist of plain radiography and axial imaging. An abdominal and chest radiograph series are procured to evaluate the presence of disease and associated findings of ascites, effusions, consolidative processes, or the suggestion of metastatic disease. These studies are followed by either computed tomography (CT) (*Fig. 59-5*) or magnetic resonance (MR) (*Fig. 59-6*) imaging techniques with axial, coronal and sagittal formatting to enable three dimensional reconstruction; and hence, the adequate documentation of tumor size, location, organ invasion, intravascular involvement, lymphadenopathy, the presence of contralateral disease, the presence of a solitary kidney, horseshoe kidney, or other anatomic variant, the presence of metastatic disease, and the suggestion of tumor spillage or rupture at diagnosis. Furthermore, one must make sure that the tumor in question truly arises from the kidney and not from simply the retroperitoneum or an adjacent organ (germ cell tumor, sarcoma or neuroblastoma). Differentiating this fact can be difficult, especially with neuroblastoma, but with primary renal tumors the parenchyma is splayed out around the mass ("claw sign" (*Fig. 59-7*)) as opposed to simply being compressed or indented. Delayed sequences can also be ordered to evaluate for the presence of tumor within the collecting system (CT-ureterogram). Ureteral involvement can also be documented on an intravenous pyelogram. One has to consider the risk of radiation-induced malignancy when contemplating which exam to

Figure 59-5

CT images (axial (a) and coronal (b)) showing left renal mass with renal vein and IVC extension with evidence of pulmonary metastasis (c).

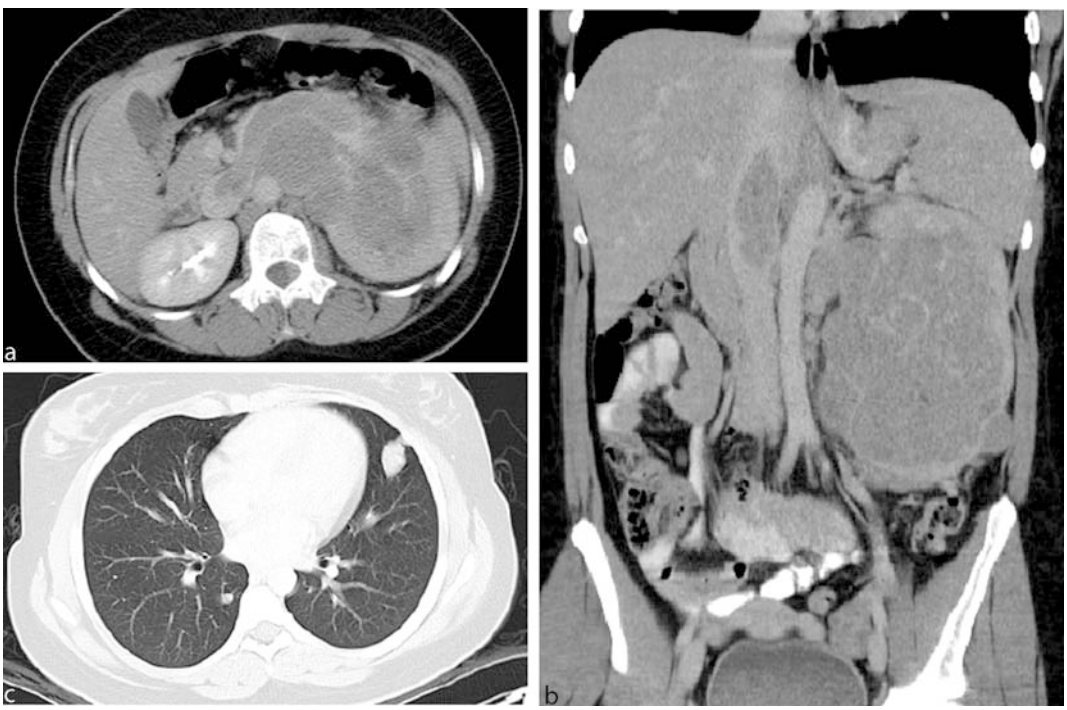
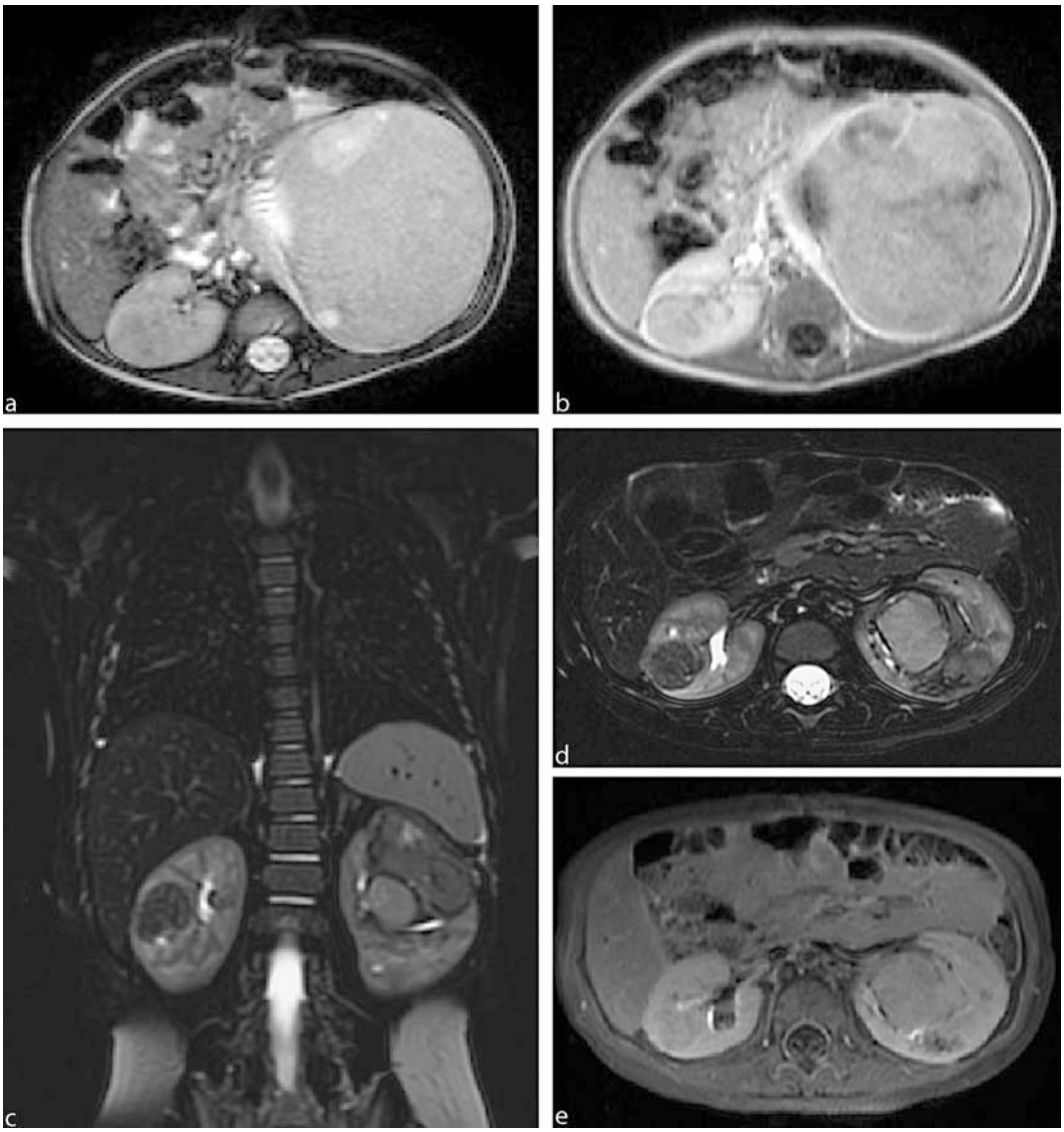


Figure 59-6

MRI Bilateral Nephroblastoma. (a) [T1] and (b) [T2] at diagnosis showing bilateral masses. After 2 months of chemo Rx masses have decreased in size (c) [Coronal T2], (d) [Axial T2], and (e) [Axial T1].



order (CT vs. MR) (184), but accurate preoperative planning must take precedence over the concern for secondary malignancies if there is any question. Adjuvants to these studies include vascular ultrasonography (US) that is probably more sensitive for diagnosing inferior vena cave and renal vein involvement. If renal vein and/or IVC involvement is discovered, then further studies may be warranted to document the degree of tumor thrombus progression including the presence of atrial (echocardiogram) or suprahepatic vein IVC involvement (CT venogram). Furthermore, US is also an excellent modality to investigate the kidney parenchyma to better define the architecture and assist in defining the characteristics of the mass and the contralateral kidney. Nuclear imaging studies do not have a role in these patients except to possibly document the baseline glomerular filtration rate and renal function contributed by each kidney in anticipation of a nephrectomy. However, these tests are seldom performed preoperatively and often do not change preoperative management of the patient. A bone scan (*Fig. 59-8*)

Figure 59-7

CT images (a) [axial], (b) [axial], and (c) [coronal] of right renal mass with evidence of a "claw sign" (white arrow) and with extension into the renal pelvis/proximal ureter causing obstruction.

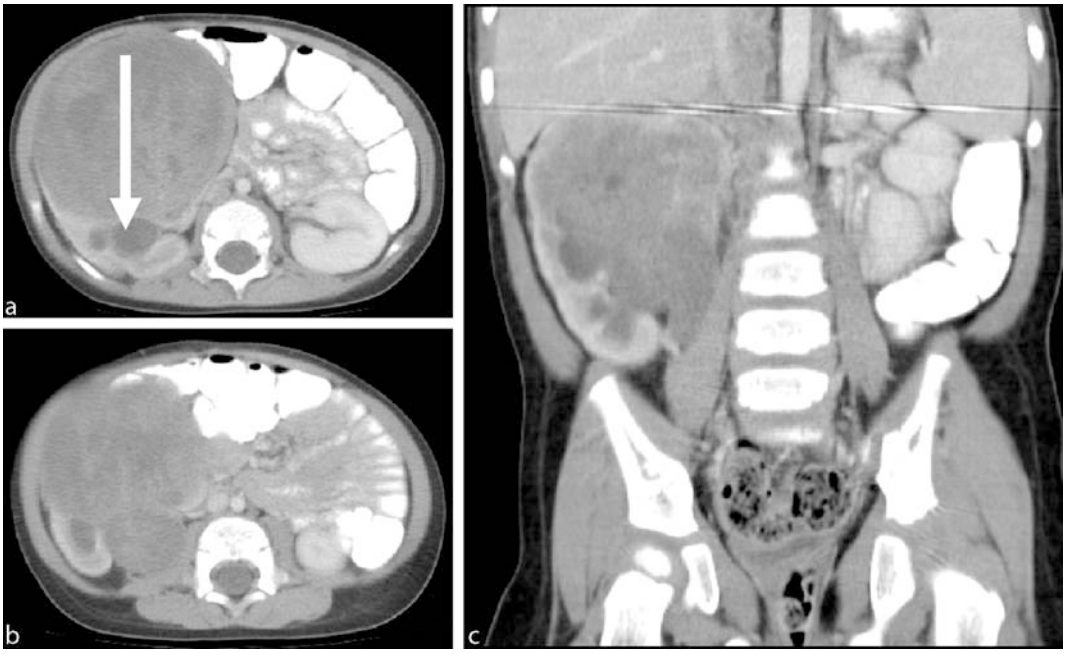
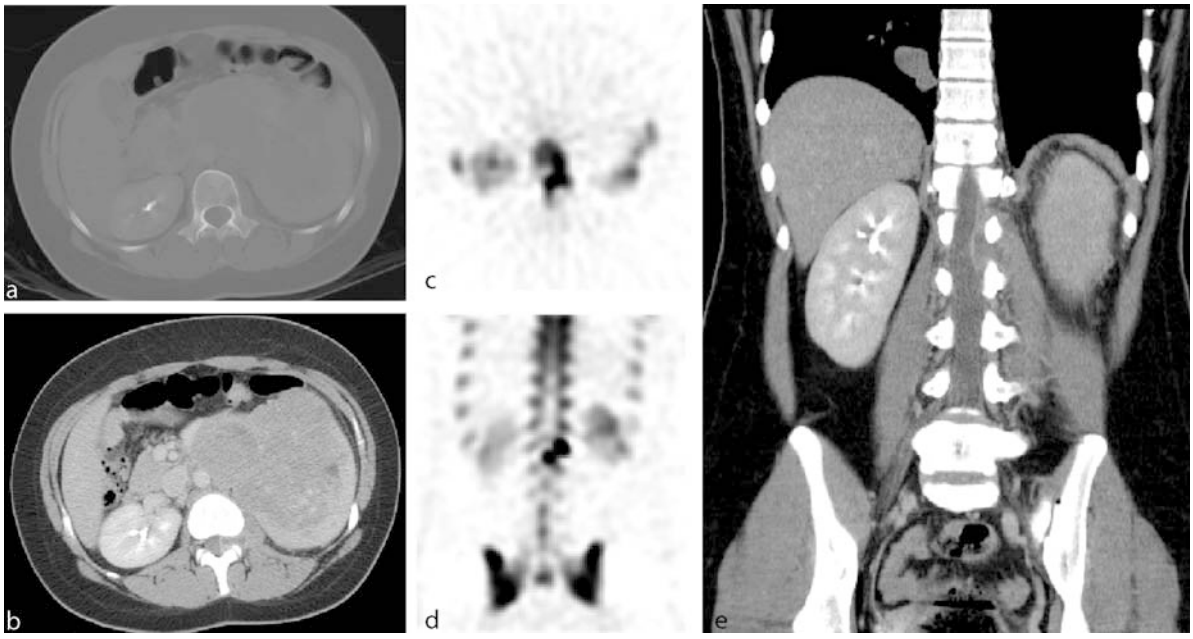


Figure 59-8

CT images (a) [bone window] and (b) [abdominal window] showing intraspinal extension of tumor and bone metastasis, (c) (axial) and (d) (coronal) display corresponding bone scan images, and (e) CT coronal image shows extent of intraspinal spread.



may be ordered to document the presence of bony metastasis if the mass is thought to be a clear cell sarcoma of the kidney. Bone scan is not routinely done in WT. A CT of the brain is often used to document metastasis in renal rhabdoid tumors, but not in other variants as the other tumors are not prone to neural involvement.

Anesthetic Evaluation and Perioperative Considerations

Once the child has been properly assessed and evaluated, primary resection as opposed to biopsy and neoadjuvant chemotherapy is espoused by most North American centers. This pathway differs from the regimens proposed by SIOP, and this issue will be discussed in greater detail below. Prior to surgery, anesthetic preparation concerning the child's pulmonary function, renal function, degree of anemia and hypertension, and the extent of the planned resection is performed. Discussions between the surgical team and the anesthesia team are critical to assess the risk of hemorrhage and the probable conduct of the operation. An intraarterial line is of vast important for hemodynamic monitoring and arterial blood gas sampling during the operation to ensure optimal respiratory function. A nasogastric tube and transurethral bladder drainage catheter ("foley" catheter) are inserted as well to drain the gut and to monitor urine output. Furthermore, gross hematuria can also be assessed during the operation with the use of a transurethral bladder catheter. Several intravenous lines are also placed in the upper extremities for rapid fluid administration and resuscitation during the operation, and the largest bore intravenous catheters that can be placed are recommended. Furthermore, the lower extremities are avoided as IV sites if possible, so as to ensure resuscitation can proceed in the event that the IVC is clamped during the operation. Previously-typed blood products should be available in the operating room prior to beginning the operation. Finally, an epidural catheter is recommended for intraoperative hemodynamic stability and postoperative pain management. This catheter blunts the body's physiological response to the operative insult by controlling the sensation of pain from the beginning of the case. The patient's hemodynamics are generally more stable with fewer swings in the patient's blood pressure and heart rate. Furthermore, with the incisional pain blunted postoperatively by the epidural catheter, the patient can be extubated at the end of the operation assuming that there were no intraoperative complications and hemorrhage was kept to a minimum. The epidural catheter can then be used in the postoperative period for all analgesia needs, and it can remain in until there is return of bowel function and an oral pain regimen can be started (usually within 5–7 days).

Operative Considerations

The operation then begins with the child positioned either supine or slightly raised on an ipsilateral flank roll to 15–20 degrees of elevation from the operating room table. These positions afford the greatest exposure to the abdomen – retroperitoneal and intraperitoneal spaces. A report from the NWTSG documented the importance of the type and size of the incision with which to remove the involved kidney without subsequent rupture or intraoperative complications (185). A generous transverse or bilateral subcostal incision is generally recommended as opposed to flank or paramedian incisions secondary to the higher reported rates of rupture of the tumors (185). Furthermore, an ipsilateral thoracoabdominal incision centered over the greatest diameter of the mass to ensure adequate exposure to the entire retroperitoneum and adjacent chest cavity can also be employed. This latter approach allows for a wide field of view via the abdominal portion of the incision, and the thoracic extension of the incision provides the unique exposure to the retroperitoneum superiorly and posteriorly where the tumor is most likely to be adherent to the diaphragm and surrounding soft tissues. Furthermore, the entire IVC can be exposed (right-sided tumors) for adequate proximal and distal control if a caval or renal vein thrombectomy is needed, or for left-sided tumors the incision can be carried over to the right anterior superior iliac spine to allow for adequate exposure of the IVC from the left side. At the conclusion of the operation, a thoracostomy tube may be required, but not always as the pneunothorax can usually be evacuated without issue. Regardless of the type of incision, upon entering the abdomen, a peritoneal survey is conducted to look for evidence of occult metastatic disease. The entire peritoneal surface is palpated, as is the liver and contralateral kidney. Any free fluid is removed and sent for cytology, especially if there is evidence of rupture. Once these maneuvers are performed, the ipsilateral colon is mobilized and brought to the center via the medial visceral rotation technique. Care should be taken to define the tissue planes appropriately so as not to injure the colonic mesentery or to dissect too closely to the renal capsule so as to increase the risk of inadvertent perforation. Early recommendations had advocated dissecting the hilum first to gain vascular control, but this led to increased intraoperative hemorrhage

and other morbidities. It is now recommended to defer the renal hilar dissection until the kidney has been fully mobilized and the renal pedicle is easier and safer to manipulate, isolate and divided. The kidney and tumor should be circumferentially dissected off the retroperitoneal structures. The ipsilateral adrenal gland is often removed as well. The utmost care must be taken so as not to injure the tumor capsule and allow for iatrogenic rupture and tumor spillage. A generous margin of soft tissue should be included with the kidney and mass if needed and possible to decrease the risk of perforation. This may even necessitate removing a portion of the diaphragm. Prior to dividing the renal vein, directly palpate the renal vein and IVC to ensure there is no tumor thrombus. If present, then complete resection of all tumor including a caval thrombectomy is in order so as to not cut across the tumor and create iatrogenic intraperitoneal spillage. If the mass on exploration is too extensive as to require adjacent organ resection (colon, spleen, liver, etc.), or intravascular involvement precludes safe tumor thrombectomy, then only a biopsy of the mass should be performed and adjuvant therapy begun prior to formal resection and local control. If the kidney and tumor can be removed, then the ipsilateral lymph nodes should be sampled so as to adequately stage the tumor, regardless of histopathological type. The lymph node areas involved should be hilar, paraaortic, and paracaval. A formal retroperitoneal lymph node dissection is neither warranted nor indicated as earlier studies have confirmed (186). All renal tumor types necessitate lymph node sampling, though data are only available for nephroblastoma. Studies conducted through NWTSG have shown that gross inspection of the lymph nodes by the operative surgeon is not adequate to define lymph node involvement in nephroblastoma (187). Inadequate lymph node sampling has led to understaging patients, and hence undertreatment with an increased incidence of local recurrence as demonstrated in NWTSG 4 (185). Finally, the ureter should be transected as close to the bladder as possible without forming a diverticulum or outpouching that can then serve as a source of infection. Generally, the ureter is carefully traced to the pelvic rim and dissected anteriorly to the junction with the bladder, and then transected where convenient. Palpation of the ureter should also take place prior to transaction to ensure there is no intraureteral tumor involvement. If the ureter is transected across tumor, then it is considered spillage with subsequent possible upstaging of the tumor and resultant increased therapy. Cystoscopy and ureteroscopy (or retrograde ureterograms) are only advocated for those with preoperative gross hematuria to define the possibility or bladder or ureter involvement (188).

At the conclusion of the operation, and under the same anesthetic, consideration should be given to the placement of an intravenous vascular access device for adjuvant therapy. An intraoperative frozen section can be performed on the tumor prior to abdominal closure to determine the histopathological subtype. Once known, a discussion between pathologist, oncologist and surgeon should be held to determine the need for adjuvant chemotherapy. If the tumor type is amenable to chemotherapy, then permanent vascular access should be placed at the same operative setting. If there is any doubt about the diagnosis or if the tumor is not amenable to adjuvant therapy (renal cell carcinoma), then the placement of a vascular access device is deferred.

Postoperative Course

Postoperatively, the patient is generally extubated in the operating room if there are no intraoperative complications, significant resuscitation with crystalloid or colloid fluids, and a functioning epidural catheter. The patient is transferred to the intensive care unit for monitoring for 24 h, and then he is transferred to the floor the next day. The nasogastric tube is left in place until there is adequate bowel function and enteral intake is begun. The epidural catheter is generally left in until an oral pain management regimen is started, or the catheter is not functioning. Once the epidural catheter is removed, the bladder catheter is removed. Attention should be directed to ensure adequate hemodynamic parameters, urine output and euvolemia, and normal renal function by laboratory monitoring during the postoperative period. Once the patient is adequately ambulating, eating, drinking, has normal renal function, and is on an oral pain control regimen, he is discharged or transferred to the oncology service for the administration of chemotherapy. This usually occurs within 7 days after surgery. NWTSG reviews in the past 30 years have shown that small bowel obstruction (SBO) is the most frequent postoperative complication (185, 189). However, a special note should be made concerning early postoperative SBO in these patients as an intussusception can occur in these patients for an as yet unknown reason. It will present early in the postoperative period with signs and symptoms consistent with a SBO. However, worsening abdominal pain and increasing nasogastric tube output should alert the surgeon to order abdominal radiographs and an US to document the presence of this condition. If needed, reoperation is indicated to relieve the obstruction, or depending on the age of the patient, an air contrast enema can be used to reduce the intussusception.

Surgical Questions and Controversies

Neoadjuvant Therapy Versus Upfront Resection

Primary nephrectomy with subsequent adjuvant therapy has been and continues to be the NWTSG/COG recommendation for all renal masses. This view is not shared by SIOP, however, and they have recommended neoadjuvant chemotherapy – with or without a tissue biopsy – for over 3 decades. Both approaches have merit, and both have resulted in comparably good overall outcomes. From a surgical perspective, the SIOP recommendation is based on many factors, but not the least of which is a greater risk of tumor spillage and rupture in patients undergoing upfront nephrectomy (190). SIOP has reported greater rates of these complications than NWTS studies (191).

Another argument for surgical resection prior to chemotherapy is that this approach allows for procurement of untreated tissue for full histologic and biologic assessment, as well as complete surgical staging, including biopsy of suspicious sites and lymph node sampling. NWTSG review of surgery related factors predicting local recurrence revealed that failure to sample lymph nodes was an adverse prognostic factor, even when compared to patients with documented nodes positive for tumor (185). The hypothesis to explain this is that a subset of patients that did not have lymph node sampling were understaged, and therefore undertreated. Results of SIOP 6, where adjuvant radiotherapy was withheld in a cohort with negative lymph nodes after resection but with preoperative chemotherapy, demonstrated the importance of accurate lymph mode sampling. The study was stopped midway after there was an increase in local recurrence in this cohort when compared to the radiotherapy cohort, and these results seem to support this concern (192, 193). The SIOP perspective on this "loss of staging information" however views that patients who respond well to therapy and are found to be lower stage at the time of resection may appropriately be treated with less intensive therapy, particularly with avoidance of anthracycline and radiotherapy (194).

Another controversy in WT management involving the surgeon is the use of neoadjuvant chemotherapy with or without a tissue biopsy. The SIOP approach allows neoadjuvant chemotherapy based on diagnosis made from imaging studies and does not mandate a pretherapy biopsy. A series of reports from the NWTSG and SIOP addressed concerns with this approach (191, 195). Namely, without a tissue biopsy, the mass could be benign or a different malignancy, and it was in almost 7–10% of cases reported in these studies (191, 195). Furthermore, a study from the United Kingdom further highlighted this result where almost 12% of there patients would have had different pathological findings in one report (196). An additional concern that is often raised is that histopathology may change after pretreatment, and patients may not be appropriately treated.

Even for those who advocate upfront nephrectomy in the majority of cases, it is clear that not all patients with WT should undergo primary resection. In order to preserve as much renal tissue as possible, patients with WT occurring in a single kidney, horseshoe kidney, or bilaterally should not undergo upfront nephrectomy. Patients presenting with significant respiratory compromise from extensive metastatic disease are not appropriate for initial surgery. Two studies by the NWTSG have further addressed which renal masses should receive a primary biopsy and neoadjuvant therapy. These reports documented the intraoperative and perioperative morbidity from surgery for nephroblastoma, especially intraoperative hemorrhage, adjacent vascular or organ injury, and mortality. Tumors that were very large (>10 cm), had extensive IVC involvement (above the hepatic veins and into the right atrium), and required resection of adjacent organs should all undergo biopsy and neoadjuvant chemotherapy prior to resection for local control. These studies also pointed out that resections performed through suboptimal incisions (flank or paramedian laparotomy) and by non-pediatric specialists were also at greater risk of intraoperative perforation and spillage and increased morbidity (185, 197). Tumors that appear to have perforated with free intraperitoneal spillage at diagnosis may also warrant biopsy to confirm the pathological diagnosis and then neoadjuvant chemotherapy.

The technique of biopsy, percutaneous versus open, as also been an area of debate and study (198). Biopsies performed via a percutaneous core needle technique increase the risk of discordant pathology and seeding of the needle tract. Open biopsy with lymph node sampling at the time of diagnosis is another method that has traditionally been used. Based on results of NWTS studies demonstrating that patients undergoing any type of prechemotherapy biopsy had higher rates of relapse, all patients undergoing initial biopsy will be considered stage III in the COG staging system (199).

Gross Hematuria at Presentation

Ureteral extension of nephroblastoma is a rare phenomenon present in only 2% of cases in a recent NWTSG review (188). This correlates with the few reports in the literature to date. In reviewing the NWTSG reports, the

authors found that of the cohort of children with ureteral involvement, 49% had evidence of gross hematuria. This symptom serves as a significant clue to the presence of tumor extension into and through the collecting system, and hence, due diligence should be undertaken by the treating medical personnel. Preoperative imaging studies may find evidence of ureteral tumor thrombus in almost 63% of patients, and CT was the most helpful modality. Prior to resection, however, cystoscopy, retrograde ureterograms, and direct palpation of the ureter to determine the presence of tumor thrombus are all warranted so as to define the extent of disease and have a complete resection. If the tumor thrombus is inadvertently missed and transected at surgery, then this would be considered intraoperative tumor spillage with subsequent tumor upstaging.

Pulmonary Metastases

Patients with WT and pulmonary metastasis have been shown to do better with intensified chemotherapy with or without radiation therapy (200–202). Although centers have recommended primary pulmonary metastasectomy to spare the morbidity of expanded therapy (201, 203), the NWTSG has demonstrated the superior efficacy of chemotherapy and radiotherapy over chemotherapy and surgery, regardless of pathological subtype (204, 205). Specifically, Green and colleagues demonstrated that metastasectomy did not have an effect on outcome in NWTS 1–3, nor was there a role for pulmonary metastasectomy in patients with stage IV disease. Furthermore, Ehrlich and colleagues recently demonstrated the importance of pretreatment biopsy of pulmonary lesions not radiographically consistent with metastatic disease as critical to avoiding overtreatment of children who may have other reasons for small pulmonary lesions (206).

Intravascular Extension

A minority of children present with evidence of intravascular involvement with nephroblastoma (4%) (207). Diagnosis includes a combination of axial imaging (CT and/or MR) in addition to ultrasonography, including echocardiography to establish atrial involvement if warranted. Surgical extirpation of all disease – including the entire thrombus – is recommended. Furthermore, if all intravascular disease can be resected, there is no change in prognosis (208). However, recommended timing of the resection has changed. An upfront resection followed by adjuvant therapy was initially recommended, but it has too great a morbidity in comparison to neoadjuvant chemotherapy and subsequent nephrectomy (185, 209). A recent report from NWTSG documented the success of this approach and the clear ability of neoadjuvant chemotherapy to safely facilitate the subsequent nephrectomy and thrombectomy (207). Specifically, the surgical morbidity was reduced by 50% (26–13%) with neoadjuvant chemotherapy, and the most severe complications occurred in the upfront surgery cohort.

Bilateral Disease

Bilateral renal masses in children has been defined as stage V disease. Diagnosis and staging of these patients is similar to those children who present with unilateral disease save for the overriding mandate to save renal parenchyma. Whereas North American and European centers have differed on the management of unilateral disease (neoadjuvant chemotherapy vs. upfront resection), a common pathway has emerged in the treatment of children with bilateral disease. The goal of treatment in this cohort of children has been renal preservation to avoid the need for permanent renal replacement therapy. The forthcoming Children's Oncology Group protocol will mirror SIOP's approach and dispense with the need for pretreatment biopsies (open or percutaneous). However, if tumors do not respond to neoadjuvant chemotherapy, then biopsy is recommended to ensure concordant histopathological results and adequate chemotherapeutic regimens. Discordant tumors (unfavorable on one side and favorable on the other side) exist and if missed, this scenario can allow for the undertreatment of the patient. Ideally, after neoadjuvant chemotherapy (three drug regimen assuming favorable histology), successful partial nephrectomies can be performed. The NWTSG evaluated this cohort of patients in NWTSG-4 (210), and total gross resection of disease was accomplished in 88% of cases. However, there was a higher percentage of both local recurrence (8%) and positive margins (16%) in this cohort. These results were deemed acceptable secondary to a substantial, successful partial nephrectomy rate (72%) and overall survival (81% at 4 years). The success of this pathway was also echoed by other authors (211).

The Role of Partial Nephrectomy

Nephron sparing surgery for unilateral nephroblastoma has not been adequately studied using prospective, randomized trials. Data amassed from the cohort of patients

with bilateral tumors has shown this surgical extirpative modality to be an effective procedure when married to pretreatment biopsy and neoadjuvant chemotherapy (see prior section on Bilateral Tumors). The dominant philosophy when dealing with the children with bilateral tumors is to preserve renal parenchyma and avoid permanent renal replacement therapy. Pursuant to this goal, several groups (212–214) have attempted to apply parenchymal sparing surgery to unilateral disease recognizing the long-term morbidity of radical nephrectomy for unilateral tumors, including other renal injury (trauma, infection, obstruction), decreased glomerular filtration rate and the onset of renal failure, and metachronous nephroblastoma in the contralateral kidney. Haecker and colleagues evaluated their cohort of patients undergoing partial nephrectomy in nephroblastoma and recommended that it only be used for patients with small, favorable histology tumors after neoadjuvant chemotherapy (213). There was a higher local recurrence rate in the partial nephrectomy cohort, as well as a lower survival in unfavorable histology tumors. Hence, the authors concluded that partial nephrectomy is feasible in small lesions, histologically favorable tumors that responded to neoadjuvant chemotherapy. Linni and colleagues published their result in analyzing the role of partial nephrectomy in unilateral nephroblastoma and recommended that this approach is not ready for universal application (214). However, they did stress that in specific cases it is reasonable to consider partial nephrectomy if the tumor decreases by 50% or greater in volume after neoadjuvant chemotherapy, if the tumor is easy to resect (unipolar lesion), if preservation of greater than 50% of the kidney remains after resection, and if there are pathologically negative para-aortic lymph nodes. Finally, the results of the UKW-3 trial has been reported by Arul and colleagues and the feasibility of unilateral, partial nephrectomy in favorable histology nephroblastoma was evaluated (212). The study attempted to determine the ability of the surgeon to adequately define the resection plane ("marking") on the nephrectomy specimen ex vivo in light of the following criteria: (1) clear resection margins, (2) no vascular invasion, (3) no pelvic invasion, and (4) >50% of the kidney preserved. The study was unsuccessful as there were no specimens officially "marked," but of the specimens identified by the surgeon as being a candidate for partial nephrectomy, 70% were deemed pathologically not to meet the above criteria to be eligible for a partial nephrectomy. However, whereas intraoperative ultrasound was not available during this study 18 years ago, it may facilitate partial nephrectomy by defining the proper plane of dissection today.

The Role of Laparoscopy

The role of laparoscopic partial and radical nephrectomy is well established in adult renal cancers (215). However, there is minimal data to support these approaches in pediatric patients, and especially in the case of nephroblastoma. The role of minimally invasive surgery to resect other pediatric malignancies has been reported (216), but North American centers that stressed the importance of primary resections with adjuvant chemotherapy following surgery for nephroblastoma have not encouraged primary laparoscopic radical nephrectomies. Generally the tumors are large, bulky, and the concern for extirpation without rupture is of paramount importance so as not to intensify the postoperative therapy a child should receive. A minimally invasive approach for tumor biopsy (unilateral or bilateral) in patients deemed poor candidates for primary resection is both feasible and realistic. European centers that espoused neoadjuvant chemotherapy with tumor reduction have a cohort of patients whose tumors are more amenable to this minimally invasive approach. Duarte and colleagues documented the success of laparoscopic nephrectomy after neoadjuvant chemotherapy following SIOP protocols in two separate reports (217, 218). They report on a total of 10 patients with no evidence of recurrence (mean follow-up 5–23 months), no complications, and no port-site implants. The authors document that the renal tumors are smaller and encased in a fibrous capsule after neoadjuvant chemotherapy which facilitates the ability to safely and completely resect these tumors and involved lymph nodes. However, the operative times for this approach are longer in comparison to open procedures.

Chemotherapy

Multiple chemotherapy agents have been shown to be appropriate in WT. These drugs act through a wide spectrum of mechanisms, and have both overlapping and unique toxicity profiles (*Table 59-4*). NWTS and SIOP studies have helped refine the schedules and dosages appropriate to the patient's stage and histology, balancing the risk of relapse with avoidance of the risk of toxicity from the chemotherapy. It is well established that low stage FH WT can be effectively treated with vincristine and actinomycin, with minimal acute and long-term toxicity (192, 219). Doxorubicin is added for higher stage patients. Although doxorubicin is a very active agent in all WT, it confers both a greater risk of toxicity on therapy, largely due to myelosuppression, and a small, but real risk of long-term cardiac toxicity (220). Cyclophosphamide,

Table 59-4

Chemotherapy agents commonly used in renal tumors

Drug	Category	Mechanism of action	Common toxicity[a]
Vincristine	Vinca alkaloid	Mitotic inhibitor; inhibition of microtubule assembly and cellular metaphase arrest.	Vesicant, NT, A, SIADH, hypotension, A
Dactinomycin	Antitumor antibiotics	Intercalation; DNA strand breaks (Topo II)	M, N and V, A, mucostitis, vesicant, hepatic (VOD)
Doxorubicin	Antitumor antibiotics	Intercalation; DNA strand breaks (Topo II); free radical formation	M, mucostitis, N and V, A, diarrhea, vesicant, cardiac (acute, chronic)
Cyclophosphamide	Alkylating agents	(Prodrug) alkylation; crosslinking	M, N and V, A, cystitis, water retention; cardiac (HD)
Etoposide	Plant products	DNA strand breaks (Topo II)	M, A, N and V, mucostitis, mild NT, hypotension, HSR, secondary leukemia, diarrhea (p.o.)
Carboplatin	Alkylating agents	Platination; crosslinking	M (Plt), N and V, A, hepatic (mild), HSR
Ifosfadime	Alkylating agents	(Prodrug) alkylation; crosslinking	M, N and V, A, cystitis, NT, renal; cardiac (HD)
Topotecan	Plant products	DNA strand breaks (Topo II)	M, diarrhea, mucostitis, N and V, A, rash, hepatic
Irinotecan	Plant products	(Prodrug) DNA strand breaks (Topo II)	M, diarrhea, N and V, A, hepatic, dehydration, ileus

[a]*M* myelosuppresion, *SIADH* secretion of inappropriate diuretic hormone, *a* alopecia, *N* and *V* nausea and vomiting, *nt* neurotoxicity

etoposide, and carboplatin are active chemotherapy agents with significant risk of toxicity that are reserved for high risk patients, including some patients with diffuse anaplasia, Stage IV disease, poor initial response to therapy, and as well, some patients with RTK and CCS. Toxicities of these drugs include significant myelosuppression, and subsequent increased risk of serious infections, decreased renal function, hearing loss, hemorrhagic cystitis, and secondary leukemias (*Table 59-4*). Irinotecan, topotecan, and ifosfamide are also agents used in very high risk patients, largely in the relapse setting (221, 222). Very high dose chemotherapy with stem cell transplant has been successful in some relapsed patients, but has proven to be very difficult to study given the very small number of patients (223).

Special Circumstance

Children with WT, particularly bilateral WT, are at risk for development of renal failure during treatment. Patients may become anephric if surgery is necessitated in critical areas of both kidneys. Patients with remaining renal tissue after surgery may develop renal failure due to chemotherapy agents, or radiotherapy. The three main drugs use in newly diagnosed WT, vincristine, actinomycin, and doxorubicin, can be given safely and effectively to patients with renal failure (224). In a review of 28 of 5,910 children registered on NWTS studies 1–IV, treated with chemotherapy with concomitant renal failure, it was concluded that reduction of dosing of these agents is not necessary, and that reasonable cure rates were observed. These patients do require close individual monitoring, and accurate pharmacologic and pharmacokinetic studies are vital.

Radiation Therapy

WT are very radiosensitive, and it was this modality that offered the first true cures in WT. However, radiation therapy carries the risk of significant long term toxicities, including secondary malignancies, scoliosis, radiation pneumonitis, cardiac toxicity, pregnancy related complications, and renal compromise or failure. Early NWTS and SIOP studies have both demonstrated that low stage patients could be cured without radiotherapy, therefore radiation therapy is reserved for patients with higher risk disease (192, 225, 226). The appropriate use of radiation therapy in patients with FH WT is still being investigated. Patients with stage IV pulmonary disease have been

shown to have better outcome with radiation therapy on NWTS compared to similar patients treated on a United Kingdom Children's Cancer Study Group trial (202, 225). However, SIOP studies show good outcomes while withholding radiation therapy in a subset of patients with initial pulmonary disease that resolves quickly with chemotherapy (202). The first COG FH WT High Risk protocol is designed to assess whether patients treated with a backbone of NWTS therapy that show resolution of pulmonary metastasis within 6 weeks can safely avoid radiation therapy.

Screening

For patients with genetic syndromes associated with increased risk of developing WT, routine radiologic surveillance is recommended. Although not unanimously agreed upon, a schedule of US every 3–4 months through age 7 has been shown to aid in detection of WT, and proposed to be cost-effective in identifying tumors at an earlier stage (227–229).

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Hypertension

60 Epidemiology of Hypertension

Midori Awazu

Introduction

Hypertension (HTN) is a significant global health issue. It is the major risk factor for atherosclerosis, leading to the development of cardiovascular disease (CVD). There is increasing evidence that HTN has its antecedents during childhood and that atherosclerosis is already present in adolescents. Thus early detection and intervention are crucial. HTN is also a risk factor for the progression to end-stage renal disease, a topic extremely relevant to pediatric nephrologists. While children are more likely to have secondary HTN, the prevalence of primary HTN appears to be increasing due to an epidemic of obesity. To identify children with HTN, clinicians should know normative values of blood pressure (BP) as well as the definition of HTN. Prevalence of HTN, factors influencing BP, and sequelae of childhood HTN are also reviewed in this chapter.

Normative Blood Pressure Values in Children

Casual Blood Pressure

The National High Blood Pressure Education Program (NHBPEP) Working Group established guidelines for the definition of normal BP in children in its Report of the Second Task Force on Blood Pressure in Children-1987 (1). Normative casual BP values were updated in 2004 by The Fourth Report on the Diagnosis, Evaluation, and Treatment of High Blood Pressure in Children and Adolescents (2). Since the 1996 Update on the 1987 Task Force Report (3), the working group has reexamined the childhood BP database and has added new data from the 1999 to 2000 National Health and Nutrition Examination Survey (NHANES). BP values are based on gender, age, and height, and 50th, 90th, 95th, and 99th percentiles are provided (*Tables 60-1* and *60-2*). There is minimal change from the 1996 report in the numbers that designate the 90th and 95th percentile for systolic or diastolic BP. The 90th and 95th percentile are BP levels that define prehypertension and HTN, respectively. The 99th percentile and the 50th percentile are given to facilitate clinical decision-making in the plan for evaluation and to provide the clinician the BP level at the midpoint of the normal range, respectively. To determine the height percentile, clinicians are instructed to use the CDC growth charts (4). Since these procedures are time consuming and may lead to the underdiagnosis of HTN (5), easier ways to determine childhood BP categories have been made available by calculators or modified BP tables (6, 7). The BP tables are based on auscultatory measurements and the definition of diastolic BP is the 5th Korotkoff sounds. One should note that values measured by oscillometric devices frequently used in clinical practice are not interchangeable with auscultatory values. Park et al. reported that the oscillometric systolic BP (SBP) averaged 10 mm Hg higher and the oscillometric diastolic BP (DBP) averaged 5 mm Hg higher than the auscultatory BP readings (8). The fourth report recommends rechecking oscillometric BP measurements greater than the 90th percentile with the auscultatory method. One should also note that the Task Force Report norms are based on a single BP measurement, not in accordance with the currently recommended multiple measurements (see Chapter "Evaluation of Hypertension in Childhood Diseases). In addition, it should be pointed out that BP measurements obtained with standard practice vital sign station screening may be different from those obtained by trained personnel in accordance with fourth report recommendations (9). Seventy-four percent of the readings were reported to be higher at the vital sign station, and only 12% differed by <5 mm Hg for both SBP and DBP.

Although the Task Force standards have been adopted worldwide, many local reference values are also used, especially in Europe. De Man et al. have shown that the normative values for European children are different from those for American children (10, 11). Pooling data from six European studies (Germany, France, Denmark, and the Netherlands) showed that the 95th percentile for mean BP was higher in European children by 6 mm Hg for SBP and 3 mm Hg for DBP. Menghetti et al. found that normal BP values for Italian children differed from both the American data and from de Man's (12). In Italian children, the 90th and 95th percentiles were 3–8 mm Hg

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Table 60-1

BP levels for boys by age and height percentile

Age, y	BP Percentile	SBP, mm Hg							DBP, mm Hg						
		Percentile of height							Percentile of height						
		5th	10th	25th	50th	75th	90th	95th	5th	10th	25th	50th	75th	90th	95th
1	50th	80	81	83	85	87	88	89	34	35	36	37	38	39	39
	90th	94	95	97	99	100	102	103	49	50	51	52	53	53	54
	95th	98	99	101	103	104	106	106	54	54	55	56	57	58	58
	99th	105	106	108	110	112	113	114	61	62	63	64	65	66	66
2	50th	84	85	87	88	90	92	92	39	40	41	42	43	44	44
	90th	97	99	100	102	104	105	106	54	55	56	57	58	58	59
	95th	101	102	104	106	108	109	110	59	59	60	61	62	63	63
	99th	109	110	111	113	115	117	117	66	67	68	69	70	71	71
3	50th	86	87	89	91	93	94	95	44	44	45	46	47	48	48
	90th	100	101	103	105	107	108	109	59	59	60	61	62	63	63
	95th	104	105	107	109	110	112	113	63	63	64	65	66	67	67
	99th	111	112	114	116	118	119	120	71	71	72	73	74	75	75
4	50th	88	89	91	93	95	96	97	47	48	49	50	51	51	52
	90th	102	103	105	107	109	110	111	62	63	64	65	66	66	67
	95th	106	107	109	111	112	114	115	66	67	68	69	70	71	71
	99th	113	114	116	118	120	121	122	74	75	76	77	78	78	79
5	50th	90	91	93	95	96	98	98	50	51	52	53	54	55	55
	90th	104	105	106	108	110	111	112	65	66	67	68	69	69	70
	95th	108	109	110	112	114	115	116	69	70	71	72	73	74	74
	99th	115	116	118	120	121	123	123	77	78	79	80	81	81	82
6	50th	91	92	94	96	98	99	100	53	53	54	55	56	57	57
	90th	105	106	108	110	111	113	113	68	68	69	70	71	72	72
	95th	109	110	112	114	115	117	117	72	72	73	74	75	76	76
	99th	116	117	119	121	123	124	125	80	80	81	82	83	84	84
7	50th	92	94	95	97	99	100	101	55	55	56	57	58	59	59
	90th	106	107	109	111	113	114	115	70	70	71	72	73	74	74
	95th	110	111	113	115	117	118	119	74	74	75	76	77	78	78
	99th	117	118	120	122	124	125	126	82	82	83	84	85	86	86
8	50th	94	95	97	99	100	102	102	56	57	58	59	60	60	61
	90th	107	109	110	112	114	115	116	71	72	72	73	74	75	76
	95th	111	112	114	116	118	119	120	75	76	77	78	79	79	80
	99th	119	120	122	123	125	127	127	83	84	85	86	87	87	88
9	50th	95	96	98	100	102	103	104	57	58	59	60	61	61	62
	90th	109	110	112	114	115	117	118	72	73	74	75	76	76	77
	95th	113	114	116	118	119	121	121	76	77	78	79	80	81	81
	99th	120	121	123	125	127	128	129	84	85	86	87	88	88	89
10	50th	97	98	100	102	103	105	106	58	59	60	61	61	62	63
	90th	111	112	114	115	117	119	119	73	73	74	75	76	77	78
	95th	115	116	117	119	121	122	123	77	78	79	80	81	81	82
	99th	122	123	125	127	128	130	130	85	86	86	88	88	89	90

Table 60-1 (Continued)

Age, y	BP Percentile	SBP, mm Hg							DBP, mm Hg						
		Percentile of height							Percentile of height						
		5th	10th	25th	50th	75th	90th	95th	5th	10th	25th	50th	75th	90th	95th
11	50th	99	100	102	104	105	107	107	59	59	60	61	62	63	63
	90th	113	114	115	117	119	120	121	74	74	75	76	77	78	78
	95th	117	118	119	121	123	124	125	78	78	79	80	81	82	82
	99th	124	125	127	129	130	132	132	86	86	87	88	89	90	90
12	50th	101	102	104	106	108	109	110	59	60	61	62	63	63	64
	90th	115	116	118	120	121	123	123	74	75	75	76	77	78	79
	95th	119	120	122	123	125	127	127	78	79	80	81	82	82	83
	99th	126	127	129	131	133	134	135	86	87	88	89	90	90	91
13	50th	104	105	106	108	110	111	112	60	60	61	62	63	64	64
	90th	117	118	120	122	124	125	126	75	75	76	77	78	79	79
	95th	121	122	124	126	128	129	130	79	79	80	81	82	83	83
	99th	128	130	131	133	135	136	137	87	87	88	89	90	91	91
14	50th	106	107	109	111	113	114	115	60	61	62	63	64	65	65
	90th	120	121	123	125	126	128	128	75	76	77	78	79	79	80
	95th	124	125	127	128	130	132	132	80	80	81	82	83	84	84
	99th	131	132	134	136	138	139	140	87	88	89	90	91	92	92
15	50th	109	110	112	113	115	117	117	61	62	63	64	65	66	66
	90th	122	124	125	127	129	130	131	76	77	78	79	80	80	81
	95th	126	127	129	131	133	134	135	81	81	82	83	84	85	85
	99th	134	135	136	138	140	142	142	88	89	90	91	92	93	93
16	50th	111	112	114	116	118	119	120	63	63	64	65	66	67	67
	90th	125	126	128	130	131	133	134	78	78	79	80	81	82	82
	95th	129	130	132	134	135	137	137	82	83	83	84	85	86	87
	99th	136	137	139	141	143	144	145	90	90	91	92	93	94	94
17	50th	114	115	116	118	120	121	122	65	66	66	67	68	69	70
	90th	127	128	130	132	134	135	136	80	80	81	82	83	84	84
	95th	131	132	134	136	138	139	140	84	85	86	87	87	88	89
	99th	139	140	141	143	145	146	147	92	93	93	94	95	96	97

The 90th percentile is 1.28 SD, the 95th percentile is 1.645 SD, and the 99th percentile is 2.326 SD over the mean

higher for SBP and DBP between 5 and 12 years of age, and 2–3 mm Hg higher in older males than American standards. With respect to Northern Europe, levels in Italy were similar or slightly higher in the lower ages and lower in late adolescence.

In Great Britain, reference BP values in children and young adults aged 4–23 years were established using an oscillometric device (Danamap 8100) (13). BP percentiles were based on demographic data obtained from seven national surveys that recorded three BP measurements at different occasions. The BP values are higher than those provided by the fourth report, due probably to the use of an oscillometric device. Several other countries have reported reference BP values for their own populations (10, 11, 14–16).

The second report of 1987 provided standards for infants less than 1 year old (1, 3) (*Fig. 60-1*). These values were obtained by the auscultatory method and the fourth Korotkoff sounds were used for DBP. Zubrow et al. and Kent et al. provided normative BP values obtained using the oscillometric method for newborns and infants during the first year of life respectively (17, 18).

Table 60-2

BP Levels for Girls by Age and Height Percentile

Age, y	BP Percentile	SBP, mm Hg							DBP, mm Hg						
		Percentile of height							Percentile of height						
		5th	10th	25th	50th	75th	90th	95th	5th	10th	25th	50th	75th	90th	95th
1	50th	83	84	85	86	88	89	90	38	39	39	40	41	41	42
	90th	97	97	98	100	101	102	103	52	53	53	54	55	55	56
	95th	100	101	102	104	105	106	107	56	57	57	58	59	59	60
	99th	108	108	109	111	112	113	114	64	64	65	65	66	67	67
2	50th	85	85	87	88	89	91	91	43	44	44	45	46	46	47
	90th	98	99	100	101	103	104	105	57	58	58	59	60	61	61
	95th	102	103	104	105	107	108	109	61	62	62	63	64	65	65
	99th	109	110	111	112	114	115	116	69	69	70	70	71	72	72
3	50th	86	87	88	89	91	92	93	47	48	48	49	50	50	51
	90th	100	100	102	103	104	106	106	61	62	62	63	64	64	65
	95th	104	104	105	107	108	109	110	65	66	66	67	68	68	69
	99th	111	111	113	114	115	116	117	73	73	74	74	75	76	76
4	50th	88	88	90	91	92	94	94	50	50	51	52	52	53	54
	90th	101	102	103	104	106	107	108	64	64	65	66	67	67	68
	95th	105	106	107	108	110	111	112	68	68	69	70	71	71	72
	99th	112	113	114	115	117	118	119	76	76	76	77	78	79	79
5	50th	89	90	91	93	94	95	96	52	53	53	54	55	55	56
	90th	103	103	105	106	107	109	109	66	67	67	68	69	69	70
	95th	107	107	108	110	111	112	113	70	71	71	72	73	73	74
	99th	114	114	116	117	118	120	120	78	78	79	79	80	81	81
6	50th	91	92	93	94	96	97	98	54	54	55	56	56	57	58
	90th	104	105	106	108	109	110	111	68	68	69	70	70	71	72
	95th	108	109	110	111	113	114	115	72	72	73	74	74	75	76
	99th	115	116	117	119	120	121	122	80	80	80	81	82	83	83
7	50th	93	93	95	96	97	99	99	55	56	56	57	58	58	59
	90th	106	107	108	109	111	112	113	69	70	70	71	72	72	73
	95th	110	111	112	113	115	116	116	73	74	74	75	76	76	77
	99th	117	118	119	120	122	123	124	81	81	82	82	83	84	84
8	50th	95	95	96	98	99	100	101	57	57	57	58	59	60	60
	90th	108	109	110	111	113	114	114	71	71	71	72	73	74	74
	95th	112	112	114	115	116	118	118	75	75	75	76	77	78	78
	99th	119	120	121	122	123	125	125	82	82	83	83	84	85	86
9	50th	96	97	98	100	101	102	103	58	58	58	59	60	61	61
	90th	110	110	112	113	114	116	116	72	72	72	73	74	75	75
	95th	114	114	115	117	118	119	120	76	76	76	77	78	79	79
	99th	121	121	123	124	125	127	127	83	83	84	84	85	86	87
10	50th	98	99	100	102	103	104	105	59	59	59	60	61	62	62
	90th	112	112	114	115	116	118	118	73	73	73	74	75	76	76
	95th	116	116	117	119	120	121	122	77	77	77	78	79	80	80
	99th	123	123	125	126	127	129	129	84	84	85	86	86	87	88

Table 60-2 (Continued)

Age, y	BP Percentile	SBP, mm Hg							DBP, mm Hg						
		Percentile of height							Percentile of height						
		5th	10th	25th	50th	75th	90th	95th	5th	10th	25th	50th	75th	90th	95th
11	50th	100	101	102	103	105	106	107	60	60	60	61	62	63	63
	90th	114	114	116	117	118	119	120	74	74	74	75	76	77	77
	95th	118	118	119	121	122	123	124	78	78	78	79	80	81	81
	99th	125	125	126	128	129	130	131	85	85	86	87	87	88	89
12	50th	102	103	104	105	107	108	109	61	61	61	62	63	64	64
	90th	116	116	117	119	120	121	122	75	75	75	76	77	78	78
	95th	119	120	121	123	124	125	126	79	79	79	80	81	82	82
	99th	127	127	128	130	131	132	133	86	86	87	88	88	89	90
13	50th	104	105	106	107	109	110	110	62	62	62	63	64	65	65
	90th	117	118	119	121	122	123	124	76	76	76	77	78	79	79
	95th	121	122	123	124	126	127	128	80	80	80	81	82	83	83
	99th	128	129	130	132	133	134	135	87	87	88	89	89	90	91
14	50th	106	106	107	109	110	111	112	63	63	63	64	65	66	66
	90th	119	120	121	122	124	125	125	77	77	77	78	79	80	80
	95th	123	123	125	126	127	129	129	81	81	81	82	83	84	84
	99th	130	131	132	133	135	136	136	88	88	89	90	90	91	92
15	50th	107	108	109	110	111	113	113	64	64	64	65	66	67	67
	90th	120	121	122	123	125	126	127	78	78	78	79	80	81	81
	95th	124	125	126	127	129	130	131	82	82	82	83	84	85	85
	99th	131	132	133	134	136	137	138	89	89	90	91	91	92	93
16	50th	108	108	110	111	112	114	114	64	64	65	66	66	67	68
	90th	121	122	123	124	126	127	128	78	78	79	80	81	81	82
	95th	125	126	127	128	130	131	132	82	82	83	84	85	85	86
	99th	132	133	134	135	137	138	139	90	90	90	91	92	93	93
17	50th	108	109	110	111	113	114	115	64	65	65	66	67	67	68
	90th	122	122	123	125	126	127	128	78	79	79	80	81	81	82
	95th	125	126	127	129	130	131	132	82	83	83	84	85	85	86
	99th	133	133	134	136	137	138	139	90	90	91	91	92	93	93

*The 90th percentile is 1.28 SD, the 95th percentile is 1.645 SD, and the 99th percentile is 2.326 SD over the mean

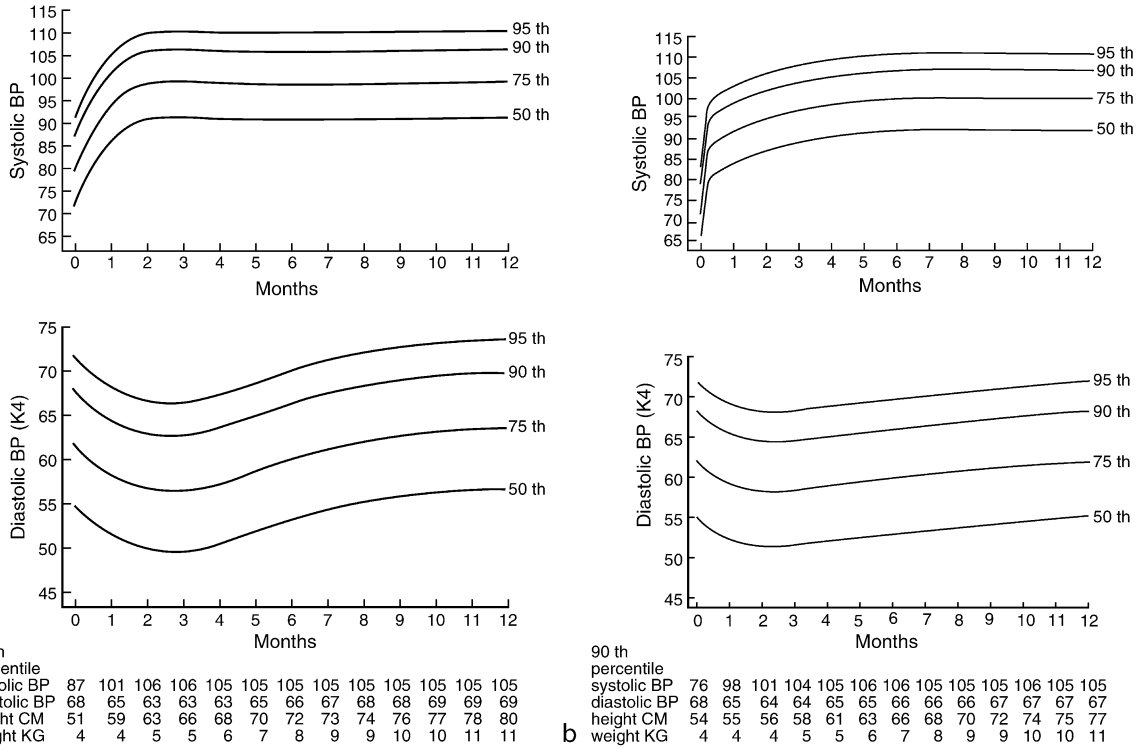
Ambulatory Blood Pressure

Ambulatory BP monitoring (ABPM) is more reliable and reproducible than casual BP as it provides multiple readings over time. It also enables computation of the mean, daytime, and night-time ambulatory BP (ABP) by measuring BP during regular activities, detects white coat effect (higher casual BP than 24-h or daytime ABP) or reversed white coat effect (lower casual BP than 24-h or daytime ABP), and provides information regarding diurnal BP pattern. The utility and tolerability of ABPM in children have been documented (19). Increasing age and higher mean arterial pressure are factors that improve the success rate of ABP measurement (20, 21). ABPM has also proved to have higher reproducibility than clinic BP in children and is now widely used (22–26).

Studies from the United States, Spain, Germany, and Taiwan reported normal values for ABPM in children by age and gender (27–30). As office BP, ABP values rise with age and tend to be higher in boys than for girls. Normal ABP values by height are also available (31–33). Wuhl et al. analyzed the data of Soergel et al. using the

Figure 60-1

(a) Age-specific percentiles of BP measurements in boys-birth to 12 months of age; (b) Age-specific percentiles of BP measurements in girls-birth to 12 months of age; Korotkoff phase IV (K4) used for diastolic BP. From: Report of the Second Task Force on Blood Pressure Control in Children–1987. Task Force on Blood Pressure Control in Children. National Heart, Lung, and Blood Institute, Bethesda, Maryland. Pediatrics 1987;79(1):1–25.



a

90 th percentile														
systolic BP	87	101	106	106	105	105	105	105	105	105	105	105	105	105
diastolic BP	68	65	63	63	63	65	66	67	68	68	69	69	69	
height CM	51	59	63	66	68	70	72	73	74	76	77	78	80	
weight KG	4	4	5	5	6	7	8	9	9	10	10	11	11	

b

90 th percentile														
systolic BP	76	98	101	104	105	106	106	105	105	105	106	105	105	
diastolic BP	68	65	64	64	65	65	66	66	66	66	67	67	67	67
height CM	54	55	56	58	61	63	66	68	70	72	74	75	77	
weight KG	4	4	4	5	5	6	7	8	9	9	10	10	11	

From: Report of the Second Task Force on Blood Pressure Control in Children–1987. Task Force on Blood Pressure Control in Children. National Heart, Lung, and Blood Institute, Bethesda, Maryland. Pediatrics 1987;79(1):1–25.

Table 60-3

90th and 95th percentile of mean day- and night-time systolic and diastolic BP, stratified according to gender and height

Height (cm)	N	Percentiles for boys (*n* = 347) 50th[a]	Percentiles for boys (*n* = 347) 95th[a]	N	Percentiles for girls (*n* = 420) 50th[a]	Percentiles for girls (*n* = 420) 95th[a]
120–129	23	105/64	119/76	36	101/64	119/74
130–139	51	108/64	121/77	51	103/64	120/76
140–149	39	110/65	125/77	61	105/65	122/77
150–159	41	112/65	126/78	71	108/66	123/77
160–169	45	115/65	128/78	148	110/66	124/78
170–179	91	117/66	132/78	46	112/66	125/79
180–189	57	121/67	134/79	7	114/67	128/80

[a]Values are systolic/diastolic blood pressure

From: Wuhl et al.: J Hypertens 20:1995–2007, 2002

least mean square (LMS) method (34) to correct for the skewed distribution of the original cohort, and provided normalized data to gender, and age or height (35) (*Table 60-3*). Although the original data by Soergel et al. have been used most widely, the data by Wuhl et al. provides the most complete and appropriate reference data for ABP in children.

It is important to use different norms for ABPM and casual BP measurement as awake ABP values are higher than BP values found in the fourth report, which are based on a single daytime auscultatory reading (36, 37).

Home Blood Pressure

While ABPM is useful, it is expensive and not available for every pediatrician. In adults, self-measurement of BP at home and work is regarded as an alternative, and recent guidelines recommend self-measurement of BP (38, 39). Readings of <130/80 mm Hg and >135/85 mm Hg are generally considered normal and hypertensive, respectively, in adults (38). Self-measurement of BP has the following advantages: (1) distinguishing sustained HTN from white coat HTN (see below), (2) detection of masked HTN (see below), (3) assessing response to medication, (4) improving patient adherence to treatment, and (5) reducing costs by avoiding ABPM or drug therapy.

In children, home BP monitoring has been utilized (40–42). Investigators in Greece confirmed the reproducibility of home BP in children (43). They first validated the Omron 705 IT oscillometric device for use in children and adolescents (44), and provided reference values for pediatric populations (45). This is currently the only reference values available in children (*Table 60-4*). The same group of investigators reported on the relationship between home BP and office measurements (46). In younger children (6–12 years), both diastolic and systolic home BP levels were higher than office BP. This difference is reduced with advancing age and eliminated after the age of 12 years. This is in contrast to adults in whom home BP tends to be lower than office BP (47).

More than 70% of the pediatric nephrologists in Germany use home BP measurement for children, and 64% consider home BP more important than office measurement, although it is less accurate than ambulatory BP (48). There were, however, wide discrepancies for standards of home BP in children and adolescents. Wuhl et al. reported that home BP is superior to clinic BP measurement but neither clinic BP nor home BP detected HTN with enough sensitivity or specificity to replace ABPM (49). The greater specificity of home BP compared to clinic BP may suggest that home BP is more suitable for diagnosis rather than screening of HTN in children.

Proper validation and regular calibration of the device by the clinician are necessary for home BP measurement. Future goals include standardization of BP devices and measurement, as well as prospective studies to demonstrate that home BP measurement better predicts cardiovascular outcomes compared to office BP recordings in children.

Definition of Hypertension in Children

Casual Blood Pressure

The definition of childhood HTN is statistically defined based on the normative distribution of BP in healthy children. This is in contrast to adult HTN defined clinically as the level of BP that makes an increase in morbidity and/or mortality (2, 3, 38). This definition could not be applied to children because data associating childhood HTN with morbid events have been scarce. Ongoing longitudinal investigations should provide new insights into the long-term significance of high BP in childhood (50–52). In the fourth report, HTN is defined as average SBP and/or DBP that is ≥95th percentile for gender, age, and height on three or more separate occasions. BP levels that are ≥90th percentile or ≥120/80 mm Hg but <95th percentile is termed prehypertension. The fourth report has added a method for staging the severity of HTN by providing the range of BP elevation for stage 1 and stage 2 HTN. Stage 1 HTN is defined as being from the 95th to the 99th percentile plus 5 mm Hg. Stage 2 HTN is 5 mm Hg or more above the 95th percentile and represents a level of BP that requires prompt evaluation.

In Great Britain, high BP is defined as either SBP or DBP above 98th percentile for gender and age, and high normal BP as BP between the 91st and 98th percentile (13). This percentile system, different from both American and European counterparts, predicts prevalence of high BP (>2 SDS) and high-normal BP (>1.33 SDS) to be 2.3 and 6.9%, respectively. These values are similar to those in other countries.

In children younger than 1 year of age, SBP has been used to define HTN. Standards for SBP and DBP for infants <1 year old have not been changed since 1987 (1).

There are several problems in using population percentiles to define normal BP (53). One relates to the evolution in the epidemiology of the reference population. BP percentile values may change as the demographics and characteristics of population change. The second problem is the need to account for secular

Table 60-4

Systolic and diastolic home BP values for clinical use

	Systolic BP				Diastolic BP			
	Day		Night		Day		Night	
Boys height (cm)	90th pct	95th pct	90th pct	95th pct	90th pct	95th pct	90th pct	95th pct
120	120.6	123.5	103.7	106.4	79.1	81.2	61.9	64.1
125	121.0	124.0	104.9	107.8	79.3	81.3	62.2	64.3
130	121.6	124.6	106.3	109.5	79.3	81.4	62.4	64.5
135	122.2	125.2	107.7	111.3	79.3	81.3	62.7	64.8
140	123.0	126.0	109.3	113.1	79.2	81.2	62.9	65.0
145	124.0	127.0	110.7	114.7	79.1	81.1	63.1	65.2
150	125.4	128.5	111.9	115.9	79.1	81.0	63.3	65.4
155	127.2	130.2	113.1	117.0	79.2	81.1	63.4	65.6
160	129.2	132.3	114.3	118.0	79.3	81.3	63.6	65.7
165	131.3	134.5	115.5	119.1	79.7	81.7	63.7	65.8
170	133.5	136.7	116.8	120.2	80.1	82.2	63.8	65.9
175	135.6	138.8	118.1	121.2	80.6	82.8	63.8	65.9
180	137.7	140.9	119.2	122.1	81.1	83.4	63.8	65.8
185	139.8	143.0	120.3	123.0	81.7	84.1	63.8	65.8
	Systolic BP				Diastolic BP			
	Day		Night		Day		Night	
Girls height (cm)	90th pct	95th pct	90th pct	95th pct	90th pct	95th pct	90th pct	95th pct
120	118.5	121.1	105.7	109.0	79.7	81.8	64.0	66.4
125	119.5	122.1	106.4	109.8	79.7	81.8	63.8	66.2
130	120.4	123.1	107.2	110.6	79.7	81.8	63.6	66.0
135	121.4	124.1	107.9	111.3	79.7	81.8	63.4	65.8
140	122.3	125.1	108.4	111.9	79.8	81.8	63.2	65.7
145	123.4	126.3	109.1	112.5	79.8	81.8	63.0	65.6
150	124.6	127.5	109.9	113.1	79.9	81.9	63.0	65.5
155	125.7	128.5	110.6	113.8	79.9	81.9	62.9	65.5
160	126.6	129.3	111.1	114.0	79.9	81.9	92.8	65.4
165	127.2	129.8	111.2	114.0	79.9	81.9	62.7	65.2
170	127.5	130.0	111.2	114.0	79.9	81.8	62.5	65.0
175	127.6	129.9	111.2	114.0	79.8	81.7	62.3	64.7

From: Stergiou et al.: *J Hypertens* 25:1375–1379, 2007

trends. The SBP and DBP among children and adolescents in the United States increased by an average of 1.4 mm Hg and 3.3 mm Hg, respectively, between 1988 to 1994 and 1999 to 2000 due, at least in part, to the increase in the prevalence of overweight and obesity (54). An increase in average BP levels among American children was confirmed by several investigators (54–56). If new BP data are collected, the 95th percentile values will increase and allow higher BP to be considered normal. In a similar manner, if separate criteria for each ethnic group are used due to ethnic differences, it may cover the cardiovascular risk associated with ethnic predispositions to HTN.

Ambulatory Blood Pressure

For ABPM values, HTN is generally defined as daytime or 24-h ABP either systolic or diastolic greater than 95th

percentile by gender, and age or height. Simple dichotomous cutoff values for normal BP versus HTN, however, may not be sufficient. Zachariah et al. developed the concept of "BP load," defined as the percentage of ABPM readings ≥140 mm Hg for SBP and ≥90 mm Hg for DBP in adults (57). In children, the BP load is defined as the percentage of total BP measurements exceeding the upper limit of normal for age, body size, and time of day. BP load has been related to target organ damage and may be more appropriate for the definition of HTN (58, 59). The acceptable limit of BP load, however, is not determined even in adults. BP loads of 25% or more are generally accepted as HTN (60).

Epidemiology of Hypertension in Children

Prevalence of Hypertension

By definition, 5% of children would fall into the category of HTN. The normative data provided by the Task Force, however, are from the first measure obtained for each child. With repeated measurement, only approximately 1% of children and adolescents proved to be hypertensive (1, 60). Adrogue et al., using the 1996 criteria, reported that systolic HTN was found in 2.7% and diastolic HTN in 2% of children after the screening (61). After the rescreening, systolic HTN had fallen to 0.8% and diastolic HTN to 0.4%. Over the last decade, however, HTN has increased, affecting up to 5% of adolescents in the United States (60–63). The childhood obesity epidemic appears to be associated with the increasing prevalence of HTN (54, 64–66). Chiolero et al., however, after reviewing literature published over the past 20 years, reported that a definite conclusion will depend on future studies (66). More recently, Din-Dzietham et al. assessed high BP secular trends in children and adolescents enrolled in US national surveys, and concluded that the increase was largely attributable to the increase in obesity (56).

The prevalence of HTN in other countries has been reported to be between 1 and 22% (62, 67–78). Many of these studies also documented the association of HTN with obesity. In Great Britain, which utilizes a different criteria, the prevalence of HTN is reported to be 2.3% (13). The prevalence of HTN seems to be increasing in association with BMI in Canada as well (69). In Northern Ireland, however, large decreases in BP have occurred among adolescents aged 12 and 15 years over the decade from 1990. The magnitude of the trends could not be accounted for by confounding factors including BMI (79).

The majority of hypertensive children have mild HTN, most often primary. A small group of children have higher BP that is usually due to a secondary cause. Younger children generally have secondary HTN, whereas essential HTN is most common in adolescents. The prevalence of secondary HTN is estimated to be 28% in children with HTN as opposed to 5% in adults with HTN (80), and 0.1% in the general population (81).

The prevalence of prehypertension ranges from 5.4 to 15.7% (13, 63, 69, 78). A recent study showed that prehypertension also increased 2.3% between 1988 and 1999 (56). Obesity increase partially explained the rise in prehypertension similar to the rise in HTN.

White Coat Hypertension

White coat hypertension (WCH) is a condition where casual BP is high but the BP during ABPM is normal (82), whereas white coat (WC) effect refers to a pressor response in the medical setting. While WC effect has been described in children and adolescents (83, 84), the diagnosis of WCH has become possible only after ABP normative values in children became available (85). Subsequent studies showed that the prevalence of WCH in children ranged between 12.9 and 88% depending on the criteria used (86–89). In the fourth report, WCH is defined as BP measured in a physician's office >95th percentile, whereas average BP is <90th percentile outside of a clinical setting (2).

In adults, the risk of CVD is thought to be lower in patients with WCH than in those with persistent HTN and higher than in those with normotension (39). In children, however, WCH may represent a prehypertensive state. Increased LVMI were reported in children with WCH (90) in contrast to a previous study which showed no significant difference in LVMI and intima-media thickness of the carotid arteries (88). Further follow up of these patients is needed.

Masked Hypertension

Masked HTN (MH), a high ABP in the presence of normal office BP, is being recognized as a risk factor for cardiovascular complications in adults (91). It has previously been called "reverse WCH" or "WC normotension". MH has been associated with more extensive target organ damage than is seen in true normotensive subjects (92, 93). MH has been found to be prevalent in the

pediatric population ranging from 7.6 to 26% (88, 89, 94–96) consistent with the values found in adults ranging from 5 to 23% (91). Studies showed high prevalence of MH in renal transplant recipients (97, 98).

Children with MH are more likely to have higher body mass indexes, a parent with HTN, greater left ventricular mass, or higher prevalence of left ventricular hypertrophy compared with normotensive controls (88, 89, 95, 96).

Factors Influencing Blood Pressure

Age

The Task Force Report and other studies demonstrate that age is the major determinant of BP (2, 3, 13). SBP increases sharply between birth and age 2 months (1). There is no significant difference between SBP at ages 2 months and 1 year. BP then increases progressively with age and more rapidly during puberty (1, 13). The prevalence of HTN also increases progressively with age (69).

Gender

BP levels, especially after 12–14 years, are higher in boys than in girls, consistent with findings in adults. The mechanisms responsible for the gender differences in BP levels are not clear, although interactions between sex hormones and the kidneys are speculated to play a role (99).

The incidence of HTN is greater among boys than girls (56, 100, 101) with the relative risk of HTN 1.50 (confidence interval: 1.15, 1.95) (62).

Height

Height is a major determinant of BP and predicts BP independent of age (102, 103), which led to the inclusion of height in normative BP tables (2). The more important role of height than weight has previously been reported (102, 104).

Obesity

Currently, the most important factor influencing BP is obesity. Weight was a strong determinant of BP even after adjustment for height (105). In contrast to the previous reports, a recent study suggests a greater effect of weight on BP than height (13). There is a strong association between HTN and weight/obesity (13, 106). The prevalence of HTN in obese children is higher and ranges from 11 to 30% (62, 69–71, 107, 108). As described earlier, the childhood obesity epidemic appears to be associated with an increase in prevalence of HTN in childhood (54, 65).

Brion et al. reported that lean mass and total body fat mass independently and positively correlated with increased BP in 9-year-old children (109). The correlation was stronger for SBP than DBP. Sugiyama et al. confirmed a positive association between BMI and SBP (110), but contrary to adult studies (111), a negative association between BMI and DBP was observed. While some pediatric studies have shown a positive association between BMI and DBP (112), other studies have found no significant association (62). Sorof and Daniels suggested that obesity HTN appears to be characterized by a preponderance of isolated systolic HTN in adolescents (64). In a similar manner to adults, central body fat is more strongly related to BP than peripheral fat in children (113). It has been hypothesized that insulin resistance may play a role in the development of high BP in children and adolescents as well (114). A population-based study in children has demonstrated a relationship between serum insulin concentration and BP (115). A positive relationship has also been reported between insulin resistance and HTN in children and adolescents.(116–118).

Some authors have reported a prevalence of systolic HTN by ABPM ranging between 50 and 62% among obese children and adolescents (119, 120). Obese youths had not only higher BP levels, but also higher BP variability. Among obese youths, 6.6% had WCH, 9.2% had MH, and 5% had sustained HTN (108). Maggio et al. showed that mean ambulatory SBP and DBP are significantly higher in prepubertal obese children than in lean subjects, whereas casual BP levels are not different (96). Lurbe et al. further reported a relationship between insulin resistance and nocturnal elevations of SBP (118).

A systematic review demonstrated that interventions to promote weight loss lowered BP in adults (121). Body weight loss by diet significantly reduced BP in obese children, although the effect was greater when exercise was added (112, 122). BP has been shown to decrease with weight reduction and increased activity even among children in the general population (101, 123).

Physical Activity

The effects of physical activity in the prevention and treatment of high BP have been well described in adults (124). The effects of exercise on resting BP in children and

adolescents, however, are conflicting (125–130). Several studies reported no association, whereas others reported inverse associations, but not consistently for both SBP and DBP or in both genders. This may be partly explained by the difference in method (questionnaire or use of mechanical techniques). A meta-analysis concluded that short-term exercise does not appear to reduce resting BP in children and adolescents (131). Recent randomized controlled trials in overweight children were designed to assess the influence of combined lifestyle changes including both diet and exercise on multiple cardiovascular risk factors in overweight adolescents. Although it is difficult to interpret the influence of exercise alone on BP, most studies suggest that physical activity can effectively reduce BP in children (132). Interestingly,, BP was not reduced any further with exercise in normotensive children.

The pediatric behavioral literature suggests that sedentary activities are more than the opposite of physical activities. They are positively associated with SBP after adjustment for confounding factors (110). In obese children, the amount of time spent watching TV is associated with both HTN and the severity of obesity (133).

As for the intensity of exercise, moderate activity has been shown to be more effective than vigorous activity in reducing SBP (134). Aerobic exercise in adolescence and high-intensity exercise throughout life were associated with low DBP in adulthood (135). A recent study suggests that the volume of activity may be more important than the intensity, in lowering BP (130).

Race and Ethnicity

In adults, HTN is more common in African Americans (non-Hispanic blacks) (32%) compared with non-Hispanic whites (23%) and Mexican Americans (23%) (136). In children and adolescents, ethnic group differences in BP are not so clear. Of approximately 50 studies that examined ethnic differences in BP in children and adolescents, approximately half reported higher BP for African Americans (62, 74, 137–139). The observed differences, however, are often explained by differences in body size, fat distribution, sexual maturation, and socioeconomic status. Furthermore, 14 studies reported no consistently significant differences in BP across ethnic/racial groups (61, 138, 140–142). The ethic differences seem to become apparent at an older age (142). NHANES data indicate slightly higher levels of BP among Mexican American youth compared to their non-Hispanic white counterparts (54). A recent study that examined 8- to 17-year-old non-Hispanic blacks and whites and Mexican Americans demonstrated that non-Hispanic blacks (4.2%) and Mexican Americans (4.6%) had a greater prevalence of HTN and prehypertension than non-Hispanic whites (3.3%) (56). The trends in obesity and high BP have a greater effect on non-Hispanic blacks and Mexican-Americans, consistent with the report by Muntner et al. (54).

Higher BP in African Americans is associated with sodium intake and lower plasma rennin activity (143). Although a recent report demonstrated a higher prevalence of HTN in African American youths in parallel with an increase in obesity (56), obesity was not as closely related to BP in African American children as in white children. Thus, at lower levels of BMI, African American youths had higher SBP and DBP, whereas at higher levels of BMI, white youths had higher SBP and DBP (138, 144).

Family History

A family history of HTN is present in approximately 50% of hypertensive children (145). HTN has long been known to cluster within families (146, 147), and the reason is thought to be due to shared environmental exposures (obesity, salt intake, life style etc) and genetic susceptibility. BP concordance between spouses is greater than that between nonspouses (148) and among biological siblings than among adoptive siblings living in the same household (149, 150). Concordance is also greater among monozygotic twins than among dizygotic twins (151). Longitudinal studies have identified parental history of HTN as a risk factor for HTN in children (152–154). A recent study by Wang et al. examined the association of parental HTN with BP change and HTN risk from young adulthood through the ninth decade of life in a longitudinal cohort (155). The results showed that HTN in both mothers and fathers has a strong independent association with elevated BP levels and incident HTN over the course of adult life. Li et al. also showed that parental HTN independently predicted children's resting BP and BP reactivity along with BMI and BMI z score (156).

Genetics

The heritable portion of BP is estimated to range between 35 and 65% (147, 157). Robinson et al. performed a retrospective case-control analysis concerning the heritability of primary and secondary HTN in children (158). The study concluded that primary HTN in children and adolescents is likely due to a large number of additive

contributions of genes and that secondary HTN may be related to just a few genes.

HTN is rarely caused by monogenic disorders which include Liddle's syndrome (a mutation in the gamma subunit of the amiloride-sensitive epithelial sodium channel SCNN1G), type 1 and type 2 pseudohypoaldosteronism (mutations in the mineralocorticoid receptor gene or SCNN1G in the former and WNK kinases 1 or 4 in the latter), glucocorticoid remediable HTN (a chimeric gene formed from portions of the 11ß-hydroxylase gene and the aldosterone synthase gene), apparent mineralocorticoid excess (mutations in the gene encoding the kidney 11ß-hydroxysteroid dehydrogenase), 11ß-hydroxylase deficiency (mutations of the *CYP11B1* gene), 11α-hydroxylase deficiency (CYP17 mutation), and others (159). The rare alleles responsible for the monogenic trait do not associate with essential HTN, but more common variants of the same genes may exhibit less severe phenotypes. In fact, polymorphisms in SCNN1G have been associated with variation in SBP (160–162). WNK1 gene polymorphisms have also been associated with ambulatory BP variations in the general population (163). On the other hand, mutations of genes that cause rare recessive diseases featuring altered salt handling and reductions in BP may protect against the development of HTN. Ji et al. screened members of the Framingham Heart Study for variation in three genes, *SLC12A3* (NCCT, the thiazide-sensitive sodium-chloride cotransporter), *SLC12A1* (NKCC2, Na-K-2Cl cotransporter gene), and *KCNJ1* (ROMK, the inward rectifier K^+ channel gene) that are responsible for Gitelman's syndrome (NCCT) and Barter's syndrome (NKCC2 and ROMK) (164). Mutation carriers showed reduced BP and low prevalence of HTN compared with non-carriers. These studies show that BP variation is affected by rare alleles.

There are two other approaches to map genetic variants i.e., linkage analysis and association studies. Until recently, genome-wide scans were performed by linkage only, whereas association studies aimed at candidate genes. Linkage analyses have been effective in detecting rare monogenic HTN, whereas association studies were expected to be better for analyses of complex traits such as essential HTN. Genome-wide linkage studies using families or siblings reported significant or suggestive linkage for BP or HTN (165). The results are variable, however, probably due to different populations, small sample sizes, lack of stratification, misclassification of phenotype, and others. Association studies (case-control studies) compare frequencies of alleles of candidate genes between hypertensive patients and normotensive controls. Many association studies with candidate genes for HTN have been reported, although most positive findings failed to replicate as linkage studies. The low reproducibility may be explained by insufficient statistical power of association studies with candidate genes (166). Nevertheless, there are promising genes such as the angiotensinogen gene (*AGT*) (167), angiotensin-converting enzyme gene (168), ß2 adrenergic receptor (*ADRB2*) (169), G protein ß3 subunit (*GNB3*) (170), and adducin gene (*ADD1*) (171).

Recently, genome-wide association studies have been applied to analyze common diseases, including essential HTN. A genome-wide association study is an approach that involves rapidly scanning markers across the complete sets of DNA, or genomes, of patients and controls to find genetic variations associated with a particular disease. This approach became possible with progress in sequencing of the human genome and detection of millions of single nucleotide polymorphism (SNP) markers. In 2007, the Wellcome Trust Case Control Consortium and the Framingham Heart Study reported the results of genome-side association studies for HTN. The former found no strong association and six SNPs with moderate associations (172). None of the six association regions overlap with genes previously suggested through association studies with candidate genes. The Framingham Heart Study also failed to detect strong signals (173). Genome-wide association studies might have problems such as misclassificaiton of subjects with only single measurement of BP. Also, a common disease and common variant hypothesis, a major premise for using genotyping of common variants, may not apply to HTN. Thus HTN may have fewer common risk alleles of larger effect sizes (a statistical measure of the strength of the relationship between two variables). Furthermore, genuine common susceptibility variants of large effect size may be poorly represented on the chip used for genotyping. In support of this possibility, resequencing target regions of rare variants of responsible genes led to the identification of mutations in *SLC12A3, SLC12A1*, and *KCNJ1* associated with HTN as stated above (164). Further studies with multiple approaches are required to clarify HTN-susceptible genes.

Birth Weight

The findings of Barker et al. relating low birth weight (LBW) to increased risk of death from CVD in late adult life have led to many studies exploring this relationship (174, 175). The majority of studies that examined the relationship reported an inverse association between BP and birth weight, supporting the 'fetal origins of adult

disease' or 'programming' hypothesis (2, 176). Systematic reviews have concluded that an inverse association is present with a magnitude ranging from −1.5 to −2.0 mm Hg/kg (177, 178). In a recent meta-analysis, the magnitude of the effect of birth weight varied by study size, with a weaker association in the larger studies (175). The inverse association between birth weight and BP has been found to be independent of potential confounding factors such as socioeconomic status, maternal and participant smoking, maternal age, birth order, maternal BP, and alcohol intake (179–184).

A systematic review found that accelerated postnatal growth (either linear growth or accelerated weight gain) in LBW individuals was associated with increased BP in adulthood (178). Law et al., on the other hand, found that accelerated growth in infancy corrected for regression to the mean had no important effect on BP in early adulthood (185). Poor height gain in early childhood and shorter leg length were reported to be associated with increased adult BP (186), whereas greater growth in height around puberty may be associated with increased BP in later life (187). These studies suggest that different growth trajectories and different patterns of growth at sensitive times in the life course may affect BP in later life. The association between birth weight and childhood BP seems to vary, depending on ethnic group (188–190).

Nephron Number

Brenner et al. proposed that LBW may be associated with a congenital deficit in nephron number, which would predispose to reduced renal sodium excretion and increased susceptibility to HTN (191). This hypothesis was supported by studies that showed a relation between birth weight and nephron number (192–194). The most direct confirmation is the finding by Keller et al. They showed that hypertensive white patients had significantly fewer glomeruli per kidney than matched normotensive controls (195). The association between nephron number and BP was observed in White Europeans, White Americans and Australian Aborigines but not African Americans. Studies in additional populations are required (196).

Salt Intake

BP is positively associated with sodium intake in adults (3, 111, 197–200). In children, however, evidence for a direct relation between sodium and BP has been less conclusive. A review of 25 observational and 12 intervention studies of sodium and BP in youth by Simons-Morton and Obarzanek (201) indicated that only three included children with elevated BP (202–204). The studies that were methodologically stronger yielded conflicting results (202–209). The small or null effect of sodium on BP in most studies may be explained by poor compliance to dietary regimens, inadequate adjustment for confounding factors, variable quality in BP measurements, lack of power to detect differences, and possible differences in sodium sensitivity across groups. In a recent meta-analysis of ten trials looking at the effect of reducing salt intake on BP in infants, children, and adolescents, He and MacGregor showed that a modest reduction led to an immediate and significant fall in BP (210). Long-term and larger intervention trials are needed, however, to conclude that the observed benefits can be maintained during childhood and adolescence and have clinical relevance.

Dietary sodium intake early in life may affect BP in later life. Hofman et al. compared infants receiving a low or normal sodium-containing infant formula for the first 6 months of life (211). A small but significant decrease in BP was found at 6 months of age in infants fed the lower sodium formula. When BP was measured at 4 years, there was no BP difference between groups (212). At 15 years of age, however, BP levels were significantly lower in the low sodium group (213). The mean difference was 3.6 mm Hg in SBP and 2.2 mm Hg in DBP, an effect larger than that reported for sodium reduction in adults (198, 199, 214). Subsequent to this study, the sodium content of most formulas has been reduced (215).

Potassium supplementation is associated with a decrease in BP in adults (216, 217). A review of 12 observational and 2 intervention studies of potassium and BP in children by Simons-Morton and Obarzanek did not provide a clear relationship (201). Higher intakes of dietary potassium in children have been associated with either lower SBP (218) or DBP (219, 220) in half of the observational studies. However, results of one study indicated the opposite effect (221). Potassium supplementation at a level of 1,500 mg given for 4 weeks did not reduce BP in normotensive children (222). Longer-term supplementation trials with potassium in hypertensive children induced lower age-related BP increases in SBP and DBP, compared with nonsupplemented children (202).

The negative association of calcium with BP has been documented in several studies in children (201, 223). Calcium is a dietary component of which suboptimal intakes in children have been associated with higher DBP (224) and SBP (225), although not in all cases (201, 226, 227). Of the randomized intervention trials conducted, supplementation with calcium at a

level ranging from 600 to 1,000 mg/day resulted in either a small non-significant decrease in SBP in normotensive children (228) or a significant decrease in DBP in children with mild HTN (229).

Potassium and calcium supplementation has been shown to enhance urinary sodium excretion that is likely to result in a hypotensive effect (230, 231). At present, the evidence is too limited to support clinical recommendations for potassium and calcium supplementation in BP management in children.

Breast Feeding

Breast feeding has recently been found to be associated with reduced BP in later life (232–234), although the findings have not been consistent (235–237). Two large cohort studies prospectively examined the relation between breast feeding and BP, and found that SBP and DBP were lower in breast-fed children compared with formula-fed children (232, 233). The relation remained significant after controlling for potential confounding factors. A longer duration of breast feeding was associated with a more significant reduction in BP (233). Martin et al. conducted a meta-analysis of studies published after 1980 reporting on BP levels in breast- and bottle-fed subjects (234). Pooled estimates based on a random effects model indicated that breast feeding was associated with a 1.4 and 0.5 mm Hg reduction in SBP and DBP, respectively. The magnitude of the association is similar to those reported for salt restriction and physical activity (2). There was some evidence that the effect on BP is amplified with the increasing age at which the BP is measured (183).

The association of breast-feeding and lower BP may be explained by a hormonal effect, lower sodium intake, or a protective effect of long-chain polyunsaturated fatty acids that are not present in most formula. A recent follow-up of a randomized controlled trial found that BP at age 6 was lower among breast-fed children and formula-fed children who had a formula supplemented with a long-chain polyunsaturated fatty acid than among those randomized to an unsaturated formula (238).

Environment

Adverse socioeconomic status during childhood is associated with increased CVD in later life, independently of adult socioeconomic status (239). Children from the low socioeconomic status are reported to have higher BP in adulthood than those from high socioeconomic status (240–244). Adjustment for risk factors including birth weight, breast feeding, adult body mass index, smoking, and alcohol consumption had little effect on the association between socioeconomic status and BP. In some studies, on the other hand, the association decreased or disappeared after adjusting for body mass index in adulthood (245). Other studies do not support the association between childhood socioeconomic status and adult BP (246–249).

Sleep Disordered Breathing

Sleep disorders including sleep apnea are associated with HTN and cardiovascular morbidity in adults (250, 251). Findings from pediatric studies suggest that elevated BP is associated with the presence of sleep disorders (252, 253). Several cross-sectional studies have described trends of increasing BP with greater frequency of apnea and hypopnea during sleep in children (254, 255). Obese children with high apnea and hypopnea index had a higher prevalence of HTN than obese children with low index (255). This relationship was not found in nonobese children.

The severity of sleep-disordered breathing in children was also associated with increased BP variability, decreased nocturnal dipping, and nocturnal HTN (256–258). Amin et al. further demonstrated that sleep-disordered breathing was independently associated with an increase in morning BP surge, BP load, and 24-h ABP (259).

Two meta-analysis studies provided conflicting results. Ng et al. suggested a significant association between sleep-disordered breathing and HTN, although sleep-disordered breathing may affect BP in either direction in children (260). According to the more recent analysis by Zintzaras et al. no evidence exists that moderate to severe sleep-disordered breathing in childhood increases the risk of elevated BP (261). However, the conclusions were based on relatively small numbers of cases recruited for a few studies, and large population-based studies are needed.

Since approximately 15% of children snore and at least 1–3% have sleep-disordered breathing (252), the fourth report recommends obtaining a brief sleep history during the initial evaluation (2).

Uric Acid

In 1993, uric acid levels were suggested to be useful indicators of adolescents at risk for HTN (262). Recently,

uric acid has been proposed to play a role in essential HTN by contributing to endothelial dysfunction leading to microvascular and inflammatory injury to the kidney (263). Data from the Bogalusa Heart Study determined that childhood uric acid predicts adult BP, suggesting that early elevation in serum uric acid levels may predispose to the development of HTN (264).

Uric acid may provide the link between LBW and childhood HTN. In a retrospective chart review of 95 hypertensive children, Feig et al. showed that birth weight inversely correlated with serum uric acid (265). In 113 randomly selected children aged 8–13 years, Franco et al. confirmed uric acid levels were significantly higher in association with impaired endothelial function in children with LBW compared with those with normal birth weight (266). Uric acid levels correlated with SBP in children of the entire cohort.

Homocysteine

In adults, homocysteine (Hcy) is a well-known risk factor for CVD and was found to be related with BP regardless of the presence of signs and symptoms of CVD (267). Plasma Hcy concentrations were shown to be correlated with BP in healthy children and elevated in obese schoolchildren with HTN and dyslipidemia (268, 269). Glowinska et al. also showed that Hcy correlated with BP and that hypertensive children had the highest concentrations (270). Papandreou et al., on the other hand, reported no association of BP and Hcy in young age groups composed of normal and obese children (271). Franco et al. demonstrated that there was a significant association between the circulating levels of both Hcy and nitric oxide with BP levels and vascular function in children aged 8–13 years, born small for gestational age (272).

Sequelae of Childhood Hypertension

Tracking of Blood Pressure

Tracking of BP refers to the process by which individuals remain in the same BP percentile over time. It is well recognized that childhood BP tracks (273–277) and predicts adult BP (105, 137, 278). The tracking correlations are reported to increase with increasing age, being 0.1 between birth and age 2, 0.5 between ages 3 and 4, and 0.6 between age 9 and 10 (106, 279) reaching a maximum of 0.7 around late adolescence/early 20s and remaining constant thereafter (280). The Bogalusa Heart Study showed that 40% of individuals with SBP and 37% of individuals with DBP levels above the 80th percentile at baseline had levels above this percentile 15 years later(273). Tracking is apparent in children with higher BP, family history, obesity, lower socioeconomic status, and increased left ventricular mass (152, 243, 273, 281–283).

End-Organ Damage

High BP in children is associated with the development of atherosclerosis (50, 51, 284–286). Autopsy studies demonstrated a relationship between HTN and early development of atherosclerosis in adolescents and young adults (285, 287, 288). Nowadays, atherosclerosis can be detected by measuring carotid intima-media thickness and large artery compliance in young adults (50, 51, 289). Other markers of target organ damage include left ventricular mass, retina, urinary albumin excretion, and renal function in patients with kidney disease.

ABPM is considered superior to office BP as a predictor of hypertensive end-organ damage (290). Similar to observations in adults, studies of pediatric subjects found greater correlation of end-organ damage, left ventricular hypertrophy and left ventricular mass index, with ABPM parameters such as 24-h mean SBP (291), mean nighttime SBP (292, 293), and daytime ABP (294) than with casual BP values. Several studies in adults had associated a generally worse prognosis with "non-dipping," which is also shown in children (295).

There appears to be ethnicity difference in target organ damage. African Americans are more likely to develop target organ damage, particularly renal disease, in adulthood (296). In normotensive adolescents, microalbuminuria has been shown to be more common in African Americans compared with whites (297). In a study in African Americans and whites aged 7–30 years, it was reported that "nondipping", a marker strongly associated with higher rates of target organ damage, begins by age 10 years and that this blunted decline is exacerbated with age during the years of adolescence (298). In hypertensive children, LVH may be more prevalent in Hispanic children than in other ethnic groups (299).

Left Ventricular Hypertrophy

In adults, increased left ventricular mass is a known risk factor for CVD (300).

Among children and adolescents with borderline or established essential HTN, left ventricular hypertrophy (LVH) is present in approximately 34–47% of cases (301–305). The fourth report recommends performing echocardiography to assess LVH in children with HTN (2).

Increased left ventricular mass indexed to height to correct for body size (LVMI) seems to correlate with casual BP or systolic ABP (301, 304, 306, 307). A recent large study, however, did not demonstrate the relationship between LVH and severity of BP elevation at the initial visit of a child with confirmed HTN (308), underscoring the importance of including echocardiography in the initial examination. "Non-dipping" status and 24-h pulse pressure have been associated with higher LVMI in adolescents with essential HTN (301, 307).

Similar to adults, children with MH have increased LVMI when compared with normotensive children (88, 95). Stabouli and colleagues demonstrated that children with WCH also tended to have higher LVMI than normotensive children (88).

Carotid Artery Intima-Media Thickness

Measurement of the carotid artery's intima-media thickness (cIMT) has gained acceptance as a way to evaluate the degree of atherosclerosis in adults (309). Elevated BP is associated with increased cIMT in children and young adults also (50, 51, 289, 304, 305). On the other hands, several studies failed to show a correlation between office SBP or DBP and cIMT after adjusting for BMI (120, 305, 310). A strong correlation, however, was found between cIMT and several ABPM parameters such as daytime systolic BP load and daytime systolic BP index (310).

Only one study has assessed cIMT in children with MH (88). The study demonstrated that masked hypertensive children tended to have greater cIMT than normotensives, but lower than hypertensive subjects.

Retina

Retinal arteriolar narrowing is a recognized consequence of chronic HTN in adults and independently predicts cardiovascular mortality (311). Retinal abnormalities have been reported in infants with HTN (312) and in 50% of hypertensive children and adolescents (313). Recently, Mitchell et al. examined the relationship of retinal vascular caliber and BP levels in two population-based cohorts among children aged 6–8 years in Australia and Singapore (314). They demonstrated that higher BP is associated with retinal arteriolar narrowing in healthy children and that the effect was continuous across the range of BP. This study provides further evidence that the risk of cardiovascular disease may have its origins early in life.

Microalbuminuria

While urinary albumin excretion is a powerful marker to identify adults with a risk for CVD, data in children with HTN are limited. In children and adolescents with type 1 and type 2 diabetes, microalbuminuria was clearly linked to elevated 24-h DBP, nocturnal BP, loss of nocturnal dip, or increases in BP load (315–320). Assadi examined the relation of LVH to microalbuminuria in hypertensive subjects and documented that urinary albumin excretion was increased in children and adolescents with HTN in correlation to LVH (321). Nguyen et al. recently examined the association between cardiovascular risk factors and microalbuminuria in a nationally representative US sample and demonstrated that microalbuminuria was associated with HTN among overweight adolescents (322).

Renal Function

Renal damage other than microalbuminuria has rarely been reported in children with HTN except for those with accelerated HTN or those with renal diseases. Harshfield et al. examined the relationship between ABP patterns and renal function in healthy, normotensive African American and white youths (323). The relationship between creatinine clearance and BP was not significant for casual BP or daytime BP for either group, or with night-time BP in the Anglo-American subjects. In contrast, creatinine clearance was related negatively to both night-time SBP and night-time DBP in the African American subjects. Mitsnefes et al. examined the role of HTN in the progression of chronic renal failure in children by using the North American Pediatric Renal Transplant Cooperative Study (NAPRTCS) chronic renal insufficiency database that consists of 3,834 patients aged 2–17 years with an estimated glomerular filtration rate (GFR) less than 75 ml/min/1.73 m^2 (324). Systolic HTN proved to be a significant independent predictor of the progression of chronic renal insufficiency in children. In a study of 61 children with chronic pyelonephritis or vesicoureteral reflux, the extent of scarring was strongly correlated

with ABP values (325). Lama et al. also showed that 24-h SBP and DBP correlated with the decline of GFR (326), during a 5-year follow up of 100 normotensive children with reflux nephropathy.

ABPM provided valuable information about pediatric renal transplant recipients (327, 328). ABPM had stronger correlations than office BP with GFR in children with chronic renal failure (329). In pediatric post-renal transplant patients, first year SBP and DBP correlated positively with values at 56 months, and GFR at 1-year and at the end of study was significantly lower in patients with high BP. Moreover, patients who maintained a normal SBP throughout the study had a significantly higher final GFR than those who were hypertensive both times (330).

In the adult renal transplant population, HTN has been linked with accelerated graft failure (331, 332). The same associations have been observed among pediatric recipients. Sorof et al. reviewed NAPRTCS data and showed higher graft failure associated with the use of antihypertensive medications (333). SBP has been found to be an independent predictor of renal function at 1-year post-transplant (334) while increases in both DBP and SBP have been linked to worsening graft survival (291). Two different studies, however, failed to reveal any relationship between graft dysfunction and ABPM measures (327, 335, 336).

Conclusion

During the last decade there have been increasing interest and progress in the field of HTN in children and adolescents. Recent accomplishments include the development of normative data, updated clinical practice guidelines, knowledge of the prevalence of HTN in association with obesity, widespread use of ABPM, and newly applied techniques to detect early target organ damage. Future studies should aim at defining HTN in relation to target organ damage. For this goal, it may be necessary to establish the role of ABPM and home BP measurement in predicting early target organ damage. The efficient way of detecting children with high BP should also be sought. At present, population-based screening of HTN in the pediatric age group is not recommended (337). While routine measurement of BP in the clinic is performed in the Unite States, it is not the case in many other countries. Even in the United States, a large number of children with HTN and prehypertension are undiagnosed (5). Finally, large population-based studies are needed to investigate whether various intervention methods prevent the development of HTN.

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61 Pathophysiology of Hypertension

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Introduction

Blood pressure can fundamentally be viewed as a function of cardiac output (CO) and systemic vascular resistance. Cardiac output depends on cardiac stroke volume and heart rate. Stroke volume in turn depends on myocontractility and preload. Systemic vascular resistance is dependent on vessel elasticity, myocontractility and afterload. Recent research has begun to unravel relationships at the cellular and molecular levels that exert important effects on blood pressure. The endothelium, intima, vascular smooth muscle and extracellular matrix interact with each other and with vasomotor, growth factors, transcriptional, hormonal and neuronal factors that affect signal transduction, gene transcription, ion transport, tissue remodeling and cellular response, all in the context of the patient's genetic background.

As will be discussed, derangements in any of these myriad processes can influence either cardiac output or systemic vascular resistance, thereby leading to the development of hypertension.

Mechanisms of Blood Pressure Regulation

Cardiac Output

Since the mean arterial blood pressure (MAP) is a function of cardiac output (CO) and systemic vascular resistance (SVR), hypertension is fundamentally a hemodynamic disorder indicating a disturbance in CO and/or SVR. CO is a function of stroke volume (SV) and heart rate (HR). The preload of cardiac myocytes determines SV, and HR is regulated by the sympathetic nervous system (SNS), as well as by the cardiac autonomic pacemaker. The SNS receives homeostatic feedback from baroreceptor/chemoreceptor reflexes and regulates the distribution of cardiac output over short-term. Increased SNS activity is most likely responsible for the mild tachycardia seen in many patients with primary hypertension (1).

Cardiac Index (CI) is a function of CO and body surface area (BSA). Young patients with mild hypertension have significantly higher CO, CI, and sympathetic nerve burst frequency than age-matched controls (1). Increased muscle sympathetic nerve activity also appears to be involved in development of complications of primary hypertension such as left ventricular hypertrophy (2). In borderline HTN, CI and HR are higher, and calculated SVR is normal (3). However, in nearly all forms of established HTN, SVR is increased and CO is reduced. Moreover, in progressive hypertension, a further increase in SVR occurs and CO continues to fall, indicating reduced vascular compliance (4).

However, it is clear that not all hypertensive patients start out with a state of increased cardiac output. In some patients, hypertension may develop because of an increased SVR. In addition, some patients may develop hypertension because of primary renal disease or a genetic defect that increases circulating blood volume through sodium retention.

Systemic Vascular Resistance/Vascular Wall

When the cross-sectional area of a vessel decreases, resistance to flow increases. This makes systemic vascular resistance mainly a function of small, peripheral arterioles, whose medial layer characteristically increases in response to sustained hypertension. The earliest structural change observed in the arterioles of experimental animals after the onset of hypertension is thickening of the media due to matrix deposition, smooth muscle cell hypertrophy (increase in cell size without division), and hyperplasia (increase in cell number) (5). The medial smooth muscle cells then rearrange themselves to create a smaller lumen without change in cell number or size, a process that has been termed remodeling. Later, there may be development of a neointima with appearance of smooth muscle cells inside the internal elastic lamina. There are also local areas of endothelial cell denudation and inflammatory cell infiltration, which can progress to atherosclerotic plaque formation (6). Finally, there may be resorption and loss of blood vessels in the periphery, a process termed "rarefaction," which has been described in both the early and later stages of hypertension (7).

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In larger vessels, the content of elastin and collagen in the media increases and the number of smooth muscle cells decreases (via medial atrophy, necrosis, or apoptosis) (8), leading to a loss of elasticity and the development of increased vessel "stiffness." Significant increases in the size and number of vasa vasorum (tiny vessels in the adventitia that supply nutrients and oxygen to the deeper layers of the media) can be observed in larger, conduit vessels, which is probably an adaptive process in the sense that the increased smooth muscle cell mass in the hypertensive vessel wall requires more oxygen and nutrients. In summary, the hypertensive vessel wall is generally characterized by increased medial thickness or an increased media/lumen ratio of the resistance arterioles. This structural change is biomechanically adaptive because wall stress is normalized as defined by Laplace's law, but functionally, this means that there is greater resistance for a given contractile stimulus (9).

Altered vascular structure in hypertension is also accompanied by functional changes in the cellular components of the vessel wall, specifically decreased relaxation and increased contraction (9). Decreased relaxation has been attributed primarily to endothelial dysfunction, and increased contraction has been attributed to enhanced smooth muscle cell vasoreactivity. Sensitivity to vasoconstrictors may also be increased (7). Decreased relaxation is an effect of impaired endothelial production of vasodilatory substances (mainly nitric oxide and prostacyclin) or increased production of vasoconstricting substances (endothelin, PDGF), or both.

Increased smooth muscle cell responsiveness is caused by alterations in the ability of vasodilating substances to exert their effects, changes in the ability of smooth muscle cells to respond to vasodilators, increased responsiveness to vasoconstrictors because of increased numbers of receptors or an augmented contractile machinery, or all of the above. Moreover, vasoconstrictors frequently stimulate smooth muscle cell growth and, conversely, many growth factors have vasoconstrictor activity (i.e., PDGF is a vasoconstrictor (10) and angiotensin II is a potent smooth muscle cell growth factor (11). These functional alterations act in concert with the structural changes discussed above to perpetuate hypertension (12), emphasizing the importance of the vasculature in the pathogenesis of hypertension.

Sympathetic Innervation/CNS

Renal vessels, tubules, and the juxtaglomerular apparatus are innervated by the renal sympathetic nerves (13). Renal sympathetic nerve activity (RSNA) influences renal hemodynamics, solute and water handling, and hormonal release. Increased RSNA is found in animal models of hypertension and also in hypertensive humans (14). Sympathetic nervous system activation, as confirmed by increased circulating noradrenaline, muscle sympathetic nerve traffic and systemic noradrenaline spillover, is almost universally present in primary hypertension (15, 16), and has been recently demonstrated in patients with renovascular hypertension (17). It appears to be particularly pronounced in younger patients (16). Renal denervation prevents or alleviates hypertension in virtually all animal models of hypertension.

Increased RSNA constricts the renal vasculature, and decreases GFR and renal blood flow (18). The hypertensive response to chronic renal adrenergic stimulation is associated with a sustained increase in plasma renin activity, and is dependent on an increase in plasma angiotensin II (ANGII) concentration (19). Sympathetic nerve activation appears to enhance the response to circulating angiotensin II (20). The renal effects of ANG II on proximal tubular chloride and water reabsorption are decreased by 75% in animals after experimental renal denervation. Thus only about 25% of ANGII effect is mediated directly via type-1 angiotensin receptors, with the majority of the effect being dependent on intact renal innervation. In experimental renal sympathetic nerve stimulation, ANG II enhanced renal venous outflow of norepinephrine, an effect that was blocked by an ANG II receptor antagonist (21). This clearly demonstrates the connection between RSNA and ANG II, and suggests that ANG II exerts a peripheral, pre-synaptic action on renal sympathetic nerve terminals in renal tubular epithelial cells and vessels to enhance the release of norepinephrine (22).

Circadian Rhythm

The circadian rhythm is an intrinsic 24-h cycle that affects numerous physiologic processes. Vascular tone, systemic vascular resistance (SVR), heart rate, and blood pressure (BP) increase in the early morning hours in both normotensive and hypertensive subjects. This rise corresponds to increased plasma renin activity and a surge in secretion of catecholamines. BP reaches its peak at approximately 9 A.M., and falls to its lowest at 3 A.M. A significant rise in BP occurs prior to awakening. Diurnal variation of BP has been clearly demonstrated in normotensive children (23); in hypertensive children, data suggest that diurnal variation is preserved in children with primary hypertension but is abnormal in those with secondary forms of

hypertension (24). Animals with disordered circadian variation due to point mutation in the circadian regulatory gene develop cardiomyopathy and renal disease (25) and human patients with blunted blood pressure dipping have increased rates of cardiovascular events such as stroke and myocardial infarction (26).

The circadian pacemaker in mammals is located in the suprachiasmatic nucleus (SCN) of the brain, which is itself located in the anterior hypothalamus, immediately above the optic chiasm (27). It receives input about light/dark cycles from the retina via the retino-hypothalamic tract, and produces several neuropeptides, including vasopressin and vasoactive intestinal peptide (VIP), that appear to have diurnal peaks of expression and release (28). Animal experiments have demonstrated that the SNC is linked to multiple sympathetic pathways that affect many components of the cardiovascular system (29). It plays a crucial role in synchronizing the peripheral oscillators that are found in all tissues, including VSMCs and the aorta, and in the execution of rhythmic behavior. The primary mediator of SCN action appears to be melatonin, which is secreted by the pineal gland under control of the SCN. Melatonin appears to transmit information about light/dark cycles from the SCN to the peripheral oscillators as well as to other centers in the hypothalamus that regulate cardiovascular activity (29, 30).

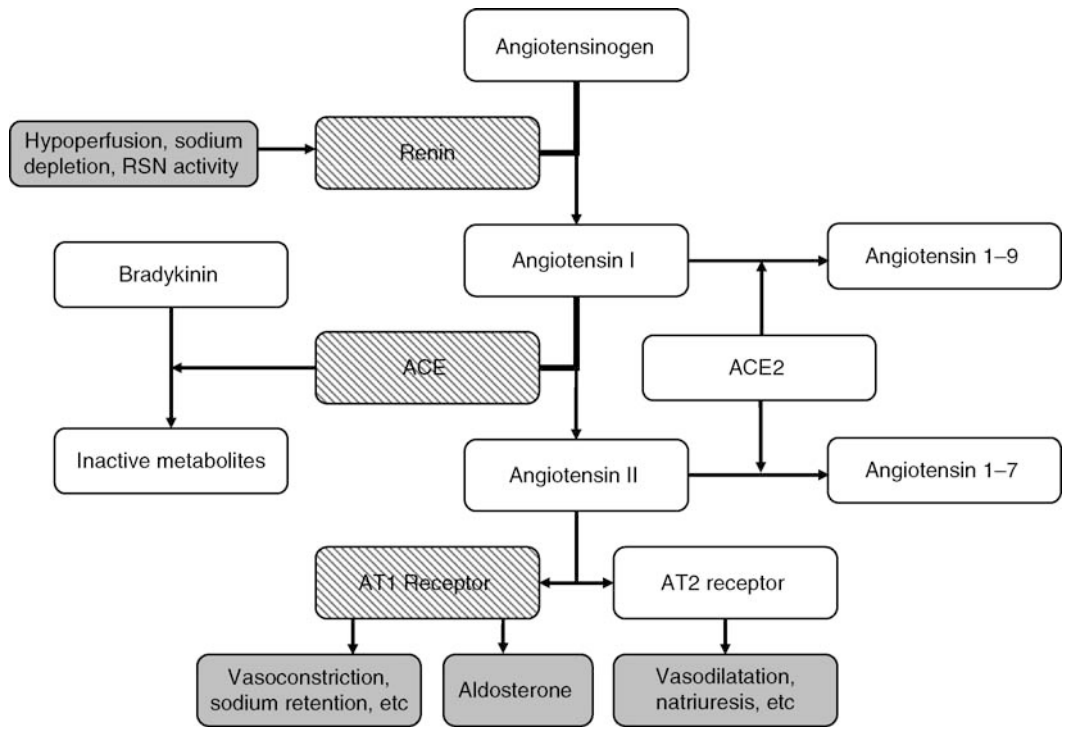
Supporting evidence for the role of the SNC and melatonin in blood pressure regulation comes from studies demonstrating that melatonin levels are decreased in hypertensive individuals, and that administration of melatonin lowers blood pressure (29). Autopsy studies of patients with primary hypertension have demonstrated that SCN neuronal populations responsible for producing vasopressin and VIP are reduced, suggesting that in at least some individuals with hypertension, normal function of the intrinsic biologic clock is impaired (31). Melatonin appears to modulate activity of the sympathetic nervous system, has antioxidant activity and may prevent endothelial dysfunction (32).

Renin–Angiotensin System

The renin–angiotensin system (RAS) (*Fig. 61-1*) is the major hormonal system affecting blood pressure. Renin, the first active molecule in the RAS, is produced in the

Figure 61-1

Renin–angiotensin system. Current targets of antihypertensive medications are indicated in *red*. *ACE* angiotensin converting enzyme; *ACE2* angiotensin converting enzyme-2; *AT1* angiotensin II type 1; *AT2* angiotensin II type 2; *RSN* renal sympathetic nerve.



juxtaglomerular cells of the afferent renal arteriole in a series of steps: following transcription of preprorenin in the cell nucleus, prorenin is further processed in the endoplasmic reticulum then packaged into secretory granules by the Golgi apparatus, where a portion appears to be converted into active renin (33, 34). Extra-renal sites of prorenin to renin conversion undoubtedly exist, as both prorenin and renin are released when the juxtaglomerular cells are stimulated. Until recently, it was thought that prorenin had no intrinsic activity. However, that view is now changing with discovery of the prorenin receptor, described below.

Major stimuli for secretion of renin from the juxtaglomerular apparatus include glomerular underperfusion or reduced sodium intake, and activity of the sympathetic nervous system. Except for renin, all the components of a local vascular wall renin–angiotensin system appear to be present in normal vessels (35), and their activity is dynamically regulated. In the classical systemic RAS, circulating renal-derived renin cleaves hepatic-derived angiotensinogen to form the decapeptide angiotensin I (ANG I), which is converted by angiotensin-converting enzyme (ACE) in the lungs to the major active component of the RAS, angiotensin II (ANG II). ACE also degrades bradykinin, a potent vasodilator, into inactive metabolites. ACE exists in plasma, in the interstitium, and intracellularly. Tissue ACE is present in all major organs, including the heart, brain, blood vessels, adrenals, kidney, liver, and reproductive organs (36), and is already functional in utero (37).

A homologue of the angiotensin converting enzyme, termed ACE2, has been recently discovered (38). ACE2 hydrolyzes ANG I to angiotensin 1–9 and ANG II to angiotensin 1–7 (*Fig. 61-1*). The functions of angiotensin 1–9 are not completely understood but angiotensin 1–7 has vasodilatory effects (39). ACE2 was initially found in heart, kidney, and testis, as well as the lungs, colon, small intestine, and ovary (40). Reduced ACE2 levels could contribute to the pathogenesis of primary hypertension via impaired degradation of ANG II, and reduced formation of vasodilator by-products at the level of the renal endothelium (41). Alterations in ACE2 activity have also been shown to have a potential role in the development of diabetic nephropathy in animal models (40).

ANG II is a potent vasoconstrictor and thus increases blood pressure. It also stimulates the release of aldosterone from the zona glomerulosa of the adrenal gland, which results in a further rise in blood pressure from aldosterone-mediated sodium and water retention. In addition, it plays an important physiologic role in the regulation of sympathetic nervous activity and the thirst response. The varied effects of ANGII in hypertension provide the physiologic foundation for the use of angiotensin converting enzyme inhibitors and angiotensin II receptor antagonists in the treatment of patients with hypertension.

ANG II effects are primarily mediated through the plasma membrane receptors AT1 and AT2 (*Fig. 61-1*). The AT1 receptor is composed of 359 amino acids and belongs to the seven-membrane-spanning G protein-coupled receptor family (42, 43). In rodents, the AT1 receptor has two functionally distinct subtypes, AT1A and AT1B, with >95% amino acid sequence homology (44). The human AT1 receptor gene has been mapped to chromosome 3 (45). Expression of the AT1 receptor is a subject to "negative feedback" by ANG II (46). AT1 receptor activation stimulates vasoconstriction, vascular cell hypertrophy and hyperplasia, sodium retention, reactive oxygen species generation via NADPH induction (47) and induction of inflammatory (48), thrombotic (49), and fibrotic processes (50). Some of the pathophysiologic effects of ANG II may be mediated through activation of the transcription factor nuclear factor-B (NF-B) (51), which participates in a variety of inflammatory responses (52).

Independently of its effect on blood pressure, ANG II via its interaction with the AT1 receptor contributes to left ventricular remodeling, alterations in the morphology and mechanical properties of the vasculature, and the development of endothelial dysfunction (53, 54). Many polymorphisms of the AT1 receptor gene have been identified, but the A1166 → C polymorphism has been the most extensively studied. It has been associated with the development of primary hypertension in various populations (55).

The AT2 receptor is a seven-transmembrane-type, G protein-coupled receptor comprising 363 amino acids. It has low amino acid sequence homology (~34%) with AT1A or AT1B receptors (42, 43). The expression of the AT2 receptor is upregulated by sodium depletion (56) and is inhibited by ANG II and growth factors such as PDGF and EGF (57). Under physiologic conditions, the AT2 receptor mainly antagonizes AT1-mediated actions (58) by inhibiting cell growth and by inducing apoptosis and vasodilatation (59, 60). Cardiovascular effects of the AT2 receptor appear to be opposite to those of the AT1 receptor (61). In the kidney, stimulation of the AT2 receptor promotes natriuresis through interactions with the renal dopaminergic system (62). It also has proinflammatory effects, via induction of NF-B (51), and trophic effects, leading to vascular (63) and cardiac (64) hypertrophy. The functions of the AT1 and AT2 receptors are summarized and compared in *Table 61-1*.

Table 61-1
Comparison of the effects of AT1 and AT2 receptors

AT1	AT2
Vasoconstriction	Vasodilatation
Tubular sodium reabsorption	Natriuresis
Aldoseterone, endothelin and vasopressin secretion	Nitric oxide production
Cellular dedifferentiation, proliferation and hypertrophy	Cellular differentiation
Sympathetic nervous system activation	Inhibition of cellular proliferation and matrix formation
Thrombosis, inflammation and fibrosis	Apoptosis
Superoxide production	Collagen synthesis
Lipid peroxidation	Suppression of renin secretion
Adhesion molecule expression Vascular matrix expansion	Anti-angiogenesis

ANG II also acts centrally on brainstem nuclei that are important in the control of peripheral sympathetic vasomotor tone, e.g., the rostral ventrolateral medulla of the hypothalamus (RVLM) (65). Since circulating ANG II is not able to cross the blood–brain barrier, it acts on circumventricular organs in which the normal blood–brain barrier is lacking. Those consist of the subfornical organ (SFO), the organum vasculosum of the lamina terminalis (OVLT), the median eminence, and the area postrema (AP) (66). Activation of the AP by circulating ANG II may in turn increase peripheral sympathetic nerve activity via a direct excitatory connection from the AP to the RVLM. Experimental microinjection of ANG II into the RVLM results in increases in arterial pressure (67) and peripheral sympathetic nerve activity (68), and facilitates arterial baroreflex modulation of RSNA (69). All those effects are blocked by AT1, but not by AT2 receptor antagonists (70). Moreover, the RVLM contains ANG II immunoreactive nerve terminals, AT1 receptor mRNA and AT1 receptor binding sites (71). This suggests that angiotensin peptides of brain origin may have a local paracrine or autocrine action on the RVLM that regulates RSNA and its arterial baroreflex control.

As mentioned earlier, both prorenin and renin are secreted by the juxtaglomerular cells. Prorenin has no intrinsic enzymatic activity due to the presence of a 43-amino acid N-terminal propeptide that covers the enzymatic cleft, obstructing access of angiotensinogen to the active site of renin (34). However, prorenin may have a role in the development of hypertension through its interactions with the prorenin receptor, which is found on the surface of vascular smooth muscle cells and the distal tubule, among other locations (72, 73). The prorenin receptor activates prorenin, resulting in generation of angiotensin I from angiotensinogen (34). Rats overexpressing the prorenin receptor develop hypertension, glomerular sclerosis and proteinuria and have elevated aldosterone concentrations (72). The prorenin receptor may explain some of the local/vascular effects of the RAS, and its existence and actions have been put forward as an explanation for the "renin escape" phenomenon seen in patients treated with agents affecting the RAS. Further research will be needed to fully define the role of prorenin and the prorenin receptor in the pathogenesis of hypertension.

Aldosterone

Aldosterone is produced from deoxycorticosterone by the mitochondrial enzyme CYP11B2 (aldosterone synthase) encoded by the CYP11B2 gene (74). As discussed later, mutations in the CYP11B2 gene lead to glucocorticoid-remediable aldosteronism, a genetic form of hypertension. Aldosterone synthase is expressed in the zona glomerulosa of the adrenal glands, aorta, endothelial cells and vascular smooth muscle cells (VSMCs), hypothalamus, hippocampus, amygdala, cerebrum, and cerebellum (75, 76). Angiotensin II and III (ANG II, ANG III) induce aldosterone synthesis and potassium, endothelin, adrenocorticotropic hormone (ACTH) and vasopressin stimulate its secretion (77). Inhibitors of aldosterone secretion include atrial natriuretic peptide, somatostatin and dopamine. Dietary sodium restriction increases aldosterone secretion in order to restore plasma volume.

Aldosterone acts via intracellular, high-affinity type I mineralocorticoid receptors that are present in the kidney, brain, heart, colon and vessel walls (78). Aldosterone induces retention of sodium and potassium excretion. It also activates the sympathetic nervous system by preventing the uptake of norepinephrine by the myocardium and causes baroreceptor dysfunction (79, 80) and eventually myocardial and vascular fibrosis (81). The importance of aldosterone in hypertension and cardiovascular disease is highlighted by recent studies demonstrating that the aldosterone receptor antagonists spironolactone and eplerenone improve endothelial dysfunction and resistance artery stiffness, increase nitric oxide bioactivity in chronic heart and renal failure, reduce cardiac fibrosis

and prevent the development of nephrosclerosis and cerebrovascular lesions (82–85).

Dopamine

Dopamine is an important modulator of systemic blood pressure with direct actions on the heart, arteries and veins. Dopamine is synthesized in noradrenergic and dopaminergic nerves, and also in non-neural tissues (e.g., kidney, gastrointestinal tract) (86). Five dopamine receptor sub-types have been identified, which can be divided in to D1-like and D2-like (87). Dopamine modulates renal epithelial transport, and gastrointestinal sodium uptake (88). It can indirectly modulate blood pressure via release of hormones and humoral agents such as aldosterone, catecholamines, endothelin, prolactin, pro-opiomelanocortin, renin, and vasopressin (89). Dopamine modulates fluid and sodium intake via "appetite" centers in the brain (90), and in addition, controls blood pressure by direct action on neuronal cardiovascular centers.

Dopamine increases cardiac output by stimulating myocardial b-adrenergic receptors, and plays an important role in modulating vascular smooth muscle tone. Stimulation of D1 and D5 dopamine receptors, which are expressed in vascular smooth muscle cells, can cause vasorelaxation, whereas stimulation of D3 receptors produces either vasodilatation or vasoconstriction, depending on the degree of renal nerve activity (87). These properties of the dopamine receptors are illustrated by the drug fenoldopam, an intravenously administered antihypertensive agent, which lowers blood pressure by stimulation of peripheral D1 receptors (91). At extremely high concentrations, dopamine produces vasoconstriction by occupation of a1-adrenergic receptors (92). Dopaminergic blockade is associated with the development of hypertension in rats and potentiates the renal effects of nitric oxide inhibition in humans (93).

Moreover, dopamine also regulates the expression of other receptors involved in BP regulation (e.g., endothelin-B, AT1). Both D1- and D2-like receptors downregulate AT1 receptor expression and function (94). Disruption of the D3 receptor gene in mice is associated with renin-dependent hypertension and a decreased ability to excrete an acute saline load (95). Aberrant dopaminergic regulation of aldosterone secretion (via D3 receptors) may be involved in some forms of hyperaldosteronism and hypertension (96, 97).

Interindividual variation in systolic blood pressure has been shown in one study to be influenced by the activity of the gene that encodes the type 1A dopamine receptor (98). A polymorphism in the noncoding region of the D1 receptor has been reported to be associated with human essential hypertension (99). Several D2 receptor polymorphisms have been reported, one of which is associated with hypertension (100). It is notable that all of the dopamine receptor subtypes identified to date have some effect on renal sodium handling (87). Recent studies have demonstrated that genetic variation in the dopamine D1 receptor promoter are correlated with renal sodium handling and blood pressure (101). These data support the concept that dopamine may result in human hypertension through altered renal sodium handling.

Atrial Natriuretic Peptide

Atrial natriuretic peptide (ANP) belongs to a family of natriuretic peptides that also includes the B-type (BNP) and the C-type (CNP) natriuretic peptides (102). The biological functions of BNP and CNP do not differ from those of ANP. ANP is involved in regulation of salt and water balance, blood pressure homeostasis, and has an antiproliferative effect at the cellular level in the cardiovascular system (103–105). ANP is primarily expressed in cardiac myocytes, but is also found in brain, vessels and kidneys. ANP has potent diuretic, natriuretic and vasorelaxant effects. In the kidney, ANP increases glomerular filtration rate (GFR) through vasodilatation of the afferent arteriole and vasoconstriction of the efferent arteriole (106), and promotes natriuresis by inhibiting tubular sodium reabsorption (107, 108) and renin and aldosterone biosynthesis (109, 110).

The combined systemic hemodynamic effects of ANP result in reduction of both preload and afterload (111, 112). Administration of endogenous ANP results in a significant decrease of blood pressure in man and in animal models of hypertension by reducing systemic vascular resistance (106, 113). At high plasma levels, ANP decreases heart rate, central venous pressure and stroke volume (114).

Mutations in the ANP gene appear to be associated with the development of hypertension in humans. A significant association has been demonstrated between an intronic variant of the ANP gene (HpaII) and salt-sensitive hypertension in African-Americans (115). A mutation located within the 5′ untranslated region of the ANP gene has been linked with the occurrence of both hypertension and left ventricular hypertrophy in a Japanese population (116). In mice, absence of corin, a transmembrane serine protease that converts pro-atrial natriuretic peptide (pro-ANP) to active ANP, results in salt-sensitive hypertension (117).

Endothelin-1

Endothelin-1 (ET-1) is an endothelial-derived, potent vasoconstrictive peptide containing 21 amino acids (118). Three isopeptides of endothelin (ET-1, ET-2, ET-3), encoded by separate genes, have been identified (119). Endothelial ET-1 synthesis is activated by vasoactive hormones, growth factors, hypoxia, shear stress, lipoproteins, free radicals, endotoxin and cyclosporine, and is inhibited by nitric oxide, natriuretic peptides, heparin and prostaglandins (120). Apart from endothelial cells, ET-1 is also produced by the heart, kidney, posterior pituitary, central nervous system, and vascular smooth muscle cells (120). ET-2 is produced in endothelial cells, heart and kidney (121), but it has not been detected in human plasma. ET-3 is selectively expressed in the endocrine, gastrointestinal and central nervous systems, but not in endothelial cells (122).

ET-1 primarily appears to be a locally acting paracrine substance. ET-1 closes membrane K^+ channels (123), which prevents efflux of K^+ from the cell, thereby favoring membrane depolarization, leading to smooth muscle cell contraction. In the kidney, ET-1 causes contraction of both afferent and efferent glomerular arterioles, thereby reducing both renal plasma flow and glomerular filtration rate (124, 125). It blocks reabsorption of sodium by inhibiting tubular Na^+/K^+-ATPase activity in the proximal tubule and collecting duct (126). Endothelin-1 inhibits release of renin from isolated rat glomeruli (127) but stimulates release of aldosterone from isolated cortical zona glomerulosa cells (128). ET-1 also stimulates production and release of atrial natriuretic peptide (ANP) by cultured atrial myocytes (129).

Endothelin signals through two receptor subtypes, ETA and ETB. The ETA receptor is preferentially activated by ET-1 but not by ET-3, whereas the ETB receptor is activated equally by ET-1 and ET-3 (130, 131). The overall cardiovascular effect of endogenous ET-1 depends on the balance between ETA- and ETB-mediated effects. Activation of vascular smooth muscle ETA receptors causes vasoconstriction and tends to elevate blood pressure, while activation of endothelial and renal ETB receptors promotes vasodilatation and natriuresis and tends to decrease blood pressure (132). ETB knockout mice have hypertension secondary to renal sodium retention and there is evidence of marked enhancement of vascular expression of ET-1 in salt-sensitive hypertensive rat models (133). ET-1 appears to potentiate sympathetically mediated vasoconstriction only in hypertensive and not normotensive subjects (134).

Hypertensive patients with normal renal function-have similar concentrations of immunoreactive E-1 as normotensive patients (135); however, plasma concentrations of ET-1 are raised in patients with accelerated hypertension, in which case they are positively correlated with creatinine clearance (136). Plasma concentrations of ET-1 in patients with chronic renal failure are 1- to 2-fold greater than normal, whereas values in those undergoing hemodialysis are 2- to 4-fold greater than normal (137). In renal transplant patients, production of ET-1 is stimulated by cyclosporine A (138), which also increases the number of renal ET-1 binding sites (139). Tacrolimus also appears to increase secretion of ET-1 in vitro (140). These findings suggest that endothelin may be involved in the pathogenesis of calcineurin-induced hypertension.

Genetic variation in ET-1 expression may be involved in the pathogenesis of essential hypertension. African-Americans with hypertension have much higher concentrations of immunoreactive endothelin than do Caucasians (141). There is also strong correlation between diastolic blood pressure and polymorphisms of the ET-1 precursor pre-proendothelin-1 between patients with essential hypertension and normotensive controls (142). Recently an association between the G/T genotype of the ET-1 gene was demonstrated in a population of obese adolescents, suggesting a possible role for polymorphisms in the ET-1 gene in the development of obesity hypertension in the young (143).

Nitric Oxide, Adenosine

Nitric oxide (NO) is an endothelium-derived gas, synthesized from the amino acid L-arginine by the endothelial isoform of nitric oxide synthase (NOS) (144). Nitric oxide is extremely labile, with a half-life of <4 s in biological solutions. It is rapidly oxidized to nitrite and then nitrate by oxygenated hemoglobin before being excreted by the kidneys (144). Synthesized nitric oxide diffuses across the endothelial cell membrane and enters vascular smooth muscle cells, activating guanylate cyclase, leading to production of the second messenger cyclic guanosine-3′,5-monophosphate (cGMP) (144). cGMP in turn mediates control of vascular tone and platelet function.

Nitric oxide is a vasodilator, and the balance between nitric oxide and various endothelium-derived vasoconstrictors and the sympathetic nervous system maintains physiologic blood vessel tone (145). In addition, nitric oxide suppresses platelet aggregation, leukocyte migration, and cellular adhesion to the endothelium. It attenuates vascular smooth muscle cell proliferation and migration, as well as inhibits activation and expression

of certain adhesion molecules, and has an influence on production of superoxide anion.

Endothelium-dependent relaxation is decreased in patients with essential hypertension (146) and appears to be related to defective L-arginine transport. Similar abnormalities have been found in normotensive subjects with a family history of hypertension (147). Treatment with inhibitors of nitric oxide synthesis induces a hypertensive response, while L-arginine treatment prevents the development of hypertension in animals prone to this disease (148), and also causes a rapid reduction in systolic and diastolic pressures when infused into healthy humans and patients with essential hypertension (149). Methylated L-arginine derivatives, including NG-NG-dimethylarginine (asymmetric dimethylarginine (ADMA)), an endogenous inhibitor of nitric oxide synthase, and symmetric dimethylarginine, its inactive isomer, are present in human plasma and urine. Elevated levels of ADMA and other markers of oxidative stress have been demonstrated in patients with primary hypertension and have been postulated to contribute to the endothelial dysfunction that accompanies hypertension (150).

Adenosine is produced from the hydrolysis of adenosine 5′-monophosphate (AMP) by 5′-nucleotidase (5′-ND) (151, 152). In the kidney, it regulates GFR, tubuloglomerular feedback (TGF), tubular reabsorption, and renin secretion. Adenosine acts via several cell surface receptors that have been designated A1, A2A, A2B, A3, A4, all of which differentially stimulate or inhibit the production of cyclic adenosine-3′,5′-monophosphate. Selective A1 antagonists lead to diuresis and natriuresis and uncouple glomerular filtration and tubular reabsorption (153). A2A and A2B receptors have been described on vascular smooth muscle cells and the endothelium, where they are associated with vasodilatation (153). A2A knockout mice have increased blood pressure and heart rate, and increased platelet aggregation (154).

Other Regulatory Hormones

Thyroid hormone plays an important role in the regulation of blood pressure. Administration of exogenous thyroid hormone results in a decrease in systemic vascular resistance, which in turn results in renin release, activation of the angiotensin–aldosterone axis, increase in renal sodium reabsorption, increase in intravascular volume, cardiac output and cardiac contractility. Thyroid hormone also stimulates erythropoietin secretion (155). Triiodothyronine (T3) acts directly on arterioles (VSMC) and promotes relaxation and vasodilatation by local heat production, and β2-receptor stimulation (156).

T3 enters the cell by a specific transporter and forms a complex with nuclear T3 receptors. This complex binds to thyroid hormone response elements of several genes and regulates their transcription. Genes upregulated by T3 include adrenergic receptors, Na^+/K^+-ATPase, and voltage-gated potassium channels, among others. Genes downregulated by T3 include those for the Na^+/Ca^{2+} exchanger, adenyl cyclase types V and VI, and the T3 nuclear receptor. In the short term, T3 modulates sodium, potassium, and calcium channels activities in the heart that leads into changes in intracellular calcium and potassium with subsequent increase in inotropy and chronotropy (157). Increased heart rate, widened pulse pressure, and increased cardiac output despite normal or low serum concentrations of catecholamines is a characteristic feature of patients with hyperthyroidism (158).

Adrenomedullin peptide (ADM), a 52-amino acid peptide, has been found in the adrenal medulla, cardiac atrium, lung and kidney. In the kidney, ADM is found in glomeruli, cortical distal tubules, and medullary collecting duct cells (159, 160). ADM is also secreted from endothelial cells and vascular smooth muscle cells (161). ADM leads to vasodilatation, natriuresis, diuresis, inhibition of aldosterone production, and enhancement of tumor cell growth (162, 163). Increased levels of ADM have been documented in patients with high-renin primary hypertension (164), and variations in the 19-repeat allele of the human ADM gene have been postulated to be involved in the pathogenesis of hypertension and diabetic nephropathy (165).

Renal Mechanisms

The kidneys have the ability to respond to changes in arterial pressure by altering the renal excretion of salt and water; increases in renal perfusion pressure lead to decreases in sodium reabsorption and increases in sodium excretion (pressure natriuresis) (166). The intrarenal mechanism of pressure natriuresis is related to medullary blood flow, renal interstitial hydrostatic pressure (RIHP), and renal autocoids (nitric oxide, prostaglandins, kinins, and angiotensin II). Increased renal perfusion pressure is associated with increases in RIHP, nitric oxide, prostaglandin E2, and kinins, and a decrease in angiotensin II (166–169). Increased blood flow to the renal medulla, which normally receives only 5–10% of the total renal blood flow, contributes to washout of the urea gradient. This results in an increase of renal interstitial fluid

pressure, which in turn reduces tubular reabsorption of sodium and water, leading to a natriuresis and diuresis, lowering blood pressure (170). Norepinephrine infused into the renal medulla results in a pressure naturesis and arterial blood pressure lowering (171). This depressor response is abolished by chemical medullectomy (172), suggesting the presence of an as-yet-to-be-characterized depressor hormone in the renal medulla (173).

Renal autoregulation, or the kidney's intrinsic ability to maintain constant renal blood flow and GFR despite changes in renal perfusion pressure, serves to protect nephron function. However, major variations in renal perfusion can still influence renal excretory functions, renin release and arterial blood pressure. Autoregulation occurs in interlobular arteries, side branches of arcuate arteries, and afferent and efferent arterioles. It depends on myogenic mechanisms present in those vessels, and is blocked by the smooth muscle relaxant papaverine (174), and by calcium channel blockade (175). Tubuloglomerular feedback (TGF) also plays an important but not essential role in autoregulation (176).

Renal autoregulation is reset in animal models of spontaneous hypertension (177). ANG II plays an important role in the resetting of the autoregulation limits (178). Higher pressures are needed to trigger the vasoconstrictive response in the afferent arteriole (179), but in the intermediate portion of the interlobular artery of the spontaneously hypertensive rat, a lower threshold pressure stimulates a greater myogenic response (180). In the Dahl salt-sensitive hypertensive rat, both the afferent arterioles and the interlobular arteries have reduced myogenic responsiveness to increases in perfusion pressure (181). Studies demonstrating that abnormalities in renal autoregulation are more pronounced in patients with severe hypertension than those with moderate hypertension (182) suggest that chronic hypertension can induce alterations in renal autoregulation. Thus, disturbed renal autoregulation may contribute to the perpetuation of blood pressure elevation in patients with established hypertension.

Finally, the kidney also controls salt and water balance through regulation of the number and function of critical renal sodium transporters and channels. For example, in an animal model of primary hypertension, the Dahl salt-sensitive hypertensive rat, AngII/AVP receptor dysfunction produces hypertension through increased tubular sodium and fluid reabsorption (183). Expression of many of these proteins is influenced by aldosterone and other hormones that affect blood pressure (184, 185). As discussed later in this chapter, all monogenic forms of human hypertension described thus far can be characterized by alterations in renal ion channels leading to either increased sodium reabsorption or decreased excretion.

Primary Hypertension

Most hypertension in adults has no identifiable underlying etiology; therefore, the term "essential hypertension" has been utilized for those individuals without secondary hypertension. However, since "essential" implies that the patient's level of blood pressure is necessary in some way to maintain normal function, this term is misleading, and should be abandoned in favor of "primary" hypertension. It is likely that as our unraveling of the human genome progresses and more genetic forms of hypertension are identified (see below), fewer and fewer patients will be diagnosed with primary hypertension; for now, however, primary hypertension remains an important entity, accounting for most human hypertension.

In children, primary hypertension has long been considered uncommon, accounting for less than 25% of hypertensive children in referral series published through the early 1990s (186). However, more recent series from referral centers have demonstrated that primary hypertension in children is likely more common than previously thought, now accounting for between 48 and 69% of children referred for evaluation of elevated blood pressure, at least in the United States (187, 188). Furthermore, survey data indicate that the prevalence of hypertension in children is increasing (189), with many children developing hypertension as a manifestation of obesity (see following discussion), which is also becoming increasingly prevalent in childhood (190).

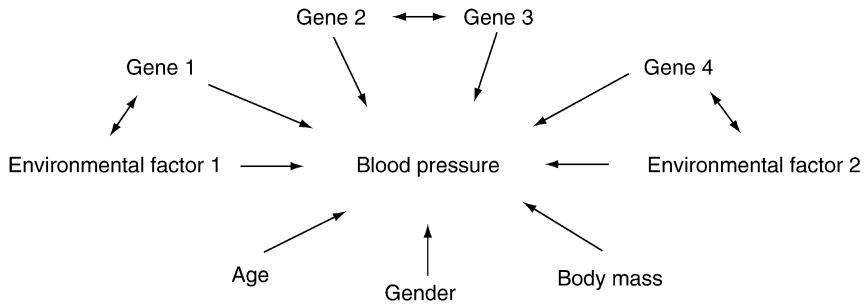
Primary hypertension is a multifactorial disorder in which elevated blood pressure is likely to be caused by different combinations of factors in different individuals (see *Fig. 61-2*). The following sections will highlight some of the more important mechanisms likely to contribute to the development of primary hypertension in children.

Genetic Influences

There is substantial evidence for genetic influence on blood pressure. Years ago, twin studies documented greater concordance of blood pressures among monozygotic than dizygotic twin (192), and population studies have demonstrated greater similarity of blood pressure within families than between families (193). Studies demonstrating a lack of correlation of blood pressure between parents

Figure 61-2

Multifactorial model of blood pressure determination, demonstrating the potential influence of genes, environmental factors, and demographic factors on blood pressure. The potential interaction of these determining factors is represented by *arrows* linking different determinants. Reproduced with permission from (191).



and their adoptive children would also support the contention that blood pressure is genetically determined (194). However, despite intensive efforts over nearly three decades, the genetic basis of hypertension has not been fully elucidated thus far.

Advances in molecular genetics have led many researchers to focus on determining how genetic variation may explain blood pressure elevation in some individuals (191). Many of these studies have focused on identifying candidate genes involved in the pathogenesis of hypertension. To date, however, with the exception of the single-gene forms of hypertension discussed elsewhere in this chapter, most of these studies have failed to demonstrate how the various genetic mutations identified lead to elevated blood pressure. For example, while the studies of Jeunemaitre (195) and Inoue (196) have identified a link between polymorphisms of the angiotensinogen gene and hypertension, and while other studies have shown that angiotensinogen levels are higher in individuals with higher levels of blood pressure (197) we still do not know how these variations cause hypertension (191). The same holds true for all of the many other allelic variants identified to date in individuals with primary hypertension.

Recent studies have demonstrated that in some people, blood pressure elevation may be the result not of a single mutation, but rather the product of multiple genetic variants interacting with each other (198). Similarly, it is probable that the role of some genetic variants in the pathogenesis of primary hypertension may not be to increase blood pressure in and of themselves, but rather to increase the susceptibility of an individual to environmental factors that can elevate blood pressure (199). This would be consistent with the scheme illustrated in *Fig. 61-2*.

Clearly, although progress has been made, delineation of the pathogenesis of primary hypertension from the genetic standpoint remains incomplete. Recent mapping of the human genome, the development of new strategies and technologies in molecular genetics, including differential gene expression, expressed sequence tags and DNA biochips provide hope that the challenge of elucidating the genetic basis of primary hypertension will be met (200). However, it should be noted that although genes are likely to be involved to some extent in nearly every hypertensive individual, the genetic contribution to an individual's hypertension may not be as significant as that of other factors, such as their body habitus or their environment.

Influence of Birth Weight and Perinatal Factors

Population studies conducted by Barker and others have demonstrated an inverse correlation between birth weight and adult blood pressure (201, 202). A relationship between birth weight and coronary heart disease and type 2 diabetes has also been noted (203). Proposed explanations for these findings include deficient maternal nutrition (201, 204), possibly leading to acquisition of a reduced number of nephrons (205). Autopsy studies demonstrating a reduced number of nephrons in patients with primary hypertension (206) have added intriguing evidence to the latter hypothesis. Other investigators have demonstrated that maternal smoking during pregnancy and bottle-feeding of newborns also may lead to hypertension later in life (207, 208), thus widening the spectrum of possible influences on later blood pressure from in utero factors to postnatal factors as well.

Animal studies have generally supported the epidemiologic evidence cited above. Pregnant rats fed low protein diets bear young that are small at birth and later develop

hypertension (209). Maternal diets low in protein result in decreased nephron number as well as suppression of the renin–angiotensin system (210), leading to elevated blood pressure and reduced GFR. In addition, various pharmacologic manipulations of the fetal milieu also lead to changes in fetal renal development that promote the appearance of hypertension after birth (211), lending further credence to the theory that perinatal events have an important influence on later blood pressure.

In contradiction to the studies demonstrating an inverse relationship between birth weight and adult blood pressure, other epidemiologic studies have found that adult blood pressure is more closely related to gestational age (212) or to early childhood growth (213) than to birth weight. In addition, recent studies have shown that twins have lower systolic blood pressure than singletons despite their lower birth weights (214). These challenges to the fetal origins hypothesis indicate that further research will be needed to determine the effects of pre-and perinatal factors on future blood pressure, and also support the concept illustrated in *Fig. 61-2* that the development of primary hypertension depends on multiple factors that will vary among different individuals.

Sympathetic Nervous System Activation

The role of the sympathetic nervous system (SNS) in blood pressure regulation has been discussed in detail above. Many lines of evidence suggest that SNS activation/overactivity is the primary mechanism by which blood pressure elevation is initiated in young individuals with otherwise unexplained primary hypertension. Children with primary hypertension have been shown to have resting tachycardia compared to normotensive children (215), and resting heart rate has been found to correlate with blood pressure in large epidemiologic studies such as the Bogalusa Heart Study (216). And while this characteristic has recently been "rediscovered" in obese adolescents with primary hypertension (217), there is a large, established body of literature that links tachycardia not only with elevated blood pressure but also with increased cardiovascular risk in general (218).

Another notable manifestation of SNS activation in hypertensive children is heightened cardiovascular reactivity to stress. Early studies by Falkner demonstrated that hypertensive adolescents had significantly greater increases in heart rate, systolic BP and diastolic BP during mental stress (performance of difficult arithmetic problems) than normotensive adolescents (215). Increased cardiovascular reactivity to the cold pressor test has also been shown to predict the subsequent development of hypertension (219). An interaction between genetic factors and SNS activation is suggested by differences in the cardiovascular response to stress in children with different racial backgrounds or family histories of hypertension (220).

SNS activation in primary hypertension suggested by clinical and epidemiologic data has been confirmed experimentally. Both children and young adults with primary hypertension have elevated plasma norepinephrine levels compared to normotensives (15, 16). Elevated catecholamines are more commonly found in younger individuals with primary hypertension than older individuals (16), and in young individuals with borderline hypertension who later go on to develop more significant blood pressure elevation (221). Abnormalities in the SNS have been demonstrated in normotensive children of hypertensive parents (222), suggesting that SNS activation is not only present in prehypertensives, but may also be genetically determined.

Direct recordings of nerve activity provides additional laboratory evidence for SNS activation in primary hypertension. Increased SNS activity has been found in recordings from both peripheral and renal sympathetic nerves (223) in hypertensive individuals. Differences in patterns of sympathetic activation have been found in obese and non-obese hypertensives, with higher cardiac sympathetic activity in lean hypertensives (224). There is also evidence to suggest that SNS overactivity may be involved in the development of the vascular hypertrophy and other systemic cardiovascular effects of established hypertension (225, 226). In sum, there is strong evidence that abnormalities of SNS activity are involved in the pathogenesis of primary hypertension, especially in the early phases of blood pressure elevation, which is clearly significant as far as pediatric hypertension is concerned.

The SNS is likely also involved in the pathogenesis of white coat hypertension (WCH), in which an individual's office blood pressure readings are elevated but whose out-of-office readings are normal (227). WCH is most likely a manifestation of the "flight or fight" response; individuals with WCH are typically anxious and demonstrate a mild resting daytime tachycardia (228). There appears to be an imbalance between parasympathetic and sympathetic tone in WCH similar to that seen in established primary hypertension (229), and increased muscle sympathetic nerve activity has been demonstrated in subjects with WCH. While further investigation of the role of SNS activation in the development of WCH is needed, it is a plausible underlying mechanism.

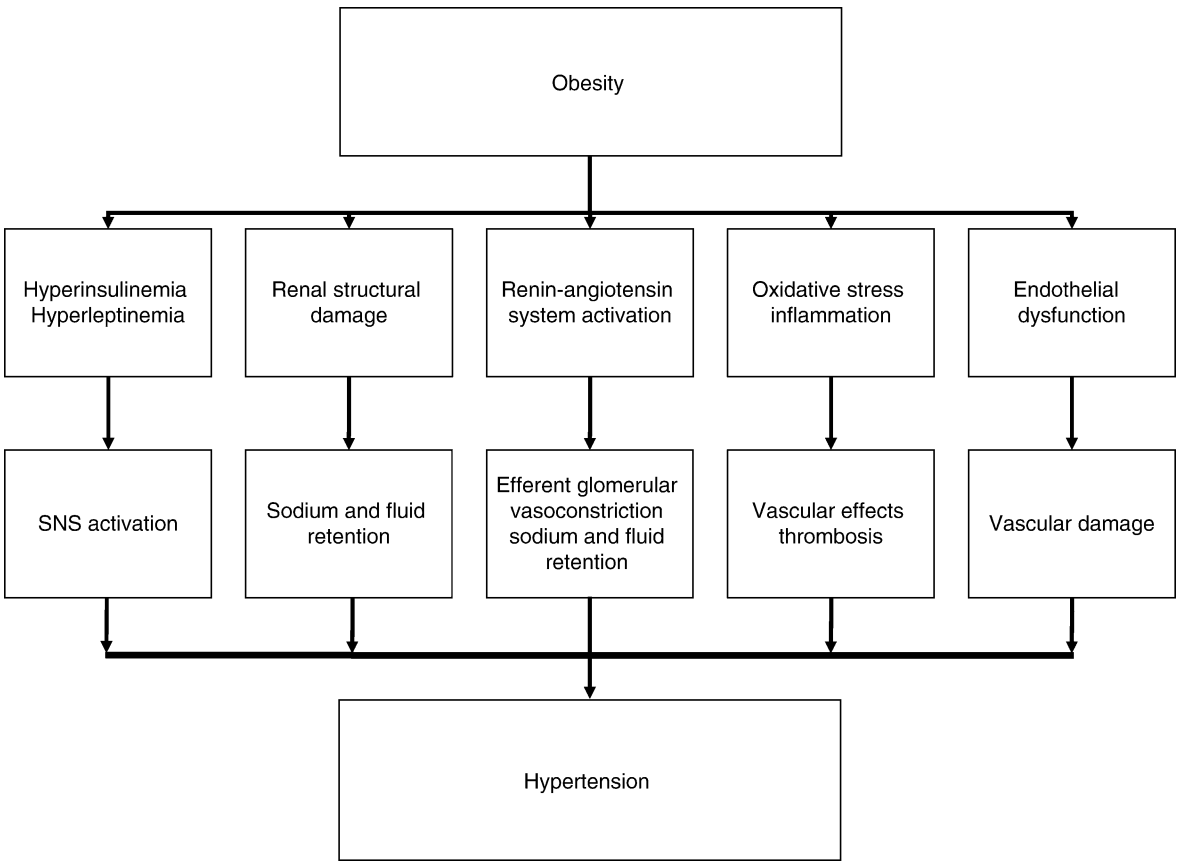
Mechanisms of Obesity Hypertension

Given the epidemic of childhood obesity (190), understanding the mechanisms of obesity hypertension is crucial for understanding the pathogenesis of hypertension in the young. While the exact mechanisms by which obesity causes hypertension remain incompletely understood, significant advances in our understanding have been made in recent years. *Fig. 61-3* presents a simplified summary of the many mechanisms of hypertension that have been demonstrated to occur in obese individuals. Selected mechanisms illustrated in the figure will be summarized in the following discussion. Others are discussed elsewhere in the chapter. Additionally, we recommend that the interested reader should consult updated reviews, as there is much active research in all of these areas.

Two major hormonal abnormalities in obesity hypertension are hyperinsulinemia, a consequence of peripheral insulin resistance, and hyperleptinemia, a consequence of the increased mass of visceral adipose tissue. Landsberg has noted that "...insulin resistance in the obese is a mechanism evolved for limiting further weight gain. Like any compensatory mechanism, however, there is a price to pay. In this situation, that price is the hyperinsulinemia and sympathetic activation which, via effects on the blood vessels, the heart and the kidneys, exerts a pro-hypertensive effect that, in susceptible individuals, causes hypertension (230)." There are several lines of evidence linking hyperinsulinemia with increased sympathetic nervous system activation and hypertension, including the finding of elevated levels of plasma catecholamines, and abrogration of hypertension after adrenergic blockade (231, 232). The hyperinsulinemia associated with insulin resistance in obese individuals probably contributes to the development of hypertension via additional mechanisms other than SNS activation, which are discussed later in the chapter.

Figure 61-3

Mechanisms of hypertension in obesity. *SNS* sympathetic nervous system.



Leptin is a peptide hormone encoded on chromosome 7 in humans and primarily produced by adipose tissue whose major function seems to be regulation of energy stores. With weight gain, leptin levels increase, leading to release of melanocyte-stimulating hormone which in turn binds to and activates the melanocortin-4 receptor which induces physiologic changes that counteract obesity (233). In obese hypertensives, leptin levels are elevated and correlate with both blood pressure and heart rate (234). The major link between hyperleptinemia and hypertension appears to be increased sympathetic nervous system activity, as demonstrated in animal studies documenting increased renal sympathetic nerve activity with intraventricular leptin infusions. Intravenous leptin infusions in animals also increase blood pressure and heart rate (234, 235). In obese humans, leptin appears to modify muscle sympathetic nerve activity as well as central SNS activity; the latter effect appears to be mediated primarily through activation of hypothalamic pathways (236, 237).

Leptin also has significant intraglomerular effects, including increased TGF-β synthesis and increased type IV collagen production by glomerular endothelial cells, and stimulation of type I collagen synthesis by mesangial cells. This, in turn, may lead to glomerular sclerosis via extracellular matrix deposition (238), perhaps contributing to or even accounting for the secondary FSGS that can be seen in obesity. Losartan treatment appears to ameliorate the renal effects of leptin in experimental animals (239).

Of the other mechanisms illustrated in *Fig. 61-3*, activation of the renin–angiotensin system and the role of inflammation and oxidative stress merit further discussion. Children with primary hypertension have been noted to have elevated plasma renin activity in single-center studies that correlate with diastolic but not systolic blood pressure (188). Similar peripheral elevations of plasma renin activity and other components of the renin–angiotensin system have been noted in obese adults (240). Elevated renin despite the presence of other mechanisms that promote volume overload (*Fig. 61-3*) point to a generalized dysregulation of the renin–angiotensin system in obesity that likely contributes not only to the development of hypertension, but also to obesity-related renal damage.

Adipose tissue is increasingly being viewed as an endocrine organ; it secretes several hormones, particularly leptin (see above) and adiponectin, and a variety of other proteins that are collectively known as adipokines. Unlike most adipokines, adiponectin expression and serum concentration are reduced in obesity (241), but there is increased expression of pro-inflammatory cytokines such as TNF-alpha and interleukin-6. This contributes to the insulin resistance seen in obesity (242), and thereby to blood pressure elevation and other adverse cardiovascular effects. Obesity is also associated with increased markers of oxidative stress such as urinary 8-epi-prostaglandin F2alpha in both experimental and population studies (243, 244).

Finally, obesity produces direct effects on the kidney that contribute to the development of hypertension (231, 245). There is compression of the kidneys by the perirenal fat, leading to increased interstitial pressure, reduced medullary blood flow and tubular compression, all of which lead to increased sodium reabsorption. Over time, hyperfiltration injury and increased glomerular transforming growth factor-beta1 expression (246) lead to glomerular sclerosis and nephron loss, further exacerbating the trend toward sodium retention, as well as activation of mechanisms operative in chronic kidney disease (CKD) such as activation of the renin–angiotensin system.

Insulin Resistance in Nonobese Hypertensives

Insulin resistance has been linked to several physiologic mechanisms that can elevate blood pressure. First and foremost among these mechanisms is likely to be an altered renal handling of sodium, leading to hypertension through an expansion of plasma volume. Insulin increases renal sodium reabsorption, possibly in the distal nephron, although this is not completely certain (247). It is likely that increased activity of renal sympathetic nerves is responsible at least in part for this effect (248). Elevated circulating levels of aldosterone, which have been demonstrated in salt-sensitive obese adolescents, may also be involved (249). Importantly, these effects of hyperinsulinemia on renal sodium handling can be reversed with weight loss (249).

The second major mechanism by which hyperinsulinemia may elevate blood pressure is through effects on vascular structure and function. Although insulin when infused directly into local vascular beds acts as a vasodilator (250), in hypertensive subjects this effect is probably offset by vasoconstriction mediated by increased sympathetic nervous activity (250, 251). In addition, impaired vasodilatation in response to insulin infusion has been demonstrated in obese individuals (252). Alternatively, insulin may act to stimulate vascular smooth muscle proliferation in resistance vessels (253), thereby leading to increased peripheral vascular resistance due to vascular medial hypertrophy. In this way, hyperinsulinemia would lead to hypertension by increasing SVR.

Not all hypertensive individuals with insulin-resistance are obese (251, 254), suggesting that insulin resistance is a primary mechanism in the development of primary hypertension. Supporting this hypothesis are studies demonstrating a significant inverse relationship between insulin-mediated glucose uptake and blood pressure levels in lean normotensive and borderline hypertensive individuals (255). The mechanisms responsible for the development of elevated blood pressure in lean individuals with insulin resistance are most likely similar to those discussed above. What remains unknown at this point is what causes insulin resistance in non-obese hypertensives (251).

Uric Acid

That serum uric acid concentrations are frequently elevated in hypertensive children and adults has been known for decades (256, 257). Serum uric acid levels have also been shown to predict the development of hypertension in community-based studies (258, 259), although other studies have failed to demonstrate that such a relationship exists (260). Given the conflicting epidemiologic data, routine measurement of uric acid levels has not been advocated in patients with hypertension, except to monitor for drug-induced hyperuricemia, which can be caused by thiazide diuretics.

However, an interesting body of work has recently emerged on a possible pathophysiologic link between uric acid and the development of hypertension. Rats given the uricase inhibitor oxonic acid developed mild hyperuricemia, elevated blood pressure, microvascular changes in the kidneys and increased expression of renin in the juxtaglomerular apparatus (261, 262). Administration of the xanthine oxidase inhibitor in the same animal model lowered serum uric acid levels and prevented the development of hypertension and vascular changes (262). The postulated mechanism for development of hypertension appears to involve endothelial dysfunction, specifically reduced levels of endothelial-derived NO (263).

Findings in this animal model appear to correlate with experimental data in humans. Uric acid levels correlate with elevated plasma renin in hypertensive adolescents (264) and with endothelial dysfunction and atherosclerosis in adults (265, 266). Lowering serum uric acid with allopurinol has been shown to improve flow-mediated dilation, an indicator of endothelial function, in patients with hyperuricemia (265), and in a recent small trial, allopurinol reduced blood pressure in adolescents with primary hypertension (267). Although further confirmatory studies are clearly required, some have speculated that uric acid-lowering strategies may find a role in the treatment and even prevention of hypertension (268).

Dietary and Environmental Influences

Numerous dietary constituents have been examined as possible contributors to the development of hypertension. Of these, the two that have received the most interest have been sodium and potassium. Population studies of sodium consumption have demonstrated that populations with lower sodium intake have lower blood pressures than those with higher sodium intake (269, 270). It is noteworthy that typically, populations with high dietary sodium intake also have lower potassium intake (269, 271), suggesting a potential pathogenic role for potassium as well. Interventional studies of sodium restriction have generally shown that reducing sodium intake lowers blood pressure in both adults (272) and children (273), whereas the data for potassium supplementation are less clear (273, 274). The "DASH" diet, which is low in sodium and saturated fats and high in potassium and calcium has been shown to be able to substantially lower blood pressure in hypertensive adults (275) as well as adolescents (276); this diet has also been advocated as a method of hypertension prevention.

However, these dietary influences on blood pressure, although present when large populations are studied, may not apply at the individual patient level (269, 277). This is because some hypertensive patients are salt-sensitive and others are not; for example, in obese hypertensives, salt sensitivity is likely present and can be reversed with weight loss (249), but lean hypertensives are less salt-sensitive. Similarly, salt sensitivity of blood pressure has been demonstrated in children with a history of low birth weight, but not in children whose birth weight was appropriate (278). The public health implications of dietary sodium restriction on a population basis cannot be ignored – a modest reduction in sodium intake resulting in a 3 mmHg reduction in systolic blood pressure has been calculated to have significant potential to reduce the incidence of stroke and other cardiovascular disease (272).

The other environmental factor felt to play a role in the development of primary hypertension is stress. Acute stress has been shown to increase blood pressure in experimental settings, most likely through activation of the sympathetic nervous system (215, 219, 220). At the population level, stress has also been advanced as an explanation for the increased incidence of hypertension among

African-Americans compared to Caucasians (279). An individual's reaction to stress may lead to other changes in their environment that are themselves related to blood pressure (280), such as obesity from increased caloric intake, which would then cause hypertension by the mechanisms discussed above.

Secondary Hypertension

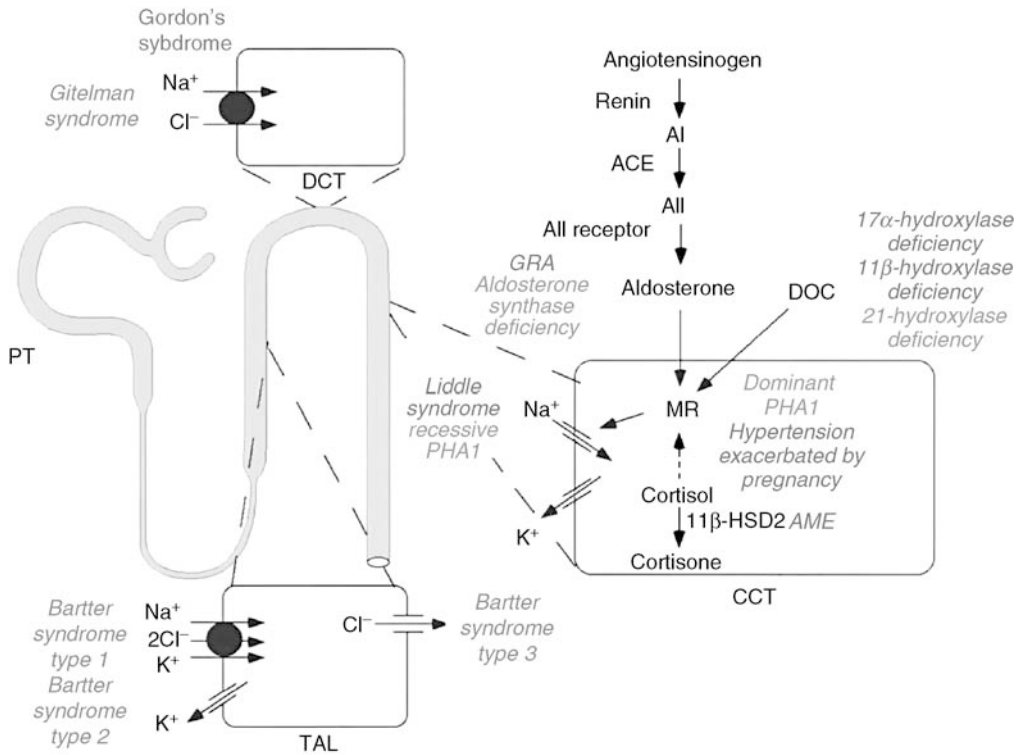
Mendelian Forms of Hypertension

Molecular genetic studies have now identified many gene mutations that affect blood pressure. Most of the mutations found to date affect sodium handling in the renal distal tubules. Below, we will mainly discuss well characterized monogenic hypertensive disorders (Fig. 61-4) (200).

Glucocorticoid-remediable aldosteronism (GRA) or *familial hyperaldosteronism type 1* (FH-I) is an autosomal dominant disease characterized by early onset of hypertension with normal or elevated aldosterone levels despite suppressed plasma renin activity (191). GRA is caused by a chimeric gene that results from unequal crossing over between the aldosterone synthase and 11β-hydroxylase genes on chromosome 8. Aldosterone synthase is the rate-limiting enzyme for aldosterone biosynthesis in the adrenal glomerulosa and 11β-hydroxylase is an enzyme involved in cortisol biosynthesis in the adrenal fasciculata whose expression is regulated by adrenocorticotropic hormone (ACTH). The resulting chimeric gene is expressed in the adrenal fasciculata and encodes a protein product with aldosterone synthase enzymatic activity whose expression is regulated by ACTH. Consequently,

Figure 61-4

Single-gene mutations altering blood pressure. A diagram of a nephron, the filtering unit of the kidney, is shown. The molecular pathways mediating NaCl reabsorption in individual renal cells in the thick ascending limb of the loop of Henle (TAL), distal convoluted tubule (DCT), and the cortical collecting tubule (CCT) are indicated, along with the pathway of the renin–angiotensin system, the major regulator of renal salt reabsorption. Inherited diseases affecting these pathways are indicated, with hypertensive disorders in *red* and hypotensive disorders in *blue*. *Abbreviations: AI* angiotensin I; *ACE* angiotensin converting enzyme; *AII* angiotensin II; *MR* mineralocorticoid receptor; *GRA* glucocorticoid-remediable aldosteronism; *PHA1* pseudohypoaldosteronism, type-1; *AME* apparent mineralocorticoid excess; *11 HSD2* 11-hydroxysteroid dehydrogenase-2; *DOC* deoxycorticosterone; *PT* proximal tubule. Reprinted and modified from (200).



aldosterone synthase activity is ectopically expressed in the adrenal fasciculata under control of ACTH rather than angiotensin II or potassium. Aldosterone secretion becomes linked to cortisol secretion, and maintenance of normal cortisol levels results in constitutive aldosterone secretion, which leads to expanded plasma volume and hypertension (281, 282). The expanded plasma volume suppresses secretion of renin, but this fails to diminish secretion of aldosterone.

Familial hyperaldosteronism type II (FH-II) is a similar to GRA (FH-I) with excess production of miniralocorticoids, but is not suppressed by dexamethasone. FH-II may be associated with a family history of adrenal hyperplasia or adenoma and appears to be more common than FH-I. FH-II is autosomal dominant, and linkage analysis suggests an association with chromosome 7 (283, 284), but the molecular basis of the disorder remains determined.

Hypertension Exacerbated in Pregnancy by mutations in the mineralocorticoid receptor is an autosomal dominant form of hypertension that is markedly accelerated in pregnancy (285). A systematic screening of patients with early-onset hypertension detected a missense mutation in the ligand-binding domain of the mineralocorticoid receptor MR S810L. MR-L810 carriers all developed hypertension before age 20, a finding absent among their unaffected relatives. Steroids lacking 21-hydroxyl groups (progesterone, spironolactone) that normally bind but do not activate MR are all potent agonists of the mutant receptor. Because progesterone levels rise 100-fold during pregnancy, all pregnancies among patients harboring this mutation have been complicated by dramatic acceleration of hypertension associated with complete suppression of the renin–angiotensin system (285).

Congenital adrenal hyperplasia from the two known defects in either 11β-hydroxylase or17α-hydroxylase activity may cause hypertension. These defects lead to overproduction of 21-hydroxylated steroids, which activate mineralocorticoid receptors, resulting in increased sodium reabsorption in distal tubules (286, 287).

Primary glucocorticoid resistance represents the partial resistance of the glucocorticoid receptor, resulting in excessive ACTH-dependent 11-deoxycortisone and corticosterone production. A cortisol level too high to be controlled by 11β-hydroxysteroid dehydrogenase type II enzyme activity activates mineralocorticoid receptors and increases sodium reabsorption in the distal tubules (286–290).

The syndrome of *apparent mineralocorticoid excess* (AME) is an autosomal recessive disease characterized by early-onset hypertension with hypokalemia and metabolic alkalosis, accompanied by suppressed plasma renin activity and the virtual absence of circulating aldosterone. In this disease, steroids other than aldosterone activate the mineralocorticoid receptor (MR). Affected patients have a lack-of-function mutation in the gene encoding the renal isoenzyme of 11-hydroxysteroid dehydrogenase (11HSD), resulting in impaired conversion of cortisol to cortisone (291). In vitro, cortisol activates MR with potency similar to aldosterone. However, in vivo almost all activation of MR is mediated by aldosterone, despite its low plasma concentration. The specificity of MR for aldosterone in vivo is mediated indirectly by 11HSD activity, "protecting" the MR from cortisol by metabolizing it to cortisone, which does not activate MR (292). In AME, the absence of this enzyme allows cortisol to activate the MR, resulting in hypertension mediated by increased sodium retention. Confirmation of the pathogenesis of AME came with the cloning of 11HSD-2. This gene, expressed in the same cells of the nephron that express ENaC, shows homozygous loss-of-function mutations in AME patients (291, 293).

Liddle's syndrome is an autosomal dominant disease characterized by severe hypertension, metabolic alkalosis, and hypokalemia with low renin and aldosterone (284). Activating mutations in the β or γ subunit of the epithelial Na channel (ENaC) are responsible for constitutively elevated renal sodium reabsorption in Liddle's syndrome (294–297). ENaC is the main sodium channel responsible for sodium reabsorption in the renal collecting ducts. Clearance of the normal channel from the membrane depends upon the sequence PPPXY in the cytoplasmic C termini of the β and γ ENaC subunits. Mutations of this sequence in either subunit prolong the cell-surface half-life (295) and increase the number of channels (297), by eliminating interaction with WW domain-containing proteins such as the ubiquitin ligase Nedd4 and thereby reducing channel ubiquitylation and internalization (294, 298–303).

Gordon's Syndrome, also known as pseudohypoaldosteronism type II, is an autosomal dominant disorder characterized by hypertension, hyperkalemia, hyperchloremic metabolic acidosis, and normal glomerular filtration rate (304–307). The WNKs ("with no lysine" (K)) are a family of serine/threonine protein kinases, and mutations of WNK1 and WNK4 genes located on human chromosomes 12 and 17, respectively, are found to be responsible for Gordon's syndrome (308–312). Deletions in the first intron of the WNK1 gene increase gene expression. This increases sodium reabsorption via ENaC and the Na^+/Cl^- cotransporter (NCC); the latter effect is caused by reducing inhibition of NCC by WNK4. Increased WNK1 expression also decreases potassium

excretion by inhibiting the renal outer medullary potassium channel (ROMK). Missense mutations of the WNK4 gene have several effects on renal ion transport. In general WNK4 inhibits NCC and prevents Na^+ reabsorption in the distal convoluted tubules. The mutated WNK4 does not inhibit NCC, leading to constitutively increased Na^+ reabsorption. WNK4 mutations also inhibit potassium excretion by ROMK, which contributes to hyperkalemia (287, 302, 313). Recent studies suggest that WNK1 may also play a role in the pathogenesis of salt-sensitive hypertension (314, 315).

Hypertension with brachydactyly features severe hypertension with abnormal skeletal development in the hand and wrist. There are no associated distinguishing biochemical features. This syndrome has been mapped to chromosome 12p12.2-11.2 (316, 317), but the responsible gene has not yet been identified.

Other Genetic Forms of Hypertension

Mutations in peroxisome proliferator-activated receptor γ (PPARγ) are dominant-negative and lead to loss of function. These mutations result in insulin resistance, type 2 diabetes, and hypertension at an early age (318). A syndrome of hypertension, hypercholesterolemia, and hypomagnesemia shows maternal inheritance of a homoplasmic mutation caused by a cytidine substitution in the mitochondrial tRNA (319).

Hypertension in Renovascular Disease

Hypertension in the one-clip, two-kidney model (analogous to unilateral renal artery stenosis in humans) is renin-dependent (320). One-clip, two-kidney animals also have increased norepinephrine release and SNS activity (321), implicating a role for the SNS in the development of renovascular hypertension. This has been disputed in animal models (320), but as mentioned previously, studies in humans with renovascular hypertension have clearly demonstrated elevated sympathetic nerve activity (17).

Renal perfusion pressure starts to be affected when renal artery stenosis reaches 50%. However, the stenosis needs to occlude at least 70% of the lumen before it begins to reduce renal blood flow and raise arterial pressure (322). In the late phase, hypertension persists despite removal of the stenosis or ischemic kidney, due to damage to the contralateral kidney (323), and probably also to systemic vascular changes as discussed elsewhere. In fact, in the one-clip, two-kidney model of renovascular hypertension, the vascular, glomerular and tubulointerstitial damage in the unclipped kidney is greater than in the clipped kidney. The clipped kidney is exposed to elevated angiotensin, but protected from hypertension (324). Activation of the renin–angiotensin system also results in an increase in oxidative stress and accelerated atherosclerosis (325).

Renovascular hypertension accounts for 5–10% of hypertension in children. The causes of renovascular hypertension are diverse, including fibromuscular dysplasia, syndromes such as neurofibromatosis type 1, tuberous sclerosis, Williams' syndrome, and Marfan syndrome, vasculitis, extrinsic compression, radiation, umbilical artery catheterization, trauma, congenital rubella syndrome, transplant renal artery stenosis, and others (326). Eleven to sixty percent of renovascular hypertension cases are familial, showing autosomal-dominant inheritance with variable penetrance (327, 328).

The renin–angiotensin system is important for not only blood pressure regulation but also for vascular remodeling and vascular smooth muscle growth. Bofinger et al. note that polymorphisms of the angiotensin-converting enzyme (ACE) allele associated with lower circulating ACE levels might predispose to defective vascular remodeling (328). In addition, elastin gene mutations in Williams' syndrome and the mutations of JAGGED 1 (JAG1) in Alagille's syndrome may be responsible for renal artery stenosis (329). Vasculitis including Takayasu disease, polyarteris nodosa, Kawasaki disease, other systemic vasculitis, and transplant renal artery stenosis are also important causes of renovascular hypertension (328).

Hypertension in Acute Kidney Injury and Chronic Kidney Disease

Hypertension resulting from renal parenchymal disease is multifactorial in origin. Acute and severe insult to the kidneys either impairs excretion of salt and water, reduces renal blood flow, or both. Dysregulation of salt and water excretion leads to volume expansion and thereby increases cardiac output. Both the reduction in renal bloodflow and the volume expansion activate the renin–angiotensin–aldosterone axis (330). For example, autosomal dominant polycystic kidney disease (ADPKD) presents with significantly increased renin, likely due to cyst expansion reducing renal blood flow, leading to ischemia (331). CKD also causes abnormal activation of the adrenergic system and baroreceptor dysfunction (332) and leads to accumulation of the NOS inhibitors (333) and endothelial dysfunction (334). There is also decreased production of vasodilators, including kinin

and prostaglandins, and increased production of the vasoconstrictor ET-1 (335). Recombinant human erythropoietin treatment induces hypertension by several mechanisms: a direct vasoconstrictive effect, diminished response to nitric oxide, increased plasma endothelin, a marked increase in intracellular calcium in vascular smooth muscle cells, arterial remodeling, and others. Abnormal parathyroid/calcium homeostasis secondary to chronic kidney diseases may increase intracellular calcium concentrations in the myocardium and platelets, which subsequently leads to hypertension (336, 337) (*Fig. 61-5*).

Hypertension in end-stage renal disease (ESRD) is primarily volume- and salt-dependent and results from impaired sodium excretion. In ESRD as well as in advanced CKD, there is expansion of the extracellular water volume, as well as in total body water (330). The renin angiotensin system is also stimulated, which in turn enhances sodium retention and produces an increase in systemic vascular resistance.

Increased sympathetic tone, as evidenced by elevations in plasma noradrenaline levels, contributes to increased systemic vascular resistance and cardiac output (338). As discussed previously, ET-1 and ANP are elevated and are associated with high blood pressure in CKD (137, 339). Administration of non-steroidal anti-inflammatory drugs in CKD increases arterial pressure and reduces renal prostaglandins (340) suggesting an important role for prostaglandins in CKD-associated hypertension. Patients with uremia secondary to impaired renal clearance also have elevated levels of the NOS inhibitors asymmetric and symmetric dimethylarginine (333, 341). Patients with ESRD are known to lose the normal nocturnal dip in blood pressure, have increased pulse pressure and isolated systolic hypertension, and may develop increased aortic stiffness (342, 343). The mechanisms of these changes have not been elucidated.

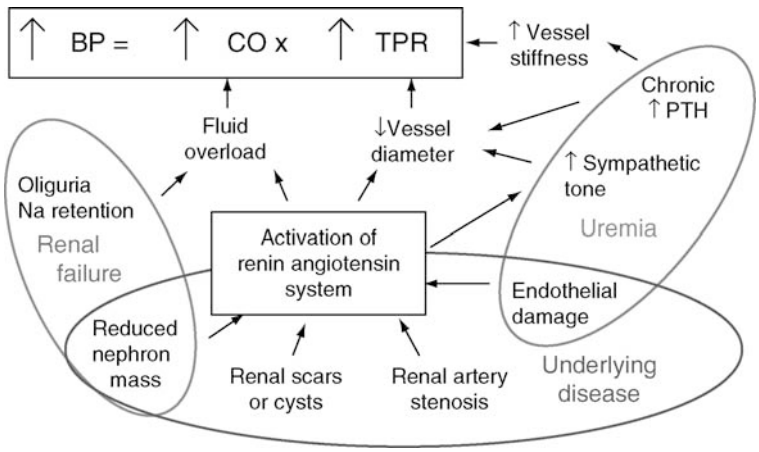
Hypertension in Dialysis Patients

Children receiving chronic dialysis have a significant incidence of hypertension: 53–65% of children receiving hemodialysis and 45–58% of children receiving peritoneal dialysis in the North American Pediatric Renal Transplant Cooperative Study (NAPRTCS) dialysis database were receiving antihypertensive medications at the time of the 2007 NAPRTCS annual report (344). It was noted that the use of antihypertensive medications among PD and HD patients decreased over 3 years (344). The major pathophysiologic mechanism in most dialysis patients seems to be volume overload related to sodium and water retention. This may be more significant in peritoneal dialysis patients than in hemodialysis patients (345). Evidence in favor of fluid overload being the major mechanism can be found in the many studies that demonstrate correction of hypertension by increased fluid removal in both peritoneal dialysis and hemodialysis patients (346, 347).

However, numerous other factors have been implicated, including overactivity of the sympathetic nervous system, activation of the renin–angiotensin system,

Figure 61-5

Interplay of different factors in the generation of hypertension in chronic kidney disease (*BP* blood pressure, *CO* cardiac output, *TPR* total peripheral resistance, *PTH* parathyroid hormone, *Na* sodium). Reprinted from (337).



erythropoietin, parathyroid hormone, and nocturnal hypoxemia (348). Recent studies have also implicated nitric oxide and endothelin as being involved in the pathogenesis of dialysis hypertension (348, 349). Endothelin and other vasoconstrictor factors have also been shown to be involved in the pathogenesis of erythropoietin-related hypertension (350). Post-dialysis hypertension in hemodialysis patients is likely renin-mediated and responds to administration of an angiotensin converting-enzyme inhibitor following dialysis (351–355).

Post-transplant Hypertension

Hypertension following renal transplantation is a common phenomenon. Eighty-five percent of deceased donor recipients and 79% of live donor recipients are receiving antihypertensive medications at the time of transplantation. The percentage receiving antihypertensives is decreased in 5 years for both deceased and live donor recipients, to 69 and 59%, respectively (344). The major contributing factors include history of pre-transplant hypertension, persistent native kidney hypertension, effects of immunosuppressive medications, transplant renal artery stenosis, and chronic allograft dysfunction (356–358).

As in hypertension of any cause, pre-transplant hypertension leads to chronic vascular changes that increase peripheral vascular resistance, perpetuating hypertension after transplantation. Although such vascular remodeling can be reversed with effective antihypertensive treatment (359), many patients come to transplant hypertensive despite therapy, so pre-existing vascular remodeling remains a significant factor following transplantation. Remaining native kidneys may also contribute to post-transplant hypertension, presumably via persistent release of renin (360).

Immunosuppressive medications, particularly glucocorticoids and calcineurin inhibitors, may both contribute to post-transplant hypertension. Although prednisone may be a significant contributing factor to the development of hypertension in the immediate post-transplant period, many current pediatric immunosuppression protocols rapidly reduce the dose of prednisone in order to maximize post-transplant growth, making prednisone a much less important contributing factor later on (360, 361). This may be even less of a problem in pediatric transplantation in the future as the use of steroid-free immunosuppression increases.

Calcineurin inhibitors affect blood pressure in a variety of ways, including increased production of the vasoconstrictor endothelin (138, 140, 362, 363), decreased production of vasodilatory substances (361), and activation of the sympathetic nervous system (364). Cyclosporine increases renal vascular resistance by stimulating afferent artery vasoconstriction. Calcineurin inhibitors also cause sodium retention (365). Many studies suggest that calcium channel blockers can minimize calcineurin-induced vasoconstriction (366, 367), thereby ameliorating hypertension and preventing chronic graft injury.

Transplant renal artery stenosis (TRAS) has been reported to occur in up to 20% of hypertensive pediatric renal transplant recipients (360), although improved surgical techniques have probably decreased its incidence more recently (361). Causes may include surgical trauma, prolonged ischemia, suturing technique or reactions to suture material, atheromatous plaques in the donor kidney, and cytomegalovirus infection (361, 368–370). Elevated renin secretion has been seen in patients with TRAS (371), suggesting that the mechanisms responsible for hypertension in TRAS are similar to those discussed elsewhere in this chapter for renovascular hypertension.

Allograft dysfunction has long been recognized as a significant cause of hypertension in both children and adults with post-transplant hypertension (360, 361). Chronic allograft nephropathy presents with a slow, progressive arteriolopathy and glomerulosclerosis. Whether resulting from the sequelae of rejection or from calcineurin nephrotoxicity, the primary mechanism of hypertension related to allograft dysfunction appears to be renal sodium retention (361, 372). Other mechanisms seen in patients with renal insufficiency of other causes may also be responsible.

Hypertension in Coarctation of the Aorta

Hypertension associated with aortic coarctation may occur in three clinical settings: prior to surgical repair, in the immediate post-operative period following surgical repair, and years after repair. Pre-repair hypertension is the result of renal hypoperfusion and activation of the renin–angiotensin system. There may also be increased activity of the sympathetic nervous system (373). In the immediate post-surgical period, paradoxical hypertension may be seen, probably resulting from increased activity of the renin–angiotensin and sympathetic nervous systems (374). This phenomenon appears to be rare following balloon angioplasty (375), which has largely replaced primary surgical repair as the initial procedure of choice.

Despite surgical repair of coarctation, many patients will later be found to have abnormal blood pressure at rest. Ambulatory blood pressure monitoring is probably

superior to casual blood pressures for detection of hypertension in this population (376). Proposed mechanisms for this late or persistent hypertension include altered baroreceptor function, thickening of blood vessels below the site of the coarctation, and elevation of plasma adrenaline and aldosterone levels (377, 378). Vascular dysfunction in patients with repaired coarctation has been demonstrated and appears to be related to the age of repair (379). Renal structural changes have also been demonstrated in animal models of coarctation (380). Recoarctation should also be investigated in any patient with the development of late post-repair hypertension.

Hypertension in Vasculitis

Because of the numerous vessels within the renal parenchyma, the kidneys are frequently targeted by systemic vasculitis. Glomerular inflammation, with cellular infiltration, proliferation, and immune complex deposition resulting in nephritis and renal failure has been well characterized in systemic lupus erythomatosus (SLE), microscopic polyarteritis, Wegener's granulomatosis, Henoch–Schonlein purpura, and cryoglobulinemic vasculitis. Takayasu's arteritis (TA), an inflammatory process associated with stenosis and obliteration of the aorta and its primary branches, induces hypertension by mechanisms that are similar to those seen in coarctation of the aorta, renovascular hypertension, or ischemic kidney disease, as discussed above. Necrotizing vasculitides, such as classic polyarteritis nodosa and Kawasaki's disease, may lead to hypertension secondary to thrombosis of inflamed major extrarenal and intrarenal arteries with resulting local (intrarenal) renin–angiotensin system activation and infarction of the renal parenchyma (381).

Hypertension in Endocrine Diseases

Hypertension can occur secondary to many hormonal derangements, including excess production of mineralocorticoids, glucocorticoids, and catecholamines. An excess of growth hormone or parthyroid hormone, hypo- or hyperthyroidism, a renin-secreting tumor, or diabetes mellitus may also lead to hypertension (382).

Primary hyperaldosteronism (PAL) or mineralocorticoid excess is now believed to be much more common than it was previously thought (382–384). PAL was first reported by Conn as aldosterone-producing adenoma (APA) (385) and more commonly results from adrenal hypertrophy ("idiopathic hyperaldosteronism" (IHA)). Rarely, pure aldosterone-producing adrenocortical carcinoma and ectopic aldostrone-secreting tumors cause PAL. Glucocorticoid-remediable aldosteronism (GRA), familial hyperaldosteronism type II (FH-II), and apparent mineralocorticoid excess (AME), which were previously described in this chapter, are also in this category.

APA and IHA both cause excess aldosterone secretion, and both produce hypertension with similar clinical features: hypokalemia, renal potassium wasting, suppressed plasma renin activity, and increased plasma and urinary levels of aldosterone (386). The blood pressure elevation due to aldosterone excess results from an increase in systemic vascular resistance (SVR) due to aldosterone-mediated increased plasma sodium concentration that leads to a rise in the intracellular calcium content. The increase in intracellular calcium leads to impaired left ventricular diastolic relaxation (387) and enhanced contractility with reduced vascular compliance (388). The increased aldosterone production in IHA reflects increased activity of a rate-limiting mitochondrial cytochrome P450 isoenzyme, the aldosterone synthase gene CYP11B2 (389). Polymorphisms of the CYP11B2 gene determine interindividual variations in aldosterone activity and response to antihypertensive treatment (390, 391).

Cushing's syndrome or glucocorticoid excess has long been known to cause hypertension, but the exact mechanism or mechanisms that result in blood pressure elevation remain unknown. Increased peripheral vascular sensitivity to adrenergic agonists may play a role in the hypertension (392). Hepatic synthesis of angiotensinogen, which stimulates the renin–angiotensin system, is activated, and phospholipase A2, which releases arachidonic acid from phospholipids and plays an important role in the synthesis of vasodilatory prostaglandins, is inhibited (393). Glucocorticoids also reduce the activity of the depressor kallikrein–kinin system, enhance pressor sensitivity to endogenous vasoconstrictors (epinephrine and angiotensin II) (394, 395), and promote sodium influx into vascular smooth muscle cells (396). High levels of cortisol exert mineralocorticoid effects, and in adrenal carcinomas, DOC and aldosterone may also be elevated (397).

Pheochromocytoma. Pheochromocytomas are catecholamine-secreting tumors of chromaffin cells that may arise from cells of neural crest origin, either in the adrenal glands or in extra-adrenal sites. Sustained or paroxysmal hypertension is the most common clinical sign, although it may be less common in children than in adults (398). Although most pheochromocytomas are sporadic, there is a familial predisposition in patients with multiple endocrine neoplasia type II (MEN II), von Hippel–Lindau disease, neurofibromatosis type 1, and

familial paraganglioma (399–401). Pheochromocytoma in MEN II is associated with a germline mutation of the proto-oncogene RET (402) and pheochromocytoma in von Hippel–Lindau disease results from germline mutation of the von Hippel–Lindau gene (403). Familial paraganglioma is an autosomal dominant disorder with catecholamine hypersecretion. Paragangliomas are mostly located in the head and neck, but can be found in the thorax, abdomen, pelvis, and bladder. Mutations of succinate dehydrogenase in subunits D, B, and C are found in most cases of familial paraganglioma (404–409).

Hypertension in pheochromocytoma results from a direct effect of high circulating levels of catecholamines primarily noradrenaline, adrenaline and dopamine (398). Some tumors produce the catecholamine precursor L-dopa. Pheochromocytomas also secrete numerous other peptide hormones, among which are renin, VIP, neuropeptide Y, somatostatin, and ET-1. These substances are responsible for many of the non-hypertensive symptoms seen in patients with pheochromocytoma, including flushing, sweating and diarrhea (398).

Acromegaly or growth hormone (GH) excess. The prevalence of hypertension in acromegaly is approximately 35% (398, 410, 411). The relationship between hypertension and growth hormone excess is not completely understood. GH causes sodium retention with volume expansion by inhibiting atrial natriuretic peptide (ANP) (412, 413). Acromegaly may also present with insulin resistance, which may impair NO production and vasodilation. GH and insulin-like growth factor-1 (IGF-1) affect growth and mitosis of endothelium and VSMC and may increase myocardial hypertrophy and contractility (414–416).

Primary hyperparathyroidism (PHPT). Whether PHPT causes hypertension is still controversial. However, potential pathogenic mechanisms of hypertension in PHPT are elevated intracellular calcium, hypomagnesemia, raised plasma renin activity, and glucose abnormalities. Impairment of endothelial vasodilation by increased intracellular calcium and vasospasm induced by neurohormonal substances due to hypomagnesemia have been reported (417, 418).

Thyroid disorders. Hypothyroidism reduces renal blood flow and glomerular filtration rate and decreases cardiac output (419). Compensatory mechanisms – primarily increased peripheral resistance mediated by increased responsiveness to adrenergic stimulation and increased SNS activity (420) – lead to diastolic hypertension. Treatment with thyroid hormone will correct the hypertension.

Hyperthyroidism, on the other hand, is associated with activation of the renin–angiotensin–aldosterone axis and increased sodium reabsorption, leading to expansion of plasma volume (154). There is also β2 receptor stimulation, which results in vasodilatation and increased cardiac output and contractility (155). These physiologic changes result in isolated systolic hypertension (157).

Renin. Juxtaglomerular cell tumors of the kidney or ectopic tumors secrete renin and induce hypertension associated with hypokalemia, hyponatremia, and other features of RAAS activation (421). This form of hypertension can often be severe and present acutely (422).

Diabetes mellitus (DM). Hypertension in patients with type I diabetes is usually correlated with the development of microalbuminuria and diabetic nephropathy. On the other hand, the majority of patients with type II diabetes have hypertension prior to the development of nephropathy. The pathogenesis of hypertension in diabetes is multifactorial, with hyperinsulinemia, sodium retention, volume expansion, and increased arterial stiffness all playing important roles. Hyperglycemia alters lipid and protein metabolism, and insulin increases sympathetic nervous system activity. DM leads to vascular changes including both macroangiopathy and microangiopathy, and autonomous neuropathy causes vascular dysfunction. Numerous studies have shown that endothelial dysfunction in DM may play a significant role in hypertension by altering vascular architecture and remodeling in response to hemodynamic changes, low NO, and prostacyclin release, increased oxygen-reactive species, and increased endothelin-1. Hyperglycemia and advanced glycosylation end-products may also be important contributing factors (423–426).

Hypertension in Pulmonary Diseases (BPD, Sleep Apnea)

Hypertension in children with chronic lung disease is most commonly seen in infants with bronchopulmonary dysplasia (BPD). Although the pathophysiology of BPD-associated hypertension is not completely understood, it is likely to be multifactorial in origin. Corticosteroids, especially dexamethasone, when used for prolonged periods will elevate blood pressure in such infants, but with withdrawal the hypertension resolves (427). Because the development of hypertension is correlated with the severity of lung disease, chronic hypoxia may also be an important mechanism (428).

Obstructive sleep apnea (OSA), characterized by episodic upper airway obstruction during sleep despite persisting respiratory efforts, leads to asphyxia, hypoxia, hypercapnea, and disruption of sleep architecture. OSA is a risk factor for systemic hypertension, myocardial infarction, stroke, and sudden death (429). Acute episodes

of hypoxia result in stimulation of peripheral chemoreceptors and increased sympathetic outflow with subsequent activation of the renin–angiotensin system. Other important contributing factors include endothelial dysfunction, oxidative stress and inflammation (430). Cardiac output may also change secondary to fluctuation of intrathoratic pressure (431, 432). Since many patients with OSA are obese, coexisting conditions such as the metabolic syndrome are probably also involved in the development of hypertension (433). Effective therapy of OSA will often result in an improvement in blood pressure in affected individuals.

Hypertension in Neurologic Disorders

Systemic hypertension can be seen in a variety of neurologic disorders, including seizures, poliomyelitis, hydrocephalus, head trauma, pseudotumor cerebri (idiopathic intracranial hypertension), Guillain–Barre syndrome, and with space-occupying lesions (tumor, bleeding, and abscess). Many of these are associated with increased SNS outflow, leading to peripheral vasoconstriction and renal sodium retention (see earlier sections).

In Guillain–Barre syndrome, for example, microneurographic studies have demonstrated increased SNS activity in the acute phase of the illness (434). Increased levels of circulating renin and catecholamines have also been reported (435). These abnormalities, and the hypertension, resolve as the neurologic abnormalities subside. Increased sympathetic activity is also seen in patients with spinal cord injury. In familial dysautonomia, HTN is caused by increased circulating catecholamines and increased responsiveness to circulating catecholamines (436).

Increased intracranial pressure (ICP) causes systemic HTN that can be quite labile. The mechanism of hypertension is believed to be the stretching of receptors located under the floor of the fourth ventricle (437). Sympathetic nervous outflow is increased in most patients, and baroreceptor function is disrupted. Circulating levels of catecholamines may be increased. Hemodynamically, HTN is primarily due to increased SVR. In many instances, especially when ICP is elevated due to trauma, systemic hypertension is an important compensatory mechanism for the maintenance of normal cerebral perfusion (438).

Pregnancy-Induced Hypertension and Pre-eclampsia

Pregnancy-induced hypertension (PIH) may affect up to 8% of all first pregnancies in the United States, and the incidence of preeclampsia has increased by 40% in recent years (439, 440). As with many of the forms of hypertension discussed in this chapter, PIH is most likely a multifactorial disorder with many contributing mechanisms. However, because the hypertension associated with pre-eclampsia develops during pregnancy and remits after delivery, the placenta is thought to play a central role in the disease development. Abnormal cytotrophoblast invasion of spiral arterioles leading to reduced uteroplacental perfusion and ischemia appears to be the initiating event (441, 442).

Placental ischemia results in activation/dysfunction of the maternal vascular endothelium that in turn increases synthesis and release of vasoactive factors such as soluble fms-like tyrosine kinase-1 (sFlt-1), cytokines, and angiotensin II type 1 receptor autoantibodies (AT_1-AA). Increased production of sFlt-1 triggers an imbalance between pro-angiogenic factors (VEGF and PlGF) and anti-angiogenic factor (sFlt-1). This imbalance reduces active angiogenesis, which is necessary for the placental vascular network. Ischemia of the placenta also increases thromboxane and AT_1-AA, leading to increased production of reactive oxygen species and ET-1, and decreases formation of vasodilators such as nitric oxide and prostacyclin (443, 444). There is also increased synthesis of inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), and IL-6, which is activated by TNF-α (441). Those cytokines, in turn, induce structural and functional alterations in endothelial cells, enhance endothelin production, and reduce acetylcholine-induced vasodilatation (441, 445–447).

Another important mechanism may be defective nitric oxide (NO) production. During normal pregnancy, maternal cardiac output and blood volume increase by 40–50%, whereas SVR and BP tend to decrease. In addition, GFR and renal plasma flow increase 30–40%. There is substantial evidence that NO plays an important role in mediating those changes. Plasma and urinary levels of cGMP, the second messenger of NO, increase during pregnancy in rats, and urinary nitrate/nitrite excretion has also been reported to be increased. Chronic NOS inhibition in pregnant rats produces hypertension associated with peripheral and renal vasoconstriction, proteinuria, intrauterine growth retardation, and increased fetal morbidity, a pattern that closely resembles the symptoms of human pregnancy-induced hypertension (445–449).

Drug-Induced Hypertension

Numerous therapeutic and illicit drugs, as well as some commonly used dietary supplements, may influence

Table 61-2

Mechanisms of drug-induced hypertension

Drug	Potential mechanism(s)
Caffeine	Vasoconstriction, increased SNS activity, decreased baroreceptor sensitivity (450, 451)
Calcineurin inhibitors	SNS activation (364), increased ET-1 secretion (138–140)
Cocaine	Increased heart rate and myocardial oxygen demand, direct vasoconstriction, increased matrix synthesis, glomerular inflammation, and glomerulosclerosis (452)
Ephedra/ephedrine	Stimulation of catecholamine release, stimulation of α-, β1-, and β2-receptors, CNS excitation and stimulation (453)
Erythropoietin	Increased erythrocyte mass, changes in production of or sensitivity to endogenous vasopressors, dysregulation of production of or responsiveness to endogenous vasodilatory factors, direct vasopressor effect, arterial remodeling through stimulation of vascular cell growth (350, 454)
Ethanol	Abnormal response of blood pressure and plasma renin activity to variations in salt intake (455)
Glucocorticoids	Stimulation of the renin–angiotensin system, inhibition of phospholipase A2, reduced activity of the kallikrein–kinin system, enhanced pressor sensitivity to endogenous vasoconstrictors, mineralocorticoid effects (394–397)
NSAIDs	Prostaglandin inhibition, decreased glomerular filtration rate, salt and water retention, tubulointerstitial injury (456)
Oral contraceptives	Alteration in sodium balance and the renin–angiotensin–aldosterone system (457, 458)
Tobacco	Increased SNS outflow, attenuated endothelium-dependent vasodilatation, increased arterial wall stiffness (459–461)
Yohimbine	Presynaptic alpha-2-adrenergic blockade; increased plasma norepinephrine levels (462)

Abbreviations: ET-1 endothelin-1; *NSAID* non-steroidal anti-inflammatory drug; *SNS* sympathetic nervous system

blood pressure significantly, especially in individuals whose blood pressure regulation is already disturbed. A detailed discussion of the many substances with effects on blood pressure would be beyond the scope of this chapter. However, some of the more commonly seen drug-blood pressure effects are summarized in *Table 61-2*.

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62 Evaluation of Hypertension in Childhood Diseases

Eileen D. Brewer

Introduction

Evaluation of hypertension in the pediatric age group should be guided by the age at presentation, the severity of hypertension at presentation and whether the hypertension is sustained or transient. The commonest causes of hypertension differ by age group (› *Table 62-1*). Hypertension is more likely to be secondary and sustained in the younger age groups, whereas primary (essential) hypertension and white coat hypertension are more likely to be the etiology in adolescents (1–3). The global epidemic of childhood obesity has been associated with an increased incidence of primary hypertension in adolescence (3–5), although the evidence base is not robust enough to know the full extent of this problem (5–7). Obese children have a 3-fold higher risk for developing hypertension compared to non-obese children (4). The frequent occurrence of primary hypertension in obese adolescents dictates an entirely different evaluation than for younger or non-obese children. Renal disease must be considered in every child with hypertension, because of the prevalence of renovascular and renal parenchymal disorders as the etiology in any age group. Secondary hypertension from renal disorders is more likely to be severe at presentation than primary hypertension. Endocrine causes of hypertension are rare, so special diagnostic studies for these disorders should be reserved for those patients whose history, physical examination and preliminary evaluation warrant further specific investigation. This chapter outlines the steps in evaluation of childhood hypertension, starting with the confirmation of the diagnosis and evaluation of its duration and severity.

Confirmation of the Diagnosis of Hypertension in Children and Adolescents

Recommended techniques for measuring blood pressure in children are discussed in detail in Chapter 61. A few points will be re-emphasized here because confirming the diagnosis of hypertension is the first step in evaluation. Standards for blood pressure in children and adolescents were updated in 2004 in the Fourth Report on the Diagnosis, Evaluation, and Treatment of High Blood Pressure in Children and Adolescents (1) and incorporated new height percentile data and new information about normal blood pressure from the 1999–2000 National Health and Nutrition Examination Survey (NHANES). Like the previous revision in 1996 (8), the updated 2004 tables classify blood pressure by gender, age and height for children 1–17-years old, with the height assessment included to minimize misclassifying children as hypertensive, when they are very tall or very short for age. Adolescents 18 years of age and older are considered adults and addressed in the 2003 Seventh Report of the Joint National Committee (JNC 7)(9), which set the guidelines for all adults aged 18 years or older, but did not take into account their age, gender or height. The 2004 Fourth Report update for children and adolescents includes normative data for multiple blood pressure percentiles to allow classification of childhood hypertension into stages parallel to those recommended for adults in the JNC 7 Report. The JNC 7 Report set the upper limit of normal blood pressure for all adults at 120/80. Pre-hypertension was classified as blood pressure between 120/80 and 139/89; stage 1 hypertension, as between 140/90 and 159/99; and stage 2 hypertension, as greater than or equal to 160/100. The 2004 Fourth Report recommended a similar classification of childhood hypertension as follows: normal blood pressure is defined as less than the 90th percentile blood pressure from the tables, or less than 120/80 for adolescents no matter what the 90% percentile; pre-hypertension is blood pressure between the 90th and 95th percentiles, or greater than or equal to 120/80 up to less than the 95th percentile for adolescents; stage 1 hypertension is blood pressure between the 95th percentile to 5 mg Hg higher than the 99th percentile; and stage 2 hypertension is greater than 5 mm Hg higher than the 99th percentile. Adding the 99th percentiles to the tables facilitates identification of stage 2 hypertension, which is the most severe and dictates more rapid evaluation and

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Table 62-1
Commonest Causes of Hypertension by Age at Presentation

Age Group	Cause
Newborn	Renal artery thrombosis or embolus (umbilical artery catheter)
	Renal vein thrombosis
	Congenital renal malformations
	Coarctation of aorta
	Renal artery stenosis
	Bronchopulmonary dysplasia
Infancy to 6 years	Renal parenchymal disease
	Renal artery stenosis
	Coarctation of the aorta
	Medications (corticosteroids, albuterol, pseudoephedrine)
	Endocrine causes
6–10 years old	Renal parenchymal disease
	Renal artery stenosis
	Primary (essential) hypertension
	Endocrine causes
Adolescence	Primary (essential) hypertension
	White coat hypertension
	Renal parenchymal disease
	Substance abuse (cocaine, amphetamines, methamphetamines, phencyclidine, methylphenidate, caffeine)
	Endocrine causes

therapy. As before, auscultation of the fifth Korotkoff sound (point at which the sound disappears) is still recommended to determine diastolic blood pressure in all age groups. In some young children no fifth sound can be heard, because sounds are still audible at 0 mm Hg, even with less pressure applied to the stethoscope head. The new recommendation for these children is to use the fourth Korotkoff sound (point at which the sound muffles) as the diastolic blood pressure (1).

The Fourth Report does not address blood pressure for infants less than 1 year of age, and very little data is available for this age group (10). The 1987 Second Task Force on Blood Pressure (11) includes standards for both systolic and diastolic blood pressure for awake infants, ages birth to 12 months, based on measurements obtained with either Doppler instruments or sphygmomanometers and the fourth Korotkoff sound to determine diastolic blood pressure. Blood pressure is lower in sleeping infants, so correction factors of 7 mm Hg for systolic and 5 mm Hg for diastolic blood pressure are recommended when using sleeping measurements to assess for hypertension.

Normal blood pressures for children and adolescents (1) or for adults (9) are based on measurements made manually with a sphygmomanometer and auscultation using a stethoscope. Even though automatic oscillometric devices, such as the Dinamap™, are in wide spread use in pediatric hospitals in North America, especially for neonates, infants and toddlers, little data and no established reference standards exist for normal blood pressure in children using these devices (12–15). In practice, values obtained with automatic oscillometric devices are often treated as if they were obtained manually and compared to the standard blood pressure tables. In the few studies comparing measurements with oscillometric devices to conventional manual sphygmomanometry in children, the blood pressures were not equivalent and the errors were not systematic for all ages (13, 14). In addition, few of the oscillometric devices on the market have actually been found to fulfill strict criteria for accuracy (15). Therefore, in cases of pre-hypertension and stage 1 hypertension, manual sphygmomanometry should be performed to confirm the diagnosis.

The conditions under which blood pressure is measured are also important, but sometimes not able to be followed precisely in a busy outpatient clinic or emergency room. The 2004 Fourth Report (1) recommends that blood pressure be taken on three separate occasions in a controlled environment, preferably in the right arm with the cubital fossa at heart level when the patient is seated, has rested quietly for 5 min, and has avoided stimulant drugs or foods. The inflatable bladder of the cuff, not the entire cuff, should be an appropriate size for the patient. The bladder width should be approximately 40% of the arm circumference midway between the acromion and olecranon; the length of the bladder should cover 80–100% of the circumference of the arm without overlapping; and the bladder width to length ratio should be at least 1:2. If the only cuffs available are either too small or a bit too large for the patient, the larger cuff should be chosen, because the error will be less than when the cuff is too small. Cuff sizes are not yet standardized as recommended by the 2004 Fourth Report (1), so determination of appropriate cuff size should be made according to the above guidelines and not by the name written on the cuff; for example, a small adult cuff might be just the right size for a tall or obese 7-year old. Measuring blood pressure accurately may be difficult in a severely

obese adolescent, unless a large adult thigh cuff is applied to the arm in a "cone" configuration. Sometimes, particularly in a small or ill child, blood pressure is most conveniently measured in the leg with an arm cuff. When abnormal, the blood pressure should be retaken in the right arm to be certain of the diagnosis of hypertension. If a patient has coarctation of the aorta, blood pressure may be greatly reduced in the legs and possibly the left arm, depending on the location of the coarctation, but will still be elevated in the right arm.

High blood pressure readings may be transient. A blood pressure reading in a crying, moving or anxious child is likely to be high, but not reliable for diagnosing hypertension. Blood pressure should be repeated several times when the child is calm. If initial values indicate pre-hypertension or stage 1 hypertension, blood pressure should be taken on repeated visits over days to weeks before diagnosing hypertension or embarking on a detailed diagnostic evaluation (1). Blood pressure tends to decrease on subsequent visits as anxiety subsides and the patient becomes more accustomed to the measurement. If white coat hypertension is suspected after focused detailed history and physical examination and minimal screening laboratory tests, ambulatory blood pressure monitoring (ABPM) is the next step in the evaluation, both for diagnosis (1, 5, 16) and cost-effectiveness (17). ABPM is measured with a wearable oscillometric blood pressure device usually placed on the non-dominant arm, which automatically measures and records blood pressure at prescribed frequent intervals (e.g., every 20 min when awake and every 30 min during sleep) over an entire 24-h period. ABPM allows evaluation of blood pressure throughout the day in the patient's own environment to reduce anxiety-induced elevations in blood pressure. Frequency of hypertension throughout the day (blood pressure load) and changes in the normal circadian pattern of blood pressure are assessed, which cannot be done by casual blood pressure measurements in the clinic or at home. In addition, ABPM sometimes uncovers masked hypertension, a condition in which the blood pressure is mostly normal in the clinic setting, but high during ABPM (5, 16). ABPM is discussed in detail in Chapter 61.

Assessment of Severity and Duration of Hypertension

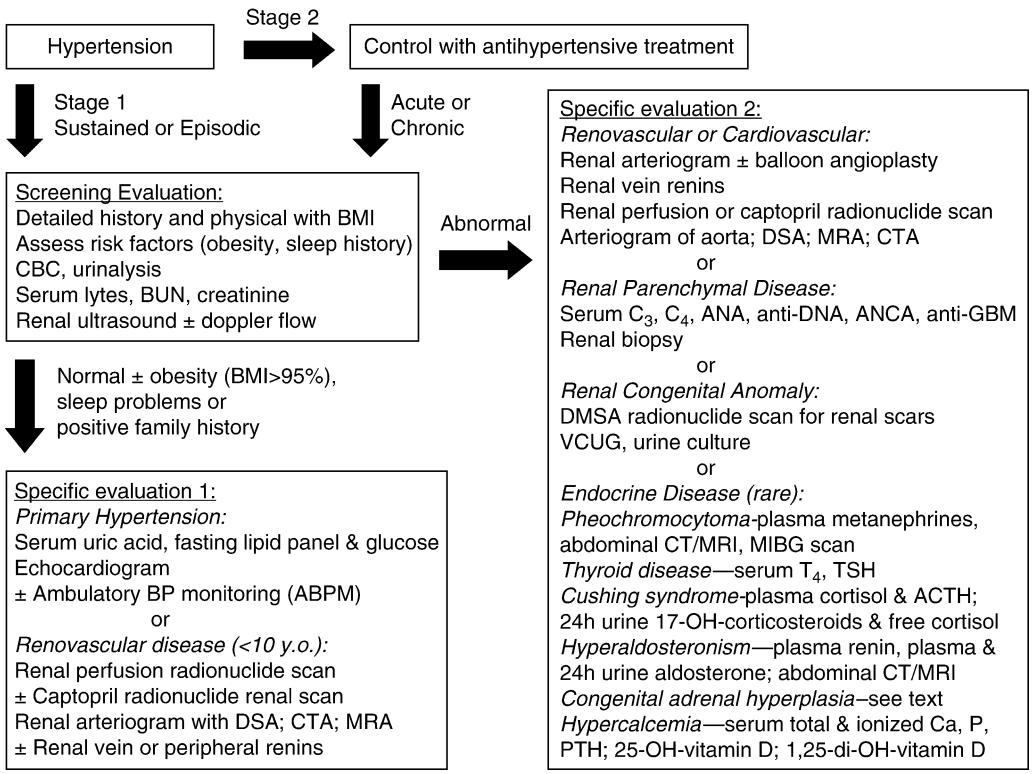
When repeated blood pressure measurements taken under appropriate conditions satisfy the definition of hypertension (1, 9), the stage, either 1 or 2, will direct further evaluation (*Fig. 62-1*). A quick guide for assessment of the stage of hypertension by age, gender and height percentile is given in *Table 62-2*. For stage 1 hypertension, diagnostic evaluation may continue before treatment is instituted. For stage 2 hypertension, emergent treatment should be undertaken to reduce the level of severe hypertension before further diagnostic evaluation (*Fig. 62-1*). If possible, laboratory tests that might be affected by treatment, such as peripheral plasma renin levels, should be drawn prior to initiating therapy.

Whether hypertension is acute or chronic, transient or sustained in duration, or episodic will help direct further evaluation. Transient or unsustained hypertension may need only clinical follow-up to be sure it does not recur. Episodic hypertension is best evaluated at the time of an occurrence, as evaluation may be unrevealing between attacks. Acute hypertension is often secondary to other diseases, which may have specific signs and symptoms to direct specific evaluation at the time of presentation.

Duration of hypertension is first assessed by a thorough hypertension-oriented history and physical examination (1). Headaches, blurred vision, seizures, chest pain and frequent epistaxis are usually associated with acute onset of hypertension. Episodic symptoms and hypertension are associated with pheochromocytoma or autonomic nervous system dysfunction. A detailed medication history for both prescription and over-the-counter medications, including herbal medicines, may help identify the etiology of acute, transient or episodic hypertension. Recent introduction of medications for which hypertension is a known side effect, like high dose corticosteroids (18) or albuterol for asthma, large (adult) doses of pseudoephedrine for cold symptoms, or phenylephrine eye or nose drops, may be the cause of new-onset, but transient hypertension. Adolescents are at risk for substance abuse and should be interviewed in private in order to maximize the chance of obtaining a history of the use of illicit drugs, tobacco or high-caffeine drinks, all of which can cause or contribute to hypertension (1, 19–21). Restless sleep, sleep apnea, irritability, anorexia and poor school performance are subtle signs of chronic hypertension and should be specifically elicited in the history. Poor growth may also be a sign of chronic hypertension or underlying chronic kidney disease. Obesity with BMI greater than the 95th percentile for age and gender is often associated with chronic primary hypertension (1, 3–5). The presence of peripheral edema, pleural effusion, rash, or swelling and tenderness of joints suggest acute onset or exacerbation of underlying renal or systemic disease. Retinal changes usually indicate long-standing, untreated hypertension and include arteriolar narrowing or tortuosity, arterio-venous nicking and hemorrhages and exudates (*Fig. 62-2*).

Figure 62-1

Algorithm for evaluation of hypertension in children and adolescents. Abbreviations: CBC, clinical blood count; BUN, blood urea nitrogen; DSA, digital subtraction angiography; MRA, magnetic resonance angiography; C_3 and C_4, complements 3 and 4; ANA, anti-nuclear antibody; anti-DNA, anti-double stranded desoxynucleic acid antibody; ANCA, anti-neutrophil cytoplasmic antibody; anti-GBM, anti-glomerular basement membrane antibody; DMSA, dimercapto-succinic acid; VCUG, voiding cystourethrogram; DSA, digital subtraction angiography CTA, computed tomographic angiography; MRA, magnetic resonance angiography; MIBG, metaiodobenzylguanidine; T_4, thyroxine; TSH, thyroid stimulating hormone; ACTH, adrenocorticotrophic hormone; OH, hydroxy; DOC, deoxycorticosterone; Ca, calcium; P, phosphorus; PTH, parathyroid hormone.



Screening Evaluation of Hypertension

Episodic or sustained, acute or chronic hypertension, whether stage 1 or stage 2, merits further screening and specific detailed evaluation as dictated by the screening. A convenient algorithm for evaluation of pediatric hypertension is shown in *Fig. 62-1*. The direction of further detailed laboratory and radiographic or biopsy evaluation of hypertension should become apparent after taking a detailed personal and family history, performing a careful physical examination and ordering a few simple laboratory tests.

The personal history should include specific questioning regarding the occurrence of headaches, sleep disturbance, visual symptoms, nosebleeds, palpitations, episodic rapid pulse, pallor or flushing, joint pains, rash, edema, gross hematuria, excessive weight gain or loss or decreased height growth. Neonatal history of low birth weight or the use of umbilical artery catheters may provide clues to the diagnosis of primary hypertension in an adolescent (22–24) or hypertension from renal artery thrombosis or emboli in an infant, respectively. Detailed dietary history may reveal excessive intake of sodium or caffeinated beverages, especially soft drinks or energy drinks (21). A medication history should include specific questions about over-the-counter drugs like pseudoephedrine or herbal preparations like ephedra, St. John's Wort or licorice (25, 26), as well as prescription drugs. Adolescents should be questioned in private to obtain a history of substance abuse or the possibility of pregnancy. Family history of hypertension, heart attacks or stroke is particularly important for children with primary hypertension.

Table 62-2

Quick Guide for Assessment of Stage of Hypertension (HTN) by Age, Gender & Height (Ht) Percentile

Age Group	Percentiles of Blood Pressure (mm Hg) for Ht Percentile			
	Stage 1 HTN[a]: 95%		Stage 2 HTN[a]: >99% + 5	
Boys	*Ht 5%*	*Ht 95%*	*Ht 5%*	*Ht 95%*
Newborn[b]	90/–	90/–	>106/–	>106/–
1–12 months[b]	98/55	106/59		>115/75
1 year	98/54	106/58	>110/66	>119/71
3 years	104/63	113/67	>115/76	>125/80
5 years	108/69	116/74	>120/82	>128/87
10 years	115/77	123/82	>127/90	>135/95
13 years	121/79	130/83	>132/92	>142/96
15 years	126/81	135/85	>139/93	>147/98
17 years	131/84	140/89	>144/97	>152/102
18 years or older[c]	No data	140/90	No data	>160/100
Girls				
Newborn[b]	88/–	88/–	>106/–	>106/–
1–12 months[b]	101/57	107/60		>115/75
1 year	100/56	107/60	>113/69	>119/72
3 years	104/65	110/69	>116/78	>122/81
5 years	107/70	113/74	>119/83	>125/86
10 years	116/77	122/80	>128/89	>134/93
13 years	121/80	128/83	>132/92	>140/96
15 years	124/82	131/85	>136/94	>143/98
17 years	125/82	132/86	>138/95	>144/98
18 years or older[c]	No data	140/90	No data	>160/100

BP blood pressure; *HTN* hypertension; *Ht* height

[a]Adapted from blood pressure tables in the 2004 Fourth Report on Diagnosis, Evaluation and Treatment of High Blood Pressure in Children and Adolescents (1) for blood pressure measured by auscultation with a sphygmomanometer on at least 3 separate occasions; if systolic and diastolic categories differ, categorize by the higher value

[b]Adapted from the Report of the Second Task Force on Blood Pressure Control in Children – 1987 (11);

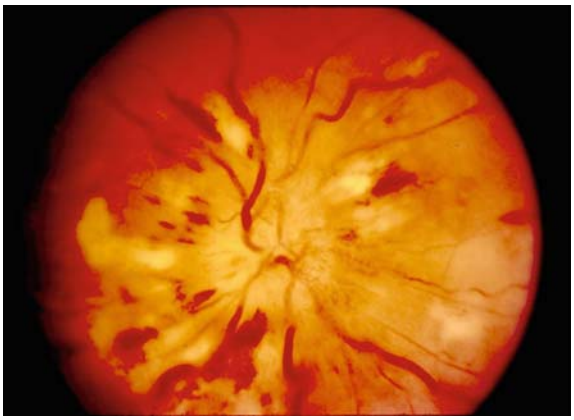
[c]Adapted from the 2003 Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (9).

A large percentage of children with primary hypertension will have a close relative with the same disease (2, 3, 8, 11).

Physical examination may reveal specific signs of genetic disease, such as the elfin facies of Williams syndrome, the café-au-lait spots and small skin neurofibromata of neurofibromatosis type I, the physical features of Cushing syndrome, or ambiguous genetalia associated with congenital adrenal hyperplasia or Denys-Drash syndrome. Rarely, unilateral facial paralysis (Bell's palsy of the seventh cranial nerve) can be the initial presentation of severe hypertension in a child (27, 28). Careful auscultation of the abdomen may reveal a bruit suggestive of renovascular disease, but a bruit is audible only about 50% of the time in these patients (29). Femoral pulses may be diminished in patients with coarctation of the aorta or middle aortic syndrome. Determination of height, weight and BMI with comparison to percentiles for age and gender should always be included as part of the screening physical examination.

Screening laboratory evaluation should be minimally invasive and cost-effective. Screening for rare endocrine causes of hypertension should not be done initially in every pediatric patient with hypertension. Screening studies for all patients should include clinical blood count, urinalysis, and serum electrolytes, creatinine and BUN (1–3). In adolescents with suspected primary hypertension and

Figure 62-2
Severe hypertensive retinopathy in a child with long-standing untreated hypertension showing arteriolar narrowing, hemorrhages and exudates and papilledema. (See color plate 38)



no suspicion for endocrine abnormalities, serum electrolytes may not be that useful. However, a low potassium concentration with metabolic alkalosis may indicate a rare, but treatable disorder, like primary or secondary hyperaldosteronism or Liddle syndrome. Elevated serum potassium in conjunction with metabolic acidosis may suggest chronic renal disease, which is confirmed by the presence of an increased serum creatinine concentration. Creatinine clearance can be estimated from serum creatinine (new Schwartz formula) (30) or measured directly with a 24-h urine collection. Since renal disease is the most common cause of hypertension in children and the kidney is also a target organ for damage from untreated hypertension, urinalysis is an important screening test. Hematuria and red cell casts with or without proteinuria suggests glomerular disease. Isolated hematuria also may be associated with dilatation of the urinary tract or trauma. Proteinuria may be seen in non-glomerular conditions such as reflux nephropathy, obstructive uropathy, or interstitial nephritis, as well as glomerular diseases like focal segmental glomerulosclerosis or membranoproliferative glomerulonephritis. Proteinuria alone may be a sign of end-organ damage from hypertension, but must be distinguished, especially in adolescents, from orthostatic (postural) proteinuria, which is a benign condition.

A renal ultrasound examination is a simple, non-invasive test that is appropriate to perform early in the evaluation of any hypertensive child (1–3). Although it may not be provide much information for the adolescent in whom primary hypertension is strongly suspected, a renal ultrasound helps exclude congenital anomalies that have previously gone undetected. The renal ultrasound provides information about the size and architecture of each kidney and the lower urinary tract. Abnormal kidneys may be small or asymmetric (renovascular disease, vesicoureteral reflux, or dysplasia); hyperechoic, symmetric and normal or large (renal parenchymal disease like glomerulonephritis); or large with or without cysts (polycystic kidney disease, multicystic dysplastic kidney). Hydronephrosis and/or hydroureters may be associated with congenital obstructive uropathy or vesicoureteral reflux.

Color-coded Doppler analysis with B-mode ultrasonography is termed duplex ultrasonography (31) and provides information about the patency and flow within the main renal vessels, including the more distal segments of the main renal artery, to help diagnose renovascular hypertension. During Doppler evaluation care should be taken to scan the entire artery from origin at the aorta to renal hilum to improve sensitivity and accuracy. Obtaining measurements in two views, both anterior and oblique, improves identification of fibromuscular dysplasia in the middle to distal segments of the main renal artery. The limited amount of body fat in many children and the proximity of renal vessels to the skin surface allow the use of high-frequency transducers to improve the technical quality (32); obese patients provide a technical challenge. Color Doppler images can demonstrate global perfusion as well as patency of flow through large renal arteries and veins. Turbulence at a stenotic site alters peak systolic and end diastolic blood flow velocities and creates a characteristic pattern of flow detected by the spectral Doppler waveform (*Fig. 62-3*). The sensitivity of the Doppler flow study is limited for detection of stenosis in accessory renal arteries, intrarenal arteries or mildly stenotic arteries (32, 33). A positive Doppler flow study is helpful in directing further evaluation, but a negative study does not rule out significant renovascular disease.

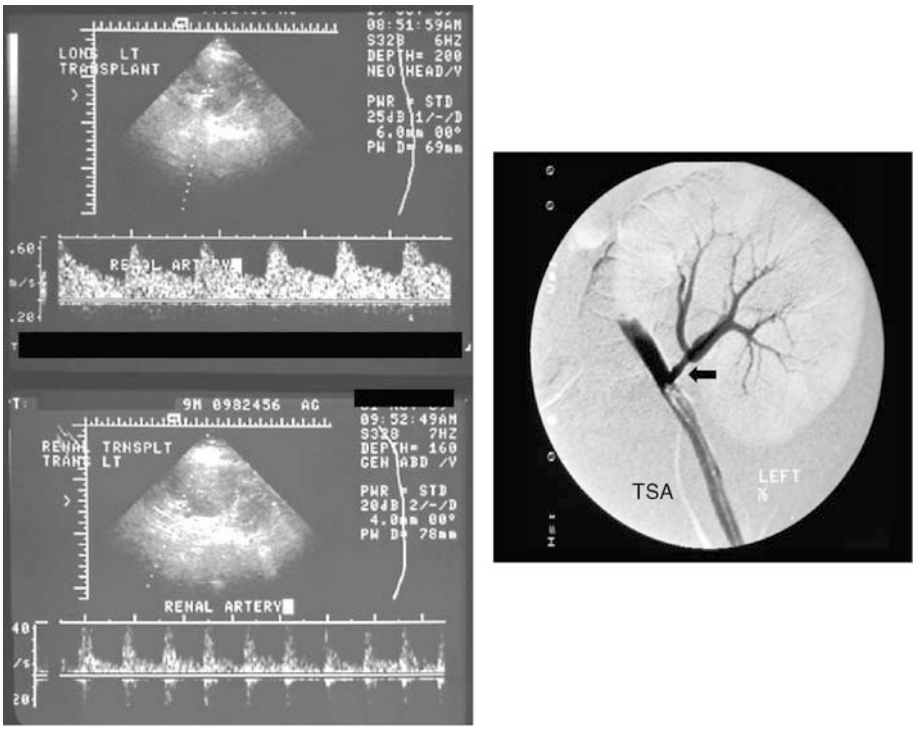
Directed Specific Evaluation of Hypertension after Screening

Specific Evaluation 1: If the screening evaluation is normal or only positive for obesity, sleep problems or a positive family history for essential hypertension, detailed evaluation should be directed toward the diagnosis of primary hypertension (*Fig. 62-1*). Since primary hypertension is largely a diagnosis of exclusion and less common in young children, renovascular disease must always be ruled out, especially if the child is less than 10-years old.

Primary hypertension: The specific evaluation of primary hypertension is designed to determine the presence

Figure 62-3

Renal transplant ultrasound with Doppler flow evaluation in a child. *Left upper panel:* The resistive index is high (0.6) and the flow pattern turbulent, suggesting transplant renal artery stenosis, which was confirmed by digital subtraction angiography (DSA) (arrow) shown in the *Right panel*. *Left lower panel:* Normalization of the Doppler flow study with resistive index lower (0.4) and no turbulence after successful balloon angioplasty of the stenosis.



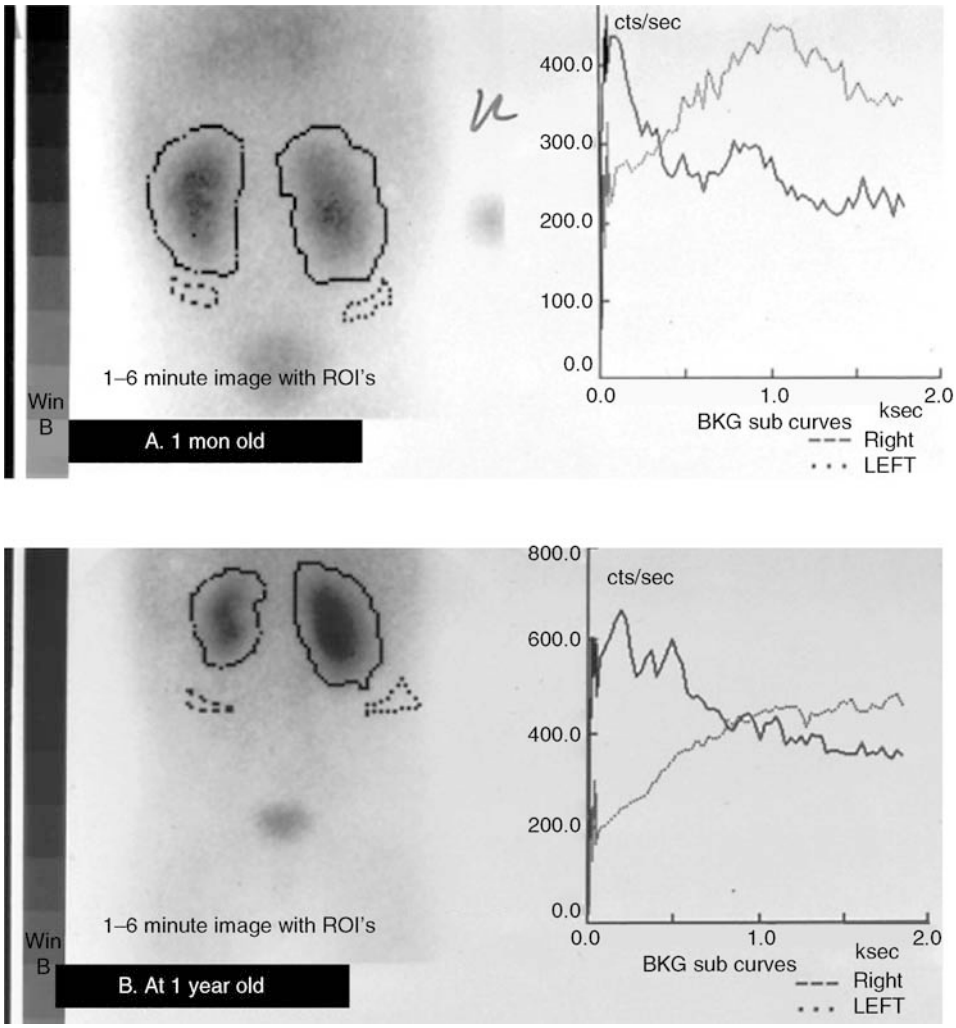
of cardiovascular risk factors, such as hyperlipidemia and insulin resistance, and to assess any end-organ damage to the heart or kidneys (1). In children elevation of serum uric acid level may help distinguish primary from secondary hypertension. Many decades ago increased serum uric acid concentrations were noted in children with primary hypertension, but largely ignored until recent reports that serum uric acid is significantly higher at presentation in children with primary hypertension compared to children with secondary hypertension (34). Elevated uric acid likely plays an early pathogenetic role in childhood hypertensives by activating cellular pathways that lead to increased renovascular tone, fibrosis and irreversible arteriolosclerosis (34). ABPM may help distinguish primary from secondary hypertension, the latter of which is more likely to have greater daytime diastolic blood pressure load, greater nocturnal systolic blood pressure load and blunted nocturnal dipping (35).

End-organ damage may already be evident at presentation of primary hypertension in adolescents. An echocardiogram is the best way to screen for left ventricular hypertrophy and will also serve as a baseline study for comparison later in the course of therapy (1). ABPM may also be useful to identify which children may be at greatest risk for developing end-organ damage from hypertension (5, 16, 36).

Renovascular disease: Renovascular disease is the cause of hypertension in about 10% of the children referred for diagnosis (37, 38). Children with renovascular hypertension are frequently asymptomatic (38). Their hypertension is often severe and may be hard to control with medication before proceeding with specific evaluation (38). An angiotensin converting enzyme inhibitor (ACEI) or angiotensin-receptor blocker (ARB) may provide the best control, but with prolonged use, can also lead to loss of function in the affected kidney from reduced arterial flow in an area of severe stenosis (*Fig. 62-4*). Patients with bilateral renal artery stenosis are at risk for developing acute renal failure at the onset of ACEI or ARB therapy. A child's response to an ACEI, such as captopril, during radionuclide imaging, with either diethylenentriamine pentaacetic acid (DTPA), mercaptoacetyltriglycine

Figure 62-4

Sequential ^{99}Tc-DTPA radionuclide renal scans in an infant with unilateral left renal artery stenosis treated medically, because body size was too small to allow transluminal balloon angioplasty. A. At diagnosis at 1 month old; 40% function left kidney, 60% function right kidney. B. At 1 year old after 11 months of oral ACEI therapy for left main renal artery stenosis; 26% function left kidney, 74% function right kidney.



(MAG3) or dimercapto-succinic acid (DMSA), can be used in the evaluation for renal artery stenosis and is referred to as a captopril renal scan (38–41). Although a captopril renal scan may help lead to a diagnosis, the test is invasive and only moderately sensitive (50–70%), especially if disease is bilateral (38–41). Since a renal arteriogram is both diagnostic and potentially therapeutic when coupled with balloon angioplasty, a captropril renal scan may not be a reasonable step in the evaluation of children with a high likelihood of renal artery stenosis at centers with skilled pediatric interventional radiologists. A renal perfusion scan with DTPA or MAG3 may be useful to identify segmental areas of hypoperfusion or infarction, especially those associated with an embolus from an umbilical artery catheter used in the neonatal period (42, 43). Intravenously administered MAG3 is initially filtered by the glomerulus, then taken up into the kidney primarily by proximal tubular secretion, so MAG3 can be used for estimation of tubular function as well as renal plasma flow.

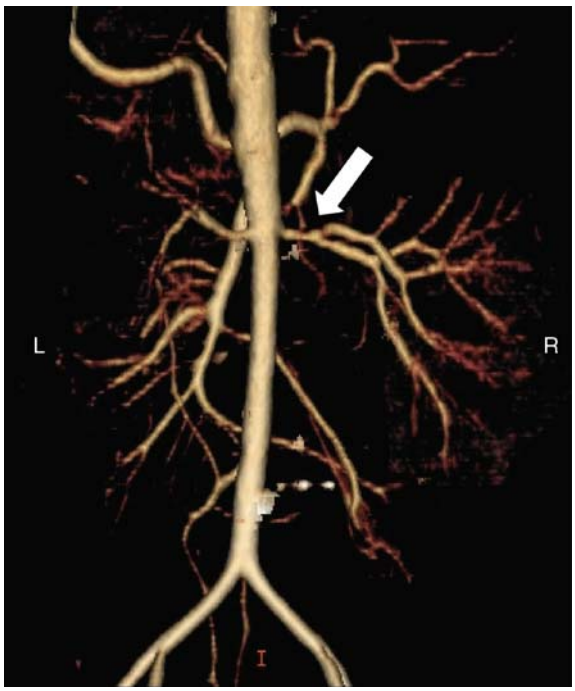
Selective renal arteriography with or without digital subtraction angiography (DSA) enhancement is the gold standard for diagnosis of renal artery stenosis. In a retrospective review of 15 years experience with renal

arteriography for pediatric hypertensive patients, Shahdadpuri and colleagues (44) showed that renal arteriography had a high positive yield of 40% of cases with diagnostic findings, when performed for the indications of severe hypertension or inability to control hypertension effectively with one drug. Renal arteriography has the added advantage that therapeutic transluminal balloon angioplasty (45–50) or placement of bridging stents (38, 49, 50) can be performed during the same procedure. Balloon angioplasty may be possible even in small children, using balloon catheters designed for coronary artery angioplasty (51). The disadvantage of renal arteriography is that it is invasive, exposes the child to nephrotoxic radiocontrast agents and usually requires general anesthesia for small or uncooperative children (39). Deep conscious sedation may be effective for cooperative older children and adolescents. If performed by an experienced interventional radiologist, the renal arteriogram can be done safely as a one-day outpatient procedure in most children. The incidence of severe complications, such as intimal tear or injury, renal artery thrombosus or branch embolus, is very low in experienced hands. Patients need to be observed for 4–6 h post-procedure for possible complications. Arterial puncture carries the risk of hematoma formation at the site and vascular compromise to the extremity.

Computed tomographic angiography (CTA) and magnetic resonance angiography (MRA) are 80–90% accurate alternatives to renal arteriogram for evaluation of renovascular disease (52–54). Stenosis of accessory renal arteries or of the more distal portions of renal arteries, as in patients with fibromuscular dysplasia, are more likely to be missed with these techniques. Both modalities allow scans of high resolution within a 10–30 s breath hold (52), so movement artifact is minimized for children, especially with the shorter 5–10 s breath hold for CTA. CTA requires only a peripheral intravenous approach, produces excellent images of the renal vasculature and allows 3-dimensional (3D) visualization with appropriate software (52, 55) (*Fig. 62-5*). A limited CT scan of the abdomen may be performed before CTA to provide additional information about the anatomy of the kidneys, adrenals and surrounding structures (31). CTA is very useful for evaluation of children and adolescents, in whom the need for invasive renal angiography is not clear-cut (55). The disadvantage of CTA is that it uses ionizing radiation and requires nephrotoxic iodinated radiocontrast agents (31, 52). MRA has the advantages of being minimally invasive and avoiding ionizing radiation, but requires the use of gadolinium contrast enhancement, which puts patients with chronic renal failure, especially those with less than 30% renal function, at risk for nephrogenic systemic fibrosis (56–58). At our center MRA has been a useful evaluation technique for infants and children too small to undergo balloon angioplasty, as well as for neonates (59), but currently we avoid MRA for any patient with even mild renal failure.

Figure 62-5

Computer reconstructed 3D CTA image from a 2.5-year old boy with failure to thrive and severe hypertension, showing mild narrowing of the distal aorta and stenosis of the right main renal artery and its proximal branches (arrow). (See color plate 39)



Plasma renin activity measured in samples from peripheral veins or especially from selective renal veins may provide additional useful information for evaluation of renovascular disease (38, 60). Selective sampling of renal vein renins may be done conveniently at the time of renal arteriogram to provide supporting evidence for unilateral or bilateral disease. The inferior vena cava above and below the renal veins should be sampled at the same time as the renal veins. If the selective renal vein renin concentration is at least 1.5 times greater than concentration in the contralateral kidney, the result is diagnostic for renin-mediated hypertension from that kidney (60). If the contralateral kidney is normal, renal vein renin concentration should actually be decreased due to down-regulation of renin production in the normal kidney from increased circulating angiotensin II (61, 62).

If the ratio between renal vein renin from the contralateral kidney and caudal inferior vena cava is <1.3, then renin production is suppressed in the contralateral kidney (60). The combination of results may be useful to determine whether a patient might benefit or be cured by surgery (60), in the event that angioplasty is only partially successful or not able to be performed.

Peripheral vein renin levels obtained before initiation of antihypertensive therapy may also be useful in evaluation of renovascular hypertension, if they are abnormal (1). Levels must be interpreted within the age-specific normals for the laboratory performing the test and in light of estimated sodium intake at the time the level was drawn. Furthermore, the levels may be adversely affected if the blood is not handled properly from the time of the blood draw. The specimen should be drawn into an EDTA tube, immediately placed on ice, spun in a refrigerated centrifuge and then kept frozen for later assay. A peripheral renin level that is elevated for age suggests renovascular or renal parenchymal hypertension. A normal level, however, does not exclude significant renovascular hypertension. Low levels of plasma renin in the presence of hypokalemia and elevated plasma and urinary aldosterone suggest primary aldosteronism or one of the forms of congenital adrenal hyperplasia as the cause of hypertension (see Endocrine section below).

Specific Evaluation 2: When the history, physical examination or screening laboratory evaluation is abnormal, further evaluation should be directed by those results. If the history, physical, urinalysis and other laboratory tests suggest renal artery stenosis or other vascular disease (*Table 62-3*) or renal parenchymal disease (*Table 62-4*), evaluation should proceed along the lines outlined in the algorithm (*Fig. 62-1*). If initial screening rules out renal disease, but suggests a rare endocrine cause (*Table 62-5*), evaluation should proceed in a different way (*Fig. 62-1*).

The need for evaluation for other causes of childhood hypertension, such as neurological abnormalities, teen pregnancy, drugs or other illnesses (*Table 62-6*), should become apparent after a detailed screening history and physical examination. The rest of the discussion in this chapter will be divided into evaluation of hypertension by disease category.

Renovascular and Cardiovascular Disease

Renovascular Disease: Multiple types of renovascular abnormalities can lead to hypertension (*Table 62-3*), but fibromuscular dysplasia is the most common, occurring in approximately 70% of pediatric cases (37, 63, 64). Most often, the medial layer of the artery is affected, although rarely the intima may be involved (65), especially in the case of neurofibromatosis type 1 (66) or Williams syndrome (67). Disease may be bilateral or unilateral, but if unilateral, may manifest itself with hypertension from the other kidney at a later date. Multiple stenotic lesions followed by post-stenotic aneurysms can resemble a string of beads on a selective angiogram (*Fig. 62-6*). Branch vessels, as well as peripheral intrarenal vessels, may be involved. Other vessels, including the abdominal aorta may be stenotic or hypoplastic, especially in association with neurofibromatosis type 1 (66) (*Fig. 62-7*) or Williams syndrome (67, 68). Typically, the lesions of

Table 62-3
Renovascular and Cardiovascular Causes of Hypertension

Intrinsic renal artery disease
Fibromuscular dysplasia
Intimal fibromuscular dysplasia (neurofibromatosis type 1, Williams syndrome)
Arteritis (Kawasaki, Takayasu, or Moyamoya disease)
Renal transplant artery stenosis
Chronic renal allograft nephropathy
Newborn with umbilical vessel catheters
Arterial or venous thrombosis
Segmental infarction from embolus
Renal transplant renal artery or venous thrombosis
Renal trauma
Extrinsic compression
Neoplasia
Wilms tumor
Neuroblastoma
Pheochromocytoma, paraganglioma
Neurofibroma
Lymphoma
Perirenal hematoma, trauma
Retroperitoneal fibrosis
Congenital fibrous bands
Cardiovascular
Coarctation of aorta
Middle aortic syndrome (hypoplastic abdominal aorta syndrome)
Williams syndrome
Turner syndrome

Table 62-4

Renal Parenchymal Diseases Associated with Hypertension

Glomerulonephritis
Acute post-infections glomerulonephritis
IgA nephropathy
Membranoproliferative glomerulonephritis
Rapidly progressive (crescentic) glomerulonephritis
Focal segmental glomerulosclerosis
Systemic vasculitis with renal involvement
Henoch Schonlein Purpura
ANCA vasculitis
Polyarteritis nodosum
Systemic lupus erythematosis
Hemolytic uremic syndrome
Interstitial nephritis (chronic pyelonephritis)
Hereditary diseases
Autosomal recessive or dominant polycystic kidney disease
Medullary cystic disease; juvenile nephronophthisis
Denys-Drash syndrome
Sickle cell disease
Liddle syndrome
Congenital renal anomalies
Vesicoureteral reflux
Obstructive uropathy
Multicystic dysplastic kidney
Horseshoe kidney
Segmental hypoplasia (Ask-Upmark kidney)

Table 62-5

Endocrine Abnormalities Associated with Hypertension

Tumors secreting vasoactive substances (catecholamines, renin)
Pheochromocytoma, paraganglioma
Sporadic or hereditary (associated with von Hippel Lindau disease, multiple endocrine neoplasias type II, neurofibromatosis)
Neuroblastoma, ganglioneuroblastoma
Juxtaglomerular cell tumors, Wilm's tumor
Thyroid disorders
Hyperthyroidism (Grave's disease)
Hypothyroidism
Cushing syndrome
ACTH-dependent
Iatrogenic (exogenous glucocorticoid therapy)
Adrenocortical tumor (adenoma, carcinoma)
ACTH-independent
Iatrogenic (ACTH therapy)
Hypothalmic or ectopic CRH-producing tumor
Pituitary or ectopic ACTH-producing tumor
Hyperaldosteronism
Adrenal tumor (adenoma, carcinoma)
Idiopathic adrenal hyperplasia
Congenital adrenal hyperplasia
11β-hydroxylase (P450c11) deficiency
17α-hydroxylase (P450c17) deficiency
Hypercalcemia
Vitamin D intoxication
Hyperparathyroidism
Williams syndrome
Malignancy

ACTH, adrenocorticotropic hormone; CRH, corticotropin-releasing hormone; P450c11 or 17, cytochrome P450 enzyme

neurofibromatosis are near the origin of the renal arteries (*Fig. 62-7*), whereas the lesions of fibromuscular dysplasia are often more distal (37, 45) (*Fig. 62-8*). Duplex ultrasonography with color Doppler flow may suggest the diagnosis of these disorders, but renal arteriogram is needed for diagnosis and delineation of the extent of the renovascular disease. If the lesion is in the main renal artery and does not involve a long segment of the vessel, transluminal balloon angioplasty may be performed at the same time (45–50) (*Fig. 62-8*). If the child is too small for balloon angioplasty, hypertension may be controlled with medical therapy, especially ACEI or ARB. Caution should be exercised when using ACEI or ARB in the presence of bilateral disease, because of the risk for inducing renal failure. A radionuclide renal scan is a useful tool to sequentially evaluate growth and function of the affected kidney during long-term follow-up (*Fig. 62-4*).

Duplex ultrasonography with color Doppler is a good screening evaluation test for renal transplant artery stenosis (69), which usually occurs at or near the surgical anastomosis (*Fig. 62-3*). The incidence is as high as 10% in children and adults (69, 70). Confirmatory arteriography is always necessary (*Fig. 62-3*), and therapeutic balloon angioplasty may be done at the same time if indicated. Duplex ultrasonography is also useful for follow-up to determine the continued success or recurrence of stenosis after balloon angioplasty or surgical intervention (69, 70) (*Fig. 62-3*).

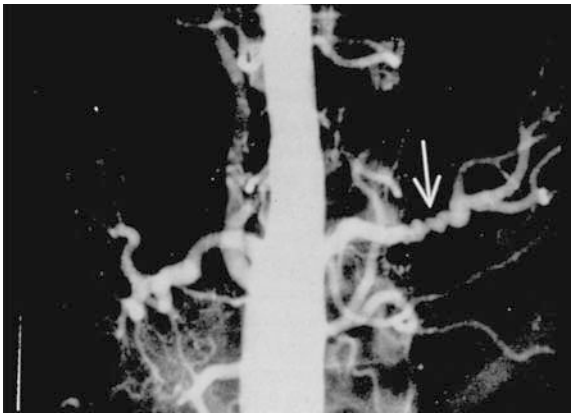
Table 62-6

Miscellaneous Causes of Hypertension

Neurologic abnormalities
Elevated intracranial pressure (associated bradycardia)
Recent seizure, status epilepticus
Familial dysautonomia (Riley-Day syndrome)
Quadriplegia with autonomic nervous system dysfunction
Femoral nerve traction
Cyclic vomiting syndrome
Polycythemia; recombinant erythropoietin therapy
Anesthetic drugs
Ketamine
Naloxone
Drug abuse
Cocaine
Amphetamines
Methamphetamines (e.g., Ecstasy)
Phencyclidine (PCP)
Methylphenidate
Oral contraceptives
Teen pregnancy

Figure 62-6

Renal arteriogram showing typical "string-of-beads" appearance (arrow) of fibromuscular dysplasia in the left main renal artery.



Mild to severe hypertension in transplant patients is associated with chronic renal allograft nephropathy, which includes chronic rejection, donor vascular disease and chronic calcineurin inhibitor nephrotoxicity, all of which lead to arterial fibrous intimal thickening, interstitial fibrosis, tubular atrophy and glomerulosclerosis late in the course after renal transplantation (71). The small arteries are affected, so imaging is not useful for diagnosis. The diagnosis is made by renal transplant biopsy.

Rarely, hypertension in a child results from extrinsic compression of a renal artery or segment by an extrinsic mass, such as a tumor or traumatic perirenal hematoma. Renal ultrasound may identify the mass, but CTA or MRA technology allows extensive 3D detail to establish the diagnosis (*Fig. 62-9*) and direct corrective therapy. More than half of patients with Wilms' tumor have concomitant hypertension secondary to either intrarenal vessel compression or renin production by the tumor (63).

Cardiovascular disease: Coarctation of the aorta accounts for 2% of secondary hypertension in childhood and adolescence, but accounts for approximately one-third of patients seen in the first year of life (72, 73). Aortic coarctation may be focal (juxtaductal), diffuse (hypoplastic aortic isthmus) or complete (aortic arch interruption) (74). Screening evaluation of the hypertensive child suggests coarctation when blood pressure is at least 10 mm Hg higher in the right arm or both arms than in the legs, and the femoral pulses are diminished. A systolic ejection murmur of low intensity may be heard over the base of the heart and precordium and radiate into the left interscapular region. The murmur may be louder over the back. The older child who has developed collateral circulation may have a continuous murmur over large collateral intercostal arteries. When coarcation is suspected on the basis of screening evaluation, the patient should be referred to a pediatric cardiologist for further evaluation.

Echocardiography with color Doppler flow may identify a characteristic wedge-shaped band of tissue to confirm the diagnosis and measure any gradient across the coarcted segment (75). The transverse aortic arch also should be imaged to evaluate for hypoplasia. MRA (74, 76–78) and multidetector CTA (79) are being used increasingly for primary evaluation of thoracic coarctation in children over 4–5 years of age or for follow-up after angioplasty or surgical correction. Echocardiography is difficult after surgical repair because of scar tissue and thorax deformities limiting the acoustic window. 3D gadolinium-enhanced MRA demonstrates excellent anatomy of the aorta, estimates pressure gradient based on peak flow velocity across the stenosis, assesses collateral aortic circulation, and allows a 3D view of the full extent of the aorta (74, 76, 78). Conventional angiography still remains the gold standard and allows direct measurement of pressure gradient across the coarctation, but MRA and multidetector CTA provide alternative non-invasive ways

Figure 62-7

Abdominal arteriogram in a child with neurofibromatosis type 1 showing long segment tubular stenosis of the aorta with collateral flow as well as severe stenosis of the left main renal artery near its origin (arrow). Post-stenotic dilatation of the renal artery and delayed uptake of contrast in the left kidney compared to the right are also present.

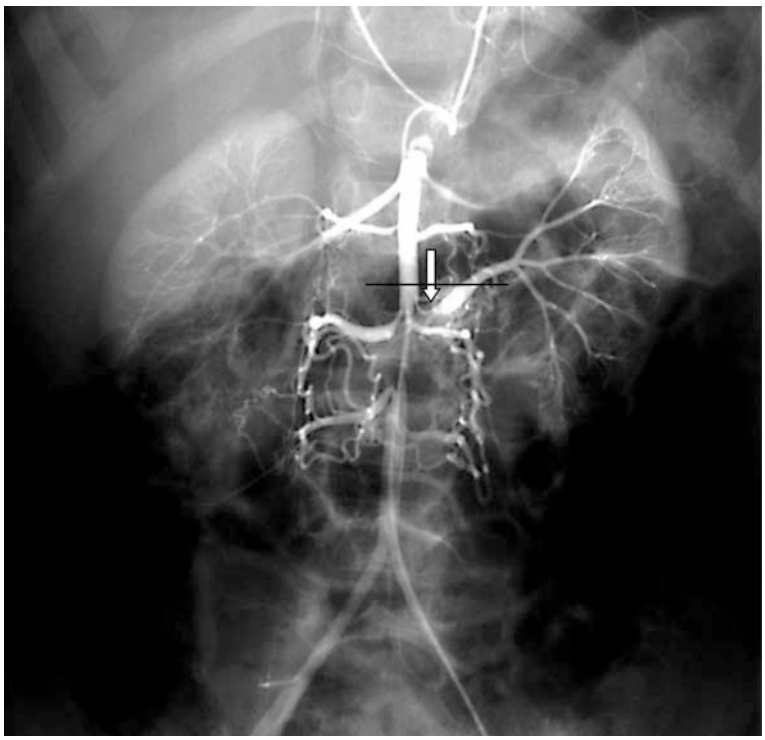
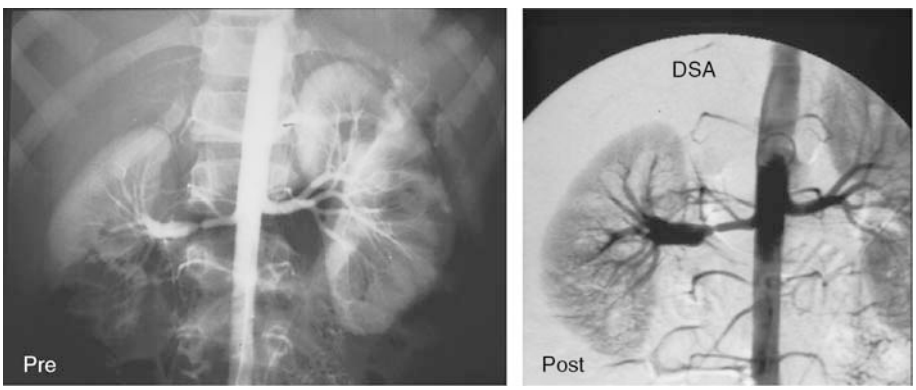


Figure 62-8

Renal angiogram in a child showing right main renal artery stenosis from fibromuscular dyplasia pre- and post-transluminal balloon angioplasty. The post image shows residual stenosis, but considerable improvement in flow of radiocontrast.

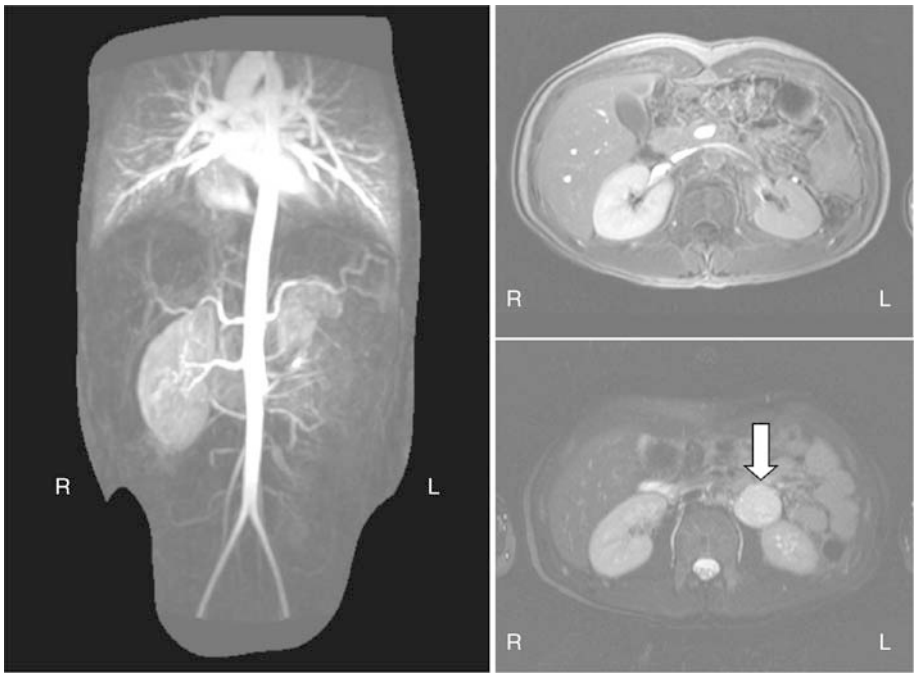


to diagnose, follow and evaluate anatomy for preoperative planning (74, 77, 79).

Other uncommon forms of cardiovascular disease lead to hypertension in infants and children (*Table 62-3*). Middle aortic syndrome, a rare entity which may be congenital, acquired from aortitis/vasculitis or associated with genetic diseases such as neurofibromatosis (*Fig. 62-7*) or Williams syndrome, may lead to severe

Figure 62-9

Images from a 3D gadolinium-enhanced MRA for a teenager with new onset severe hypertension. MRA shows that each kidney is supplied by two renal arteries (left panel). The right kidney and left upper pole kidney show prompt nephrograms, but perfusion of the lower two-thirds of the left kidney is delayed (left and right upper panel) secondary to compression of the left lower renal artery by a 4 cm paraspinal soft tissue mass (arrow, right lower panel).



life-threatening hypertension as well as lower extremity claudication and mesenteric ischemia (28, 37, 80–84). Diagnosis is made by conventional abdominal angiography showing tubular stenosis of the aorta and its visceral branches. 3D CTA is much less invasive and provides excellent detail of the aorta and its branches, but is not yet ready to replace angiography for preoperative planning of surgical therapy (81).

Williams syndrome or Turner syndrome should be suspected during screening evaluation because of typical physical findings, including the elfin facies of Williams syndrome and short stature and webbed neck of Turner syndrome. A variety of cardiovascular abnormalities that can cause hypertension are associated with Williams syndrome, including supravalvular aortic stenosis and thoracic coarctation (67, 84). Patients with Turner syndrome have a high incidence of renal anomalies (40%) and of cardiovascular malformations (20–50%), including coarctation of the aorta, bicuspid aortic valve, and aortic root dilatation (85–88). They also have a high incidence of cardiovascular disease morbidity and mortality as adults (86). Hypertension *per se* may contribute to their adult cardiovascular disease (86–88). ABPM in 75 girls with Turner syndrome revealed abnormal blood pressure circadian rhythm in 50%, suggestive of secondary hypertension (87).

Renal Parenchymal Disease

Most children with secondary hypertension (60–80%) have renal parenchymal disease (2, 89, 90). A variety of glomerular and a few tubular or interstitial renal disorders may cause hypertension in children and adolescents (*Table 62-4*). The most common renal parenchymal disorders associated with hypertension are glomerulonephritis and reflux nephropathy (90–92). Further evaluation for renal parenchymal disease is dictated by findings of the screening history and physical examination or an abnormal CBC, urinalysis, BUN, serum creatinine or electrolytes (*Fig. 62-1*). Hematuria, proteinuria, red blood cell casts on urinalysis, and edema suggest glomerulonephritis. Other systemic signs and symptoms, such as purpuric or malar rash, arthritis and abdominal pain, are suggestive of systemic vasculitis. Anemia and short stature for age in the presence of

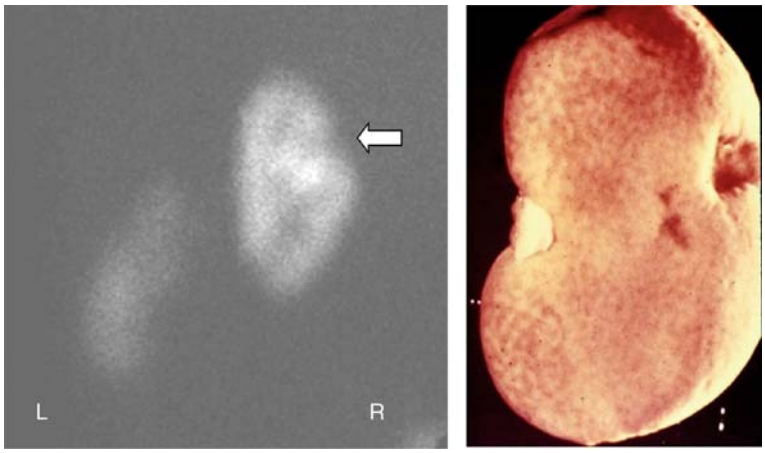
elevated BUN and serum creatinine are characteristic of chronic kidney disease with renal failure, which can result from most of these disorders. Additional laboratory studies, including serum C_3 and C_4, anti-glomerular basement membrane antibody (anti-GBM), anti-nuclear antibody (ANA), anti-double stranded desoxynucleic acid (anti-DNA), and anti-neutrophil cytoplasmic antibody, may help make the diagnosis of a specific glomerulonephritis or systemic vasculitis, but a renal biopsy is almost always needed for definitive diagnosis. The diagnosis of hemolytic uremic syndrome may be made clinically, if microangiopathic hemolytic anemia, thrombocytopenia and uremia are present. These diseases are all discussed in detail in other chapters of this book.

Some hereditary diseases are frequently associated with hypertension (*Table 62-4*). These include autosomal recessive and dominant polycystic kidney disease, medullary cystic disease, juvenile nephronophthisis, Denys-Drash syndrome and sickle cell disease. Diagnostic evaluation of these disorders is discussed in detail in other chapters of this book. Liddle syndrome (pseudoaldosteronism) is an autosomal dominant disease characterized by early onset severe hypertension, hypokalemia, metabolic alkalosis and decreased plasma renin activity and aldosterone (93–95). Originally thought to be an endocrine disorder, Liddle syndrome actually results from mutations/deletions in the gene for an epithelial sodium channel (ENaC) located in the luminal membrane of the distal convoluted tubule and collecting duct. Normal endocytosis of ENaC is impaired leading to increased numbers of ENaC channels and avid unregulated renal sodium reabsorption, volume expansion, hypertension and renal potassium wasting (93). The ENaC is sensitive to amiloride or triamterene suppression, which in combination with dietary sodium restriction forms the basis of therapy for Liddle syndrome (95).

Hypertension associated with congenital renal anomalies, especially vesicoureteral reflux, typically presents in late childhood or early adolescence and may be the first sign of disease (27, 91). Screening renal ultrasound may be diagnostic and at least will direct further specific evaluation, which should include a detailed history for undiagnosed fevers or documented urinary tract infections in early childhood and a voiding cystourethrogram (VCUG). The VCUG will both diagnose and grade reflux. Urinalysis and urine culture should be negative for urinary tract infection before proceeding with a VCUG. The absence of reflux does not negate the possibility that reflux was present in infancy and early childhood and then spontaneously resolved, but left the child with renal scars, reflux nephropathy and the potential for developing hypertension at an older age (91, 92). Unfortunately, no test is available to predict the risk for developing hypertension, so regular long-term follow-up of blood pressure is recommended (91). To detect the presence of renal scars, a DMSA radionuclide scan is preferred over conventional urography or ultrasonography (96, 97). DMSA is filtered then reabsorbed by the renal tubule to become fixed in the parenchyma, providing a static image of functional renal tissue (98). Absence of uptake is consistent with a non-functioning scar as illustrated by *Fig. 62-10*.

Figure 62-10

DMSA radionuclide scan for evaluation of renal scars. *Left panel:* ^{99m}Tc-DMSA radionuclide scan of a 9-year old, who presented with hypertension and Bell's palsy, showing right upper pole renal scar (arrow) and poorly functioning smaller left kidney (relative activity 29%) from vesicoureteral reflux. *Right panel:* Kidney from a different patient showing an upper pole scar from vesicoureteral reflux. (See color plate 40)



Segmental hypoplasia with atrophy, known as the Ask-Upmark kidney (Fig. 62-11), is a scarred, shrunken segment of the kidney characterized histologically by the presence of colloid filled tubular microcysts and few or absent glomeruli (99, 100). Whether this defect is congenital or the result of injury from vesicoureteral reflux or urinary tract infection is uncertain (100). Partial nephrectomy cures the associated hypertension. Renal arteriography with selective renal vein renin sampling is helpful to identify the exact location and extent of the hypoplastic segment before undertaking surgery (101).

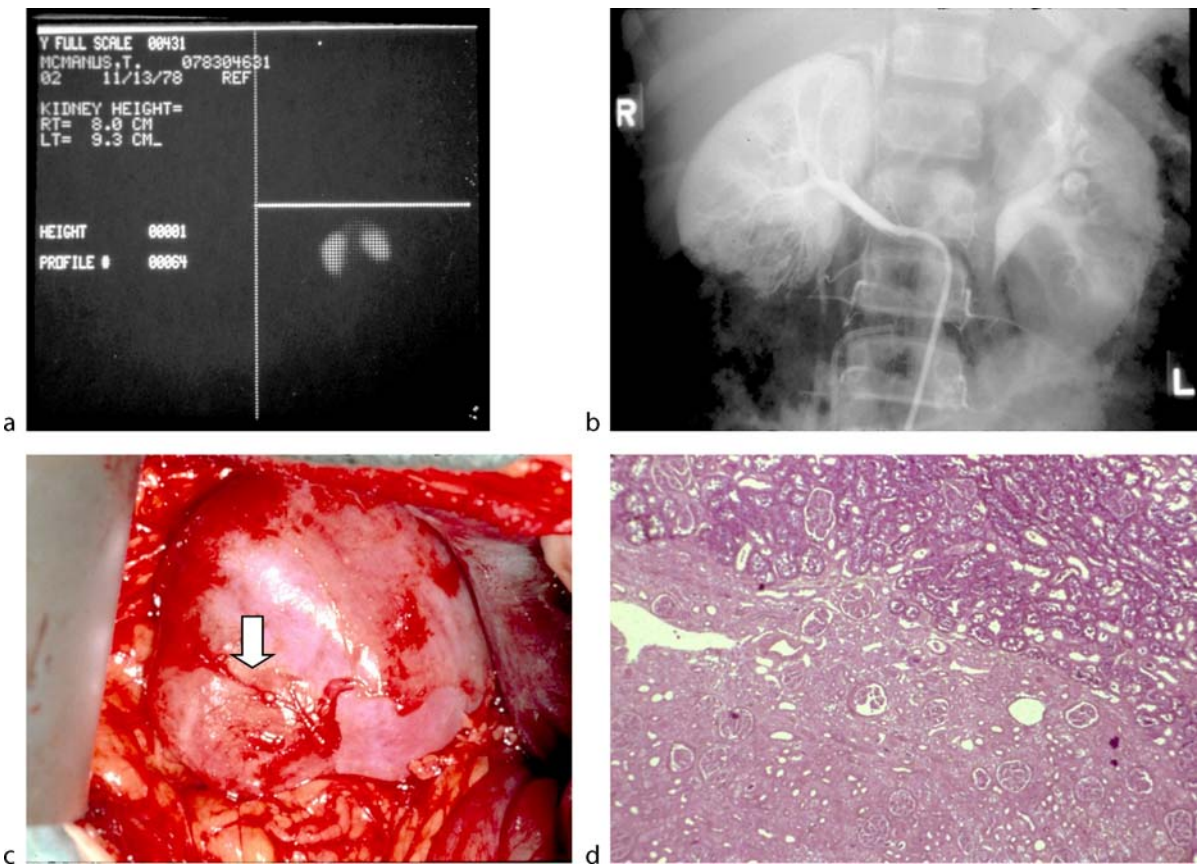
Endocrine Abnormalities Associated with Hypertension

Endocrine causes of hypertension in childhood are rare, but usually treatable and often curable (102). Specific evaluation should be directed by the suspected disorder (Fig. 62-1). The combination of a good history and physical examination, accurate interpretation of screening laboratory data and sequential ordering of diagnostic tests will lead to a diagnosis and still be efficient and cost effective.

Pheochromocytoma: The incidence of pheochromocytoma is quite rare in all age groups (102, 103), and accounts for about 1% of the cases of secondary hypertension in childhood (72, 103). Children are more likely to have multiple tumors, recurrence of tumors and a positive family history for related tumors than adults (102, 103)). Pheochromocytomas are chromaffin cell tumors called paragangliomas that arise from the adrenal gland (103). When they are not adrenal in origin, but arise in the autonomic nervous system of the abdomen, thorax, neck or head, they are termed extra-adrenal paragangliomas (103). Children with pheochromocytomas

Figure 62-11

Ask-Upmark kidney. A. ^{99}Tc-DTPA radionuclide scan showing right kidney smaller than left. B. Selective right renal arteriogram showing no filling of lower pole of right kidney; left kidney with late nephrogram of previous radiocontrast injection. C. Intraoperative photograph showing small lower pole of right kidney. D. Light microscopy of the junction of the normal portion of the kidney with the hypoplastic lower pole segment (PAS stain). (See color plate 41)



may present with the classical clinical triad of episodic hypertension, headache and sweating, but hypertension is sustained about 80% of the time in children (102, 103). Other symptoms include anxiety, flushing, visual disturbances, abdominal pain, nausea, vomiting and weight loss. Physical examination may reveal hypertensive retinopathy, features of associated syndromes like the café-au-lait spots of neurofibromatosis, or rarely an abdominal mass, the palpation of which may lead to an abrupt increase in blood pressure and other symptoms of catecholamine release (102).

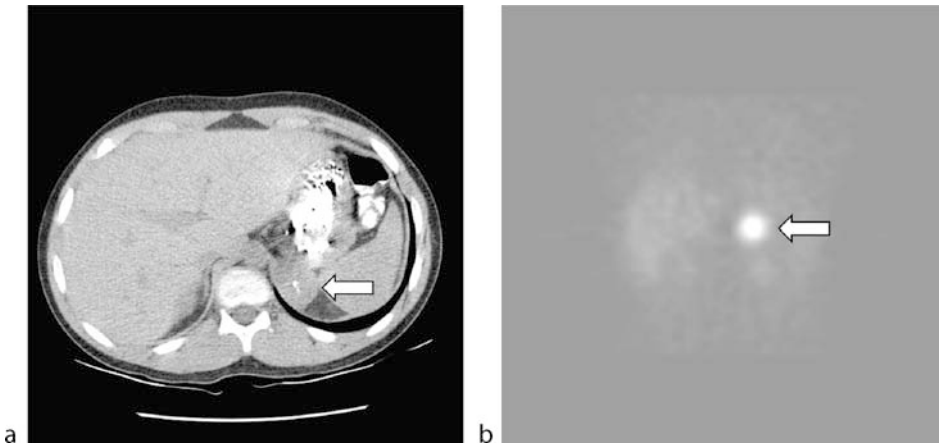
Pheochromocytomas in childhood may be sporadic, but are hereditary about 40% of the time (103). Advances in genetic mutation analysis have improved identification of familial disease, allowing detection of affected children at an early age, before typical signs and symptoms occur (103). The most common hereditary syndromes are von Hippel-Lindau disease, multiple endocrine neoplasia type 2 (MEN 2) and neurofibromatosis. If pheochromocytoma is the primary manifestation of the family disease, a von Hippel-Lindau gene mutation is the most likely cause, whereas most of the patients with MEN 2 have medullary thyroid carcinoma.

Pheochromocytoma is a potentially curable cause of severe and sometimes life-threatening bouts of hypertension (102, 104), so a thorough evaluation to make the diagnosis is important. Specific evaluation for pheochromocytoma (*Fig. 62-1*) should start with measurement of plasma metanephrine and normetanephrine, which are elevated in 80% of patients even when they are asymptomatic (105). Blood should be obtained when the child is in the supine position for better diagnostic sensitivity (103), and results compared to age and gender appropriate reference ranges, since adult normals are higher than those for children (106). Periodic screening with this test can lead to early diagnosis in children with familial disease (103). If the levels are normal, the diagnosis may be excluded. If the results are only marginally increased, further evaluation with repeat plasma metanephrines plus plasma catecholamines or a clonidine-suppression test should be performed, but only by a clinician experienced with these tests (103). Imaging studies may also be indicated for further screening in cases of known familial disease. High levels of plasma metanephrines indicate the presence of a pheochromocytoma with 100% specificity and warrant proceeding directly to imaging studies to locate the tumor (103).

The first step in imaging is usually a CT or MRI scan of the abdomen (103, 104). CT detects adrenal pheochromocytomas with 93–100% sensitivity (*Fig. 62-12*), but has only 90% sensitivity for extra-adrenal tumors. MRI has high sensitivity for detecting both. CT and MRI cannot delineate whether a mass is a pheochromocytoma versus other adrenal or soft tissue tumor. As an example, the paraspinal tumor depicted by MRA in *Fig. 62-9* was unexpectedly identified as an extra-adrenal paraganglioma histologically after surgical removal. In order to identify pheochromocytomas pre-operatively, a meta-iodobenzyl guanidine (MIBG) scan should be done (*Fig. 62-12*). MIBG, which localizes in storage granules in adrenergic tissue of neural crest origin, is very specific (95–99%) for pheochromocytoma and neuroblastoma in children (107, 108). ^{123}I-MIBG offers superior imaging quality compared to ^{131}I-MIBG (109). False-negative

Figure 62-12

Diagnostic imaging for pheochromocytoma. A. Abdominal CT scan showing a 3 cm mass with central calcifications (arrow) located superior to the left kidney in the left adrenal gland. B. ^{123}I-MIBG scan showing uptake in right adrenal mass (arrow).



MIBG results can occur, particularly if tumors are small or in the thorax, neck or head. Repeated examinations may be necessary to finally localize small tumors. Once localized, the pheochromocytoma may be surgically removed and will cure hypertension in 90% of cases (102). In series of childhood pheochromocytomas, as few as 5% up to 45% of tumors have been reported to be malignant (103).

Renin-producing tumors: The possibility of a renin-secreting tumor, such as a juxtaglomerular cell tumor (110, 111) or Wilms' tumor (112, 113), should be entertained in a hypertensive child with hypokalemia, elevated plasma renin activity and absence of other obvious renal or renovascular disease. For juxtaglomerular cell tumors, abdominal CT or MRI imaging will identify small masses better than selective renal arteriography, which is normal in about 50% of reported cases (110).

Thyroid disorders: Mild hypertension may be associated with hyperthyroidism from Grave's disease or rarely thyroid adenoma (102). Acquired hypothyroidism in adults is associated with hypertension and is reversible with thyroid replacement therapy in about 50% (114). The incidence of hypertension from hypothyroidism in children is unknown. The first step in specific evaluation of thyroid disease is assessment of serum T_4 and thyroid stimulating hormone concentration to make the diagnosis of hyper- or hypothyroidism (*Fig. 62-1*). Further evaluation is probably best made by referral to a pediatric endocrinologist.

Cushing syndrome: Exogenous glucocorticoid or adrenocorticotropic hormone (ACTH) therapy, including that used for treatment of asthma, renal disease, rheumatologic disease, neurologic disorders and cancer or transplantation immunosuppression, is the most common cause of Cushing syndrome and endocrine-associated hypertension in pediatrics (102). On the other hand, excess endogenous production of glucocorticoids or ACTH, from an adrenal or pituitary tumor, is unusual in childhood. Obvious physical changes of glucocorticoid excess, including round facies, plethora, truncal obesity, acne, easy bruisability and abdominal striae, may direct the physician to the diagnosis. If the patient is receiving no glucocorticoid medications, screening with an 8 a.m. plasma cortisol, ACTH level and 24-h urinary 17-hydroxycorticosteroids and free cortisol may be appropriate, if referral to a pediatric endocrinologist is not readily available.

Hyperaldosteronism: Hypertension associated with adrenal tumors or idiopathic adrenal hyperplasia is usually mediated by aldosterone or other compounds with mineralocorticoid effects (102). Mineralocorticoid excess leads to salt and water retention, volume expansion and renal potassium wasting. Hypertension in the presence of hypokalemia and metabolic alkalosis found on screening evaluation, should direct the specific evaluation to that for hyperaldosteronism (*Fig. 62-1*). If plasma renin activity is low, plasma and 24-h urinary aldosterone should be measured. Urinary aldosterone is elevated in the presence of high plasma aldosterone concentration and decreased in the presence of other compounds with mineralocorticoid effect. Imaging of the adrenal glands with CT or MRI should be the next step for diagnosis and localization of adrenal adenoma or carcinoma in patients with hyperaldosteronism (102).

Congenital Adrenal Hyperplasia (CAH): Only two enzyme deficiencies in the cortisol pathway are associated with hypertension, 11 β-hydroxylase (102, 115–118) and 17 α-hydroxylase (102, 117, 119, 120). 11 β-hydroxylase deficiency is the second most common cause of CAH, but only accounts for about 5% of cases (102). 11 β-hydroxylase deficiency is an autosomal recessive disorder caused by mutations of the CYP11B1 gene located on chromosome 8q21-q22 (116). 11 β-hydroxylase deficiency results in decreased conversion of 11-deoxycortisol to cortisol and 11-deoxycorticosterone (DOC) to corticosterone, and excess production of androgens from precursors more proximal in the pathway. Decreased cortisol production stimulates secretion of pituitary ACTH resulting in adrenal stimulation and excess production of DOC, which has mineralocorticoid effects in high concentration. The excess androgen production results in virilization and ambiguous genitalia in female infants and penile enlargement in males. Hypertension, hypokalemia and low plasma renin activity are present in most, but not all affected patients. Referral to a pediatric endocrinologist for more extensive laboratory evaluation is an appropriate next step. Characteristic findings are elevated plasma concentrations of 11-deoxycortisol and DOC and the androgens, dehydroepiandrosterone (DHEA) and testosterone, as well as increased urinary excretion of 17-hydroxycorticosteroids, tetrahydro-11-deoxycortisol and 17-ketosteroids (118). ACTH stimulation testing may be needed to make or confirm the diagnosis, especially in adolescents.

17 α-hydroxylase deficiency is very rare, occurring in less than 1% of cases of CAH (102). The trait is autosomal recessive and caused by mutations in the CYP17 gene on chromosome 10q24-q25 (117). This enzyme has both 17 α-hydroxylase and 17,20-lyase (desmolase) activity (119, 120). 17 α-hydroxylase normally facilitates conversion of progesterone to 17-hydroxyprogesterone and pregnenolone to 17-hydroxypregnenolone, which lead to the production of cortisol. 17,20-lyase is required for the synthesis of androgens and estrogenic C18 steroids.

Deficiency of the enzyme limits synthesis of cortisol as well as androgens and estrogens. Unlike patients with 11 β-hydroxylase deficiency, patients with 17 α-hydroxylase deficiency are not virilized, so may not be diagnosed before discovery of their hypertension, hypokalemia and hypogonadism in adolescence. Girls have primary amenorrhea and absent secondary sexual characteristics. Boys usually have complete male pseudohermaphroditism with female external genitalia, a blind vagina, absent uterus and intrabdominal testes. Laboratory evaluation reveals increased serum progesterone and DOC and reduced plasma cortisol, 11-deoxycortisol, DHEA, testoterone and estradiol concentrations. These cases are very rare and best evaluated by referral to a pediatric endocrinologist.

Hypercalcemia: Hypercalcemia is well-known to cause hypertension as well as polyuria, constipation, abdominal pain, anorexia and mental status changes. In pediatric cases, the etiology is most often vitamin D intoxication, Williams syndrome or malignancy and rarely primary hyperparathyroidism (102). Evaluation should include measurement of serum total and ionized calcium, phosphorus, parathyroid hormone, 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D (*Fig. 62-1*).

Miscellaneous Causes of Hypertension

Table 62-6 lists a number of miscellaneous causes of hypertension, including some drugs that should be considered in the evaluation of the hypertensive child. In adolescents or younger children at high risk for drug abuse, a urine drug screen should always be obtained as part of the initial evaluation. A few of the other causes of hypertension in children will be discussed in more detail here.

Neurologic abnormalities: A variety of neurologic disorders give rise to hypertension (*Table 62-6*). In the critically ill child with rising blood pressure and falling heart rate, evolving increased intracranial pressure (ICP) should always be suspected. The incidence of ICP is especially high with acute meningitis or encephalitis (121). Children with seizures and status epilepticus may present with hypertension (122). If blood pressure in the postictal period is high, but less than 4 standard deviations above the mean, hypertension is more likely to be transient and seizure-induced rather than caused by hypertensive encephalopathy with seizures (122). Disorders of the autonomic nervous system may also lead to hypertension. Familial dysautonomia is the best know of these disorders (123–125). Clinical features include paroxysmal hypertension, swallowing difficulties, speech and motor incoordination, and pain insensitivity. Clinical diagnosis is based on Ashkenazi Jewish heritage, diminished tear production, lack of an axon flare after intradermal histamine, lack of lingual fungiform papillae and decreased deep tendon reflexes. The gene for this disorder, IKBKAP, has been isolated and mapped to chromosome 9q31.

Teen pregnancy: Pregnant teenagers are at high risk for developing hypertension, which increases their risk for poor outcomes for the baby, including low birth weight, pre-term delivery and congenital malformations (126–128). When hypertension occurs early in gestation, hospitalization with strict supervision and control of blood pressure may be required. Ultrasound imaging for evaluation of hypertension is acceptable, but conventional radiographic studies and radioisotopic scans should be avoided because of potential adverse effects on the fetus. ACEI and ARB are teratogenic and their use in pregnancy is contraindicated (129, 130). Pubertal and post-pubertal girls with antecedent hypertension should be advised to protect against pregnancy while taking these medications for control of hypertension (130). When preeclampsia or severe hypertension occurs after 36 weeks' gestation, delivery is the therapy of choice. If a teenager with known hypertension becomes pregnant, evaluation should always include serial reassessment of maternal renal function throughout pregnancy.

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63 Management of the Hypertensive Child

Demetrius Ellis

Overview

There has been a marked rise in the prevalence of childhood hypertension (HTN) over the last 10–15 years. This has mirrored the rise in obesity and sleep disorders in children (1–4). With an increasing number of children becoming candidates for management of HTN, pediatricians must become much more familiar especially with the pharmacologic management of this important disorder. The extent of interventions to treat HTN will depend greatly upon the demonstration of a link between blood pressure (BP) values and the pathophysiological changes observed in target organs of HTN. Thus, longitudinal investigations are needed in hypertensive children to determine the benefits and risks of lifestyle and/or pharmacologic interventions in preventing cardiovascular and other short and long-term end-organ injury resulting from any level of BP.

In children with HTN, as many as 90% of hypertensive preadolescent children have secondary HTN where as the majority of adolescents have primary (essential) HTN (5–7). Despite the relatively low prevalence of HTN especially in the preadolescent population, there are multiple and unique hereditary or acquired renal and urologic causes of HTN. This underscores the need to develop a therapeutic plan tailored for each child. The age, size, and lifestyle of the child are just a few of the considerations in selecting pharmacologic agents to manage the HTN. Careful attention must also be given to the means of administration, dosage guidelines, and adverse effects of antihypertensive agents.

In addition to providing a general approach to the management of the hypertensive child, including nonpharmacologic measures and a review of the major classes of antihypertensive agents, a major objective of the current treatise is to provide a framework for the rational use of antihypertensive agents based on an understanding of causality and of the pathophysiologic mechanisms. The ultimate aim of such targeted approach to therapy is to provide efficient and effective control of HTN and to minimize adverse effects.

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Recommendations for Treatment

Pediatricians are at the forefront for detecting risk factors for HTN through regular and proper measurement of BP and through assessment body mass index, dietary, and exercise habits, prescribed or over-the-counter medications and drugs, and lifestyle measures in general, that may impact BP. Such evaluation followed by counseling on healthy habits is essential in preempting the potentially devastating consequences of HTN. This is particularly important in the prehypertensive population with BP between the 90th and <95th BP percentiles in whom BP may be measured more frequently. Tables listing BP by age, gender, and height percentiles must be consulted in order to define the level of BP that should raise concern (8).

The current guidelines for managing childhood HTN are summarized in the fourth report of the National High Blood Pressure Education Program (NHBPEP) Group on High Blood Pressure in Children and Adolescents (8). In brief, all asymptomatic hypertensive children may benefit from nonpharmacologic management. Pharmacologic treatment is indicated in children with BP ≥95th percentile, i.e., Stage I HTN (95th–99th percentile) plus 5 mm Hg who are unresponsive to changes in life style, and in those with Stage II HTN (≥99th percentile) plus 5 mm Hg. Also, children with secondary hypertension, and those with end-organ injury are candidates for pharmacotherapy.

Goal of Therapy

In asymptomatic children, the NHBPEP (8) recommends achieving target BP of:

1. Less than the 95th percentile if there is no other coexisting disorder.
2. Less than the 90th percentile in children with evidence of coexisting cardiovascular risk factors, diabetes or end-organ damage. HTN in children with overt proteinuria or progressive renal insufficiency is also an indication for lowering BP below the 90th percentile.

Of immediate concern is the management of acute symptoms of HTN. For example, aggressive and immediate pharmacologic control of BP is often required in hypertensive children with overt evidence of end-organ injury in the form of encephalopathy, seizures, Bell's palsy, cerebrovascular accident, congestive heart failure, or hypertensive retinopathy.

The long-term objective of HTN control is the prevention of cardiovascular disease and heart failure which remain to be the leading causes of morbidity and mortality in adults in the USA. Currently there are no longitudinal studies in children assessing the relationship of the level and the duration of HTN, and cardiovascular risk. While numerical goals are often used to assess the efficacy of therapy, isolated BP values obtained at clinic settings may be unreliable. Thus, many experts recommend more reliable means of predicting cardiovascular complications, including ambulatory BP monitoring (ABPM) (9–11) which requires special authorization for reimbursement by health care insurers in the USA, or, home blood BP monitoring (12).

Reversal of the symptoms and signs of HTN and of markers of HTN is an even more important goal of therapy. Left ventricular hypertrophy (LVH) on echocardiography is perhaps the most sensitive means for detecting end-organ injury in children with well-established HTN. LVH is detected in 40% of such children (13–15). Microalbuminuria, as a marker of microvascular disease, is another early clinical indicator of end-organ damage in adults with HTN, but its importance has not been investigated in children except as it relates to type 1 diabetes.

Lifestyle Measures and Nonpharmacologic Management of HTN (See *Table 63-1*)

While many children present with established HTN, prevention of HTN and its complications is highly desirable and also plausible. A heightened awareness of environmental or medical factors, such as obesity, and renal disorders may indicate a need for close monitoring to detect HTN such that timely intervention can be implemented. Thus, the pediatrician and other health care providers must inquire about environmental or modifiable risk factors as well as to assess genetic risks. It is essential that education and counseling must then be provided to the family and not just the child so as to enhance motivation and compliance with the proposed treatment strategy. Intervention may be needed in the management of overweight, sleep disorders, in intake of exogenous agents that may provoke HTN, and in limiting psychobehavioral factors including school stress and excessive use of computer games. Attention must be given to the sodium content of processed food along with the amount of fat in the diet, as well as the amount of vegetables and fruits consumed by the child. An overview of the amount and type of exercise and activity may also be part of the medical history.

Table 63-1

Lifestyle modifications and approximate range of systolic BP reduction (mm Hg)[a]

Exercise/limit TV and other sedentary activities (4–9)
Weight reduction (smaller portions/exercise) (5–20/10 kg weight loss)
Consume more fresh vegetables and fruits, low-fat dairy products and low content of saturated fats (2–8)
Reduce Na intake (2–8)
Avoid alcohol (2–4)
Limit caffeine
Avoid tobacco (cigarettes/chewing)

[a]Obtained from adult studies

Lifestyle changes are essential in managing all children with HTN. An initial conservative, nonpharmacologic treatment approach is also recommended in children with Stage 1 HTN and no end-organ injury. The aim of treatment is not only to lower blood pressure but to also diminish other consequences of unhealthy lifestyle which may impact cardiovascular disease such as obesity and hyperlipidemia, and to reduce the use of caffeine, drugs, alcohol, tobacco, and high salt intake, as well as to balance food intake and exercise.

Setting realistic and measurable goals over specified periods of time for achieving weight reduction, correction of metabolic disturbances, and numerical targets of BP, are useful adjuncts in managing the hypertensive child.

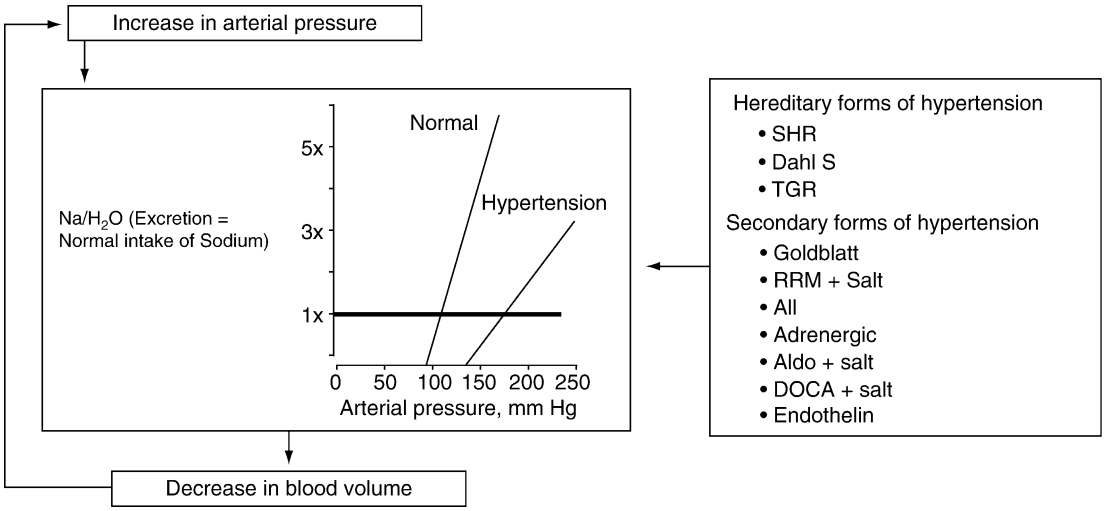
Diet

Salt Restriction

The link between a high salt intake and HTN is indisputable (16–18) *Fig. 63-1* shows the mechanisms implicated in salt-sensitive HTN (19). Several epidemiologic studies as well as a well-controlled study, the INTERSALT study (20), confirmed the relationship between salt and HTN. Also, a meta-analysis of multiple studies indicates that salt reduction lowers BP in individuals with HTN (21). More importantly, the randomized Dietary Approaches to Stop Hypertension (DASH) study showed that the lower the salt intake the greater the BP reduction

Figure 63-1

Etiopathogenesis of salt-sensitive hypertension. Schematic representation of the pressure-natriuresis hypothesis for the long-term control of arterial pressure. In normal animals, any elevation in arterial pressure would be expected to increase sodium and water excretion via pressure natriuresis. Because sodium and water intake is habitually determined and remains relatively fixed, the increase in sodium and water excretion slowly lowers blood volume sufficiently until arterial pressure returns exactly to control. The pressure-natriuresis relationship either exhibits a reduced sensitivity (slope reduction) or is shifted in a parallel manner toward a higher set point, or in every experimental model of hypertension studied to date. Thus, sodium and water are retained until arterial pressure is elevated sufficiently to restore sodium and water balance. SHR indicates spontaneously hypertensive rats; Dahi S, Dahi salt-sensitive rats; TGR, transgenic renin gene rats; RRM, reduce renal mass; Aldo, long-term administration of aldosterone; DOCA, long-term administration of deoxycorticosterone acetate; and gray bar, normal level of sodium intake.



in both normotensive and hypertensive individuals on a normal North American diet. Also, this study demonstrated the benefit of a diet rich in fruits and vegetables and low in saturated fats in lowering BP (22).

North American children have a high intake of salt derived mainly from processed food rather than salt added to food by the individual. Sodium restriction is particularly useful in African–Americans with salt-sensitive HTN. This is because such individuals have an inheritable impairment in sodium excretion at any level of renal perfusion pressure. When combined with an acquired taste for salt leading to ever increasing salt intake, this eventually leads to sustained HTN. For any level of sodium intake, salt-sensitive individuals may experience a 16-fold increase in systolic blood pressure (SBP).

The NHBPEP recommends a 1.2 g/day sodium intake for 4–8 year-olds and 1.5 g/day for older children (8). These limits may be difficult to implement. A daily intake of 1.5–2.0 g of sodium (4.25–5.0 g of salt)/m^2 body surface area may be more realistic compared to an average salt intake that is double this amount among children in North America. The DASH study recommends no added salt, as well as increased fruits, vegetables, and low fat and low dairy intake (22). This is a more palatable diet and more likely to succeed in the long term.

Potassium/Calcium/Magnesium

A higher intake of potassium, calcium, and magnesium has been shown to have an ameliorating effect on BP in both experimental models of HTN, as well as in human epidemiologic studies (17). No specific requirements for supplemental amounts of potassium, calcium, and magnesium are available for hypertensive children.

Caffeine

Acute caffeine intake may increase "central" BP which may not be evident by measuring peripheral BP, by increasing catecholamine release and thereby raising vascular resistance (23, 24). Caffeine intake has risen in children in North America in parallel to obesity. "Super-sizing" of portions by many food providers have included large

volumes of high calorie and high caffeine soft beverages, as well as less well known amounts of caffeine found in iced tea (70 mg of caffeine/12 ounces of tea compared to 55 mg/12 ounces of Mountain Dew and most Coke/Pepsi products). Caffeine-sensitive individuals may develop tachycardia that initially raises SBP independent of obesity.

Weight Loss

There are several studies linking obesity and HTN in children (3, 4). The causes of hypertension in obesity are multifactorial, complex and poorly understood. Several mechanisms are reviewed under Sect. "Obesity/Metabolic Syndrome/Sleep Apnea".

The body mass index (BMI = weight (kg)/height $(m)^2$) chart should be consulted to determine if the child is overweight (BMI > 85 percentile) or obese (BMI > 95 percentile) for age and gender. A plan for management may include modifications in diet and exercise as outlined by Rocchini (25). Participation in a formal weight management program to review and to monitor for complications of obesity, and to reenforce the need for weight control with the child and his family, is strongly recommended by the author.

Hyperlipidemia is an independent risk factor for cardiovascular disease associated with HTN. However, hyperlipidemia is also more prevalent in obese children with or without HTN. The coexistence of the two disorders is more conducive to cardiovascular injury. The American Heart Association recommends Step 1 or Step 2 diet to control hyperlipidemia (26). Exercise may also be helpful not only in reducing body weight but also by lowering triglycerides in particular.

Exercise (See Sect. "Hypertension in Athletes")

A 10 mmHg reduction in mean arterial BP may be achieved through regular exercise. The American College of Sports Medicine recommends aerobic exercise three to four times per week with the goal of achieving 60–85% of maximal heart rate (26). A daily aerobic activity lasting 30–60 min is highly recommended. Static exercises are less useful for weight control while excessive weightlifting (more than 30 min/day for more than 3 days/week) may contribute to HTN.

Drug Intake and HTN

In addition to nicotine (tobacco) and alcohol use, there is an expanding list of prescribed and illicit drugs that may either cause HTN or exacerbate preexisting HTN (*Table 63-2*). A careful medical history for such drug intake may lead to appropriate education and counseling.

Table 63-2
Exogenous substances that raise the blood pressure

Substance	Raise blood pressure	Interfere with therapy	Source of substance
Anabolic steroids	Yes	No	Patient
Caffeine	Yes	No	Patient
Cocaine	Yes	Yes	Patient
Ethanol	Yes	No	Patient
Nicotine	Yes	No	Patient
Sodium chloride	Yes	Yes	Patient
Sympathomimetic agents	Yes	No	Patient or clinician
Nonsteroidal antiinflammatory agents	Yes	Yes	Patient or clinician
Chlorpromazine	Yes	No	Clinician
Corticosteroids	Yes	Yes	Clinician
Cyclosporine	Yes	No	Clinician
Tacrolimus	Yes	No	Clinician
Erythropoietin	Yes	No	Clinician
Monoamine oxidase inhibitors	Yes	No	Clinician
Oral contraceptives	Yes	No	Clinician
Tricyclic antidepressants	Yes	No	Clinician
Strattera	Yes	No	Clinician
Adderall	Yes	No	Clinician
Wellbutrin	Yes	No	Clinician
Ritalin	Yes	No	Clinician
β-adrenergic agonists	Yes	Yes	Clinician
Theophylline	Yes	No	Clinician
Phencyclidine	Yes	No	Clinician

Behavioral Modification/Stress Management/Biofeedback

These are techniques that may be used to complement other treatment modalities. They may be especially useful in individuals with white coat HTN evident on ABPM, or those with systolic HTN.

Pharmacologic Agents Used to Manage Hypertension

Introduction

Important recent legislative initiatives such as the Food and Drug Administration Modernization Act of 1997 (FDAMA) (27) followed by the Better Drugs Act of 2002 (28), have enabled trials of antihypertensive agents in children and refined the investigative guidelines to include data on the long-term effects of antihypertensive agents on cognitive function and body growth. These legislations have been very instrumental in obtaining evidence-based data on dosing, efficacy and safety for many antihypertensive agents currently utilized in children. In return for sponsoring pediatric clinical trials, pharmaceutical companies receive labeling or exclusivity for approved indication of their agent as well as an extension of the drug patent.

Based on clinical trials, an increasing number of antihypertensive drugs have received FDA approval for pediatric use. An updated list of these drugs that received label change based on pediatric studies is maintained on the internet (29) and is summarized in *Table 63-3*. However, at best, antihypertensive trials in children are limited in scope, partly because of the relatively small number of children with specific disorders. Also, there is a large variability in the bioavailability of drugs based on the child's age and body size. A number of other areas remain problematic and include a limited number and uniformity of liquid preparations for use in younger children, the lack of information on the stability of such suspensions, and a dearth of pharmacokinetic data of antihypertensive agents in general. Thus, certain agents may not be uniformly effective despite adjustment in dosage, or the child may require more than one agent to achieve optimal BP control. Also, long-term effects of antihypertensive agents on linear growth and cognitive function remain to be assessed in longitudinal studies.

Table 63-3

FDAMA-related written requests for antihypertensive medication studies and exclusivity status in children (as of 3/2008)

Compound	Study status	Exclusivity granted
Amlodipine	Completed	Yes
Benazepril	Completed	Yes
Betaxolol	Completed	Yes
Bisoprolol/HCTZ	Completed	Yes
Candesartan	Unknown	–
Carvedilol	Completed	Yes
Enalapril	Completed	Yes
Eplerenone	Completed	Yes
Esmolol	Completed	Yes
Felodipine	Completed	Yes
Fenoldopam	Completed	No
Fosinopril	Completed	Yes
Irbesartan	Completed	Yes
Isradipine	Unknown	–
Lisinopril	Completed	Yes
Losartan	Completed	Yes
Metoprolol	Completed	Yes
Nitroprosside	Unknown	Yes
Quinapril	Completed	Yes
Ramipril	Completed	No
Timolol	Completed	Yes
Valsartan	In progress	–

Updated information available at http://www.fda.gov/cder/pediatric/exgrant.htm

Indications for Pharmacologic Treatment of HTN

The pharmacologic management of HTN is multifaceted and individualized based on the pathophysiology and on the classification of HTN. Criteria for pharmacologic management have been outlined by the NHBPEP (8). Accordingly, one of the following criteria must be present before drug therapy is initiated:

1. *Stage I* HTN (95th–99th percentile) plus 5 mm Hg who are unresponsive to changes in life style, and in those with *Stage II* HTN (>99th percentile) plus 5 mm Hg.
2. Symptomatic hypertension including headaches, changes in mental process or consciousness, increased urination, or irritability.
3. Secondary HTN.
4. Evidence that the high blood pressure is causing end-organ damage. This may be evident by left ventricular hypertrophy or increased left ventricular mass index, or by hypertensive alterations of the retinal vessels as described by Keith-Wagener.
5. Coexisting diabetes. All children with HTN and diabetes should be considered for pharmacologic therapy, independent of albuminuria status.

6. The presence of additional cardiovascular risks including elevated serum cholesterol, known heart disorders, severe obesity, etc.

General Approach to Pharmacotherapy of HTN

In general, the use of fewer medications with fewer adverse effects leads to better compliance. Single medications with extended release or long-action, or combination drugs may also enhance compliance, particularly in adolescents. It should be noted, however, that even among the best conceived studies, target BP may be achieved in only 45–63% of the children (30).

As a rule, one should start with one medication at a time using the lower dosage range. BP should be closely monitored at home, school or at the primary care physician's office. If the child is asymptomatic and BP fails to improve within 2–3 days after using the agent at the mid-range dosage, there are three general guidelines:

1. Further increase the dosage gradually to the maximum tolerable dose ("stepped" or "step-up" care), or,
2. Replace the initial agent with that of another class ("sequential monotherapy") on the assumption that it is not possible to predict how all children with a given disorder will respond to any individual agent. or,
3. Add another agent ("add-on" care).

Many clinicians are now deviating from the current paradigm of step-up care because of the higher risk of dose-dependent adverse effects, and also because adding drugs with complimentary mechanisms of action administered at mid-range dosages lowers such risks while providing an additive effect (31).

Choice of Antihypertensive Medications

Because renal disease comprises 60–70% of the etiology of HTN in preadolescent children (5, 7), the use of angiotensin converting enzyme inhibitor (ACEI) and angiotensin recptor blocker (ARB) is more prevalent in children than in adults while other classes of medications may also be effective. A survey of pediatric nephrologists noted that 47% used ACEI, 37% used CCB, and 7.6% used a beta blocker as first-line drugs to manage primary HTN (32). Pediatric nephrologists utilize ACEI as first-line drugs in 84% of children with HTN associated with renal disorders. Primary or essential hypertension is much more prevalent in adolescents. In this population the initial medication choice may be much wider (see Sect. "Primary (PH) or Essential HTN"). In general, the use of beta blockers is discouraged as an initial agent except for special circumstances, which will be discussed below. The special needs of hypertensive children participating in competitive sports must also be addressed (see Sect. "Hypertension in Athletes").

In choosing antihypertensive agents for any indication, one must always consider the presence of other medical conditions. For instance, thiazide diuretics should be avoided in individuals with gout, diabetes or hyperlipidemia; beta blockers are relatively contraindicated in individuals with bronchospasm, asthma or peripheral vascular disease; CCB should be avoided in individuals with certain cardiovascular disorders; and ACEI must be avoided throughout pregnancy.

In addition, the choice of medication within a particular class may depend on cost because insurance providers may not reimburse for certain medications, even if these have been tested in children. In addition, the psychological effects of labeling a child as "hypertensive" and in need of antihypertensive medications should be addressed with the child and the family.

Classes of Antihypertensive Agents

The author employs three essential steps to aid the decision to choose a specific class of antihypertensive medication and which agent within a class for a particular child:

Step 1. Formulate a hypothesis of the pathophysiology of HTN and of contributing factors. This will aid the selection of one or more agents with a mechanism of action that may counteract or oppose such pathophysiologic mechanisms. *Table 63-4* provides pediatric dosage guidelines for agents that are widely utilized in hypertensive children.

Step 2. Identify comorbid conditions such as diabetes and other metabolic disorders, heart disease, pulmonary or kidney disorders and consider whether or not these disorders may be negatively impacted by the proposed agent(s).

Step 3. Monitor for adverse effects associated with specific antihypertensive agents.

Diuretics

These agents promote urine production (diuresis) by reducing renal tubular sodium reabsorption (termed

Table 63-4

Antihypertensive drugs for management of hypertension in children

Diuretic		
Drug	**Dose**	**Comments**
Hydrochlorothiazide (HCTZ)	Initial: 1 mg/kg per day (q.d.; bid)	All patients treated with diuretics should have electrolytes monitored shortly after initiating therapy and periodically thereafter. Avoid thiazides in newborns with hyperbilirubinemia
	Maximum: 3 mg/kg per day upto 50 mg/day	
Chlorothiazide	Initial: 10–20 mg/kg per day	
	Maximum: 375 mg if age <2-years; 1,000 mg if age 2–12-years with 0.2–0.4 mg/kg/day	
Metolazone		Especially useful in combination with loop diuretics
Chlorthalidone	Initial: 0.3 mg/kg per day (q.d.)	May precipitate azotemia. Use with extreme cation in children with renal impairment
	Maximum 2 mg/kg per day up to 50 mg/day nephrocalcinosis	
Furosemide	Initial: 0.5–4.0 mg/kg per dose (q.d.-bid)	Avoid loop diuretics in children with hypercalciuria or nephrocalcinosis. May cause hypocalemia, hypocalcemia, hypomagnesemia. may be useful as add-on therapy in children with resistant HTN in association with renal failure
	Maximum: 6 mg/kg per day	
Bumetanide	0.015–0.06 mg/kg/day (daily or bid)	
Spironolactone	Initial: 1 mg/kg per day (q.d.-bid)	
	Maximum: 3.3 mg/kg per day up to 100 mg/day	
Triamterene	Initial: 1–2 mg/kg per day (bid)	Spironolactone, riamterence and amiloride may cause severe hyperkalemia, especially if given with ACE inhibitor or ARB
	Maximum: 3–4 mg/kg per day up to 300 mg/day	
Amiloride	Initial: 0.4–0.625 mg/kg per day (q.d.)	
	Maximum: 20 mg/day	
Epleronone	0.5–1 mg/kg per day (q.d.)	
Central α-agonist		
Clonidine*	Children ≥12 years (bid)	May cause dry mouth and/or sedation
	Initial: 0.2 mg/day	Transdermal preparation also available
	Maximum: 2.4 mg/day	Sudden cessation of therapy can lead to rebound HTN
α-metyldopa	Initial: 10 mg/kg per day (bid-qid)	Sedation is common
	Maximum: 60 mg/kg per day	
Peripheral α-agonist		
Doxazosin	Initial: 1 mg/day (q.d.)	May cause hypotension and syncope, especially after the first dose
	Maximum: 4 mg/day	
Prazosin	Initial: 0.05–0.1 mg/kg per day (tid)	
	Maximum: 0.1 mg/kg per day	
Terazosin	Initial: 1 mg/day (q.d.)	
	Maximum: 20 mg/day	
α- and β-Blocker		
Labetalol	Initial: 1–3 mg/kg per day (bid)	Should not be used in insulin-dependent diabetics
	Maximum: 10–12 mg/kg per day up to 1,200 mg/day	Asthma and heart failure are relative contraindications for all drugs in this classes

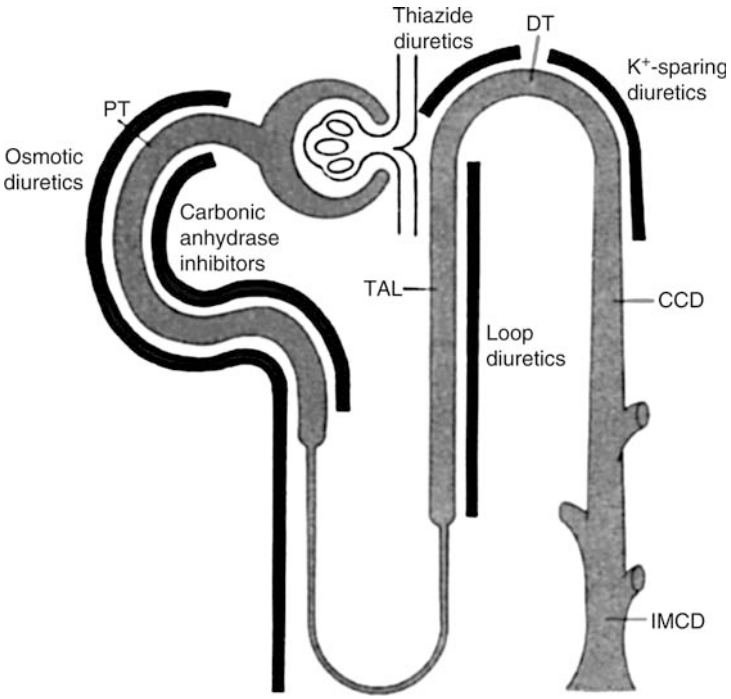
Table 63-4 (Continued)

Diuretic		
Drug	**Dose**	**Comments**
Carvedilol	Initial 0.15 mg/kg/day (bid)	Heart rate is dose limiting for all drugs in this classes
	Maximum: 0.5 mg/kg/day (bid)	All drugs in this classes may impair athletic performance
β-Blocker		
Atenolol	Initial: 0.5–1 mg/kg per day (q.d.-bid)	
	Maximum: 2 mg/kg per day up to 100 mg/day	
Bisoprolol/HCTZ	Initial: 2.5/6.25 mg/day (q.d.) (0.04 mg/kg per day is the bisoprostol component in Ziac)	
	Maximum: 10/6.25 mg/day	
Metoprolol	Initial: 1–2 mg/kg/per day (bid)	
	Maximum: 6 mg/kg per day up to 200 mg/day	
Propranolol	Initial: 1–2 mg/kg per day (bid-tid)	
	Maximum: 4 mg/kg per day up to 640 mg/day	
Vasodilators		
Hydralazine	Initial: 0.75 mg/kg per day (qid)	Tachycardia and fluid retention are common side effects
	Maximum: 7.5 mg/kg per day up to 200 mg/day	Hydralazine can cause a lupus-like syndrome in slow acetylators
		Avoid both agents in coronary artery disease, head trauma, intracranial hemorrhage and other cerebrovascular disorders
Minoxidil	Children $<$12 years: (q.d.-tid)	Prolonged use of minoxidil can cause hypertrichosis
	Initial: 0.2 mg/kg per day	Minoxidil is usually reserved for patients with resistant to multiple drugs
	Maximum: 50 mg/day	
	Children $\geq$12 years	
	Initial: 5 mg/day	
	Maximum 100 mg/day	
Calcium channel blocker		
Amlodipine (Norvasc)	0.06 mg/kg/day	Amolodipine and isradipine can be compounded into stable extemporaneous suspension
	Children 6–17 years: 2.5–5 mg once/day	
Felodipine (Plendil)	Initial: 2.5 mg/day (q.d.)	Felodipine and extended-release nifedipine tablets must be swallowed whole
	Maximum: 10 mg/day	
Isradipine (Dinacirc)	Initial: 0.15–0.2 mg/kg per day (tid-qid)	Isradipine may cause tachycardia
	Maximum: 0.8 mg/kg per day up to 20 mg/day	
Short-acting nifedipine	Initial: 0.2–0.25 mg/kg per dose	See Section VII. 12
	Maximum: 0.5 mg/kg per dose	

Table 63-4 (Continued)

Diuretic		
Drug	**Dose**	**Comments**
Extended-release nifedipine (Procardia XL, Adalat-CC, Nifedipine-ER)	Initial: 0.25–0.5 mg/kg per day (q.d.-bid)	
	Maximum: 3 mg/kg per day up to 120 mg/day	
Diltiazem (Cardizem)	1.5–2 mg/kg/day divided tid	Avoid in children with left ventricular dysfunction. Avoid in neonates and infants, or in those with sick sinus syndrome or A-V block
Verapamil (Calan-SR)	3–8 mg/kg/day divided tid	Same as for diltiazem
ACE inhibitor		
Benazepril (Lotensin)	Initial: 0.2 mg/kg per day upto 10 mg/day (q.d.)	All ACE inhibitors are contraindicated in pregnancy; females of childbearing age should use reliable
	Maximum: 0.6 mg/kg per day up to 40 mg/day	
Captopril (Capoten)	Initial: 0.3–0.5 mg/kg/dose (tid)	Cough and angioedema are reportedly less common with newer members of this class then with captopril
	Maximum: 6 mg/kg/per day	
Enalapril (Vasotec)	Initial: 0.08 mg/kg/per day up to 5 mg/day (bid)	Benazepril, captopril, enalapril, and lisinopril labels contain information on the preparation of a suspension
	Maximum: 0.6 mg/kg per day up to 40 mg/day	
Fosinopril (Monopril)	Children >50 kg: (q.d.)	Check serum potassium, creatinine periodically to monitor for hyperkalemia and renal dysfunction
	Initial: 5–10 mg/day	
Lisinopril (Prinivil, Zestril)	Initial: 0.07 mg/kg per day upto 5 mg/day (q.d.)	
	Maximum: 0.6 mg/kg per day upto 40 mg/day	
Quinapril (Accupril)	Initial: 5–10 mg/day (q.d.)	
	Maximum: 80 mg/day	
Ramipril ((Altace)	6 mg/m^2/day	
	Maximum: 20 mg/day	
Angiotensin-receptor blocker		
Irbesartan (Avapro)	6–12 years: 75–150 mg/day (q.d.)	All ARBs are contraindicated in pregnancy; females of childbearing age should use reliable contraception
	>13 years: 150–300 mg/day	
Losartan (Cozaar)	Initial: 0.7 mg/kg per day up to 50 mg/day (q.d.)	Check serum potassium, creatinine periodically to monitor for hyperkalemia and renal dysfunction
	Maximum: 1.4 mg/kg per day up to 100 mg/day	
Candesartan (Atacand)	0.23–0.35 mg/kg per day (q.d.)	
Valsartan (Diovan)	1.3 mg/kg/day (q.d.)	
	Maximum: 40 mg/day (q.d.)	
Olmesartan (Benicar)	1–5 years: 0.3 mg/kg per day (q.d.)	
	16 years: 0.3–0.6 mg/kg per day (q.d.)	

Figure 63-2
Nephron site of diuretic action.



saluresis or natriuresis). Hence, these agents are frequently prescribed for the management of HTN associated with edema or salt retaining disorders. *Fig. 63-2* shows the nephron site of diuretic action. *Table 63-5* summarizes the mechanism and duration of action as well as dosage of diuretics in children (33–35). Diuretics have a wide range of adverse effects and are often misused.

All diuretics, except spironolactone and amiloride, exert their action at the intraluminal side of the tubular epithelium. Nearly all diuretics other than mannitol are highly bound to plasma proteins; hence, they are not filtered at the glomerulus and must be secreted by tubular organic acid or organic-base secretory pathways. The site and mechanism of action determines if electrolytes other than sodium (example potassium) will be lost in the urine. Several agents acting in the late distal convoluted tubule induce limited diuresis, but may be useful as potassium sparing drugs, or they may be indicated for the management of specific monogenic hypertensive disorders.

In salt-sensitive hypertensive individuals with disturbed BP/natriuresis, diuretics such as hydrochlorothiazide may be of special benefit. Blacks and children with low birth weight and a decreased number of nephrons may also benefit from use of thiazides, especially when HTN is associated with a high body mass index.

Unlike the widespread use of diuretics in adults, such agents are utilized less frequently in the management of childhood HTN. In general, they are used as second or third line drugs to manage HTN associated with renal disorders, and loop diuretics are preferred over other classes, particularly in the setting of renal insufficiency. Diuretics have an additive and occasionally synergistic effect when used with other classes of antihypertensive agents. There are multiple antihypertensive combinations that contain thiazide diuretics so as to facilitate compliance. Osmotic diuretics, such as the nonmetabolizable sugar mannitol, expand the extracellular fluid compartment and are not utilized in the management of HTN.

Loop Diuretics

These include *furosemide*, *ethacrynic* acid, and *bumetanide*. By blocking the $Na^+/K^+/2\ Cl^-$ (NKCC2) electroneutral cotransporter in the luminal membrane of the medullary and the cortical segments of the ascending limb of the loop of Henle, these agents may increase the fractional excretion of sodium (FENa) by inhibiting the reabsorption of as much as 20% of the filtered sodium, and thereby lead to dramatic diuresis. Potassium, calcium, and magnesium depletion is also enhanced by these

Table 63-5

Site, mechanism, duration of action, and dose of diuretics used to manage hypertension

Site of action	Diuretic	Mechanism of action
Ascending loop of Henle	Fursemide (Lasix)	Inhibition of chloride reabsorption
	Bumetanide (Bumex)	
	Ethacrynic Acid (Edecrin)	
Distal convoluted tubule (early) and cortical thick ascending limb	Hydrochlorothiazide (Hydrodiuril)	Inhibition of sodium reabsorption
	Chlorothiazide (Diuril)	
	Metolazone (Zaroxolyn)	
Distal convoluted tubule (late) and collecting duct	Spironolactone (Aldactone)	Competitive inhibitor of aldosterone
	Triamterene (Dyrenium)	Direct effect by reducing electrical potential between cell and lumen
	Amiloride (Midamor)	Direct effect by reducing electrical between cell and lumen

rapidly acting agents. These agents are contraindicated in children with hypecalciuria or nephrocalcinosis. Metabolic alkalosis and volume contraction may result, especially if hypokalemia is not corrected. Loop diuretics may serve as first or second line agents in the management of HTN in disorders leading to sodium and fluid retention and HTN, such as acute poststreptococcal glomerulonephritis or other edema-forming states.

Thiazide Diuretics (Hydrochlorothiazide, Chlorothiazide, Metolazone)

Thiazide diuretics (*hydrochlorothiazide, chlorothiazide, metolazone*) directly inhibit the Na^+/Cl^- cotransporter in the cortical thick ascending limb and in the early distal tubule. The resultant increased delivery of sodium to the cortical collecting duct induces a kaliuresis similar to that seen with loop diuretics. However, the overall diuretic effect is more gentle than with loop diuretics. While thiazide diuretics are widely described as add-on or in combination with other antihypertensive agents, usually they are not effective in individuals with low GFR and low urinary flow rates. Because thiazides often cause volume contraction, more reabsorbtion of solutes such as glucose, calcium, and uric acid, as well as fluid can take place in the proximal tubule. Thus, hyperglycemia, hypercalcemia and hyperuricemia (gout) may occur, while serum potassium and magnesium concentrations may fall. These agents are contraindicated in individuals with high bilirubin concentrations, particularly newborns with hypebilirubinemia. *Chlorothiazide* may be given intravenously and has an additive effect when used concurrently with loop diuretics. This may also be further augmented by the addition of *aminophylline* infusion in children exhibiting diuretic resistance. *Metolazone* is the most powerful thiazide diuretic. It is especially effective in individuals with renal insufficiency when combined with loop diuretics. It can be given orally or enterally and has a longer duration of action than other agents in this class. Hence, once per day dosing is often adequate.

Potassium-Sparing Diuretics

Spironolactone, canrenone, and epleronone are aldosterone receptor antagonists and inhibit potassium excretion by the principle cells located at the cortical collecting duct, and to a lesser degree at the connecting tubule. Spironolactone is mainly used as a potassium-sparing diuretic and has a slow onset of action (about two days) and a relatively long half-life (20 h). All potassium sparing diuretics are relatively contraindicated in hyperkalemic disorders unless combined with another diuretic such as spironolactone/thiazide (Aldactazide). Both spironolactone and canrenone cause gynecomastia and amenorrhea and prolonged use of these agents is not recommended in children. Eplerone is a newer potassium-sparing diuretic that is relatively devoid of such side-effects and is gaining wider use in the pediatric population (see Sects. "Newer Antihypertensive Agents" and "Congenital Adrenal Hyperplasia (CAH)").

Triamterene and *amiloride* both inhibit the ENaC in the late distal tubule and collecting duct, thereby producing an electropositive change in the lumen which inhibits potassium and hydrogen secretion by transporters in principle cells which are dependent on such electrochemical gradient. These agents are often combined with loop or thiazide diuretics and may also cause hyperkalemia. They may also have a special role in the management of certain monogenic forms of HTN population (see Sect. "Monogenic Forms of HTN").

Resistance to diuretics used in the setting of HTN may be caused by several factors including a high salt intake,

progressive renal insufficiency, and use of nonsteroidal antiinflammatory agents which inhibit renal synthesis of vasodilatory and natriuretic prostaglandins.

Centrally Acting Agents

After crossing the blood-brain barrier, agents in this class stimulate alpha and/or beta imidazoline receptors in neurons of the nucleus tractus solitarius and other areas of the ventral lateral medulla. This results in inhibition of central sympathetic outflow to peripheral vessels and hence, lowers peripheral vascular resistance. Because of concurrent sympathoinhibition to the heart, vasodilation is not accompanied by reflex tachycardia. Also, cardiac output and renal blood flow, as well as glomerular filtration rate (GFR), are not affected by these agents (36). Hence, agents such as clonidine are often utilized as second or third line medications in the management of sustained HTN, particularly in children with renal disorders. Also, such agents are useful in managing disorders in which sympathetic overactivity may be playing a role, such as pheochromocytoma, diabetes, or the metabolic syndrome.

Clonidine is the most commonly utilized centrally acting agent in children. It is well-absorbed when given enterally or via the transdermal route and requires no adjustment in dosage in renal failure. It is also minimally removed by dialysis. Clonidine is especially effective in lowering BP when used adjunctively with vasodilators that cause reflex tachycardia including hydralazine and minoxidil. Because of a rapid onset of action (30–60 min), clonidine may be used to manage hypertensive urgencies, particularly if anxiety is contributing to HTN. However, transdermal delivery is not useful in this setting because the peak action occurs after 1–2 days, although effectiveness lasts for upto seven days.

Like other agents in this class, somnolence and dry mouth are the most common adverse effects of clonidine. Postural hypotension, muscular weakness and gastrointestinal symptoms are less frequent side effects. These adverse effects may particularly affect the lifestyle of children participating in competitive sports. Also, sudden withdrawal of clonidine may result in rebound HTN particularly when high dosages are utilized. With prolonged use, and particularly with high dosages of clonidine, vasodilation may occur and result in salt and water retention. This may be offset by the addition of a diuretic. Allergic dermatitis at the application site of transdermal clonidine is often encountered.

Alpha-methyldopa must be converted to the "false neurotransmitter" alpha-methylnorepinephrine, which then prevents norepinephrine from reacting with the alpha-adrenergic receptors. Because it can cause Coombs-positive hemolytic anemia and greater somnolence and depression compared to clonidine, this agent has limited use in the pediatric population. Its use in renal disorders and in dialyzed individuals is more problematic than with clonidine. It should not be used in children with hepatitis or other active liver disorders. However, alpha-methyldopa may be useful in agitated children with HTN following brain injury, and may also be utilized to manage pregnancy associated HTN because it does not interfere with placental blood flow and it is not teratogenic.

There is no appreciable experience with the use of *guanabenz* or *guanfacine* in children with HTN.

Alpha Agonists

These are fourth or fifth line drugs in children with chronic HTN. Among the drugs in this class noted in *Table 63-4*, only prazocin has had any notable use in children. However, phenoxybenzamine has specific utility in the management of pheochromocytoma (see Sect. "Pheochromocytoma/Paraganglioma, Neuroblastoma and Neuroendocrine HTN").

Beta Blockers

These agents reduce BP by several mechanisms. By binding to beta 1-adrenoceptors that predominate in cardiac myosites, they prevent epinephrine binding, thereby lowering heart rate and cardiac output. Beta blockers also decrease sympathetic outflow leading to reduced catecholamine-renin release. Peripheral vascular resistance is not greatly affected as vascular myosites possess mainly beta 2-adrenoceptors. Cardioselective beta blockers (high beta 1/beta 2 ratio) tend to lower the risk of bronchoconstriction.

Table 63-6 compares the hemodynamic and metabolic effects of beta blockers (37). There are no pediatric clinical trials comparing the hemodynamic and adverse effects of various beta blockers.

Metoprolol and *atenolol* are among the most currently utilized beta blockers. The cardioselective beta blocker, metoprolol, has shown safety and efficacy in both regular form and its extended release form (Toprol-XL) in children (38, 39). Bisoprolol in combination with hydrochlorothiazide (Ziac) is also effective in managing pediatric HTN (40).

In addition to having heterogeneous hemodynamic effects, beta blockers have very diverse effects on lipid

Table 63-6

Effects of selective antihypertensive drugs in patients with hypertension

Parameter	Ideal drug	Traditional β-blockers	Carvedilol	α1-Adrenoceptor blocker	ACE inhibitor or ARB	DHP calcium channel blocker	Thiazide diuretic
Mean arterial blood pressure	↓	↓	↓	↓	↓	↓	↓
Total peripheral resistance	↓	(↑)	(↓)	↓	↓	↓	↓
Cardiac output	0	(↓)	0	0	0	0	0
Heart rate	0/↓	↓	0/↓	(↑)	0	(↑)	0
Sympathetic nervous system activation	↓	↓	↓	(↑)	↓	↑	↑
Renin-angiotensin-aldosterone system	↓	↓	↓	0	↓	↑	↑
Lipid metabolism	0/+	–	0	0/+	0	0	–
Glucose metabolism	0/+	–	0	0	0/+	0	–

ACE, angiotensin-converting enzyme; *ARB*, angiotensin receptor blocker; *DHP*, dihydropyridine; ↓, decreased (inhibition); ↑, increased (activation); *0*, no effect; +, positive effect; and ( ), predominantly after acute administration (adapted from (143))

and glucose metabolism and other adverse effects that may reduce their cardiovascular benefit. Such negative findings reported with atenolol (41–43) have led to an increasing trend towards use of beta-adrenergic blockers which also possess alpha-blocking effects and therefore, are also capable of vasodilation. Such agents include the noncardioselective alpha/beta blockers *carvedilol* and it's once per day dosing controlled release formulation (Carvedilol CR) (44), and *labetalol*. An added advantage of these agents is that they are largely devoid of metabolic disturbances. Thus, carvedilol may be advantageous in individuals with diabetes.

Pediatric use of beta blockers is largely limited to children with compelling indications or as add-on second or third line agents. With the exception of their use to manage systolic HTN or HTN associated with obesity (45), beta blockers have limited utility as initial agents in primary HTN (40). Also, the general concept of using beta blockers to manage hypertensive children has been questioned (46). This contrasts with the frequent use of beta blockers as first line agents in the managements of adults with primary HTN (47). Beta blockers do have a special role in managing hypertensive disorders such as pheochromocytoma and postcoarctectomy HTN. Intravenous esmolol is also useful in managing hypertensive crisis (see Sect. "Management of Hypertensive Emergencies"). Beta blockers are also indicated for the management of myocardial infarction and heart failure in adults. However, these disorders are uncommon in hypertensive children, further limiting the use of beta blockers in the pediatric population, particularly as monotherapy.

Class adverse effects of beta blockers, include fatigue, nightmares, anxiety, dizziness, and bronchospasm, as well as hyperlipidemia and hyperglycemia. Thus, they are relatively contraindicated in children with asthma or diabetes, although alpha/beta blockers may be used in diabetics.

The use of beta blockers, including metoprolol, is discouraged in competitive athletes or runners with HTN because they prevent the compensatory rise in pulse rate which is necessary to maintain a higher BP during strenuous activity.

Direct Vasodilators

This class includes *hydralazine*, *minoxidil*, and *diazoxide*. The specific mechanism of action is not well understood. Hyperpolarization of arterial smooth muscle may result from opening of the K^+ channel. This leads to inhibition of IP_3-induced release of calcium from the sarcoplasmic reticulum thus preventing muscle contraction. Among drugs in this class, *hydralazine* is currently widely utilized intravenously to manage severe HTN in the Emergency Department setting particularly if the specific etiology of the HTN is not known. Common side effects include skin flushing and tachycardia which currently limit

maintenance use of oral hydralazine to manage chronic HTN. Minoxidil is a very effective vasodilator but hirsutism is a common and prominent drawback that prevents its use as a maintenance agent in children. Diazoxide can cause a precipitous fall in BP and its use has been supplanted by newer safer medications.

Sodium nitroprusside is a potent direct vasodilator which is very useful in managing hypertensive emergencies (see Sect. "Management of Hypertensive Emergencies").

Calcium Channel Blockers (CCB)

CCB antagonize the L-type calcium channels located in vascular smooth muscle. By blocking the influx of calcium into the cell CCB decrease smooth muscle contraction thus causing direct vasodilation. Several CCB also exert an important effect on cardiac muscle, sinoatrial, and atrioventricular nodes, and, hence, they may be potentially detrimental in individuals with preexisting cardiac disorders. By far, most of the CCB utilized in children belong to the more cardioselective dihydropyridine class which includes *amlodipine, nifedipine, nicardipine, isradipine, and felodipine.* The negative effect on cardiac conduction of CCB belonging to the phenylalkylamine (*verapamil)* and benzothazepine (*diltiazem*) classes limits their use in pediatric HTN.

It should be noted that the dihydropyridine CCB should not be taken together with meals that have a high fat content or with grapefruit or grapefruit juice. Such foods inhibit the enterocyte CYP3A4 system which degrades CCB and may increase their bioavailability by as much as a factor of two- to fourfold (48).

Table 63-7 highlights the hemodynamic and cardiovascular heterogeneity of CCB (49). CCBs should not be given to children with heart failure or actual damage of the myocardium. These agents may be more effective in lowering BP in African–American adults than ACEI or ARB (50) but such benefit has not been investigated in the pediatric population.

CCB are perhaps only second to ACEI and ARB in frequency of use in pediatric HTN. Most CCB are typically given twice daily. Extended release and long acting CCB formulations (*Procardia XL, Nicardipine SR, Isradipine CR, Felodipine SR, Verapamil SR*) are often dosed once daily. Generally long-acting formulations tend to produce less abrupt reductions in BP compared to agents requiring more frequent dosing. Newer preparations which combine CCB with a diuretic (example, Nifedical) may aid compliance in children requiring the use of both of these agents.

Amlodipine is very widely utilized in hypertensive children. It is inherently long-acting and probably has the least negative inotropic cardiac effects (*Table 63-7*). Studies indicate that amlodipine is equally effective to nifedipine in lowering blood pressure in children with essential HTN or in those with renal disorders (51). Pharmacokinetic studies suggest that once-daily administration may suffice in older children. Children under 13-years of age may require double the daily dosage used in older children (0.30 ± 0.16 mg/kg/day vs. 0.16 ± 0.12 mg/kg/day) and often need twice daily dosing for optimal BP control (52). It is available in tablet and in extemporaneously prepared liquid forms allowing dosage titration in small sized children. Compared with nifedipine, amlodipine use is associated with less gingival hyperplasia which occurs frequently in children with transplants managed with cyclosporine-based immunosuppressive regimens (53).

Extended release nifedipine (Procardia XL) is widely used in pediatric HTN because of its effectiveness and once or twice daily dosing. It lowers BP within 2 h.

Short-acting nifedipine is used primarily in the management of acute HTN, or in children with impaired gastrointestinal absorption, or in those who are unable to swallow medications. It lowers BP within 20 min of oral or sublingual administration. The use of short acting nifedine has declined over the past decade in large part because of safety concerns including rapid hypotension, syncope, and other cardiovascular risks mainly reported in adults, and because of the availability of newer agents thought to be safer. However, short acting nifedipine appears to be safe in children (54) and it remains to be a valuable agent for managing refractory or acute HTN in children, thus preventing the need to transfer such patients to the intensive care unit. The risk to safety ratio of short acting nifedipine may be optimized by proper selection of patients and adherence to several guidelines (see Sect. "Management of Hypertensive Emergencies").

Nicardipine also has relatively few adverse effects on the myocardium. It is especially useful in managing children with renal insufficiency who develop hypertensive emergencies (see Sect. "Management of Hypertensive Emergencies), in which the use of sodium nitroprusside is avoided because of an increased risk for cyanide/thiocyanate toxicity. The initial dosage is 1 mcg/kg/min by continuous infusion. The drug is highly effective and the dosage is readily titratable. Children should be closely monitored in the intensive care unit for hypotension and palpitations which are uncommon in the author's experience. Also, among CCB, nicardipine inhibits the metabolism of tacrolimus and cyclosporine more effectively and can lead to toxic blood levels of these drugs. Thus, close monitoring of such levels are needed if nicardipine is used to manage posttransplant HTN.

Because short acting liquid nifedipine is somewhat difficult to withdraw from the capsule and deliver

Table 63-7

Summary of CCB pharmacokinetics parameters

		Verapamil	Nifedipine	Nicardipine	Isradipine	Amlodipine	Diltiazem	Comments
Brand Names		Calan (SR) Convera-HS Isoptin (SR) Verelan	Adalat (CC) Procardia (XL)	Cardene (SR) Cardene I.V.	DynaCire (CR)	Norvasc	Cardizem (CD, SR) Cardizem Inj. Dilacor XR Tiamate Tiazac	Those with parentheses indicate medication available as immediate release as well as form/s in the parentheses
Adult dose range	Dose ranges for PO Administration	240–480 mg/day*	10–30 mg/day*	20–40 mg/day (SR – 60–120 mg/day in 2 divided doses)	5–20 mg/day	2.5–10 mg/day	180–360 mg/day (Info on SR/CD/XR not included because due to differences in dosing/schedule)	*Total daily dose is equivalent between sustained and immediate release forms (q.d. dosing)
Pediatric dose range		4–8 mg/kg/day	0.75–1.5 mg/kg/day	15–60 mg/day	0.2–0.8 mg/kg/day	0.12–0.29 mg/kg/day	1.5–3.6 mg/kg/day	Dose ranges other than immediate release forms not documented in pediatrics
Metabolism		Extensive First pass effect, Hepatic*	Hepatic*	Extensive First pass effect, Hepatic*	Hepatic	Hepatic*	Extensive First pass effect, Hepatic	*Dose adjustment needed if renal impairment
Elimination		70% Urine* 16% Feces	Urine	Urine*	Urine	Urine	Urine and bile	*Dose adjustment needed if renal impairment
Protein Binding (%)		90	90	>90	97	>93	78	
Bioavailability (%)		10–20	65	30	17	65–90	35–60	Absorption > 90% for all
Half Life		4.5–12	2	8	8–12	30–50	2–5	
Schedule		T.I.D.	T.I.D.	T.I.D.	B.I.D. (also T.I.D. for pediatric)	q.d.	T.I.D./Q.I.D.	All extended release preparations are dosed q.d.
Time to Peak Effect		1–2	0.5	0.5–2	1.5–3	6–12	2–3	
Dosage forms		Capsule, Tablet, Injection*	Capsule (liquid filled), Tablet*	Capsule (only for adults), Injection	Capsule*	Tablet*	Capsule, Injection, Tablet*	*Extemporaneous oral preparations can be made

accurately, *isradipine*, which is a second generation dihydropyridine, is often preferred in small children who cannot receive standard dosing of nifedipine. This agent is available in both tablet form as well as an extemporaneously prepared suspension formulation, and has a rapid onset of action. Because of its short half-life (3–8 h), isradipine permits smoother BP control but often requires dosing 3–4 times daily (55, 56). It is a useful agent particularly in younger children with HTN associated with renal disorders in general (see Sect. "Renal Disorders").

Common adverse effects of all CCB (about 10% each) include headache, dizziness, skin flushing, and fatigue associated with vasodilation. Lower extremity edema is also linked to vasodilation and is unresponsive to diuretics. Such edema, and tachycardia which occurs more frequently in infants and younger children receiving isradipine, are leading causes for discontinuing CCB. Nifedipine, as well as diltiazem, are associated with a higher incidence of gingival hyperplasia, particularly when used concurrently with cyclosporine.

Angiotensin Converting Enzyme Inhibitors (ACEI) and Angiotensin Receptor Blockers (ARBs)

By blocking angiotensin converting enzyme (ACE) in lungs and in vascular endothelium, ACEI inhibit the conversion of angiotensin I to angiotensin II (AT-II) thereby blunting the renin-angiotensin-aldosterone system *(RAAS)*. *Figs. 63-3* and *63-4* demonstrate the multiple renal and extra-renal actions of AT-II (57, 58). ACEI lower BP by reducing the vasoconstrictor effect of AT-II after it's binding to vascular smooth muscle AT-II type 1 receptors, and by decreasing salt and water retention through lowering of aldosterone biosynthesis. The latter effect may be much more pronounced in hypertensive individuals with reduced GFR. ACEI also block kininase thereby decreasing the degradation of bradykinin; the latter enhances the antihypertensive action of ACEI. Moreover, ACEI exert a beneficial effect on HTN by direct affects on atrial natriuretic peptide, vasopressin, and prostaglandins, and by down-regulation of adrenergic sympathetic activity (57). In addition to their effects on all arterial vessels, both ACEI and ARB lower intra-glomerular pressure by their preferential dilation of the efferent arteriole and by reducing the vasoconstrictive effect of angiotensin II (AT-II) (57–59). ARB have a more targeted action than ACEI by blocking the AT-II type I receptor. This may limit the adverse effects of ACEI related to higher bradykinin and lower plasma aldosterone concentrations, including hyperkalemia, chronic dry cough, and angioedema. Thus, ARB use is often preferred in managing HTN caused by diverse renal disorders (see Sect. "Renal Disorders") (60).

The addition of antialdosterone agents, such as spironolactone, to augment the antihypertensive action of ACEI

Figure 63-3

The renin-angiotensin system. *ACE* angiotensin-converting enzyme; *Ang* angiotensin.

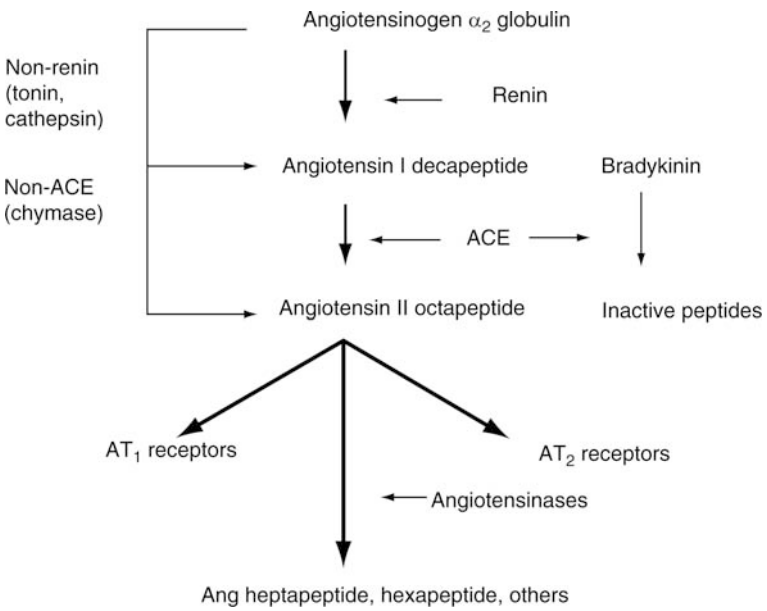
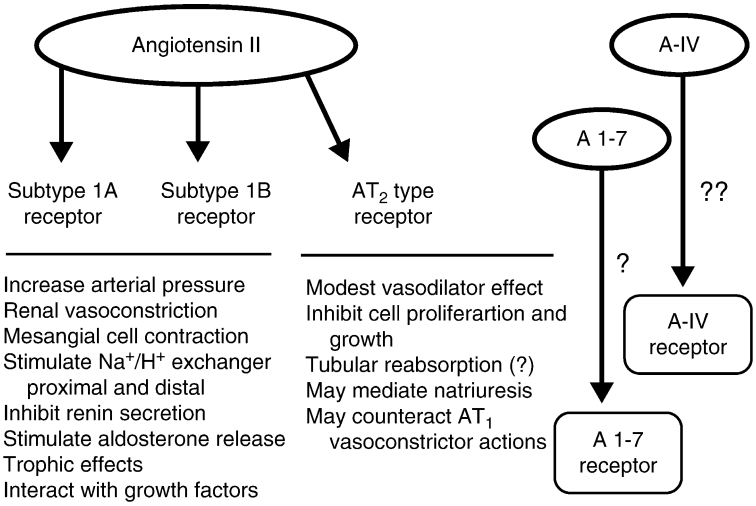


Figure 63-4

Types of angiotensin II receptor and their demonstrated or postulated actions.



and ARB in disorders associated with RAAS activation offers theoretical advantages (61). This drug combination may afford synergism and may be more nephroprotective than when either agent used alone. However, aldosterone blockade often potentiates the hyperkalemic and, possibly, the hyponatremic effects of ACEI. Thus, the combined use of spironolactone and ACEI should be avoided especially in children with reduced GFR.

Individuals with ACEDD genetic polymorphism have higher RAAS activity and may obtain a greater benefit from use of these agents (62, 63). Individuals with angiotensinogen polymorphisms may also benefit from use of the newer antirenin agents such as aliskiren (see Sect. "Newer Antihypertensive Agents"), while those with certain AT-II type 1 receptor polymorphisms may benefit from the use of ARB.

Among widely employed ACEI, the longest and more informative experience has been gained with *captopril*, *enalapril*, and *lisinopril*. When compounded into liquid formulations these agents are easier to dose and deliver in infants and younger children. *Captopril* is the only ACEI containing a disulfhydril moiety that has been credited with antioxidant and antiinflamatory action. Thus, captopril may be of special value in hypertensive children with lupus or other renal disorders. It has a short-duration of action and it usually requires three daily dosages. Titration should take place slowly over several days, particularly if it is utilized in hypertensive neonates. This is because neonates have physiologically higher renin activity which helps to maintain constant renal blood flow and GFR at a relatively low prevailing mean arterial BP in neonates. *Enalapril* and *lisinopril* have a more prolonged action enabling single or as twice daily dosing. This advantage makes these agents more suitable for use in older children and adolescents (64). Lisinopril is also available in combination with hydrochlorothiazide (*Zestoretic*). Enalapril is the only ACEI that can be administered intravenously, but it should be carefully titrated in neonates in order to lower the risk of acute renal failure.

Table 63-8 compares the pharmacologic characteristics of several ARBs. They vary greatly in their affinity for the AT-II type 1 receptor, in their half life and in bioavailability; all are highly protein-bound and therefore not easily removed by dialysis (65). *Losartan* (Cozaar) is the prototype ARB and has the widest experience of pediatric use (60). The drug is excreted by the kidneys and in bile, and is not dialyzed. *Valsartan* is also relatively short-acting and is excreted by the kidneys (30%) and bile (70%); absorption is decreased by 40% when taken with food. *Irbesartan* (Avapro) is longer acting than losartan or valsartan due to its high affinity for the AT-II type 1 receptor and is excreted mainly in bile (80%) and by the kidney (20%). *Candesartan* (Atacand) is also a long-acting AT-II receptor 1 antagonist and has 60% renal and 40% bile excretion and, therefore, may accumulate more readily than other ARB in individuals with severe renal dysfunction.

In order to offset hyperkalemia or salt and water retention associated with excessive aldosterone secretion in many renal disorders, several fixed combinations of ACEI or ARB with hydrochlorothiazide are now available (example: *Hyzaar, Diovan HCT, Avalide, Atacan HCT,* etc.).

Table 63-8

Pharmacologic characteristics of clinically available AT_1 receptor blockers

Drug/active metabolite	Compound	Relative AT_1 receptor affinity	Bioavailability (%)	Plasma half-life (h)	Protein binding (%)	Usual dosage (mg)
Losartan/*EXP 3174*	DUP-753, MK-954	50	33	2	98.7	50–100, optimal dose
		10		*6 to 9*	*99.8*	
Valsartan	CGP-48933	10	25	6	95.0	80–320, optimal dose
Candesartan/*CV 11974*	TCV-116	280	42	4	99.5	4–32, optimal dose
		1		*3 to 11*		
Irbesartan	SR-47436, BMS-186295	5	70	12	90.0	150–300, optimal dose
Eprosartan	SKF 108566	100			97.0	200– 400, twice a day
Telmisartan	BIBR 0277	10		24		40– 120, optimal dose

Common class adverse effects of ACEI include hyperkalemia and persistent nonproductive cough, while angioedema, neutropenia, and anemia are other less common adverse effects. In general, ARB have a more favorable adverse effect profile but are more expensive than ACEI. Both ACEI and ARB may cause renal functional impairment particularly in individuals with initial GFR below 60 mL/min/1.73 m^2 or in those with severe renal artery stenosis. Periodic monitoring of serum creatinine, potassium, and sodium concentrations is needed to identify important changes that may require modification in drug dosage. Also, a complete blood count may be done every 4–6 months to detect neutropenia or anemia with chronic use of these agents. These adverse effects typically resolve after lowering of dosage or discontinuing these agents. Also, both ACEI and ARB are teratogenic and their use is contraindicated during pregnancy.

Newer Antihypertensive Agents

Vasopressin Receptor Antagonists (*Table 63-9*)

Although traditionally vasopressin is not regarded as a "hypertensive hormone," it may exert a hypertensive action in African–Americans or other individuals with a blunted renin-angiotensin system. Vasopressin receptor antagonists exert their action mainly in tissues rich in V2 receptors (vascular endothelium and principal cells of the renal collecting and connecting tubules) thereby markedly increasing urine output, lowering urine osmolality, and raising serum sodium levels (64). Thus, such agents may be especially useful for managing hyponatremic euvolemic disorders such as SIADH, and hypovolemic or edema-forming hyponatremic disorders such as congestive heart failure, which are traditionally resistant to conventional diuretics. These agents do not lower blood pressure or GFR and as a rule, do not cause orthostatic hypotension, hypokalemia, hyponatremia, or reduced renal blood flow and lowering in GFR. They are also well tolerated. Such advantages over presently employed diuretic agents may make these vasopressin receptor antagonists useful adjunct agents in the management of hypertension as well. Only the intravenous agent *conivaptan* (Astellas Pharmaceuticals) has been approved by the FDA while several oral V2-selective antagonists are currently undergoing phase III trials.

Beta Blockers

Nebivolol is a beta I-selective, third generation beta blocker that received FDA approval in December 2007 for use in adults. This agent uniquely stimulates release of the potent vasodilator nitric oxide (66, 67). Its use is associated with less erectile dysfunction or impairment with lipid and glucose metabolism compared to agents such as metoprolol. Nebivolol is effective in dosages corresponding to one-tenth of dose of atenolol. There are no data of its use in children.

Table 63-9
Vasopressin receptor antagonists undergoing commercial development.

Compound	Receptor	Route	Manufacturer
Conivaptan (YM-087)	$V1_a$/V2	Intravenous	Astellas (Tokyo, Japan)
Lixivaptan (VPA-985)	V2	Oral	CardioKine (Philadelphia, PA)
Tolvaptan (OPC-41061)	V2	Oral	Otsuka (Tokyo, Japan)
Satavaptan (SR-121463)	V2	Oral	Sanofi-Aventis (Paris, France)

Aldosterone Antagonists

Eplerenone is a newer mineralocorticoid receptor blocker with less antiandrogenic and antiprogesteronic properties than spironolactone or canrenone. Thus, eplerenone may be of particular benefit to children because of less interference with sexual maturation. It is especially useful in managing HTN caused by glucocorticoid-remediable hyperaldosteronism (see Sect. "Congenital Adrenal Hyperplasia (CAH)")

Renin Inhibitors

Although ACEI are effective in lowering BP in several hypereninemic disorders leading to increased circulating angiotensin II concentrations, such agents stimulate renin release by interfering with angiotensin II type 1 receptor-induced inhibition of renin release. Ultimately, this leads to reduced sustained antihypertensive effectiveness. *Aliskiren* is the prototype of a new class of drugs that directly inhibit renin. Several randomized human trials showed that orally administered aliskiren at 75 or 150 mg once daily was not only extremely safe but also very effective in lowering BP in adults with mild-to-moderate HTN (68, 69). Its antihypertensive action was additive when combined with ramipril, amlodipine, or hydrochlorothiazide. There is limited information of the use and safety of this agent in hypertensive adults with renal disorders and there are no pediatric studies using this agent.

Vasopeptidase Inhibitors

This class of agents is capable of blocking both angiotensin converting enzyme and neutral endopeptidase (NEP) (70). NEP degrades several peptides including atrial and brain natriuretic peptides, adrenomedullin, and bradykinin. Thus, inhibition of NEP potentiates the action of these peptides resulting in more salt and water excretion, inhibition of RAAS, and peripheral vascular dilation. *Omapatrilat* is a prototype agent in this class that is undergoing extensive human clinical trials (71, 72). Despite its effectiveness in lowering BP, FDA approval has been delayed because of a relatively high incidence of angioedema and dry cough attributed to higher bradykinin levels than those encountered with ACEI.

Endothelin-1 Receptor Antagonists

Endothelin-1 receptor antagonists such as *bosentan* and *avosentan* are quite effective in lowering BP associated with endothelin release which commonly occurs in the post-transplant setting (see Sect. "Posttransplant HTN"). However, endothelin antagonists are difficult to administer and are not currently indicated for treatment of systemic HTN.

Dopamine Antagonists

Fenoldopan is a newer dopamine 1 receptor agonist which promotes smooth muscle relaxation particularly within renal and splanchnic vessels. It may be particularly valuable in managing hypertensive crisis (see Sect. "Management of Hypertensive Emergencies").

Management of Specific Disorders

Primary (PH) or Essential HTN

Over the past decade the incidence of PH has increased markedly particularly among adolescents, in large part because of the obesity epidemic and lifestyle changes (2). This underscores the need to identify and implement lifestyle measures which may lead to prevention of PH at a younger age.

There are no large, longitudinal studies to provide evidence-based guidelines for the management of PH in children (30). Because beta blockers may inhibit reflex tachycardia in children involved in vigorous activities such agents may cause syncope and other adverse cardiovascular events. Thus, unlike the JNCC guidelines for managing HTN in adults (47), diuretics and beta blockers are not commonly employed as initial agents in the control of PH in children.

The combined use of beta blocker/thiazide was associated with suboptimal BP control in one pediatric study

of PH (40). However, cardioselective beta blockers may be used selectively in children with PH resulting mainly in systolic HTN. For such purposes, atenolol or carvedilol may be used. CCB and ACEI use was examined in a retrospective study of childhood PH (64). Both classes of agents lowered systolic BP while only ACEI also lowered diastolic BP. However, when the 95th percentile for age, gender, and height was the "target value," only 45% achieved systolic BP control while 62.7% achieved diastolic BP below the 95th percentile (64).

ACEI have been shown to be as effective as beta blockers and diuretics in adults with PH. ACEI may also have greater cardioprotective benefits which extend beyond BP lowering whereas several studies indicate an increase in coronary artery disease and heart failure with use of CCB. Although, such adverse outcomes are uncommon in children managed with CCB, the author prefers starting with an ACEI or ARB for pharmacologic management of PH. In obese or other children with isolated systolic HTN, carvedilol or labetalol may be indicated. Close monitoring of BP is essential in modifying the dosages of antihypertensive agents especially in children with PH in whom improvements in lifestyle may have a drastic effect in lowering BP.

Important outcome measures of managing PH in adults include a reduction in coronary heart disease, stroke, and cardiovascular death. Such data are not available in children.

Obesity/Metabolic Syndrome/Sleep Apnea

The metabolic syndrome is almost always encountered in obese children and is associated with hyperlipidemia, insulin resistance and HTN. Sleep apnea is also nearly always associated with obesity in children. Thus, the mechanisms of HTN in both of these disorders are complex and appear to overlap.

Obesity is associated with sustained sympathetic nerve activity causing an increased heart rate, and with higher endothelin-1 expression leading to impaired vasodilation and increased systemic vascular resistance (73). Circulating insulin and leptin concentrations are often high in obese children and both of these are known to aid sodium retention (74). In addition, autonomous synthesis of aldosterone by adipocytes has been demonstrated in individuals with central obesity (75, 76). Hyperaldosteronism may be confirmed by urine aldosterone exceeding 12 mcg/24 h despite sodium loading, and a plasma aldosterone to peripheral renin activity ratio greater than 20–30 when aldosterone is measured in ng/dL and PRA in ng/mL/h. These mechanisms of HTN suggest a greater potential amelioratery effect on BP of lifestyle measures or even surgical means, such as gastric bypass, in order to maintain a sustained weight loss. In addition, the preferential use of beta blockers and antialdosterone agents may be of greater but yet unproven antihypertensive benefit in obese children. There is also recent evidence indicating that ARBs may lower the incidence of type 2 diabetes in obese hypertensive individuals with metabolic syndrome by improvement in metabolic parameters (77). Thus, carvedilol or labetalol, or an ARB may be the initial pharmacologic agent in these disorders while thiazide diuretics should be avoided (see Sect "Thiazide Diuretics").

Renovascular HTN

This category of HTN includes renal microvascular disease, renal artery stenosis due to multiple etiologies, and renal focal ischemia due to renal microthrombi or scarring after pyelonephritis. This type of HTN associated with these disorders is often resistant to antihypertensive agents that do not inhibit the renin-angiotensin-aldosterone system. A "critical" fall in perfusion to a part or to the entire kidney in these disorders stimulates renin release and AT-II, and other mechanisms tending to restore renal perfusion but in the process also raise systemic BP (78–80). Because angiotensin II plays a major role in renovascular HTN, it is targeted for pharmacologic blockade.

An example of this is *renal artery stenosis* (RAS). There are no comparisons of the complications of revascularization procedures and medical management of RAS in children. While nonmedical approaches have an associated risk for hemorrhage, vessel or renal perforation, and thrombosis leading to loss of renal function, this approach is often preferred in children as it offers the possibility of a permanent cure, particularly in children having stenosis at the ostium or abdominal aorta syndrome. Conversely, ACEI or ARB use may lead to acute renal failure in children with bilateral severe or critical RAS (greater than 80% narrowing), or in those managed with diuretics and salt restriction leading to diminished circulatory volume.

Once the location and severity of the stenosis is established by imaging studies, a decision may be made to administer oral ACEI, intravenous enalaprilat, or ARB. As a caveat, the medical management of RAS should take place with great caution so as to avoid rapid lowering of systemic BP. Salt restriction should be moderate, and diuretics should be avoided. For pharmacologic management of renovascular HTN other than bilateral RAS, in infants the author recommends starting with

low-dose captopril and titrating upwards as needed. Older children may receive labetalol or carvedilol as the initial agent, and an ACEI or ARB may then be added depending on BP response. Sodium nitroprusside or fenoldopam infusion may be administered in children with hypertensive emergency (example: vessel dissection, cerebrovascular accident, seizures, retinal hemorrhage or Bell's palsy) (see Sect. "Management of Hypertensive Emergencies"). In all instances erum creatinine concentration should be carefully monitored. If it rises by more than 20% above baseline levels, ACEI or ARB may be stopped, reduced in dosage, or substituted with intravenous nitroprusside or labetalol, until a revascularization procedure is performed.

Renal Parenchymal Disorders

Activation of the renin-angiotensin-aldosterone system (RAAS) plays a major role in the regulation of BP in humans. Classically, RAAS activation occurs with hypotension; conversely RAAS is suppressed in states associated with systemic HTN. It has been demonstrated that after initial nephron injury caused by a variety of renal disorders, the RAAS becomes inappropriately activated and often remains active, rather than become suppressed, despite persistent systemic HTN (57, 81). Chronic or persistent activation of RAAS may accelerate renal parenchymal injury by several mechanisms: (1) direct vasoconstriction by angiotensin II raising systemic and/or intraglomerular BP leading to hyperfiltration injury, (2) aldosterone-mediated increase in salt and water retention and other deleterious effects of sustained elevations in plasma aldosterone concentration, and (3) elaboration of TGF beta, plasminogen activator inhibitor-1, and vascular endothelial growth factor (VEGF-A) as well as other mediators of renal fibrosis and hypertrophy which promote arteriolar wall thickening (57, 59, 82–87).

Intrarenal activation of RAAS without systemic HTN, has also been implicated in progressive renal injury associated with diabetic nephropathy, autosomal dominant polycystic kidney disease, or multicystic dysplasia.

Expansion of the circulatory volume is a major cause of HTN in disorders such as acute poststreptococcal glomerulonephritis and other acute nephritides. In this setting the use of diuretics with or without a CCB is generally preferred because GFR is often reduced on initial presentation and may become further compromised by adding ACEI/ARB to the diuretic regimen. In contrast, in chronic kidney disease (CKD)/dialysis, HTN due to circulatory volume expansion is common and may be challenging to control especially in individuals with advanced renal insufficiency. The specificity of renal protection afforded by ACEI or ARB in the setting of HTN in CKD may be overemphasized. However, these agents are still preferentially utilized because the antihypertensive synergism afforded by RAAS blockade coupled with diuretics is often effective as both classes of drugs promote salt and water excretion. Diuretics may also reduce the risk of hyperkalemia associated with concurrent ACEI use in this setting.

Although systemic hypertension may be due to multiple etiologies, the final common pathway for hypertensive renal injury involves transmission of the elevated blood BP to the renal microvasculature. The latter is physiologically opposed by intricate autoregulatory mechanisms of renal blood flow which become impaired with sustained increases in systolic BP exceeding 180 mm Hg in adults (this value is unknown for children). Autoregulation of renal blood flow is also compromised by reduced renal mass (example: fewer nephrons at birth or after nephrectomy due to tumor), and by acquired glomerulopathies. In these disorders systolic HTN tends to correlate better with progressive renal damage. Under these circumstances vascular or glomerular disruption may occur with only modest or even transient elevations in systemic BP, which may be underestimated by BP levels obtained by casual peripheral BP measurements. Ultimately, the loss of autoregulation of renal blood flow associated with renal injury renders damaged glomeruli more susceptible to the hydraulic affects of HTN, leading to progressive glomerulosclerosis (85).

Newer investigations have uncovered multiple biological functions of aldosterone beyond its well established role in potassium and volume/BP homeostasis. The unregulated stimulation of aldosterone in many renal disorders has been implicated in progressive tubulointerstitial fibrosis and dysfunctional cardiac remodeling associated with chronic HTN. Aldosterone receptor antagonists are often helpful as add-on second or third line drugs in individuals with HTN resistant to other agents (88). Use of this class of agents may reduce BP by their diuretic action, as well as by nonvolume mediated effects (89–94). Antialdosterone agents may also offset the rebound elevation in plasma aldosterone concentrations ("aldosterone escape") noted after an initial fall in plasma aldosterone concentrations evident after starting ACEI (90). Higher serum potassium concentrations particularly in individuals with GFR below 60 mL/min/1.73 m^2 managed with ACEI may be partly responsible for stimulating aldosterone biosynthesis in this setting. Hence, in theory, with proper monitoring and prevention of hyperkalemia, the combined use of spironolactone or eplerenone together with ACEI or ARB may afford more optimal nephroprotection in children with HTN and/or proteinuria (91).

Oligonephronia or, reduced nephron number, is another important and, perhaps, underappreciated cause of HTN both in children as well as in adults suspected of having primary HTN. The number of nephrons in healthy individuals is quite variable ranging from 0.8 to 1.8 million. Infants with low birth weight or intrauterine growth retardation may be susceptible to HTN during early childhood because of an impaired ability to excrete salt and water (95–98). Thus, early detection and management of such HTN may be useful in preventing renal dysfunction and other end-organ injury in early adulthood.

In conclusion, several studies have shown that suppression of the RAAS improves the rate of progression of renal injury (99). Because of their ability to lower both systemic as well as intraglomerular HTN, ACEI/ARB are recommended as first-line agents in managing HTN in children with diverse chronic renal disorders (32, 100). These agents may also have an antifibrotic and antiinflammatory benefit which may also enhance their nephroprotective properties (59, 101) which are not observed with the use of other classes of antihypertensive agents that are equally effective in lowering systemic BP. Because proteinuria is another important marker of renal disease progression, the greater antiproteinuric action afforded by ACEI and ARB has led to wider use of these drugs in hypertensive individuals who also have proteinuria. The combination of ACEI and ARB to block RAAS more completely has also been advocated, particularly in hypertensive individuals with proteinuric disorders. In children with refractory HTN associated with circulatory volume expansion, a large array of combination formulations of ACEI or ARB with hydrochlorothiazide may be more effective (47). If BP is not optimally controlled, clonidine, labetalol or carvedilol may be added as a second line agents (in place of a diuretic), or, these drugs may serve as third line agents. Provided that renal function is relatively well preserved, an antialdosterone agent may also be used as a third or fourth line agent. In children with more advanced renal failure (CKD stages 3–5), CCB may be the first line agents with clonidine, a loop diuretic, labetalol or carvedilol, and clonidine serving as second or third line agents. Minoxidil is effective for HTN refractory to these agents but its adverse effects, particularly hirsutism, makes long term use untenable.

Posttransplant HTN

Roughly two-thirds of children with kidney and other solid organ or bone marrow transplants managed with calcineurin-based regimens that include glucocorticoids develop chronic, iatrogenic HTN (102). The transplant population may, perhaps be only second in prevalence of secondary HTN to the sum of all children with renal disorders. Steroids are the main culprit since de novo HTN after transplantation is very uncommon with tacrolimus monotherapy (103). However, cyclosporine and, to a much lesser degree, tacrolimus may potentate the effect of steroid by inducing endothelial and proximal tubular cell synthesis of endothelin- 1. This potent vasoconstrictor preferentially constricts the afferent glomerular capillary thereby promoting renin-mediated systemic HTN (104). Endothelin-1 receptor antagonists such as bosentan and avosentan are quite effective in lowering BP associated with endothelin release. Also their combined use with ACEI/ARB may be of special value in disorders associated with both HTN and proteinuria. However, endothelin antagonists are difficult to deliver and have other potential hazards that have hindered their clinical use outside of pulmonary HTN (105).

Because CCB have a preferential affect in vasodilating the afferent glomerular arteriole they are often recommended. Because nifedipine, amlodipine, and isradipine do not alter metabolism of cyclosporine or tacrolimus, and because their clearance is not influenced by renal dysfunction, they are often used to manage HTN in the posttransplant setting or in children with renal disorders. In younger children, isradipine may be easier to dose; mean dosages of 0.38 ± 0.22 mg/kg/day divided in three daily dosages proved effective in a series of children mostly under 10 years old having renal disorders or various transplants (55). These dosages were generally higher compared to those utilized in adults. Another large series comprised of children of all ages with HTN associated with glomerulonephritis or solid organ transplants, found that similar dosages were effective and well tolerated (56). CCB use in the posttransplant setting may be complicated by a high rate of dependent edema due to peripheral vasodilation, and by posttransplant erythrocytosis.

Glucocorticoids may also contribute to salt and water retention, expansion of the intravascular volume, and HTN (106). In such individuals, reduction in salt intake together with use of a diuretic may be useful in lowering BP. While loop diuretics are preferred for use in the setting of posttransplant HTN, these agents should be used with caution in children with hypercalciuria or nephrocalcinosis.

ACEI and ARB are also efficacious in the management of posttransplant HTN with or without concurrent proteinuria (107). However, caution should be taken in using ACEI/ARB. ACEI, in particular, may exaggerate hyperkalemia which occurs frequently in the posttransplant

setting because calcineurin inhibitors suppress aldosterone secretion which is a key mechanism for the elimination of potassium. Also, both of these agents may lower intraglomerular pressure and GFR leading to the mistaken diagnosis of rejection. In addition, these agents may contribute to anemia that persists after successful transplantation.

Because hydration is very important after renal transplantation diuretic use is usually avoided in the management of posttransplant HTN even when hyperkalemia is present. Fludrocortisone (Florinef) is often utilized for control of such hyperkalemia. However this mineralocorticoid may also contribute to hypertension.

The finding of a bruit over the allograft artery or the presence of abnormal blood flow by Doppler ultrasonography may suggest renal artery stenosis as the etiology of HTN. In such disorders, a pharmacologic trial with ARB may precede the use of intraluminal angioplasty of the affected artery.

Monogenic Forms of HTN (*Table 63-10*)

These disorders are detailed in several recent reviews (108–115). *Table 63-10* provides an excellent summary of the causes evaluation and management of monogenic HTN (108). Although some of these conditions are rare, they often manifest with HTN during childhood and they provide an excellent insight into the pathophysiology of HTN. The majority of these forms of HTN stimulate sodium reabsorption at different sites along the nephron and tend to be associated with metabolic alkalosis and hypokalemia.

Glucocorticoid Remediable Hyperaldosteronism (Also Known as Familial Hyperaldosteronism Type I)

This is an autosomal dominant disorder resulting from a chimeric gene in which the 11 beta-hydroxylase promoter sequence is spliced to the coding region of the aldosterone synthase gene. This chimeric gene is located in the zona fasciculata, rather than in the zona glomerulosa of the adrenal gland where aldosterone synthesis normally occurs, and the 11 beta-hydroxylase promoter is under the control of ACTH. This results in a salt-sensitive HTN at a young age with low plasma renin activity but high or normal plasma aldosterone concentrations, and secondary hypokalemia and metabolic alkalosis. This form of HTN responds to exogenous glucocorticoids which suppress ACTH release. However, prolonged treatment with such agents has unacceptable adverse effects especially in growing children. Alternative agents include amiloride and spironolactone. The latter is effective in opposing the aldosterone-mediated salt retention but it frequently causes gynecomastia and sexual dysfunction. A newer agent, eplerenone, is currently recommended because it also antagonizes the effect of aldosterone but has less interference with sexual maturation than spironolactone.

Table 63-10
Features of inherited hypertension

	Inheritance pattern	Age	K	PRA	Aldo	Aldo: PRA	GC resp.	MR-A resp.	Rx
Liddle's	AD	C,A	N or ↓	↓	↓		–	–	A,Tr
Gordon's	AD	A (C)	N or ↑	↓	N or ↑	↑	–	–	T
AME	AR	I,C,A	↓ (N)	↓	↓		–		MR-A
H-P	AD	C,A	N or ↓	↓	↓		–	reversed	A,Tr,T
GRA	AD	I,C	N or ↓	↓	↑ (N)	↑			G,A,Tr
FH II	AD	A	N or ↓	↓	↑	↑	–		MR-A
CAH	AR	I	N or ↓	↓	↓		–		MR-A
FGR	AR/AD	I	N or ↓	↓	↓		–		MR-A

AME, apparent mineralocorticoid excess; *H-P*, hypertension exacerbated by pregnancy; *GRA*, glucocorticoid-remediable aldosteronism; *FH II*, familial hyperaldosteronism type II; *CAH*, congenital adrenal hyperplasia with 11- or 17-hydroxylase deficiency; *FGR*, familial glucocorticoid resistance; *AD*, autosomal dominant; *AR*, autosomal recessive; *Age*, typical age at presentation; *I*, infancy; *C*, Childhood; *A*, adulthood; *K*, potassium; *N*, normal; ↓, decrease; ↑, increase; *PRA*, plasma rennin activity; *Aldo*, aldosterone; *Aldo: PRA*, ratio of aldosterone to PRA (<30 diagnostic if Aldo, in ng/dL, PRA in ng/mL/h); *GC resp.*, response to glucocorticoids; –, negative; +, positive; *MRA resp.*, response to mineralocorticoid receptor antagonists; R_x, treatment; *A*, anciloride; *Tr*, triamterene, *T*, thiazides

Apparent Mineralocorticoid Excess (AME) (116–119)

Mutations in the 11 beta-hydroxysteroid dehydrogenase type 2 (11 beta-HSD2) leads to loss of enzyme function resulting in decreased conversion of cortisol to cortisone in the principle cells located in the cortical collecting duct and connecting tubule. Cortisol, unlike cortisone, binds to the mineralocorticoid receptor and activates the basolateral sodium-potassium ATPase leading to sodium reabsorption, potassium wasting, hypokalemia, metabolic alkalosis, and hypertension. Plasma renin activity and aldosterone concentrations are suppressed secondary to HTN. The licorice-derived moieties, glycyrrizic acid and carbenoxolone (used to treat gastric ulcer disease), inhibit the action of 11 beta-HSD2 and can also cause HTN by the same mechanism. Individuals with apparent mineralocorticoid excess respond well to dietary sodium restriction, thiazides and mineralocorticoid receptor blockers including spironolactone, canrenone, and epleronone.

Genetic polymorhisms in the gene encoding for 11 beta-HSD2 may play a role in the development of low renin primary HTN in about 40% of individuals in the USA.

Gain of Function Mutation of the Epithelial Sodium Channel (ENaC) (Liddle's Syndrome) (120)

ENaC is the rate limiting step in sodium reabsorption. In Liddle's syndrome the mutated ENaC channels of the principle cells of the cortical collecting duct remain in the plasma membrane for a prolonged time period. This effectively increases the number of such channels, leading to increased sodium reabsorption and HTN. The clinical presentation is similar to apparent mineralocorticoid excess (infra supra). Thiazides, amiloride, and triamterene antagonize the ENaC directly and thereby lower BP in such individuals. Spironolactone does not affect the ENaC and does not reduce BP in Liddle's syndrome.

Gain of Function Mutation of the Mineralocorticoid Receptor (121)

This is a rare disorder in which a single amino acid substitution of the mineralocorticoid receptor gene in principal cells results in pathologic increases in sodium retention, volume expansion, and HTN. Such volume expansion may be enhanced by aldosterone, cortisol or progesterone. This disorder has been implicated in adults with primary HTN characterized by low plasma renin activity and low plasma aldosterone concentrations which are physiologically suppressed by the HTN. Also, women with preeclampsia or eclampsia may have a similar genetic predisposition to HTN which manifests only during pregnancy because of high plasma concentrations of progesterone prevailing during pregnancy. This is because progesterone tends to activate rather than suppress the mutant receptor. Similarly, spironolactone inhibits thewild type mineralocorticoid receptor but tends to activate the mutant receptor and may promote HTN in this disorder.

Congenital Adrenal Hyperplasia (CAH) (122)

Two forms of CAH are associated with HTN. The common form is caused by a mutation in the CYP11B1 gene encoding for 11 beta-hydroxylase. This leads to loss of feedback inhibition of adrenocorticotrophic hormone and stimulation of adrenal mineralocorticoid synthesis resulting in HTN. The rare form is caused by a mutation in the gene encoding for 17 alpha-hydroxylase. Both of the disorders lead to loss of function of these enzymes that are very important in the proper synthesis of cortisol and gonadal hormones. As a result, plasma 11-deoxycortisol and deoxycorticosterone concentrations rise resulting in sodium retention, volume expansion, and HTN.

Pseudohypoaldosteronism Type II (Gordon's Syndrome)

Several genes encoding aberrant kinases have been linked to this familial disorder which manifests clinically with HTN and hyperkalemia with associated muscle weakness. Peripheral plasma renin activity is suppressed while plasma aldosterone concentrations are inappropriately normal. Thiazides are highly effective in managing both the hyperkalemia and HTN.

Autosomal Dominant HTN with Brachydactyly (123)

Severe HTN starting in childhood, and death before age 50 due to stroke are common manifestations of this disorder. This disorder is not caused by salt sensitivity. No specific gene locus has yet been identified, but the sulfonylurea receptor-2 gene has been implicated. Such individuals have normal plasma renin and aldosterone concentrations. HTN is believed to be of neurogenic

origin. It is postulated that these individuals have a "neurovascular control anomaly" on magnetic resonance angiography demonstrating abnormalities of the posterior fossa of the brain causing compression of the ventrolateral medulla and of cranial nerves IX and X, resulting in a high peripheral vascular resistance. There is no satisfactory response of the HTN to any particular agent. However, HTN improves following decompression surgery of the affected brain area.

Pheochromocytoma/Paraganglioma, Neuroblastoma and Neuroendocrine HTN

Pheochromocytoma may be entertained in the etiology of HTN on the basis of classical symptoms of paroxysmal headache, palpitations, and skin flushing. However, younger children tend to have more sustained HTN as well as visual disturbances, emesis, sweating, tremors and anxiety. Several antihypertensive agents may stimulate catecholamine secretion and lead to false diagnosis of pheochromocytoma. Assuming that neurological symptoms are absent, funduscopic exam is normal and there are no seizures or severe encephalopathy to indicate an impending medical emergency, management of the HTN may be delayed until the appropriate plasma and urinary studies are obtained to establish the diagnosis of pheochromocytoma or other neuroendocrine disorders with similar presentation. Disorders mimicking catecholamine secreting tumors include essential HTN, diencephalic syndrome, and severe anxiety.

Catecholamine secreting tumors such as pheochromocytoma, neuroblastoma, and ganglioneuroblastoma result in a hyperadrenergic state. Thus, skin flushing and tachycardia may be signs of a high cardiac output state. Such sympathetic stimulation along with vasoconstriction and systemic HTN lead to diuresis and volume depletion. As a result, hemoconcentration and hypokalemia may ensue.

Preoperative medical management of the HTN is of great value in avoiding major intraoperative complications including hypotension, hypertensive crisis, and tachyarrhythmias associated with surges of catecholamine secretion during tumor manipulation and removal (124–128).

In the setting of tachyarrhythmias, initial management is aimed at inhibiting the release of catecholamines and chronic vasoconstriction utilizing phenoxybenzamine or alpha-2 adrenergic agonists with shorter duration of action such as clonidine or prazosin. Because of potentially rapid and profound fall in BP, the author does not endorse the use of phentolamine as a diagnostic tool or for treatment of pheochromocytoma. Instead, treatment may begin with oral *phenoxybenzamine* (Dibenzyline) at an initial dosage of 0.3 mg/kg/day divided in two daily dosages. The drug may be titrated by 25% every 48 h to an average dose of 1.2 mg/kg/day (maximal dosages may be as high as 4 mg/kg/day but are not recommended by the author), until BP slowly falls into the normal range for age, gender, and height. Precaution must be taken to prevent orthostatic hypotension especially during the early or acute phase of volume repletion. Salt restriction or high salt intake is not recommended. Beta blockers may be started about 48 h after the start of alpha-adrenergic agonists in order to reduce the risk of tachyarrhythmias or epinephrine-induced vasoconstriction from unopposed beta-adrenergic stimulation in individuals with epinephrine secreting tumors. For this purpose, carvedilol, a noncardioselective alpha/beta blocker, may be started at a dosage of 0.15 mg/kg/day divided in two dosages, with an increase in dose to 0.5 mg/kg/day divided in two dosages, as needed. Also, atenolol or metoprolol may be equally effective to carvedilol in improving the high cardiac output state.

Drugs that decrease synthesis of catecholamines such as the tyrosine analog alpha-methyl tyrosine or metyrasone (tyrosine hydroxylase inhibitor) are not utilized extensively in the population but have been shown to be helpful adjuncts in the management of adults with pheochromocytoma.

Agents known to release histamine and thereby stimulate sympathetic affects, including morphine, succinylcholine, atracurium, and related compounds should be avoided. In addition, drugs that provoke catecholamine release should be avoided along with foods containing tyramine which may displace catecholamines from storage vesicles. Alpha-1-adrenergic antagonists with shorter half-life than phenoxybenzamine may be substituted for about two days prior to surgery.

Resection of the tumor may be done safely when the child's BP, tachycardia, and cardiac dysfunction resolve, and other symptoms and signs subside or improve. This typically occurs 2–3 weeks after initiating treatment.

Endocrine Disorders

Conns Syndrome

In this disorder HTN often results from a benign aldosterone-secreting adrenal adenoma. This is an uncommon condition in childhood and presents with HTN in association with high aldosterone levels but suppressed peripheral

renin activity. The management of this disorder includes antialdosterone drugs, such as spironolactone and other less specific antihypertensive agents. Tumor removal may be useful if HTN becomes resistant to medical therapy.

Cushing Syndrome

This disorder results from prolonged exposure of body tissues to cortisol. A primary pituitary or adrenal etiology leading to overproduction of cortisol is uncommon in children. However, exogenous administration of corticosteroids for managing inflammatory disorders or to prevent transplant rejection are common causes of this condition. HTN and changes in body habitus are manifestations of this disorder. The mechanisms of HTN and its treatment are discussed in Sect. "Posttransplant HTN".

Hyperthyroidism

Hyperthyroidism raises BP through a high cardiac output state secondary to high serum thyroxine levels associated with Grave's disease or other disorders which stimulate an increase in oxidative metabolism. This disorder is uncommon in children and is managed with beta blockers to decrease the heart rate.

Hypertension in Athletes

HTN in athletes may be caused by any of the known etiologies of HTN affecting children. In addition, performance enhancing drugs such as growth hormone, anabolic steroids, erythropoietin, protein cocktails, as well as high caffeine and high salt intake, may be more frequently abused or utilized by athletes and may contribute to HTN. Excessive weightlifting and other isometric activities done repetitiously and for prolonged periods of time may result in behavioral modification that perpetuates the known exaggerated BP response to such activities, even hours after the activity has terminated. Hypertensive athletes should minimize static activities and should only perform these in combination with aerobic activities (129, 130). Thus, a detailed history to discover known inciting influences of HTN followed by proper counseling is very important.

Participation in sports should be encouraged in competitive athletes with well-controlled HTN unless there is evidence of ongoing end-organ injury or a known structural cardiovascular disorder or arrhythmia. The American Academy of Pediatrics Committee on Sports Medicine and Fitness currently encourages dynamic exercises and participation in competitive sports in children with mild or well-controlled HTN, but not in those with severe HTN (8, 130). Other recommendations of this Committee are listed in *Table 63-11*. The risk for sudden death related to HTN per se is extremely low. An echocardiogram and standardized exercise testing may be useful in identifying athletes who are at high risk for such catastrophic events.

In general, most pharmacologic agents may be utilized to manage HTN in competitive athletes. However, diuretics should be avoided. Also, the use of beta blockers are generally contraindicated as these may limit the normal compensatory tachycardia associated with dynamic exercise and may result in syncope or other consequences of hemodynamic insufficiency. Moreover, alpha adrenergic agonists, such as clonidine and other centrally acting agents, should be used with caution in athletes because sympathoihinbition may also prevent compensatory tachycardia related to exercise and thereby result in postural hypotension and muscle weakness.

A combination of aerobic and static exercises should also be encouraged. Weightlifting may start with a low

Table 63-11

Recommendations for participation in competitive sports for the hypertensive pediatric patient

1.	Patients with mild to moderate hypertension (90th to 99th percentile for age and height; 1996 Task Force) and no evidence of end-organ involvement or other cardiovascular disease can participate in all competitive sports. Blood pressures should be monitored every 2 mo to monitor the impact of participation
2.	Patients with severe hypertension (>99th percentile for age and height; 1996 Task Force) but with no evidence of end-organ involvement or other cardiovascular disease should be restricted, particularly from high-static sports, until blood pressure is controlled. They may then participate as in number 1. For those patients with severe hypertension and evidence of end-organ involvement, the same recommendation may be followed as long as sports participation does not exacerbate the end-organ involvement or place the patient at risk
3.	Patients with hypertension and coexisting cardiovascular disease may require additional restrictions based on the nature of the cardiovascular disease and associated risks

From American Academy of Pediatrics Committee on Sports Medicine and Fitness. Athletic participation by children and adolescents who have systemic hypertension. *Pediatrics* 1997;99:637–638, with permission. (ref (130))

weight and multiple repetitions and slowly advance to greater amounts of weightlifting and fewer repetitions.

HTN During Pregnancy (131, 132)

Over one million adolescents become pregnant in the USA each year. Adolescents have a significant higher risk for pregnancy associated HTN, and for having small for gestational age offspring who may also be at susceptible to HTN. Gestational HTN in adolescents may also be a forerunner for development of sustained maternal HTN 6–9 years after pregnancy.

The spectrum of HTN during pregnancy includes the common disorder, preeclampsia (associated with proteinuria), and the much less common conditions of eclampsia (proteinuria and seizures) and gestational HTN (no proteinuria). Collectively these disorders occur in 10% of all pregnancies and cause considerable maternal and infant mortality. Preexisting HTN and renal disease are important risk factors in 30% of women who develop preeclampsia.The pathogenesis of these disorders is unclear. Individuals with a gain of function mutation of the mineralocorticoid receptor may be at risk for developing preeclampsia (see Sect. "Gain of Function Mutation of the Mineralocorticoid Receptor").

Although preeclampsia usually resolves after delivery of the infant, it is desirable to manage the HTN until at least 32 weeks of gestation so as to limit the complications of prematurity to the fetus. Provided that there is no end organ damage, as a general rule, a higher threshold for treatment may be used (usually diastolic BP $>$ 100 and/or systolic BP $>$ 160 mm Hg) because lower placental blood flow may compromise growth and overall safety of the infant. The goal of therapy is a diastolic BP below 90 mm Hg. Drugs that do not affect placental blood flow are recommended (132). Alpha methyldopa may be an initial agent; hydralazine is another such agent.

In individuals nearing delivery, prophylaxis against seizures (eclampsia) is essential during labor and for 1–2 days after delivery. This consists of intravenous magnesium sulfate starting with a 4 g loading dose (over 15–20 min), followed by 2 g/h by continuous infusion thereafter. The goal is to achieve a blood magnesium level of 6 mg/dL. Serum magnesium levels must be monitored frequently as concentrations above 8–9 mg/dL may cause symptoms of magnesium toxicity.

Because both ACEI and ARB are teratogenic, their use is contraindicated during pregnancy. Birth defects have been noted as early as the first trimester of pregnancy that was previously thought to be a safe period (131). Birth defects include cardiovascular and neurologic complications, craniofacial deformities, and renal dysplasia leading to renal insufficiency and oligohydramnios.

Neonatal HTN (133)

Renovascular causes of HTN predominate in this population. Common etiologies include renal thromboemboli secondary to umbilical vascular access and right-to-left shunts due to ventricular septal defect or patent ductus arteriosus. Other causes include acute cortical necrosis associated with asphyxia, sepsis or circulatory shock leading to renal venous thrombosis or kidney injury, along with hypercarbia related to bronchopulmonary dysplasia leading to sodium and fluid retention, and HTN. Intracranial hemorrhage leading to neurogenic or "central" HTN, polycystic kidney disease, coarctation of the aorta and iatrogenic causes such as use of glucocorticoids, caffeine, dopamine, and inadequate pain control may be less common causes.

The management of neonatal HTN is highly individualized. The benefit of specific antihypertensive agents in preventing cardiac dysfunction, pulmonary insufficiency, and intracranial hemorrhage should be balanced against the risks of developing renal dysfunction or hypoperfusion of brain and viscera as BP is being lowered. *Table 63-12* shows the dosages of antihypertensive medications utilized in neonates. Gradual titration of such medications is particularly important in neonates. In the absence of renal artery stenosis, captopril at 0.01–0.1 mg/kg/dose given every 8-h is usually effective in newborns suspected of having other forms of renovascular HTN. Isradipine given at a dosage of 0.1 mg/kg/dose every 6–8 h is another useful agent particularly in newborns with bronchopulmonary dysplasia or steroid-induced HTN. Intravenous hydralazine (at 0.1–0.2 mg/kg/dose), or esmolol, are reserved for more refractory HTN in this population. Diuretics are generally avoided but may be used with caution in infants with bronchopulmonary dysplasia.

Many causes of HTN that start in the newborn period resolve spontaneously by 1-year of age. Thus, "step-down" care should be anticipated. Also, most infants tend to outgrow their medication dosages due to rapid body growth during infancy.

Management of Hypertensive Emergencies

Hypertensive emergency is defined by the presence of acute end-organ injury. The implication is that immediate

Table 63-12

Pharmacologic therapy of neonatal hypertension

Angiotensin-converting enzyme inhibitors	
Captopril	0.05–2.0 mg/kg/day PO (÷q6–12 h)
Enalaprilat	5–10 μg/kg/dose IV (q8–24 h)
Beta-adrenergic antagonists	
Esmolol	100–500 μ/kg IV load over 1–2 min, then 50–500 μg/kg/min IV continuous infusion
Labetalol	1–20 mg/kg/day PO (÷q8–12 h)
	0.2–1.0 mg/kg/dose IV bolus or 0.25–3.0 mg/kg/h IV continuous infusion
Propranolol	0.5–5.0 mg/kg/day PO (÷q6–12 h)
Calcium-channel antagonists	
Amlodipine	0.1–0.6 mg/kg/day PO (÷q12 h)
Isradipine	0.05–0.15 mg/kg/dose PO (q6 h)
Nifedipine	0.125–0.5 mg/kg/dose PO (÷q6–8 h) (max dose = 3 mg/kg/day)
Nicardipine	0.5–3.0 μg/kg/min IV continuous infusion
Diuretics	
Bumetanide	??
Chlorothiazide	20–30 mg/kg/day PO (÷q12 h)
Furosemide	0.5–4.0 mg/kg/dose IV/PO (÷q6–8 h) (max dose = 4 mg/kg/day)
Hydrochlorothiazide	2–3 mg/kg/day PO (÷q12 h)
Vasodilator	
Hydralazine	0.1–0.6 mg/kg/dose IM/IV q4–6h OR 0.75–5.0 μg/kg/min IV continuous infusion
Sodium nitroprusside	0.5–8.0 μg/kg/min IV continuous infusion

÷, divided

reduction in BP is needed. Although a large majority of children who present to the emergency department with hypertensive emergency have BP above the 99th percentile, hypertensive emergency is not strictly defined by numerical BP values.

Symptoms and Signs

Unlike adults in whom hypertensive emergencies usually occur in individuals with known and often severe pre-existing HTN, in children the discovery of HTN is often made at the time of presentation with symptomatic HTN. The severity of manifestations in children with hypertensive emergency often depends on the chronicity of HTN as well as on the magnitude of the BP elevation. In general chronic Stage I or Stage II HTN is usually well tolerated whereas the same BP level may be associated with severe headache or seizures in children with acute glomerulonephritis. Similarly children with cardiac outlet obstruction or cardiomyopathy may become symptomatic despite mild HTN.

Perhaps the most common symptoms of HTN in the majority of older children who present to the emergency department are those of hypertensive encephalopathy manifest by headache, nausea, vomiting, mental confusion, blurred vision, agitation or frank seizures. Other manifestations may include cerebral infarction, intercerebral or retinal hemorrhage, congestive heart failure, acute pulmonary edema (and shortness of breath), acute renal insufficiency, or microangiopathic hemolytic anemia. Uncommon sequelae of hypertensive emergencies in children include myocardial infarction or aortic dissection. Newborns and infants with severe HTN may present with congestive heart failure, hypertensive retinopathy, respiratory distress, apnea or cyanosis, extreme irritability, hypotonia, convulsions or coma. Vomiting or diarrhea as well as failure to thrive may be among the global chronic manifestations of HTN in this younger age group.

In the absence of renal disorders which often predispose to hypertensive emergency one must be aware of previously unrecognized cardiac and endocrinologic etiologies of HTN which may influence the therapeutic strategy.

Pathophysiology of Hypertensive Emergencies

Chronic HTN leads to compensatory, functional and structural changes in arterial vessels which tend to maintain perfusion to brain and kidneys in particular, while protecting against injury which may otherwise result from organ hyper-perfusion due to HTN (134). When systemic BP surpasses the compensatory limits of autoregulation, of blood flow arterial injury such as fibrinoid necrosis and organ ischemia may follow (135, 136). Conversely when BP is suddenly reduced below the new steady state limits of compensation, ischemic injury may be enhanced particularly in individuals with atherosclerotic or other structural hypertensive arterial changes. Many children with hypertensive emergencies do not have structural vascular lesions and are, therefore, less susceptible to organ ischemia after BP lowering. Conversely, the lack of adaptive responses in children with acute and rapid rise in BP may predispose to symptoms at BP levels

which may not be gauged as being sufficiently high to produce such symptoms.

General Principles of Management

Assuming that the clinical setting permits it, the diagnostic plasma and urinary studies shown in *Fig. 63-5* should be done prior to pharmacologic treatment because the latter my confound several of these studies.

There are no controlled clinical trials of treatment of hypertensive emergencies in children. Only a few reports address this topic in the pediatric age group (137–139). A review of multiple adult studies failed to produce statistical superiority of any single agent and the outcome was not uniform between studies (140). As a general rule, agents with short duration of action are preferred such that when BP reduction is gauged as excessive, the effect can be quickly reversed by stopping the agent and/or administering normal saline. A major long-term objective is to assure compliance with maintenance antihypertensive therapy during follow-up (141, 142).

The goal of emergency therapy is to achieve a gradual decrease in systolic and diastolic BP to near the 99th percentile, or, to reduce the mean arterial pressure (one third of the pulse pressure difference added to the diastolic BP) by 25%. With such reduction in BP over a period of minutes or hours, the symptoms and signs of HTN tend to improve or resolve. Further lowering in BP may then take place more slowly over several days or weeks such that the BP falls below the 90th percentile for age, gender, and height percentile (8).

Figure 63-5

Evaluation and guidelines for use of intravenous agents in the management of hypertensive emergenciest (Do not use hydralazine or nieardipine in children with head trauma or intracranial hemorrhage).

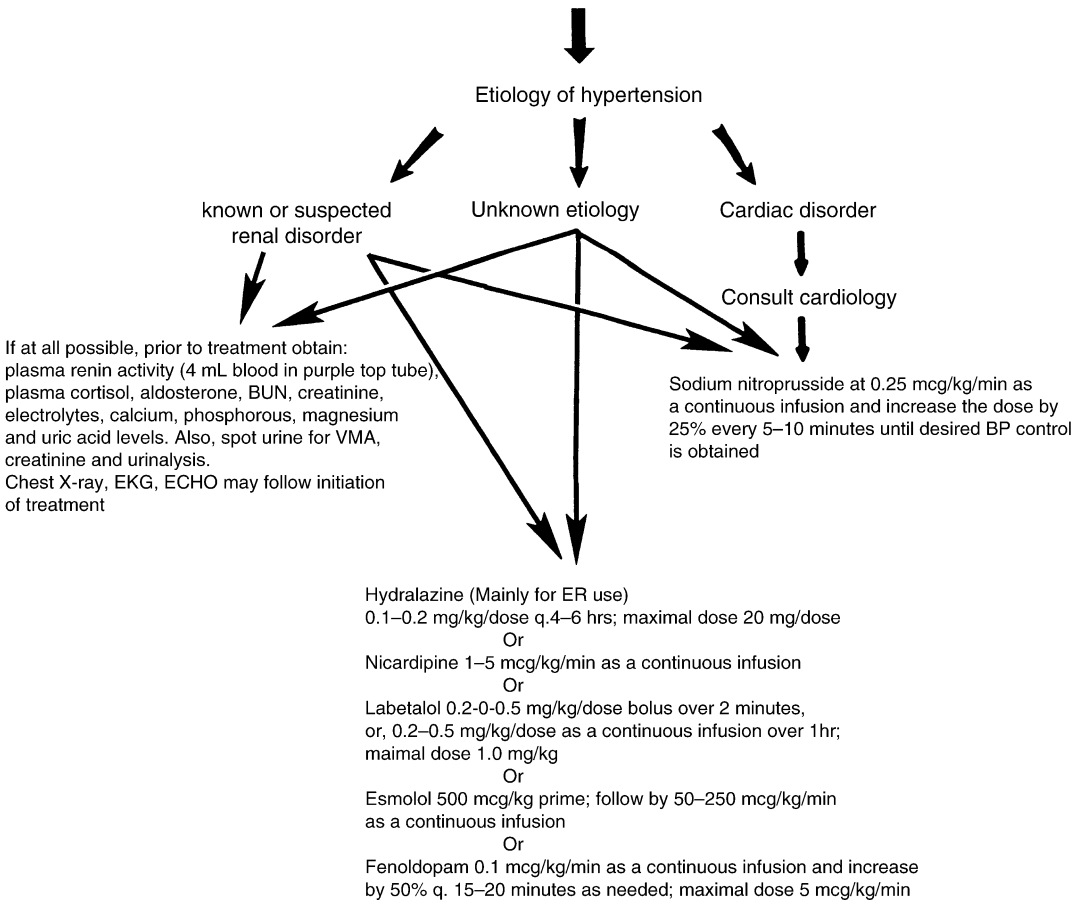


Table 63-13

Pharmacologic management of hypertensive emergencies and urgencies in infants and in children

Action	Agents (trade names)	Dose	Route	Comments
Vasodilator	Hydralazine (Apresoline, others)	0.1–0.2 mg/kg (maximum 20 mg/dose)	IV	Administer at < 0.2 mg/kg/minute; onset 5–20 minutes; lower dose can be repeated after 5–20 minutes. Duration is 2–6 hours. Can cause reflex tachycardia. Available in 20 mg/ml ampoule.
	Minoxidil (Loniten)	0.1–0.2 mg/kg (maximum 50 mg/day)	Oral	Onset ≤ 30 minutes; maximum effect @ 2–8 hours; long duration of effect, once daily dosing, contraindicated in pheochromocytoma.
	Diazoxide (Hyperstat)	1–3 mg/kg (maximum 150 mg dose)	IV push	Administer IV push over 10–30 seconds, maximum effect within 5 minutes, can be repeated after 5 minutes, duration of effect 3–12 hours. Available in 300 mg/30 ml ampoule. Alternative dosing is 1 mg/kg every 5–15 minutes until blood pressure control is achieved.
	Sodium nitroprusside (Nipride)	0.25–8 mcg/kg/min	IV drip	Administer by continuous infusion only; onset within 2 minutes, duration < 10 minutes. Start at 0.25 mcg/kg/minute and increase the dose by 25% every 5–10 minutes until blood pressure control is obtained. Obtain thiocyanate levels if used for more than 48 hours; protect from light. Available in 50 mg/5 ml vial.
Adrenergic alpha and beta blocking agents	Labetalol (Normodyne, Trandate)	0.2–0.5 mg/kg/dose (maximum 1mg/kg)	IV	Administer IV over 2–3 minutes; onset 2–5 minutes; maximum effect in 5–15 minutes, duration 2–4 hours; lower dose can be doubled and repeated at 15 minute intervals x 2; can also be given at same dose by IV infusion over 1 hr. Avoid if child has a history of asthma / bronchospasm. Contraindicated in congestive heart failure, sinus tachycardia, heart block. Available as 5 mg/ml.
	Clonidine (Catapres)	5–10 mcg/kg/day (maximum 25 mcg/kg/day)	Oral	Divide dose and administer q 6–12 hours. Also available in transdermal formulation. Avoid in cerebrovascular disorders. May cause agitation, insomnia, drowsiness, rebound hypertension after rapid withdrawal
	Esmolol (Brevibloc)	Loading dose: 500 mcg/kg IV, then use as continuous infusion 50–250 mcg/kg/min.	IV drip	Use for postoperative hypertension; administer loading dose over 1 minute; followed by continuous infusion; onset: beta blockade occurs in 2–10 minutes; duration 10–30 minutes (short). Contraindicated in congestive heart failure, sinus tachycardia, heart block.
	Isradipine (DynaCirc)	0.2–0.9 mg/kg/day (maximum 0.6 mg/kg/day)	Oral	Available in capsules (2.5 and 5 mg) and in liquid formulation (1 mg/mL). May cause tachycardia or congestive heart failure.

Table 63-13 (Continued)

Action	Agents (trade names)	Dose	Route	Comments
Calcium-channel blocking agents	Nifedipine (Procardia, others)	0.2–0.5 mg/kg (Initial dose should not exceed 0.25 mg/kg)	Oral	Swallow whole or bite capsule and swallow, or remove liquid from capsule with syringe; not effective by sublingual route (actual effect due to swallowing the drug with subsequent rapid oral absorption); initial dose can be repeated once within 30 minutes. Onset of effect within 1–5 minutes after bite and swallow dosing, contraindicated in patients with heart disease; available in 10 and 20 mg capsules.
	Nicardipine (Cardene IV)	1 mcg/kg/min (maximum 5 mcg/kg/min)	IV drip	Onset 10 minutes; duration 2–6 hours; available as 2.5mg/ml
Alpha-adrenergic blocker	Phentolamine (Regitine)	0.05–0.1 mg/kg/dose (maximum 5 mg)	IV	Diagnostic test for pheochromocytoma. Onset is immediate; maximum effect in 2 minutes, duration 30 minutes; available in 5 mg vial.
ACE inhibitors	Enalaprilat	5–10 mcg/kg/dose	IV	Administer undiluted over 5 minutes; onset ≤ 15 minutes; maximum effect @ 1 hour; duration 4–6 hours. Severe hypotension can occur in sodium or volume depleted patients. Use lower dosages in neonates.
Dopamine receptor agonist	Fenoldopam	0.1 mcg/kg/min (maximum 5 mcg/kg/min)	IV	Administer as a continuous infusion; onset ≤ 15 minutes. Especially useful in children with severe renal dysfunction.

Management of Hypertensive Emergencies

A list of antihypertensive agents and the recommended dosages in children with hypertensive emergencies is shown in *Table 63-13*. An algorithm for managing hypertensive emergencies with intravenous agents is shown in *Fig. 63-5*. Oral agents, such as nifedipine, have a limited and often controversial role in the management of hypertensive emergencies (see below). In most other children with symptoms and signs of hypertensive emergency, or in those with cardiovascular or other disorders associated with HTN in whom CCB or beta blockade may be harmful, the initial agent may include intravenous hydralazine (see *Fig. 63-5*). If HTN persists, the child may be managed in the intensive care unit where more powerful parenteral agents may be administered (see *Fig. 63-5*), and BP may be monitored continuously and directly through an intra-arterial transducer.

Not shown in *Fig. 63-5*, is the management of drug-induced HTN due to catecholamine excess (cocaine intoxication, monoamine oxidase inhibitor crisis). This disorder may be controlled with an alpha adrenergic blocker while avoiding initial use of β-blockers which may potentiate the alpha adrenergic action of catecholamines. This resembles the management of pheochromocytoma-associated HTN (see Sect. "Pheochromocytoma/Paraganglioma, Neuroblastoma and Neuroendocrine HTN"). Also, intravenous diuretics may be very useful as adjunctive agents in the setting of congestive heart failure in disorders accompanied by fluid overload, such as acute glomerulonephritis.

Oral Agents

In general, oral agents may be used in a minority of children with mild manifestations of hypertensive emergency secondary to renal etiologies, such as mild headache or other symptoms of impending organ injury, rather than those with evidence of active organ injury. In this setting, the initial therapy may consist of oral agents, and, frequently, short acting *nifedipine*. Unlike studies in adults showing that the use of this agent may be associated with precipitous fall in BP and an increased risk for myocardial infraction, stroke, and death (143), pediatric use of this agent is safe and effective (54, 144). However, both the clinical setting and the initial dose may influence safety. Guidelines for avoidance of the use of short acting nifedipine in children may include:

1. Congestive heart failure, presence of myocardial ischemia, post cardiac arrest, severe left ventricular

hypertrophy, left ventricular outflow obstruction, or cardiac arrhythmia.
2. Acute hypertension with high cardiac output states such as sickle cell crisis or other chronic anemia.
3. Cyanotic heart disease
4. Hypovolemic states including children undergoing acute diuresis, or those with acute hemorrhage.
5. Acute hypertension associated with severe pain. BP should be reassessed after administration of appropriate analgesics such as morphine.
6. Acute central nervous system injury
7. Children receiving large dosages of beta blockers, such as atenolol or labetolol.
8. Infants less than 1 year of age in whom dosing of nifedipine may be inaccurate.

In the absence of such disorders, orthostatic hypotension is usually avoided when the starting dose of nifedipine is 0.2 to 0.25 mg/kg/dose. Although it is often given sublingually, very little of the agent is actually absorbed via the oral mucosa. Thus, if the entire 10 mg nifedipine capsule is to be used, the author recommends the "bite and swallow" method. If lesser amounts are needed, the 10 mg capsule contents is drawn into a 1 mL graded syringe (0.034 mL = 1 mg) and the portion needed may be administered orally so as to speed up gastrointestinal uptake and onset of action. If needed, this dose may be repeated once within 30 min. All subsequent dosages should not exceed 0.5 mg/kg/dose with a maximum total dose of 10 mg, and the frequency of administration may be every 4–6 h. Close BP monitoring is mandatory and such children should not ambulate after receiving the medication unless they are completely free of orthostatic hypotensive symptoms and signs such as headache, dizziness or mental disturbances.

Minoxidil is an alternative oral agent with properties of smooth arterial muscle relaxation (leading to vasodilation) which may be effective in preventing the development of a full hypertensive emergency. Close BP monitoring and avoidance of ambulation is also recommended after its use.

Intravenous Agents (*Fig. 63-5*)

The majority of children with hypertensive emergencies require intravenous antihypertensive medications. Nowadays, diazoxide is rarely used for this purpose because of unpredictable or precipitous fall in BP. Also, hydralazine has become less popular because of the common occurrence of flushing, tachycardia, nausea, and headache. However, hydralazine may be useful in the Emergency Department setting, particularly if the etiology of the HTN has not been established. With more severe or refractory symptomatic hypertension more potent intravenous medications are indicated. Coadministered antihypertensive and other medications may be reviewed for possible synergism or drug interaction which may exacerbate adverse effects of the intravenous agents.

In the author's experience, the most useful parenteral agents in children include sodium nitroprusside (137, 145, 146), nicardipine (147–149), and labetalol (150, 151); esmolol may be a preferred drug in infants (137, 152–154) along with labetalol and enlaprilat (141, 155). As shown in *Table 63-13* some of these agents may be given as a constant infusion and have an onset of action of less than 5 min. Generally, one should start with the lower dosage range with stepwise increase in dosage by 25% every 5–10 min until the desired BP is reached.

Sodium nitroprusside may be the initial intravenous agent in any child presenting with hypertensive emergency with the exception of coarctation of the aorta, closed head injury or suspected high intracranial pressure. This agent along with hydralazine, and diazoxide should also be avoided in children with intracranial hemorrhage. With chronic use of this agent accumulation of its metabolic end products comprised of cyanide and thiocyanate, may cause nausea, vomiting, neurological symptoms, hypothyroidism, dyspnea and lactic acidosis. *Nicardipine* may be more suitable for children with renal insufficiency who are unable to effectively excrete these metabolites. Less frequently, nicardipine may also be given as mini-boluses. *Fenoldopam*, a vascular dopamine receptor agonist (DA1), is also effective and may be uniquely suitable for managing hypertensive emergencies in the setting of renal insufficiency (136, 140, 156). *Esmolol* may be given by constant infusion. However, esmolol, labetalol, and enalaprilat (IV enalapril) may also be administered on an intermittent basis. Intravenous labetalol or nicardipine may be particularly useful in the management of hypertension after transplantation. As with esmolol and other beta-blockers, labetalol should be avoided or used with caution in children with reactive airway disease and one should be aware of other contraindications to their use (see Sect. "Newer Antihypertensive Agents"). Infants are particularly susceptible to enalaprilat-induced inhibition of angiotensin II, and may develop significant reduction in renal blood flow and glomerular filtration rate. Hence, enalaprilat should be used with caution in infants with known or suspected renal artery stenosis. However, this agent may be useful in managing renovascular HTN due to focal renal scarring or microthrombi associated or intubation of the umbilical vessels.

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Acute Renal Failure

64 Pathogenesis of Acute Renal Failure

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Introduction

Acute renal failure (ARF) is defined clinically as the sudden loss of renal function that may result from inadequate renal perfusion associated with a decrease in effective circulation, arterial or venous obstruction, renal cell injury, or obstruction to urine flow as occurs in obstructive uropathy. Renal cell injury, commonly termed intrinsic ARF, results from an ischemic or toxicant insult that causes acute tubular damage with accompanying loss of ability to reabsorb filtered solute. The resulting decrease in glomerular filtration rate (GFR), an invariable component of ARF, is then a successful adaptive response, since continued filtration of plasma across the glomerular basement membrane without reabsorption of the filtrate by injured renal tubules would result in massive losses of salt and water (1). Thus, the decreased GFR associated with ARF prevents severe depletion of extracellular fluid.

Many definitions for ARF have been employed, ranging from dialysis requirement to subtle increases in serum creatinine (2). The term acute kidney injury (AKI) has been proposed to replace the term ARF and is garnering wide usage (3). The intent of the change in terms is to standardize the definition and to reflect both the entire spectrum of the condition as well as the understanding of mechanisms that underlie the renal dysfunction. AKI refers to a complex disorder from multiple causes with varied clinical manifestations ranging from a minimal but sustained elevation in serum creatinine to anuric renal failure. Rapidly and fully reversible causes of acute renal insufficiency, such as volume depletion, are specifically excluded from the spectrum of AKI. The unifying feature of AKI is renal cell injury, so AKI and intrinsic ARF, in a sense, can be used interchangeably.

Renal ischemia is both the most common isolated cause of AKI and most common contributor to multifactorial AKI. For decades, then, studies of ARF have focused on models of ischemic renal cell injury. From these studies it is clear that AKI manifestations can range from mild, sublethal cell injury with disrupted renal cell architecture and function to fulminant necrotic cell death. Furthermore, both extremes, along with the full spectrum of intervening cell injury features, can be present simultaneously or sequentially after the insult (*Fig. 64-1*)

This chapter, then, describes the pathophysiology of ARF, as revealed in experimental models, both *in vivo* and *in vitro*, of ischemia induced AKI. Included are classical concepts of ARF as well as a contemporary understanding of vascular, cellular, molecular, and metabolic alterations that are associated with renal cell injury. Mechanisms that lead to cell injury and death will be addressed along with processes that can result in cellular repair and renal recovery.

Renal Hemodynamics

Renal vasoconstriction in experimental and clinical ARF is intense (4). Because vasoconstriction was considered to be the dominant factor, it was suggested once that the term "vasomotor nephropathy" might be more appropriate than ARF to describe this condition (5). The hypothesis was that an insult to the renal tubular epithelium results in release of vasoactive compounds that increase cortical vascular resistance causing decreased renal blood flow (RBF), thus perpetuating injury to the tubule and furthering the cycle. Release of vasoconstrictive compounds may then diminish GFR by constricting afferent and efferent arterioles, thereby causing diminished urine output, or oliguria. Therefore, emphasis has been placed on identifying vasoactive compounds that are stimulated by an ischemic or toxic insult. Candidate systems for the vasoconstriction and vascular components of AKI include angiotensin, prostaglandins, adenosine, endothelin and nitric oxide.

Renin-Angiotensin System

The renin-angiotensin system functions in a manner consistent with the renal vasoconstriction hypothesis (6). An injury to the more proximal portion of the nephron, a typical feature of ischemic AKI, impairs reabsorption resulting in delivery of more solute and water to the distal

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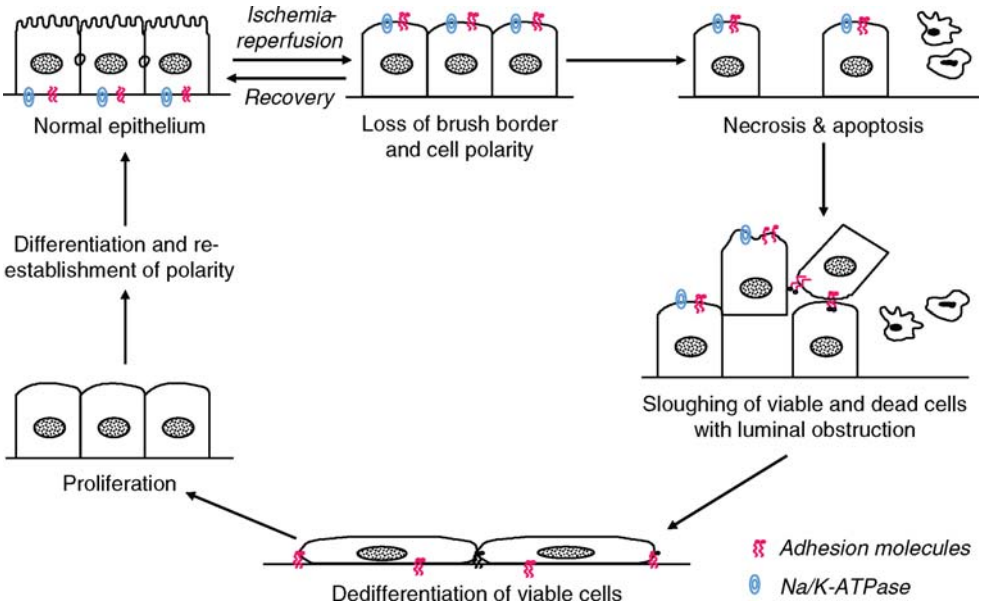
Figure 64-1

Disruption and recovery of renal tubule architecture **Ischemia disrupts normal polar distribution of membrane transport proteins and adhesion molecules, causing loss respectively of reabsorptive function and tubule integrity. Sublethally injured cells can recover normal structure and function. More severely injured cells die of apoptosis or necrosis, resulting in denudation and obstruction of the tubule. Restoration of tubule structure proceeds through dedifferentiation, proliferation, and redifferentiation processes likely mediated by growth factors and stem cells.**



nephron. This increased solute load in the distal nephron perfuses the macula densa of the juxtaglomerular apparatus, stimulating the release of renin with the subsequent generation of angiotensin II, a potent renal vasoconstrictor.

Several findings suggest that this system may have a role in the pathogenesis of ARF: (*a*) hyperplasia of the juxtaglomerular apparatus with increased renin granules is found both in patients and in experimental animals with ARF; (*b*) plasma renin activity is increased in patients with ARF; and (*c*) changing intrarenal renin content modifies the degree of renal functional impairment. For example, feeding animals a high-salt diet, which suppresses renin production, prior to a renal injury preserves renal function compared with animals fed a low-salt diet, which stimulates renin production.

However, other findings suggest that the renin-angiotensin system is not fully responsible for the changes in renal hemodynamics and function in ARF. For example, amelioration of experimental ischemic AKI also follows the induction of a solute diuresis with either mannitol or loop diuretics, neither which affect intrarenal renin. Furthermore, the degree of renal injury is not changed by immunizing animals against renin-angiotensin, treating with angiotensin converting enzyme inhibitors, or infusing with a competitive antagonist to angiotensin II. Therefore, the role of the renin-angiotensin system as the modulator of renal injury in ARF is uncertain.

Nevertheless, recent findings have renewed interest in this pathway, since it appears that a hormone downstream from renin and angiotensin, aldosterone, may play a central role in ischemia induced AKI (7). Prior mineralocorticoid receptor (MR) blockade with spironolactone was found to prevent several structural and functional manifestations of ischemia-reperfusion injury. Spironolactone administration prior to induction of renal ischemia prevented the typical reductions in RBF and GFR during reperfusion, and substantially reduced accompanying renal histomorphologic damage and tubule apoptosis. The protection conferred by MR blockade correlated with changes in other pathways that have been implicated in AKI. Primarily, it appeared that MR blockade decreased oxidative stress present during reperfusion after renal ischemia.

Prostaglandins

Prostaglandins are potent renal vasodilators. While inhibition of prostaglandin production is associated with

decreased RBF and can affect the severity of ARF, the precise role of prostaglandins in the induction and maintenance of ARF is not established (6). However, prostaglandin inhibitors, such as non-steroidal anti-inflammatory drugs, increase the risk for development of ARF and may act synergistically with other insults.

Adenosine

Adenosine, which results from catabolism of adenine nucleotides, is a potent vacoconstrictor of renal vasculature, whereas it is a vasodilator of peripheral vasculature (8). The infusion of methylxanthines, which block adenosine receptors, inhibits the tubuloglomerular feedback mechanism and the decrease in GFR that accompanies reduced tubular absorption, suggesting that adenosine may be involved in the modulation of renal vascular resistance in ARF. In some experimental models, the infusion of methylxanthines has been associated with diminished functional impairment. The proposal that adenosine is an important vasoactive compound following an acute tubule insult is attractive, because it would link alterations in cell metabolism with hemodynamic changes.

However, the evidence that adenosine is a major factor in the vasoconstrictive response is inconclusive because: (a) methylxanthines have a variety of effects in addition to the inhibition of adenosine receptors; (b) while adenosine is produced following the initiation of adenine nucleotide catabolism, it is rapidly catabolized by adenosine deaminase when released into extracellular fluid; (c) intrarenal levels of adenosine diminish very rapidly upon the establishment of reflow to the kidney, while renal vasoconstriction is continued through the early phases of reperfusion following an ischemic injury; and (d) when tissue adenosine levels are increased by inhibition of adenosine deaminase during ischemia, post-ischemic recovery of renal function is enhanced (9).

A recent study in the A1 adenosine receptor (A1AR) mouse knockout model may help reconcile these apparent discrepant effects of adenosine on renal blood flow and AKI. The increased delivery of sodium chloride to the macula densa due to dysfunction of the ischemic proximal tubule would be expected to cause afferent arteriolar constriction via A1AR activation, and thereby decrease GFR (10). However, knock out of the A1AR resulted in a paradoxical worsening of ischemic renal injury, and exogenous activation of A1AR was protective (11). Thus, tubuloglomerular feedback following ischemic injury, contributed to by activation of adenosine receptors, may be an adaptive response that limits delivery of solute to damaged proximal tubules, thereby reducing the demand for ATP-dependent resorptive processes. The effect of exogenous A1AR activation in human AKI is not known.

Endothelin

Endothelin, a 21-amino acid peptide, is a potent renal vasoconstrictor that has other important biological activities as well (12–14). It contributes to the vasoconstriction that occurs in a variety of tissues as a response to hypoxia. Raised endothelin levels in plasma and in both the cortex and medulla of the kidney have been demonstrated in experimental animals after the induction of renal ischemia (12). The post-ischemic infusion of anti-endothelin antibody or endothelin receptor antagonists provides protection from ischemic injury in animal models, but there is a lack of human data (12–15).

Nitric Oxide

Nitric oxide (NO) is a potent renal vasodilator, which is widely distributed in the kidney, and is produced by constitutive and inducible synthetases located in endothelial cells and renal tubules (16). While a specific role for these compounds in cerebral ischemia has been established (17), the effect of either stimulation or inhibition of nitric oxide synthetase (NOS) on ischemic or toxic ARF is confusing and, at times, contradictory. The apparent contradictory effects are likely attributable to the differential distribution and specific effects of each isoform on renal vasculature and tubules.

The rat kidney is protected from ischemic injury when inducible NOS is targeted with antisense oligonucleotides, suggesting that NO has a direct cytotoxic effect on renal epithelia (18). A similar cytotoxic effect of NO is found in isolated proximal tubule cells subjected to hypoxia (19). However, it has been postulated that production of NO following ischemia should help alleviate the vasoconstriction which characterizes ARF. In that regard, non-selective NOS inhibition has been found to worsen renal function and cause profound renal vasoconstriction (17). Hence the dilemma: stimulation of NO in the renal vasculature via NOS could modulate vasoconstriction and potentially lessen AKI; induction of NO via NOS in renal tubules is cytotoxic (16–18). So, this complex biological system may indeed play a role in the pathogenesis of ARF, but the specificity of action remains to be determined and may depend on whether vascular or tubular effects of NOS predominate in a particular setting.

All things considered, persistent renal vasoconstriction is a well-documented event during reperfusion following renal ischemia, but vasodilation does not consistently result in amelioration of an acute renal insult. Once AKI has been established, the infusion of potent vasodilators such as prostaglandins or dopamine does not lead to sustained improvement in GFR (20, 21). The early recovery phase of ARF is associated with an increase in renal blood flow while GFR increases more slowly (22). Confirming the findings of animal studies, several human trials of renal vasodilators such as dopamine have failed to demonstrate improvement in GFR in established ARF despite augmentation of total renal blood flow (23). Although renal hemodynamic factors play an important role in initiating ARF, alterations in renal vascular resistance and renal perfusion on a macroscopic scale may not be dominant determinants of renal epithelial cell injury. They may, in fact, be an adaptive response to ameliorate further cellular injury during reperfusion. On the other hand, microvascular alterations are now recognized to play a major role, as discussed below.

Alterations in Microvasculature

Medullary vascular congestion is a consistent observation following experimentally induced ischemic renal injury. Advances in recent years, including application of *in vivo* microscopy, has given substantial credence to the concept that injury to the vascular endothelium is central to the initiation and extension of AKI. The microvascular endothelium undergoes direct structural alterations after an ischemic insult, and is both a source of and a target for inflammatory injury (24–28).

Similar to features described years earlier in tubule epithelial cells, an ischemic insult has been found to disrupt the structural integrity of both the actin cytoskeleton within endothelial cells and the junctional complexes between endothelial cells (29, 30). Consequent endothelial cell swelling, blebbing, and cell death or detachment of viable cells occurs, and circulating endothelial cells have been found in humans with septic shock (31). Sites of endothelial denudation may be prone to prolonged vasoconstriction. Minimally invasive intravital microscopy in animals has revealed sporadic cessation and even reversal of blood flow in peritubular capillaries in the renal cortex during reperfusion (32, 33). Systemic or intra-renal administration of fully differentiated endothelial cells into post-ischemic rat kidneys has resulted in significant functional protection (33). Similar, but less impressive amelioration of AKI can be achieved using surrogate cells expressing endothelial nitric oxide synthase (eNOS). Furthermore, ischemic injury markedly increases expression of angiostatin, a well known anti-angiogenic factor that induces apoptosis of endothelial cells (34).

Ischemic AKI also increases endothelial expression of a variety of adhesion molecules that promote endothelial-leukocyte interactions. These include intercellular adhesion molecule-1 (ICAM-1), P-selectin, and E-selectin (25, 35). Ablation of the ICAM-1 gene or pre-treatment with ICAM-1 antibody rendered mice resistant to ischemic AKI, raising the promise of the latter approach in humans. However, trials with anti-ICAM-1 monoclonal antibody administered post-ischemia did not prevent AKI manifested as delayed graft function in deceased donor kidney transplant recipients (25). Similarly, initial studies of animals with gene knock-outs and treatment with monoclonal antibodies or pharmacologic inhibitor have suggested a role for endothelial E- and P-selectins in the microvascular injury (35–37). Subsequent studies, though, demonstrated that platelet P-selectin, not endothelial P-selectin, is the key contributor to AKI (38). Proposed mechanisms include initial adhesion of platelets to the endothelium with subsequent recruitment of leukocytes, or adhesion of platelets to neutrophils with consequent intra-luminal cell aggregate formation and trapping in narrow peritubular capillaries (35). Furthermore, derangements in the coagulation cascade, such as alterations in tissue-type plasminogen activator and plasminogen activator inhibitor-1 in the kidney (39), may combine with the alterations in adhesion molecules to cause the fibrin deposits characteristically found in the renal microvasculature following ischemic injury.

Despite the lack of apparent benefit from the trial of anti- ICAM-1 in limiting ARF in transplant recipients, the series of investigations in the past several years that have highlighted the important role that microvascular injury plays in ischemia induced AKI provide rationale for additional trials of pro-angiogenic agents in ARF. These may include agents that can mobilize or increase the pool of endothelial progenitor cells, such as erythropoietin, bone morphogenic protein, Vascular Endothelial Growth Factor, and statins (28).

Nephronal Factors

The term 'nephronal factors' refers to the unique susceptibilities of specific nephron segments to injury. Best studied following ischemia are the S_3 segment of the proximal tubule and the medullary thick ascending limb (mTAL). There has been controversy over the relative importance

of each of these segments in ARF (40). What is clear, though, is that studies of cellular injury and repair in both segments have provided important insights into the pathophysiology of tubule cell injury.

Tubule Segment Susceptibility to Injury

Medullary Thick Ascending Limb: The mTAL appears to be particularly vulnerable to hypoxia because oxygen tension in the medulla is low and the mTAL segment has a high rate of oxygen consumption (41). Initial observations that established this relationship were made in isolated kidneys that were perfused with cell-free perfusate. Injury to the mTAL is prevented or reversed by increased oxygen delivery through addition of red blood cells or hemoglobin to the perfusion media. The degree of mTAL necrosis in this model was modified by the work load imposed on this segment. Decreasing the work load by inhibiting mTAL solute transport with furosemide or ouabain markedly diminished the severity of injury; increasing the work load had the opposite effect.

The relationship of these observations to the pathogenesis of ischemia induced AKI in man is controversial. Reducing oxygenation in experimental animals does not reproduce the lesions seen in the isolated-perfused kidney model. The experimental models where the lesions are predominantly in the mTAL involve combining severe reductions in renal blood flow with the administration of a nephrotoxin. Nevertheless, studies of the unique vulnerability of this nephron segment to hypoxia have provided insight into the relationship between oxygen supply, work load, and the cellular targets of ischemic, hypoxic, or anoxic injury (40). They may, in fact, be most pertinent to the understanding of synergistic effects of various cellular insults that alone would not produce AKI.

Straight Segment of the Proximal Tubule: The straight segment of the proximal tubule (S_3 segment) is highly dependent on oxidative phosphorylation to supply the energy necessary for its bulk transport functions. It is particularly susceptible to ischemia and to nephrotoxins that disrupt energy supply or mitochondrial function (6, 42). The earliest alterations in the S_3 segment, after mild or brief ischemia, are found in the brush border and consist of blebbing and internalization of the luminal membrane. With more prolonged ischemia, these changes progress to a more severe form of sublethal injury characterized by cell swelling, mitochondrial condensation, and broader disruption of cellular morphology. Simultaneous with these changes in sublethally injured cells, other cells in this segment may progress to a lethal injury such that the tubule epithelium is denuded from the basement membrane (*Fig. 64-1*). These changes in the S_3 segment result in two important pathophysiologic events, intratubular obstruction and backleak.

Intratubular Obstruction and Backleak

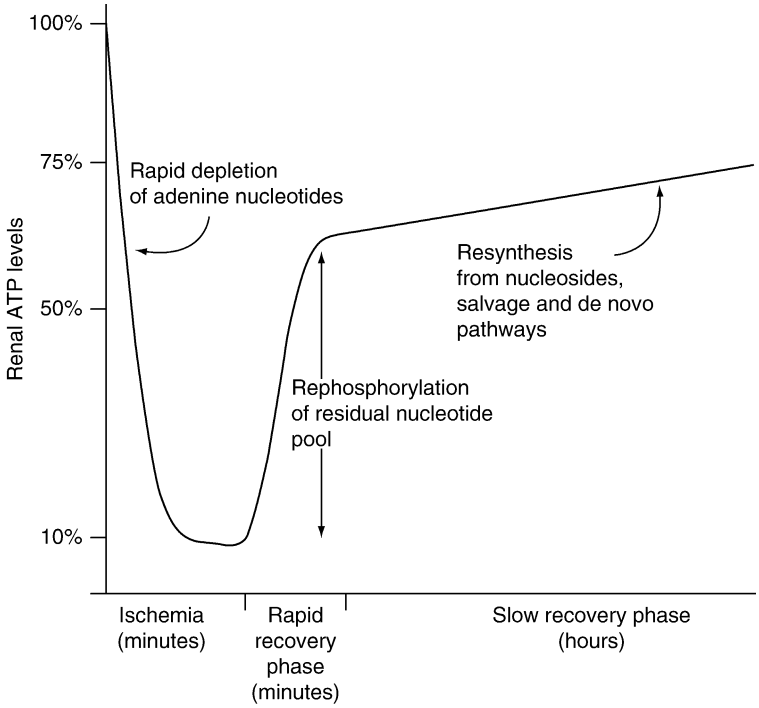
Cell and brush-border debris become impacted in the hairpin turn of the loop of Henle causing obstruction and backleak of tubular fluid in some nephron segments. Intratubular obstruction following ischemic injury has been demonstrated by histomorphology and by finding elevated proximal tubular pressure (42–44).

The backleak of tubular fluid that accompanies the loss of tubular integrity in AKI has been demonstrated in both experimental animals and patients (42–45). In animals, microinjection of inulin or horseradish peroxidase into normal nephrons results in the inulin being collected only from that kidney and the staining of horseradish peroxidase only within that tubule lumen. In contrast, inulin is found to be excreted by the contralateral kidney and horseradish peroxidase is found in both intra-and peritubular loci after microinjection into an injured nephron. In patients with ARF, backleak has been demonstrated by the differential clearance of graded dextrans (45). Since filtration is preserved and inulin, urea, and creatinine leak back into the circulation, the renal clearances of these solutes no longer represent GFR in patients with ARF. They nevertheless retain clinical utility in the global assessment of renal clearance ability.

The cellular and molecular mechanisms of tubule obstruction and backleak have been nicely defined (46, 47). Integrins are outer membrane proteins, basolaterally located in epithelial cells that attach the cell to the extracellular matrix. Following ischemia or ATP depletion, these adhesion molecules become detached from the extracellular matrix, allowing both dying and viable cells to leave the tubule basement membrane and float into the tubule lumen. In addition, these integrins become displaced from the basal to the luminal domain, which allows intraluminal sloughed cells to attach to cells that remain in place, thus contributing to tubule obstruction (*Fig. 64-1*). The integrin attachment occurs via an arginine-glycine-aspartic acid (RGD) sequence (46). This same RGD sequence may also promote binding of sloughed tubule cells to Tamm Horsfall protein produced in the mTAL and distal convoluted tubule (47). The infusion of RGD peptide abolished the characteristic increase in proximal tubule pressure and substantially improved renal function following ischemia, thus confirming the

Figure 64-2

Renal ATP depletion and recovery during ischemia and reperfusion **The initial rapid phase of ATP recovery occurs via phosphorylation of residual renal AMP and ADP. Slow phase recovery represents resynthesis and phosphorylation of adenine nucleotides.**



central role of integrin disruption to the processes of intratubular obstruction and backleak in AKI (48, 49).

Cellular and Metabolic Alterations

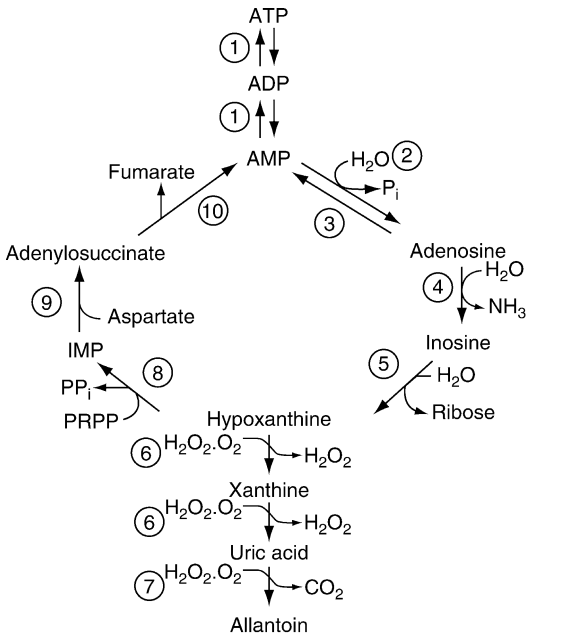
Adenine Nucleotide Metabolism

Energy depletion and restoration play a pivotal role in renal cell injury (50). *in vivo*, renal ischemia causes a profound fall in ATP; *in vitro*, metabolic inhibition of ATP production is used to induce cellular injury similar to the *in vivo* ischemia model. Within 5 to 10 minutes of inducing ischemia, nearly 90% of renal ATP has been consumed (9). With reperfusion, renal ATP levels recover in a bimodal fashion (*Fig. 64-2*). In the first few minutes of reperfusion, there is rapid, though incomplete, recovery of ATP to 50 to 70% of normal levels, depending on the duration of ischemia (51, 52). The longer the interval of ischemia, the less is renal ATP recovery during the initial, rapid phase. ATP recovery during the initial phase correlates highly with the total pool of adenine nucleotides (ATP + ADP + AMP) present in the kidney at the end of the ischemic interval. The total adenine nucleotide pool, mainly composed of AMP, decreases as the duration of ischemia increases. The catabolism of adenosine by adenosine deaminase results in progressive depletion of the adenine nucleotide pool with extended duration of ischemia (*Fig. 64-3*). Thus, ATP recovery during the initial period of reoxygenation is accomplished predominantly by oxidative phosphorylation of the residual cellular nucleotides (AMP and ADP).

A second, slow phase of ATP recovery occurs over hours following the initial rapid phase. The ATP recovery rate in the second phase also is a function of the preceding duration of ischemia; longer ischemic intervals result in slower recovery rates. The second phase requires resynthesis of ATP from purine nucleotide degradation products and salvage pathways, or from precursors provided during reperfusion (*Fig. 64-3*). A primary role for ATP depletion in ischemia induced epithelial cell injury is supported by several findings. (1) When ATP catabolism is inhibited during renal ischemia, the structural and functional manifestations of AKI are significantly ameliorated

Figure 64-3

Schematic of ATP metabolism in renal ischemia **Adenine nucleotide catabolism proceeds clockwise.**



1. Adenylate kinase
2. 5′-nucleotidase
3. Adenosine kinase
4. Adenosine deaminase
5. Hydrolase
6. Xanthine oxidase
7. Urate oxidase
8. Hgprtase
9. Adenylosuccinate synthetase
10. Adenylosuccinate lyase

(22, 42, 50). Graded reductions in cellular ATP, either *in vivo* or *in vitro*, determine the severity of cellular disruption (53, 54). Finally, augmentation of ATP recovery during reperfusion substantially enhances recovery of renal structure and function (50). Consequently, alterations in adenine nucleotide metabolism are both a consequence and predictor of renal cell injury. Furthermore, altered ATP metabolism triggers several pathways that lead to deranged epithelia structure and function: reactive oxygen molecules, increased intracellular calcium, activation of phospholipases, and disruption of tubule cell architecture.

Reactive Oxygen Molecules

Reactive oxygen molecules (ROM) have been implicated in a variety of kidney diseases, including reperfusion injury after ischemia (55). The most highly reactive oxygen molecules are free radicals such as hydroxyl radical (OH) or superoxide anion (O_2^-). The high reactivity and brief existence of these molecules result in injury close to the site of free radical generation. Other oxygen species such as hydrogen peroxide (H_2O_2) and hypochlorous acid (HOCl), not being free radicals, are less reactive. However they have longer half-lives than free radicals and may cross cell membranes to cause injury distant from their site of origin.

ROM are generated during reperfusion of kidneys (55). Ischemia induces metabolism of the adenine nucleotides to adenosine, inosine, and hypoxanthine (*Fig. 64-3*). Ischemia also may cause a conformational change in xanthine dehydrogenase (which uses NAD as an electron receptor) to xanthine oxidase, which uses oxygen (56). Once perfusion and oxygen delivery are returned, xanthine oxidase can metabolize hypoxanthine to xanthine and uric acid, generating hydrogen peroxide and superoxide anion (*Fig. 64-3*). Mitochondria, which produce reactive species though electron transport systems, when damaged by ischemia, may leak the injurious oxygen metabolites. The respiratory burst and myeloperoxidase released from activated PMNs produce superoxide anion, hydrogen peroxide, and hypochlorous acid (55). Prostaglandin metabolism may also provide reactive molecules. Iron can play a significant part by donating electrons to hydrogen peroxide to form the highly damaging hydroxyl radical (57). In this way, iron release may be responsible for the injury resulting in hemoglobin- or myoglobin-induced ARF (58).

Once generated, ROM damage cellular components and extracellular matrix (50). Intracellular proteins can be oxidized, resulting in conformational changes with loss of enzymatic activity or compromised integrity of structural proteins. Membrane lipids and lipoproteins undergo peroxidation by free radicals; these propagate and result in progressive membrane damage. Hydroxyl radicals damage DNA and oxidize matrix proteins. Reactive oxygen metabolites may contribute to ischemia by deleterious interactions with nitric oxide synthetases or with NO itself. NO can combine with superoxide to form peroxynitrate, a potent oxidant.

Because of these putative actions, much attention has focused on the role of ROM in ischemia-reperfusion injury (51, 55). While many studies have shown reactive oxygen species involved in the pathogenesis of AKI in animal models, it has long been questioned whether they play a significant role in human AKI. However, a recent study found a significant increase in oxidative stress in humans with ARF, as evidenced by depletion of plasma protein thiols and increased carbonyl formation (59).

The effects of enhancing endogenous antioxidants (superoxide dismutase, glutathione, catalase, apotransferrin,

or neutrophil gelatinase-associated lipocalin, NGAL) or administering exogenous free radical scavengers (*N*-acetylcysteine, deferoxamine, or edaravone) have been examined (60–63). Many of the animal studies have shown benefit from treatment with the anti-oxidants or free radical scavengers, but studies of humans using this approach, so far, have been at best inconclusive or have shown no benefit. So, despite the large number of studies on this topic, no final conclusion can be made as to the importance of reactive oxygen species in the pathogenesis of AKI, since strong evidence exists both for and against these compounds having a dominant role.

Intracellular Calcium

Under normal circumstances, extracellular fluid calcium concentration exceeds that of intracellular free calcium (Ca_i) by a factor of 10^4. The remarkably low Ca_i is maintained by a variety of mechanisms that exclude calcium from the cell or sequester it within intracellular compartments. Calcium is extruded from cells by plasma membrane Ca-ATPase and the Na/Ca-exchanger. Intracellular sequestration is mediated by Ca-ATPase in the endoplasmic reticulum and by calcium uptake into mitochondria. A fall in cellular ATP would be expected to reduce the activity not only of Ca-ATPase, but also Na/K-ATPase and thereby affect the Na/Ca-transporter. The net effect of cellular ATP depletion on extrusion of Ca from the cell and sequestration in the endoplasmic reticulum, then, would be to decrease calcium transport contributing to a rise in Ca_i. In addition, release of Ca from cellular organelles may contribute substantially to the rise in cytosolic Ca.

The contribution of mitochondrial damage to the observed rise in cytosolic calcium after ischemic or hypoxic injury appears to be minimal, because even damaged mitochondria are able to sequester calcium actively (51). However, the converse effect may be a mechanism in lethal cell injury. That is, the increases in cytosolic Ca that occur through the mechanisms outlined above can lead to unrestricted uptake by mitochondria. A substantial rise in mitochondrial calcium levels, then, usually occurs after cell injury is lethal.

There has been controversy whether high Ca_i levels play a central role in the pathophysiology of cell injury or whether the intracellular Ca changes are merely secondary to cell death. Increased cell and mitochondrial Ca levels are linked to cell death; but were thought not to increase until irreversible changes had occurred (64, 65). Later studies, however, have demonstrated a reversible increase in Ca_i early in the course of cell injury. After 5 minutes of hypoxia, rat proximal tubules develope a significant rise in Ca_i prior to evidence of membrane damage, and the increase in Ca_i is reversed with reoxygenation (66). In an *in vitro* model of renal cell injury from ATP depletion, graded levels of ATP depletion cause significant, early increases in Ca_i, but do not cause cell death. Furthermore, the increases in Ca_i are tightly and inversely correlated to the level of ATP depletion, and precede disruption of membrane protein-cytoskeleton interactions and induction of putative recovery mechanisms through activation of heat shock transcription factor and the stress response (54).

Therefore, alterations in intracellular Ca appear not to be merely a terminal manifestation of lethally injured cells. Increases in Ca_i may play an early role in AKI by mediating several aspects of sublethal injury, and a later role in a subsequent transition to lethal injury after a more severe insult. An increase in Ca_i may contribute to renal cell injury in several ways. It could activate calpain, contributing to degradation of the cytoskeleton; it could increase activity of other proteases and phospholipases that are active in mechanisms leading to either sublethal cell disruption or cell death; and it could inhibit mitochondrial oxidative phosphorylation. The latter effect would lead to a vicious cycle of progressive cell energy depletion, further cell injury, and eventual cell death. However, alterations in intracellular calcium also could activate recovery mechanisms, such as induction of the stress response (54). Furthermore, increased cytosolic calcium dramatically induces calcium binding proteins such as annexin A2 and S100A6, which play an important role in cell proliferation during recovery from AKI in animal models (67). Nonetheless, the relative contribution that changes in Ca_i make to specific pathophysiologic versus recovery mechanisms in AKI is yet to be defined.

Phospholipids and Lipases

The striking morphologic changes that occur in proximal tubules after ischemia, especially brush border membrane blebbing and the release of intracellular enzymes such as lactate dehydrogenase, suggest that significant membrane alterations may be part of the cellular injury in AKI. Changes in intracellular calcium flux have been shown to activate endogenous phospholipase, and some phospholipase may be activated by decreased cellular ATP. Phospholipase activation results in breakdown of membrane phospholipids into products including free fatty acids and lysophospholipids, which can disrupt

membranes and can be precursors of active metabolites that promote inflammation and further activate phospholipases. Moreover, the lack of ATP during ischemia may prevent the synthesis of new phospholipids to replenish membranes. The result would be a generalized loss of membrane phospholipids with an accumulation of degradative products that by their detergent activities would further disrupt cell membranes and eventually lead to loss of membrane integrity and cell death (68).

As discussed earlier, another mechanism that appears to contribute significantly to progressive membrane deterioration is injury by oxygen free radicals. ROM generated during reperfusion peroxidate lipids, which can propagate through other membrane lipids causing widespread membrane damage. In fact, signs of lipid peroxidation provide significant evidence for free radical generation during reperfusion. Certain phospholipase isoforms may limit, rather than enhance, membrane injury by hydrolyzing phospholipids oxidized by ROM (68). Removal of the damaged, oxidized lipids are thereby facilitated, limiting progressive membrane damage from these toxic metabolites.

Renal ischemia indeed causes activation of phospholipase (PLA_2) and a rapid fall in cortical phospholipid levels (69). The detrimental effects of PLA_2 can be offset by unsaturated free fatty acids (70) and suggest that PLA_2 may be cytotoxic by degradation of phospholipids in cell membranes or through accumulation of lysophospholipids (71). It appears, though, that the role PLA_2 plays in renal cell injury depends on both the particular isoform of PLA_2 as well as the insult applied to cause AKI (68). For example, cytosolic calcium dependent PLA_2 is implicated in mediating oxidant induced renal cell injury. On the other hand, inhibition of endoplasmic reticulum Ca-independent PLA_2 potentiates oxidant induced lipid peroxidation and necrosis in renal proximal tubule cells, suggesting that this particular isoform has a role in phospholipid repair (72). The effect, however, is specific to injury from oxidants since injury from mitochondrial inhibition (antimycin A) is not potentiated by inhibiting that PLA_2 isoform.

Just as phospholipase activity may vary according to the stimulus and domain, and either contributes to or limits the injury depending on the context, the role of lipid alterations similarly can differ in AKI (73). While free fatty acid and lysophospholipid accumulation may contribute to membrane damage, cholesterol accumulation appears to stabilize plasma membranes and limit renal cell injury in models of AKI. Acute tubule injury initially lowers, then increases, cholesterol content in renal tubule cells (74). When renal cells were treated with a variety of agents to reduce cholesterol content, including cholesterol synthesis inhibitor or stripping agent, cell susceptibility to injury from ATP depletion is substantially increased, suggesting that cholesterol is a cytoprotectant in the context of ischemic AKI (75).

So, while the understanding of lipid alterations in AKI has become more complex, the story is similar to that of changes in Ca_i. The role of these alterations may be pathologic or adaptive, depending on the type and severity of the insult, on the location of the intracellular perturbations, and on the particular isoforms active in the affected areas.

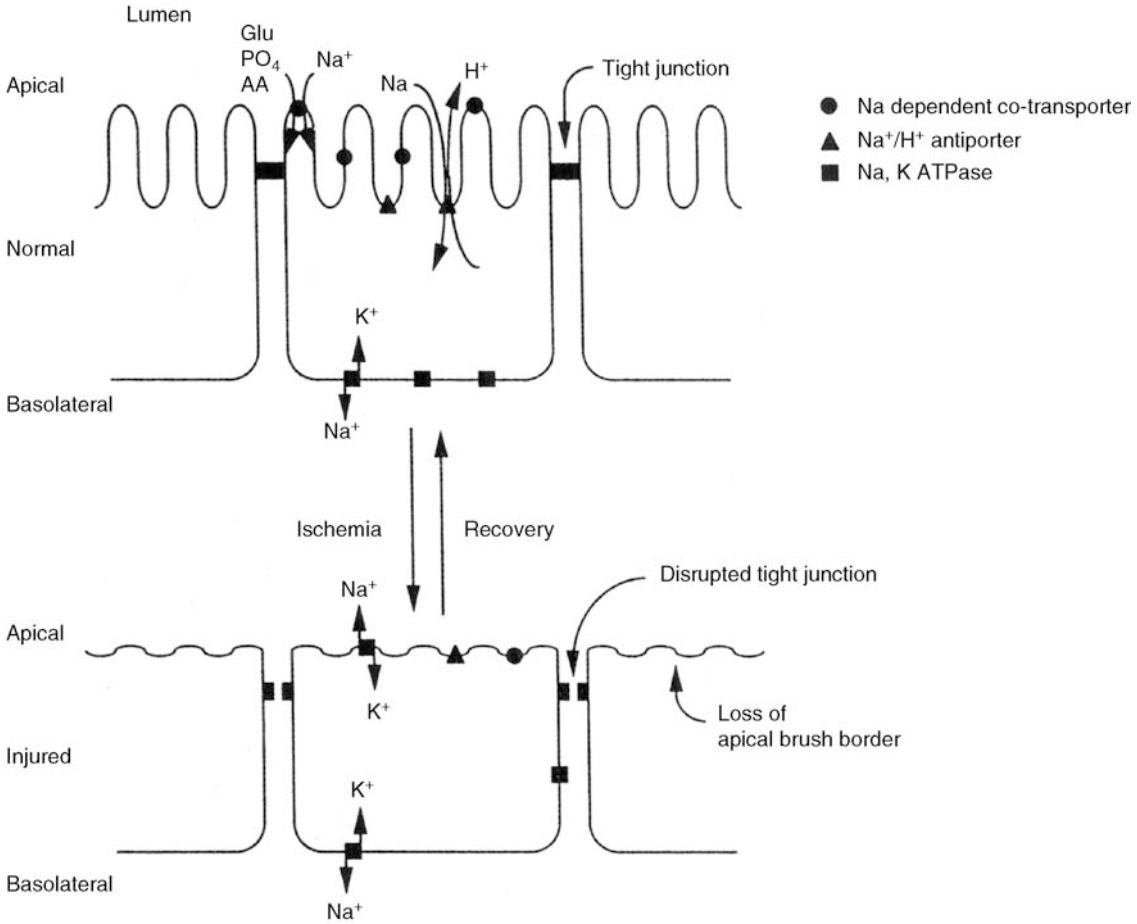
Disruption of Tubule Cell Architecture

Renal tubules are composed of highly specialized cells whose primary function of transport depends on their polar structure (*Fig. 64-4*). Changes in cell polarity that occur during acute renal failure have important effects on these functions (76). The plasma membrane of proximal tubule cells is divided into apical and basolateral domains by the tight junction that it shares with neighboring cells. The apical membrane, which faces the tubule lumen, differs from the basolateral membrane both in lipid composition and in the type of membrane proteins present. The microvilli of the apical domain are rich in sodium-dependent cotransporters and Na/H-antiporters, which effect reabsorption of glucose, amino acids, phosphate, and bicarbonate along with sodium. The sodium gradient across the apical membrane, which drives the resorption of these compounds, is established and maintained by Na/K-ATPase located on the basolateral membrane. Plasma membrane polarity is maintained by the tight junctions that prevent lateral diffusion of membrane phospholipids and proteins into the opposite domain. In addition, the cortical cytoskeleton anchors membrane proteins, such as Na/K-ATPase, in specific domains through an intricate sequence of linked proteins. The actin based cytoskeleton is a dynamic structure that maintains cellular polarity and mediates a number of processes necessary to sustain both structure and function of renal epithelia (77). Interactions between cytoskeletal proteins and plasma membrane proteins are responsible for functions that include cell adhesion, endocytosis, signal transduction and activity of ion channels.

The loss of cellular polarity is a fundamental alteration in renal epithelial injury and results in reduction of transepithelial sodium reabsorption. Renal ischemia alters this polarity in that membrane lipids and proteins, which are highly mobile, redistribute between apical and basolateral

Figure 64-4

Disruption of proximal tubule cell polarity **Ischemia causes loss of apical brush border, opening of tight junctions, and loss of cell polarity that impairs transepithelial solute reabsorption. Recovery of function parallels recovery of cell architecture.**



domains (*Fig. 64-4*). After as little as 5 minutes of renal ischemia there is blebbing, loss, and internalization of brush border membranes and, shortly thereafter, basolateral Na/K-ATPase migrates to apical membranes (76). With longer ischemia, the loss of polarity worsens. Proximal tubule tight junction integrity, an obstacle to diffusion of membrane components across domains, is lost. Membrane proteins such as Na/K-ATPase, which normally are anchored to the actin cortical cytoskeleton, can then migrate when this attachment is disrupted.

The actin cytoskeleton plays an important role in both processes. Disruption of actin microfilaments and polymerization follows ischemia and is dependent on the severity of the insult (77, 78). Actin disruption itself results in opening of tight junctions and loss of the polar distribution of those proteins that anchor Na/K-ATPase to the plasma membrane (52, 79–81). Ankyrin, which links fodrin to Na/K-ATPase, normally is limited to the basolateral domain. After ischemia, ankyrin migrates along with Na/K-ATPase to the apical domain while fodrin, which normally links actin to ankyrin and Na/K-ATPase, becomes dissociated and solubilized throughout the cytosol (81, 82).

Actin depolymerizing factor (ADF, also known as cofilin) participates in ischemia-induced actin cytoskeletal alterations and determines the rate and extent of these ATP depletion-induced cellular alterations (83). ADF/cofilin is a cytosolic protein that is normally maintained in the inactive phosphorylated form by Rho GTPases. ATP depletion inactivates Rho GTPase, which results in activation and relocalization of ADF/cofilin to the surface membrane (83, 84). Concomitantly, ATP depletion dissociates the actin stabilizing proteins tropomyosin and ezrin, allowing the activated ADF/cofilin to bind

and cleave actin, leading to microvillar breakdown (85). Finally, atypical protein kinase (aPKC) signaling, impaired during ATP depletion, participates in tight junction disassembly during cell injury. Furthermore, aPKC function is important for tight junction reassembly during recovery, a feature necessary for restoration of cell polarity and tubule integrity (86).

The reduction in transepithelial sodium reabsorption that follows renal ischemia, a hallmark of AKI and manifested clinically as increased fractional excretion of sodium is thus a function of this disruption of renal tubule cell architecture. Recovery of normal sodium reabsorption does not occur until these renal cell structural changes and membrane polarity, in sublethally injured cells, is reestablished during recovery from AKI.

Cell Death: Necrosis and Apoptosis

After an ischemic or toxic insult, cells can proceed down one of several pathways: recovery from sublethal injury or death by necrosis or apoptosis. Indeed, individual cells can simultaneously be moving through each of these pathways in AKI (*Fig. 64-5*). The hierarchy of injury that results depends on the cell type, location, and on the severity and duration of the insult (54, 87, 88). Cell death by necrosis tends to follow a severe insult that leads to metabolic collapse of the cell. Necrosis, then, is massive, rapid, and near synchronous leading to cell lysis with secondary injury to surrounding tissue. Typical features of necrosis include cytoplasmic swelling, loss of membrane integrity, and nuclear and cellular fragmentation. Cell death from apoptosis is a more organized, regulated process that leads to cytoplasmic and nuclear shrinkage, DNA condensation and fragmentation, and eventual cell breakdown into membrane-bound apoptotic bodies. These apoptotic bodies *in vivo* are cleared rapidly by other cells, which has made the assessment of how much apoptosis occurs following a renal injury difficult.

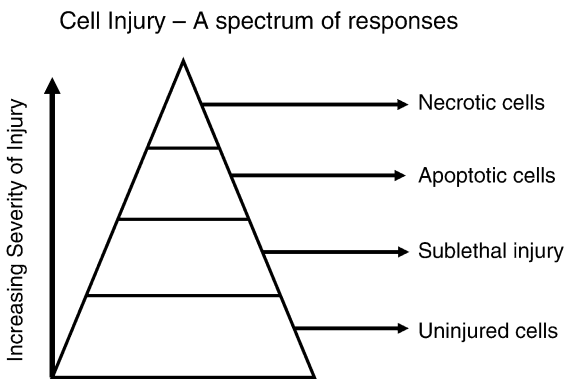
While clinical acute renal failure from ischemic or toxic injury has long been called acute tubular necrosis (ATN), this term has been questioned since biopsy of patients with ARF rarely show necrotic features. However, biopsies of patients typically are late in the course of ARF and may miss earlier signs of necrosis. Animal models of ischemic or toxic injury clearly show at least focal areas of necrosis early during reflow. Nevertheless, the current understanding that a renal insult can leads to simultaneous sublethal and lethal injury, both necrosis and apoptosis, in cells in different areas of the kidney has lead to the change in terminology from ARF or ATN to acute kidney injury (AKI) (3).

Measuring apoptotic cell death *in vivo* has been problematic, leading to controversy over whether this is an important pathway in AKI. This is an important issue since the same type of insult *in vivo* or *in vitro* can cause either necrosis or apoptosis (89). Definitive morphologic criteria have established apoptosis as a pathway to cell death in renal epithelia both *in vivo* and *in vitro* (90, 91), and apoptosis is now recognized as an important feature of human AKI (92, 93).

Biochemical assays can be helpful in quantifying apoptosis, but they are not uniformly reliable measures of apoptosis *in vivo* or *in vitro,* unless traditional cell morphology criteria are also applied in the particular setting being studied. For example, neither endonuclease activation nor DNA laddering, previously thought to be features unique to apoptosis, consistently discriminate between necrosis and apoptosis in injured renal epithelia (94, 95). Thus, the specificity of traditional *in vivo* assays for apoptosis such as the TUNEL assay, based on endonuclease activation, has been questioned. However, the specificity of TUNEL staining for apoptosis in several forms of *in vivo* renal cell injury has been determined using accepted morphological criteria (96). While not specific for apoptosis in some forms of renal injury (e.g., H_20_2), TUNEL is 99% specific for apoptosis in *in vivo* ischemic renal injury. Studies such as these have shown that apoptosis is an important mechanism of cell death in AKI, and have allowed for exploration of apoptotic pathways that might be active after a renal injury.

Figure 64-5

The spectrum of cellular outcomes in acute kidney injury **The particular outcome of an injured cell depends on the severity, duration and specificity of the insult, and on the type of cell and location. In acute kidney injury, the entire spectrum of responses can occur in different cells simultaneously (adapted from (265)).**



The process of apoptosis has been separated into three sequential phases: initiation, commitment, and execution phases. Membrane receptors and the Fas pathway can contribute to the initiation phase of apoptosis in ischemia, and interference with this pathway can ameliorate In vivo AKI (97–100). The series of intracellular derangements in cell architecture, metabolism, and function during ischemia are likely a major trigger to the initiation of apoptosis following ischemia.

Central to the commitment phase of apoptosis are several changes in mitochondria. Loss of mitochondrial transmembrane potential is pivotal to the apoptotic cascade; interventions that prevent the mitochondrial permeability transition event prevent apoptotic nuclear and cell membrane alterations (101–104). Bax translocation from its cytoplasmic pool to mitochondria contributes to opening of mitochondrial pores and the mitochondrial permeability transition with subsequent release of cytochrome C (105–107). That Bax mediated apoptosis occurs in ATP depletion injury has been demonstrated in cultured proximal tubule cells (107). There is growing evidence implicating an imbalance between the pro-apoptotic (Bax, Bid) and anti-apoptotic (Bcl-2, Bcl-xL) members of the Bcl-2 family of proteins, as well as the pro-apoptotic protein p53, that are active in the commitment phase of apoptosis in both animal (108–110) and human (92, 111, 112) conditions of ischemia induced AKI. Though cytochrome C release from mitochondria is not specific for apoptosis, it follows the mitochondrial permeability transition that is modulated by the Bcl-2 protein family, and indicates commitment to cell death. Its release is an essential factor in causing downstream activation of the effector proteins for apoptosis (88, 101, 107).

Caspase 3 is the effector protein that lies at a critical juncture in apoptosis pathways. Activation of Caspase 3 begins the execution phase of apoptosis (113–115). A series of additional caspases are activated in the execution phase that cleave multiple cellular proteins, including cytoskeletal-associated proteins and endonuclease inhibitors, resulting in the characteristic morphology of apoptosis – cell shrinkage, membrane blebbing, nuclear condensation and fragmentation (88).

Defining the relative importance of apoptosis in a particular form of AKI, and determining which portion of initiation and effector phase pathways are active after a specific renal insult, provide the hope that targeting specific sites in these apoptotic pathways for therapeutic intervention could limit renal injury in patients at risk to develop severe ARF.

Inflammatory Response

Although studies many years ago suggested that the immune system had no role at all in ischemia induced ARF, a growing body of evidence in the past several years indicates that the inflammatory response plays a major role in AKI. Inflammatory cascades initiated by endothelial dysfunction can be augmented by potent mediators generated by the ischemic proximal tubule. Both pro-inflammatory cytokines (TNF-α, IL-6, IL-1β, TGF- β) and chemotactic cytokines (MCP-1, IL-8, RANTES) are generated following renal ischemia and can enhance inflammation (24, 25, 116, 117). Toll-like receptor 2 (TLR-2), a pro-inflammatory molecule, is upregulated in renal tubules following ischemic kidney injury. Inhibition of TLR-2 by gene knock out or antisense treatment prevents ischemia induced renal dysfunction and suppress some of the cytokines in cultured proximal tubule cells (118, 119). Some of these cytokines, measured in the urine and plasma, have been found to be predictive of kidney injury or mortality in patients (120, 121).

Leukocyte infiltration into injured kidneys has been documented following ischemia; neutrophils being the first, followed closely by macrophages (25, 122). The significance of these events is not clear because (a) neutrophil depletion or blockade of function provides only partial functional protection and only in some animal models, (b) neutrophil infiltration appears not to be a prominent feature of ischemic renal injury in humans, and (c) although selective macrophage depletion ameliorates ischemic kidney injury, macrophages are dependent on coordinated action of T cells and neutrophils (123). The role of T cells has also been evaluated; they have been found in the kidney in both animal and human ischemic AKI models (25, 124, 125). Protection against injury from ischemia is observed in mice subjected to T-cell depletion as well as in mice with a double knockout of CD4/CD8 (126, 127). Reversal of protection in the latter model occurs with adoptive transfer of wild-type T cells into the knockout mice.

On the other hand, animals deficient in both T and B cells are not protected from ischemic injury (128) and depletion of peripheral CD4 T cells does not confer resistance against ischemic renal injury (129). Complicating the T cell picture further is identification of both protective (Th2 phenotype) as well as deleterious (Th1 phenotype) subtypes of T cells (130). Compared with wild type animals, B-cell deficient mice kidneys, with comparable T cell and neutrophil infiltration following ischemic renal injury, are partially protected both structurally and

functionally (131). Wild type serum transfer, but not B cell transfer, into these B-cell deficient mice restores susceptibility to ischemic AKI, implicating a soluble serum factor as a mechanism by which B cell deficiency confers renal protection (131).

Ischemia-reperfusion injury in most organs activates the classical pathway of the complement cascade. However, the alternative pathway, along with the mannose-binding lectin pathway of complement activation, appears to predominate in ischemic AKI in animals and humans (132–134). Recent studies have focused on C5a, a powerful chemoattractant for inflammatory cells, and its antagonists (135). C5a receptor is expressed in tubule epithelial cells and interstitial macrophages and is upregulated following ischemia-perfusion injury and sepsis (136–138). Inhibition of C5a generation using monoclonal antibodies prevents neutrophil and macrophage influx and protects against renal dysfunction after ischemia, and pretreatment with orally active C5a receptor antagonists in animals conferred histologic and functional protection against ischemic renal injury (137, 139, 140).

Strategies to modulate the inflammatory response by altering cytokine action have also been tried in AKI. IL-10 is a potent anti-inflammatory cytokine that provides protection against ischemic AKI by inhibiting maladaptive cytokine production by Th1 cells (141). Administration of a monoclonal antibody against the pro-inflammatory cytokine IL-6 reduces pro-inflammatory cytokine production, decreases neutrophil infiltration, and ameliorates structural and functional consequences of ischemic AKI (142). Furthermore, several agents with generalized anti-inflammatory effects are being studied as candidates to prevent or treat acute kidney injury. These include Bimosiamose (a pan-selectin inhibitor), statins and erythropoietin (with anti-inflammatory actions separate from their better known cholesterol lowering and erythropoetic effects respectively), and alpha-melanocyte stimulating hormone (143–148).

The clear involvement of the immune system in ischemic AKI suggests a mechanism underlying the consistent observation that development of acute renal failure substantially increases overall morbidity and mortality in patients suffering from a variety of diseases, including isolated cardiac disease, sepsis, and multi-organ dysfunction (149–153). In that context, it has been intriguing to see animal studies documenting distant organ effects and dysfunction in the heart, lungs, and brain after isolated ischemic AKI (154, 155). These distant effects of renal ischemia may be mediated by inflammatory signals generated in the injured kidney (156, 157).

Genetic Susceptibility, Gene Expression, and Biomarkers

In the clinical realm, it has long been observed that the incidence or severity of ARF varies widely between patients who have apparently been subjected to the same renal insult. While unrecognized environmental factors may certainly play a role, this observation has led clinicians to suspect that individual patients may have an inherent genetic susceptibility or resistance to developing ARF. A recent study of different rat strains has given credence to this concept that genetic background can substantially influence vulnerability to AKI. In that study, the inbred Brown-Norway rat strain, from two separate sources, was profoundly resistant to developing ARF following ischemia when compared with other inbred and outbred rat strains, all of which develop similar levels of renal failure (158). The rat strain specific protection appears to be on a cellular level since immediate cellular manifestations of ischemic AKI are limited, and the protection is sustained. The specific mechanism providing this protection is yet to be determined, but that it is related to differential gene expression is indicated by the finding of increased constitutive expression of inducible heat shock proteins, putative cytoprotectants, in the protected strain.

Specific human genetic polymorphisms have been linked, by several separate groups, alternately to either resistance or susceptibility to develop ARF (149). These human studies have implicated genetic polymorphisms of pro- and anti-inflammatory cytokines as well as heme oxygenase-1 (HO-1) as potential determinants of susceptibility to ischemic renal injury (149). Recent studies in pediatric patients have further supported a genetic basis underlying either resistance or susceptibility to develop ischemic ARF.

Genetic polymorphisms of the 70 kD stress protein Hsp72 appear to play a role in neonatal susceptibility to both ARF and its risk factors (159, 160). The Hsp72 (1267) GG allele has associated low inducibility of the Hsp72 protein. Very low birth weight neonates have increased prevalence of Hsp72 (1267) GG allele, and neonates in general carrying the Hsp72 (1267) GG genetic variation have increased risk of ARF (159, 160). Furthermore, an association was found between several maternal and fetal cytokine genetic polymorphisms, which increase the inflammatory response in preterm infants, and the incidence and severity of sepsis, necrotizing enterocolitis, and eventual ARF (159). Although genetic polymorphisms of the renin-angiotensin-aldosterone system have not been shown to influence directly the risk for ARF, they may

be associated with patent ductus arteriosus, poor postnatal adaptation, and heart failure, all prevalent risk factors for ARF (159, 161). A combination of high tumor necrosis factor-alpha producer and low interleukin-6 producer genotypes has been shown to increase the risk of acute renal failure in neonates as well as increase the mortality of adults in renal failure (162, 163).

Recent advances in functional genomics and cDNA microarray-based technologies have provided the opportunity to see the landscape of the numerous and intricate pathways activated by a complex process such as acute kidney injury (164–168). In combination with bioinformatic tools, these studies have identified novel genes with altered expression, new signal transduction pathways, and biomarkers of AKI. Kidney injury molecule-1 (KIM-1) protein, upregulated following ischemia on the apical membranes of proximal tubule cells, may be involved in renal tubule regeneration and is a promising non-invasive urinary biomarker of ischemic kidney injury (169) (170–173). The NGAL gene and protein is highly induced early in post ischemic kidney both in animals and humans; increased expression of this protein is found early in tubule cells undergoing proliferation, suggesting a protective role in AKI (164, 174–177). In addition, the finding that NGAL is rapidly excreted in the urine following a variety of renal insults suggests that NGAL is a novel, sensitive and early urinary biomarker of ischemic renal injury in patients (178–180). Furthermore, the demonstration in animal studies of protective effect against injury when exogenously administered makes it a potential therapeutic target (63, 181–184). Additional pathophysiologic pathways identified using these techniques include Zf9, a Kruppel-like transcription factor, which is strongly expressed during kidney development and markedly upregulated in post-ischemic tubule cells, along with its major trans-activating factor, TGF-β1(185). Thrombospondin 1 (TSP-1), a known p53-dependent pro-apoptotic and antiangiogenic molecule in malignant cells, is upregulated in postischemic proximal tubule cells, and TSP-1 null mice are partially protected from ischemia (169). Finally, microarray analyses have identified alterations in C4 complement, calcium binding protein S100A4, and matrix Gla protein, which may be involved in the long term outcome of ischemic acute renal injury (186).

Repair Mechanisms

Just as multiple pathways to cell injury are activated in AKI, the repair processes necessary to restore kidney structure and function would be expected to be multifaceted. The mechanisms to renal recovery, then, might include (1) endogenous repair of sublethally injured cells, (2) proliferation of surviving renal tubular cells, and (3) replacement of dead renal cells either by circulating stem cells or inherent stem cells within the kidney, with subsequent differentiation into tubule segment specific renal cells. The rapid initiation of repair in injured tubules supports the theory of endogenous repair of sublethally injured cells, with replacement of denuded segments of tubules with cells of either proximal or distal tubule origin (187–189). An understanding of factors that influence the recovery process would identify potential therapeutic interventions that could modulate injury or augment cellular repair to restore kidney function more quickly and fully following injury.

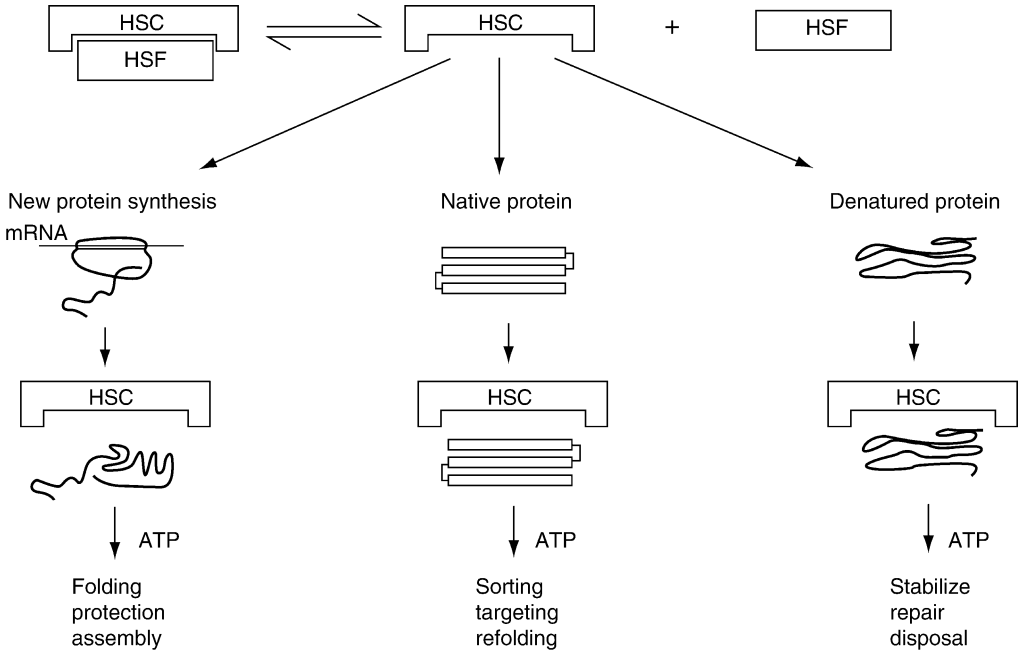
Heat Shock Proteins

Heat-shock proteins (Hsps), also called stress proteins, are highly conserved across species, being present in nearly every organism studied. They are elaborated in cells in response to injury caused by heat, anoxia, and a number of toxic agents. Hsps appear to be essential to basic cell function and survival by providing routine assistance in intracellular protein handling and trafficking (see *Fig. 64-6*) (190). These stress proteins are grouped into families according to size and apparent function. The two stress protein families most studied in AKI are the 70-kDa (Hsp70) and the small Hsp 25/27 class. Both of these Hsps act as chaperones to newly formed proteins, allowing proper folding and preventing inappropriate peptide interactions or aggregation. In like manner, these Hsps assist in the translocation of proteins across intracellular membranes and assist denatured proteins in refolding or reassembling into normal configuration as part of the process of repair (*Fig. 64-6*). Heat shock transcription factor (HSF) activation, the most proximal component of the stress response, is rapid after onset of renal ischemia, and significant Hsp induction in the kidney soon follows (53, 191, 192). The particular functions of these stress proteins in the kidney appear to have multiple aspects in AKI.

Renal tubule cells, which have suffered loss of structural integrity following injury, regain their normal architecture and polarity through remodeling (76). The recycling of cytoskeletal and plasma-membrane proteins is a primary mechanism of cellular repair following ischemia or ATP depletion (81, 193). The chaperone capacity of Hsps makes them likely candidates to be involved in the repair process. Indeed, among many potential functions

Figure 64-6

Heat shock protein functions **Shown are known actions of constitutively expressed Hsps, primarily of the 70 kD family (Hsp 70) called heat-shock cognates (HSC), in processing cellular proteins. Cell stress increases denatured protein, causing increased demand for HSC. Heat Shock Transcription Factor (HSF), reversibly bound to HSC, is released with the increased demand for HSC. HSF then rapidly initiates transcription for all inducible Hsps, including Hsp72 and Hsp25/27 (see text).**



during the recovery process, it has been found that Hsp72 and Hsp25/27 bind specifically to cellular proteins disrupted by ischemia or ATP depletion. Following renal ischemia, Hsp72 binds to aggregated cellular proteins, which include Na/K-ATPase, and is released from the aggregated proteins upon addition of ATP, a feature typical of its chaperone function (194). Furthermore, Hsp72 binds specifically to Na/K-ATPase during ATP depletion injury in renal cells (195). On the other hand, Hsp 25/27 is induced and moves from a soluble cytosolic distribution to an insoluble actin-associated state rapidly after renal ischemia, and binds specifically to disrupted actin during ATP depletion injury (196, 197).

These dynamic actions of different classes of Hsp, each with its specific function, may be essential to reestablish cellular polarity and integrity and may also provide insight into mechanisms of protection afforded by Hsps. In fact, separate overexpression of either Hsp72 or Hsp 25/27 limits the disruption of renal cell architecture that occurs with energy deprivation (195, 197). Furthermore, Hsp72 and 25/27 appear to work synergistically to preserve renal cell structure in the *in vivo* model of ischemic preconditioning (198).

Because of their well known characterization as cytoprotectants in a variety of cell types, Hsps have been examined by several groups as potential renal cytoprotectants (199). Studies of cultured renal epithelia have clearly demonstrated that stress proteins can mediate protection against specific manifestations of injury that result from specific insults (195, 197, 199–201). Whether heat shock proteins provide significant protection to the intact kidney has been controversial. Important initial studies clearly showed that stress proteins, when induced by a prior insult, do not uniformly and broadly protect the kidney against subsequent ischemic injury (199, 202, 203). However, recent studies of *in vivo* renal ischemia by several separate groups have supported a role for either Hsp72 or Hsp25/27 in providing resistance against ischemic renal injury (198, 204–206).

Furthermore, a more actively primed stress response may contribute to the phenomenon, long recognized by clinicians, that the immature kidney appears to be resistant to insults that would be expected to produce profound ARF in older patients. As observed in patients, immature rat kidneys are more resistant to ischemic injury than their mature counterparts. The neonatal

rat kidney has increased constitutive activity of HSF compared with mature animals, which translates into increased expression and induction of Hsp72 both prior and subsequent to an hypoxic or ischemic insult (207, 208). Not only does increased HSF activity and Hsp expression in the neonatal kidney correlate with resistance to hypoxia and ischemia, blocking HSF function reduces both Hsp72 expression and the tolerance of the immature renal tubules to anoxia (207–209). Heat shock proteins, then, may play a fundamental role in the tolerance of immature kidneys to ischemic or anoxic injury. So, while broad Hsp protection against all forms and severity of injury in the kidney clearly is not a fact, evidence continues to mount that these stress proteins contribute, along with other mediators of cytoprotection, to resistance against renal injury from ischemia.

Growth Factors

It has long been suspected that growth factors, either produced locally or exogenous to the kidney, are involved with the dedifferentiation, cellular proliferation, and redifferentiation of the tubule epithelial cell following AKI (*Fig. 64-1*). In the past two decades many studies have demonstrated the activity of a variety of growth factors on these aspects of cellular proliferation and differentiation following AKI. These include epidermal growth factor (EGF), insulin like growth factor-1 (IGF-1), hepatocyte growth factor (HGF), parathyroid hormone related peptide (PTHrP), basic fibroblast growth factor, osteopontin, neural cell adhesion molecule, and transforming growth factor – beta 1 (TGF-β) (188, 210–212).

Growth factor function may be intimately linked to processes involved in regulated, apoptotic cell death. In the rat model of AKI, expression of pro- and anti-apoptotic members of the Bcl-2 gene family (Bcl-2, Bcl-X(L), Bax) are temporally and spatially related to tubule expression of EGF, IGF-1, and TGF-β, all growth factors that are thought to be reparative in ARF (212). The study suggested that the distal tubule is adaptively resistant to ischemic injury through anti-apoptotic effects of Bcl-2 genes, and its survival allows expression of growth factors critical not only to the survival and regeneration of its own cell population (autocrine action), but also to the adjacent ischemia-sensitive proximal tubule cells (paracrine action).

Acute kidney injury in rats increases expression of transcription factors Ets-1 and Wnt-4 that are in pathways involved in cell cycle progression (213). Nephrogenic proteins basic fibroblast growth factor (bFGF), vimentin, neural cell adhesion molecules, paired homeobox-2, bone morphogene protein-7, Noggin, Lim-1, Engrailed, Smad, phospho-Smad, hypoxia-inducible factor-1 alpha (HIF-1 alpha), vascular endothelial growth factor (VEGF), and Tie-2 are all induced by renal ischemia in a pattern similar to that seen in normal renal development, and administration of bFGF accelerated induction of many of those nephrogenic proteins during recovery (214, 215). HIF-1 alpha is rapidly activated by renal ischemia and growth factor genes regulated by this transcription factor, VEGF and erythropoietin, appear to have important effects on renal epithelial cells, as well as endothelial cells in the renal microvasculature. Erythropoietin administration prior to renal ischemia protects the kidney against the injury through mechanisms that limit apoptosis (216).

The relative importance of the many growth factors identified to be active in AKI has rarely been addressed. One study, however, directly compared the effect of two of these growth factors in AKI using a transgenic mouse model with targeted over expression in the proximal tubule of HGF in one strain, and PTHrP in another strain. It was found that the strain with increased HGF in the proximal tubule had a fourfold increase in tubule cell proliferation and a threefold decrease in apoptotic tubule cell death along with rapid recovery from ischemic injury compared with either the mice with targeted proximal tubule over expression of PTHrP or background strain control mice (217). This study confirmed the importance of HGF during the recovery process, but raises questions about the role of the rapid induction of PTHrP observed in the proximal tubule after renal ischemia. By extension, it also raises questions about all of the growth factors identified as being induced by renal ischemia whose functional effects are yet to be defined. Some may be beneficial. Some, such as TGF-β, may have detrimental effects in the long term. A complex interaction between the growth factors and various chemokines that is dependent on the local context of the affected cells likely determines the outcome following AKI. A delicate balance of these factors may determine whether tissue remodeling following renal injury results in restitution of normal architecture or proceeds to a dead-end of irreversible fibrosis.

Stem Cells

With the advent of stem cell research, the possibility that some form of stem cell might be integral to kidney repair after AKI rapidly came to the fore. Along with the well-known embryonic stem cells, non-embryonic/adult stem cells that can differentiate into more than one type of

specialized cell come from several sources. Best characterized are hematopoetic stem cells that can differentiate into any of the blood cell lines. Mesenchymal stem cells reside in the bone marrow and can differentiate into a variety of mesenchymal tissues *in vitro* and *in vivo*. Tissue specific progenitor cells may reside in any organ and be able to differentiate into a range of cells as the need arises. Each of these potential sources of stem cells is actively being studied to determine the contribution to renal repair following acute kidney injury (218).

Both categories of bone marrow derived stem cells (BMDSC), hematopoetic stem cells (HSC) and mesenchymal stem cell/stromal cells (MSC), have been evaluated in AKI. Although, some have reported differentiation and incorporation of BMDSC into other cell types and tissues, including lungs, hepatocytes, pancreatic islet cells, endothelial cells and cardiac myocytes, not all studies have supported those findings (219–225). Biopsies of kidney transplants from a female donor to a male recipient, after acute injury to the transplant, have demonstrated the presence of Y chromosome positive tubule cells in the graft, indicating homing and differentiation of non-inflammatory bone marrow derived cells into renal tubule cells following injury (226). However, transplantation of male bone marrow into lethally irradiated female mice have demonstrated that bone marrow derived cells contribute minimally to the repair of the renal tubule and that most cells involved in repair are of endogenous tubule origin (227–230). Studies using bacterial β – galactosidase expressing BMDSC support the concept of these cells being incorporated into renal tubules after AKI, but the specificity of this technique has been questioned since injured endogenous tubule cells are able to incorporate and express this marker (231, 232). Hence, whether bone marrow derived cells participate significantly in tubule regeneration through incorporation and differentiation remains an open question.

Evidence that BMDSC have a functional role in the repair process is more consistent. The severity of renal injury in mice whose bone marrow has been ablated is worse than those without ablation and is reversible with infusion of lineage negative bone marrow cells (231). Studies designed to differentiate the function of MSCs from HSCs found MSC incorporation into regenerating tubules associated with protection from injury, but no such findings with HSCs (233, 234). Although other studies have not confirmed that MSCs engraft into renal tubules, they have found functional effects attributable to MSCs such as blunted rise in serum creatinine and alteration in pro-inflammatory cytokine and growth factor expression (230, 235). Similar findings in other reports suggest that protection by MSC may be mediated by paracrine or endocrine effects (235, 236). The number of MSCs infused appears to be a critical determinant of their protective function (229–231, 233). Recent studies suggest further that mobilizing endogenous BMDSCs is a therapeutic option in AKI using stem cell factor or colony-stimulating factors (macrophage-CSF or granulocyte-CSF) (237, 238). The mechanisms underlying the observed renal protection using these agents might be through ameliorating injury pathways detailed earlier in this chapter, along with affecting recovery pathways through BMDSC mobilization.

Since developmental biology has found that most renal epithelial cells proximal to the collecting duct originate from mesenchymal cells that transform into the specialized cells of the glomeruli and renal tubules, it is possible that these mesenchymal stem cells may persist in the adult renal interstitium, providing a reservoir of tubule cell progenitors (218, 239). Evidence for this concept comes from using the cell proliferation marker BrdU to identify slow cycling stem cells in rodent renal interstitium during late organogenesis that disappear following transient ischemia (240). Stem cells can be isolated from adult renal interstitium with help of surface markers and they express endothelial or epithelial cell markers when cultured under the influence of specific growth factors (241, 242). Called side population (SP) cells, the stem cells isolated from renal interstitium have been shown in mice to differentiate toward epithelial lineage, supporting the possibility of their involvement in the repair process. Furthermore, infusion of SP cells in rodents ameliorates renal injury induced by cisplatin (243, 244). These SP cells, following injury, have increased expression of several growth factors that appear to play a role in renal repair (as discussed earlier). So, while the relative contribution that stems cells make through direct repopulation of the nephron is unclear, evidence is growing that stems cells make an important contribution to the overall repair of the tubule after AKI via several pathways.

Long-Term Sequelae

The focus in the clinical realm, and in experimental models of ARF, typically is on the severity of the acute renal manifestations and the speed and completeness of early recovery. Little attention commonly is given to long-term sequelae in the kidney following an episode of ARF, particularly since many, if not most, patients who survive the triggering event appear to recover renal function completely in the short term. However, this aspect of

AKI is garnering progressively increased recognition. Decades ago it was found that many adult patients who suffer ARF never recover renal function completely, manifested by decrements in GFR and urinary concentrating ability months to years later (245–249). More recent reports in young pediatric patients have documented late renal insufficiency after recovery from an episode of ARF (250–253). The finding of significant and progressive renal insufficiency in adolescence and early adulthood after recovery from ARF as a neonate is of particular interest. Though the neonatal kidney may be more resistant to AKI, it may be more prone to develop late sequelae once AKI has been established.

The rat model of AKI has revealed how a late decline in renal function may follow apparent complete initial recovery as measured by GFR. One mechanism may be that many nephrons may not be able to regenerate completely after the injury, leading to disconnection of intact glomeruli from residual tubule segments with eventual later decline in overall renal function (254, 255). Another is that inflammatory mediators of renal injury, initiated by AKI, may persist and cause progressive renal dysfunction (256).

Finally, the early alterations in the renal microvasculature that accompany ischemia induced AKI appear to have substantial long term consequences (257). It has been found that peritubule microvessel density is reduced by half, weeks after uncomplicated recovery from ischemic AKI (258). The renal microvascular system, then, seems to lack the profound regenerative ability of the renal tubule system. The peritubular capillary dropout has the accompanying functional effect of reduced renal oxygenation, particularly in the outer medulla, the region of the kidney most susceptible to an ischemic insult (259). That chronic renal hypoxia may contribute to progressive renal dysfunction in patients is suggested by finding impaired renal oxygenation in kidney transplants affected by chronic allograft dysfunction (260).

The principal cause of the renal microvascular dropout after AKI, whether it be endothelial cell necrosis or apoptosis, lack of regenerative potential, endothelial-mesenchymal transition, or progressive interstitial fibrosis is yet to be defined. TGF-β, though, may be a central player in the initial injury as well as in potentiating the later progressive renal injury. Blockade of TGF-β, which is highly expressed after AKI, preserves microvascular density (261). TGF-β may be pivotal in a vicious cycle triggered by AKI, since it can stimulate apoptosis in endothelial cells (262), stimulate endothelial-mesenchymal transition (263), and is itself a fibrinogenic factor that is triggered by hypoxia (264). Blocking TGF-β, then, could intervene on several processes that may contribute to chronic, progressive renal injury after AKI.

Conclusion

The study of acute kidney injury that leads to clinical acute renal failure continues to advance rapidly. The progressive discovery of the many facets in the pathogenesis of the injury, markers of the injury, and potential recovery mechanisms provides hope that therapy targeted to the kidney injury specifically, along with ongoing advances in supportive therapy, will substantially improve survival and wellness of patients affected by acute kidney injury. Suggested by the many interactive pathways active in AKI outlined here, no single intervention alone is likely to alter outcome substantially. An integrated approach may be required to limit injury or facilitate renal recovery. Having a clear understanding of the pathogenesis of acute kidney injury from a particular renal insult should help direct when to intervene with a specific therapy, and which intervention would be most likely to benefit the patient.

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65 Clinical Evaluation of Acute Kidney Injury in Children

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Introduction

The incidence of acute kidney injury (AKI) (previously called acute renal failure) has increased over the past several years and AKI leads to substantial morbidity and a high mortality rate (1–4). The epidemiology of AKI has changed to multifactorial causes particularly in hospitalized patient and many forms of AKI such as ischemic/hypoxic AKI which were thought to be reversible are now known to be significant contributors to chronic kidney disease (CKD) (1–4). There are many causes of AKI and the more common etiologies are listed in *Table 65-1*. Some diseases, such as the tumor lysis syndrome, drug-induced interstitial nephritis, aminoglycoside nephrotoxicity, and other toxic nephropathies, usually present as AKI and recovery is usually complete while other diseases, such as rapidly progressive glomerulonephritis (RPGN), may present as AKI but rapidly evolve into chronic kidney disease (CKD). Several renal diseases, such as the hemolytic-uremic syndrome (HUS), Henoch-Schönlein purpura, and obstructive uropathy with associated renal dysplasia, may present as AKI with improvement of renal function to normal or near-normal levels, but the child's renal function may slowly deteriorate, leading to CKD several months to years later.

Children with AKI due to hypoxic/ischemic insults, HUS, acute nephritis as well as other causes are more likely to demonstrate oliguria or anuria (urine output less than 500 ml/24 h in older children or urine output less than 0.5–1.0 ml/kg/h in younger children and infants). Children with acute interstitial nephritis, nephrotoxic renal insults including aminoglycoside nephrotoxicity, and contrast nephropathy are more likely to have AKI with normal urine output. The morbidity and mortality of nonoliguric AKI are substantially less than oliguric renal injury (5, 6). This chapter will review the common causes of AKI, evaluation of AKI and potential future therapies. The previous chapter discussed the pathophysiology of AKI while the next chapter focuses on medical management and renal replacement therapy for AKI.

Definition

Currently, there is not a uniform definition of AKI in adult or pediatric patients and AKI is defined in multiple ways but the majority of definitions of AKI currently in use involve a change in the serum creatinine level. Increases in the serum creatinine and blood urea nitrogen are typical in AKI but each may be affected by factors other than a decline in the glomerular filtration rate. Since the serum creatinine is a reflection of muscle mass, children with decreased muscle mass may have substantial decreases in their glomerular filtration rate with minimal to modest changes in the serum creatinine concentration. The blood urea nitrogen (BUN) is affected by multiple factors including the state of hydration of the child, gastrointestinal bleeding, protein intake, a hypermetabolic state and the administration of catabolic medications such as corticosteroids each of which tend to increase the BUN irregardless of renal function. In contrast, the BUN may be lower than expected in AKI in a child with severe liver disease or in a child with protein malnutrition.

It is accepted that the serum creatinine is an insensitive and delayed measure of decreased kidney function following AKI (7–14). Multiple studies have focused on the development of sensitive and specific biomarkers of AKI; biomarkers under investigation include changes in plasma Neutrophil Gelatinase-Associated Lipocalin (NGAL) and cystatin C levels and urinary changes in NGAL, Interleukin-18 (IL-18) and Kidney Injury Molecule-1 (KIM-1) (7). NGAL was found to be one of the most rapidly induced proteins in the kidney following experimental AKI and NGAL appeared in the urine of animals before other biomarkers (8, 9). In a prospective study of children undergoing cardiac surgery, plasma and urine NGAL measurements increased hours after surgery in those who developed AKI compared to 2–4 days for an increase in serum creatinine to occur (9). NGAL was also shown to be an early predictor of contrast induced nephropathy in children (10). Other biomarkers that have shown promise to detect AKI at an early stage include

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Table 65-1
Common causes of acute kidney injury

Pre-renal failure
Decreased true intravascular volume
Decreased effective intravascular volume
Intrinsic renal disease
Acute tubular necrosis (vasomotor nephropathy)
Hypoxic/ischemic insults
Drug induced
Toxin mediated
Endogenous toxins - hemoglobin, myoglobin
Exogenous toxins - ethylene glycol, methanol
Uric acid nephropathy and tumor lysis syndrome
Interstitial nephritis
Drug induced
Idiopathic
Glomerulonephritis
Vascular lesions
Renal artery thrombosis
Renal vein thrombosis
Cortical necrosis
Hemolytic Uremic Syndrome
Hypoplasia/dysplasia with or without obstructive uropathy
Idiopathic
Exposure to nephrotoxic drugs in utero
Hereditary renal disease
Autosomal dominate polycystic kidney disease
Autosomal recessive polycystic kidney disease
Alport's syndrome
Sickle cell nephropathy
Juvenile nephronophthisis
Obstructive uropathy/lower tract lesions
Obstruction in a solitary kidney
Bilateral ureteral obstruction
Urethral obstruction
Blaldder rupture

KIM-1 and urinary cystatin C in adult and pediatric patients undergoing cardiac surgery (11, 12). Recently, liver fatty acid-binding protein (L-FABP) has also been shown to be a sensitive and early predictor of AKI in pediatric patients undergoing cardiac surgery (13). As described later the development, testing and successful implementation of therapeutic strategies in AKI is going to require the development of sensitive biomarkers so that therapy can be initiated in a timely manner. Reliable biomarker(s) for early detection of AKI should facilitate early identification, help stratify risk and contribute to informative diagnostic classification so interventions to improve outcome of AKI can be developed (14).

As described above, the definition of AKI in adults and pediatric patients has been quite variable. A new classification system entitled the RIFLE criteria (R = Risk for renal dysfunction, I = Injury to the kidney, F = failure of kidney function, L = loss of kidney function, and E = end stage renal disease) has been proposed as a standardized classification of acute kidney injury in adults (15) and has been adapted for pediatric patients (16). The pediatric RIFLE was found to better classify pediatric AKI and to reflect the course of AKI in children admitted to the ICU (16). The pediatric RIFLE criteria have been validated in children and appears quite promising for better characterization of AKI (16). The RIFLE criteria have been used by the Acute Kidney Injury Network (AKIN) which is a group of adult nephrologists, pediatric nephrologists, critical care physicians and societal organizations interested in AKI research; the focus of AKIN is to facilitate international, interdisciplinary and intersociety collaborations to ensure progress in the field of AKI (17). In addition, the definition of AKI has a substantial impact on studies to determine the incidence and epidemiology of AKI in adult and pediatric patients (18).

Some definitions of AKI include a decline in urine output. While a decline in urine output is a common clinical manifestation of acute kidney injury, many forms of acute kidney injury are associated with normal urine output. Children with acute kidney injury due to hypoxic/ischemic insults, hemolytic uremic syndrome, acute glomerulonephritis, rapidly progressive glomerulonephritis as well as other causes are more likely to demonstrate oligo/anuria (urine output less than 400–500 cc/24 h in older children or urine output less than 0.5–1.0 cc/kg/h in younger children and infants). Children with acute interstitial nephritis, nephrotoxic renal insults including aminoglycoside nephrotoxicity and contrast nephropathy are more likely to have acute kidney injury with normal urine output. Studies have shown that the morbidity and mortality of nonoliguric acute kidney injury is substantially less than oliguric renal injury (19, 20).

Epidemiology of Acute Kidney Injury

The epidemiology of AKI is quite different in developed and developing countries. AKI has become increasingly prevalent in both developed and in developing countries with

significant morbidity and mortality (21). In developed countries, AKI was more common in intensive care units in older individuals with multi-organ failure, sepsis and multiple co-morbid conditions. In urban areas of developing counties, AKI occurred under similar epidemiology conditions as in developed counties. In contrast, AKI in rural areas of developing countries is usually a result of a single disease or infection such as gastroenteritis, malaria, leptospirosis or hemolytic uremic syndrome in younger other wise healthy individuals (21). Due to the limited resources to treat AKI in rural area and the high mortality of AKI, strategies for the prevention of AKI in rural areas is paramount to decrease morbidity and mortality.

While the precise incidence and causes of AKI in pediatric patients is unknown, recent studies suggest that the incidence of AKI in hospitalized children is increasing (22–27). An important cause of AKI in hospitalized children is in the setting of post cardiac surgery and in children undergoing stem cell transplantation. AKI in such children is frequently multifactorial with ischemic/hypoxic injury and nephrotoxic insults being important contributors. No epidemiology studies using an established definition of AKI have been conducted in pediatric patients. In a large study of adult patients, the incidence of AKI was 209 per million population and the most common cause of AKI was prerenal in 21% of patients and acute tubular necrosis in 45% of patients (28). Similar epidemiologic studies have not been performed in pediatric patients but hypoxic/ischemic and nephrotoxic induced AKI has been shown to be an important cause of AKI in neonates, children and adolescents (5, 21–27). In a study of pediatric patients in a tertiary care center, 227 children received dialysis therapy during an eight-year interval for an overall incidence of 0.8 per 100,000 total population (6). In a study of neonates, the incidence of AKI ranged from 8 to 24% of newborns and AKI was particularly common in neonates who had undergone cardiac surgery (29). Other studies have demonstrated that very low birth weight (less than 1,500 g), a low Apgar Score, a patent ductus arteriosis and maternal administration of antibiotics and nonsteroidal anti-inflammatory drugs was associated with the development of AKI (23). A low Apgar score and maternal ingestion of non-steroidal anti-inflammatory drugs has been associated with decreased renal function in preterm infants (23, 30). The incidence of AKI in newborns in a developing county was 3.9/1,000 live births and 34.5/1,000 newborns admitted to the neonatal unit (24).

Several studies have demonstrated that newborns and children may have genetic risks factors for AKI independent of environmental risk factors (31–35). Several candidate polymorphisms have not been associated with AKI while other polymorphisms have been found to be associated with AKI. Polymorphism of the ACE gene or the angiotensin receptor gene with resultant alterations in activity of the renin angiotensin system does not appear to play a role in the development of AKI (31). In studies in newborns, polymorphisms of tumor necrosis factor alpha, interleukin 1b, interleukin 6 and interleukin 10 genes were investigated in newborns to determine if polymorphisms of these genes would lead to a more intense inflammatory response and predispose newborns to AKI (32). The allelic frequency of the individual genes did not differ between newborns with AKI and those without AKI but the TNFa/IL-6 AG/GC haplotype was present in 26% of newborns who developed AKI compared to 6% of newborns who did not develop AKI. The investigators suggested that the combination of these polymorphisms might lead to a greater inflammatory response and the development of AKI in neonates with infection (32). As described later, future therapies for AKI might involve strategies to interrupt the inflammatory response. In other studies, the incidence of ACE I/D allele genotypes or the variants of the angiotensin I receptor gene did not differ in neonates with AKI compared to neonates without AKI but they may be associated with patent ductus arteriosis and heart failure and indirectly contribute to CKD (31–34). AKI occurred more commonly in very low birth weight neonates carrying the heat shock protein 72 (1267) GG genetic variation which is associated with low inducibility of heat shock protein 72 (33). Given the important role of heat shock proteins in ischemic renal injury, these findings suggest that some neonates are more susceptible to ischemic injury (34). Another very interesting study of demonstrated that NADPH oxidase p22phos and catalase gene variants are associated with biomarkers of oxidative stress and adverse outcomes in acute renal failure (35). Since oxidant stress plays a role in the pathophysiology of AKI, it is interesting that genetic variants of pro-oxidant and anti-oxidant molecules are associated with susceptibility to AKI (35, 36). Future studies of the genetic background of the child at risk for AKI due to medication exposure, toxin exposure, ischemic hypoxic insults or other insults will likely impact the management of the child at risk for AKI and the management of AKI.

Etiology of Acute Kidney Injury in Children

AKI can be divided into prerenal injury, intrinsic renal disease including vascular insults, and obstructive uropathies

(see *Table 65-1*). Some causes of AKI, such as cortical necrosis and renal vein thrombosis, occur more commonly in neonates, whereas HUS is more common in young children, and RPGN generally occurs in older children and adolescents (*Table 65-2*). Hypoxic ischemic injury is an important cause of AKI at all ages (*Table 65-2*). An important cause of AKI in neonates is exposure to maternal drugs in utero that interfere with nephrogenesis such as angtiotensin converting enzyme inhibitors, angiotensin receptor blockers and non steroidal anti-inflammatory drugs (37–41). The history, physical examination, and laboratory studies including a urinalysis and radiographic studies can establish the likely cause(s) of AKI. In many instances, such as AKI occurring in hospitalized children, multiple factors are likely to be implicated in the etiology of AKI.

Prerenal Injury

In prerenal failure, renal function is decreased due to decreased renal perfusion and the kidney is intrinsically normal. Restoration of renal perfusion results in a prompt return of renal function to normal while acute tubular necrosis as described below implies that the kidney has suffered intrinsic damage. However, the evolution of prerenal injury to intrinsic renal injury is not sudden and a number of compensatory mechanisms work together to maintain renal perfusion in the face of adverse renal hemodynamic conditions (42). When renal perfusion is compromised, the afferent arteriole relaxes its vascular tone to decrease renal vascular resistance and maintain renal blood flow. Decreased renal perfusion results in increased catecholamine secretion, activation of the renin angiotensin system and the generation of prostaglandins. During renal hypoperfusion, the intrarenal generation of vasodilatory prostaglandins including prostacyclin mediates vasodilatation of the renal microvasculature to maintain renal perfusion (42). Administration of aspirin or non-steroidal anti-inflammatory drugs can inhibit this compensatory mechanism and precipitate acute renal insufficiency during renal hypoperfusion. Similarly, when renal perfusion pressure is low as in renal artery stenosis, the intraglomerular pressure necessary to drive filtration is in part mediated by increased intrarenal generation of angiotensin II to increase efferent arteriolar resistance (42). Administration of angiotensin converting enzyme inhibitors in these conditions can eliminate the pressure

Table 65-2

Common causes of acute kidney injury according to age group

Age	Glomerular diseases	Vascular diseases	Developmental/tubulointerstitial
<2 years		Cortical Necrosis	In utero exposure to ACEI, ARBs, NSAID
		Renal Artery Thrombosis	Obstructive Uropathy
		Renal Vein Thrombosis	Dysplastic Kidneys
		Hypoxic/ischemic AKI	Hypoxic/ischmeic AKI
			Nephrotoxic AKI
2–6 years	Post-infectious glomerulonephritis	Hemolytic Uremic Syndrome	Obstructive Uropathy
	Henoch Scholein Purpura	Hypoxic/ischemic AKI	Hypoxic/ischemic AKI
			Nephrotoic AKI
			Tumor lysis syndrome
6–12 years	Post-infectious glomerulonephritis	Hemolytic Uremic Syndrome	Obstructive Uropathy
	Henoch Scholein Purpura	Hypoxic/ischemic AKI	Hypoxic/ischemic AKI
	RPGN		Nephrotoxic AKI
			Tumor lysis syndrome
			Interstial Nephritis
12–18 years	RPGN		Obstructive Uropathy
	Systemic Lupus Erythematosis		Hypoxic/ischemic AKI
	Henoch Schonlein Purpura		Nephrotoxic AKI
			Interstitial Nephritis

gradient needed to drive filtration and precipitate acute kidney injury (43, 44). It was originally thought that selective COX-2 inhibitors would be renal sparing but it has been recognized that the selective COX-2 inhibitors can adversely affect renal hemodymanics similar to the effects of non selective COX inhibitors (45). In addition, clinical use of selective COX-2 inhibitors has been associated with acute kidney injury (45). Thus, administration of medications that can interfere with compensatory mechanisms to maintain renal perfusion can precipitate acute kidney injury in certain clinical circumstances.

Prerenal injury results from renal hypoperfusion due to true volume contraction or from a decreased "effective" blood volume. True volume contractions results from hemorrhage, dehydration due to gastrointestinal losses, salt wasting renal or adrenal diseases, central or nephrogenic diabetes insipidus, increased insensible losses as occurs in burns, and in disease states associated with third spaces losses such as sepsis, nephrotic syndrome, traumatized tissue and capillary leak syndrome. Decreased effective blood volume occurs when the true blood volume is normal or increased but renal perfusion is decreased due to diseases such as congestive heart failure, cardiac tamponade, and hepatorenal syndrome. Whether prerenal injury is caused by true volume depletion or decreased effective blood volume, correction of the underlying disturbance will return renal function to normal.

The urinalysis can help differentiate ATN from prerenal injury; the presence of casts and renal tubular epithelial cells was found to highly correlate with ATN while the absence of such findings was associated with prerenal disease (46). The urine osmolality, urine sodium concentration, the fractional excretion of sodium, and the kidney failure index have all been proposed to be used to help differentiate prerenal injury from vasomotor nephropathy (ATN). This differentiation is based on the concept that the tubules are working appropriately in pre-renal injury and are therefore able to conserve salt and water appropriately while in vasomotor nephropathy tubules have progressed to irreversible injury and are unable to appropriately conserve sodium (47, 48). During prerenal failure, the tubules are able to respond to decreased renal perfusion by appropriately conserving sodium and water such that the urine osmolality is greater than 400–500 mOsm/L, the urine sodium is less than 10–20 meq/L, and the fractional excretion of sodium is less than 1%. The fractional excretion of sodium is calculated by the formula urine sodium/plasma sodium divided by urine creatinine/plasma creatinine. Since the renal tubules in newborns and premature infants are relatively immature compared to older infants and children, the corresponding values suggestive of renal hypoperfusion are urine osmolality greater than 350 mOsm/L, urine sodium less than 20–30 meq/L, and a fractional excretion of sodium of less than 2.5% (47, 48). When the renal tubules have sustained injury as occurs in acute tubular necrosis, they cannot appropriately conserve sodium and water such that the urine osmolality is less than 350 mOsm/L, the urine sodium is greater than 30–40 meq/L and the fractional excretion of sodium is greater than 2.0%. However, the use of these numbers to differentiate prerenal injury from acute tubular necrosis requires that the patient has normal tubular function initially. While this may be the case in some children, newborns with immature tubules, children with pre-existing renal disease or salt wasting renal adrenal disease as well as other diseases may have renal injury with urinary indices suggestive of ATN. Therefore, it is essential to consider the state of the function of the tubules prior to the potential onset that might precipitate vasomotor nephropathy/ATN.

In adult patients it has been shown that calculation of the fractional excretion of urea nitrogen is a more sensitive test to differentiate prerenal injury from acute tubular necrosis than the fractional excretion of sodium particularly in patients who have received diuretic therapy (49). The fractional excretion of urea nitrogen was found to be at least as sensitive and specific as the fractional excretion of sodium in prerenal azotemia but the fractional excretion of urea nitrogen did not lose its sensitivity or specificity in differentiation prerenal injury and ATN when patients had received diuretic therapy. Weather the fractional excretion of urea will be more sensitive or specific compared to the fractional excretion of sodium in pediatric patients is unknown and such studies need to be performed to determine if this is a more sensitive measure of tubular function in children.

Intrinsic Renal Disease

Acute Tubular Necrosis

Acute tubular necrosis (ATN) can evolve from prerenal injury if the insult is severe and sufficient enough to result in vasoconstriction and acute tubular necrosis. The pathophysiology of ischemic/hypoxic ATN is thought to be related to early vasoconstriction followed by patchy tubular necrosis. ATN may also result from injury of tubular epithelial cells from drugs including aminoglycoside antibiotics, cisplatin, ifosfamide, amphotericin B,

acetaminophen and radiocontrast dye and exogenous toxins such as ethylene glycol, methanol, bromate, or endogenous toxins such as myoglobinuria and hemoglobinuria (*Table 65-1*).

Hypoxic/ischemic ATN

Hypoxic/ischemic acute tubular necrosis evolves from prolonged prerenal injury or following severe hypoxic insults. The urinalysis is likely to demonstrate casts and renal tubular epithelial cells and casts with or without low grade proteinuria casts while urine indices of tubular function demonstrate an inability to conserve sodium and water (46). The serum creatinine typically increases by about 0.5–2.0 mg/dl per day depending on the severity of the insult. Radiographic studies demonstrate kidneys of normal size with loss of corticomedullary differentiation while a radionucleotide renal scan with technetium−99-MAG3 or technetium−99-DTPA will demonstrate normal or slightly decreased renal blood flow with poor function and delayed accumulation of the radioisotope in the renal parenchyma without excretion of the isotope in the collecting system (*Fig. 65-1a*).

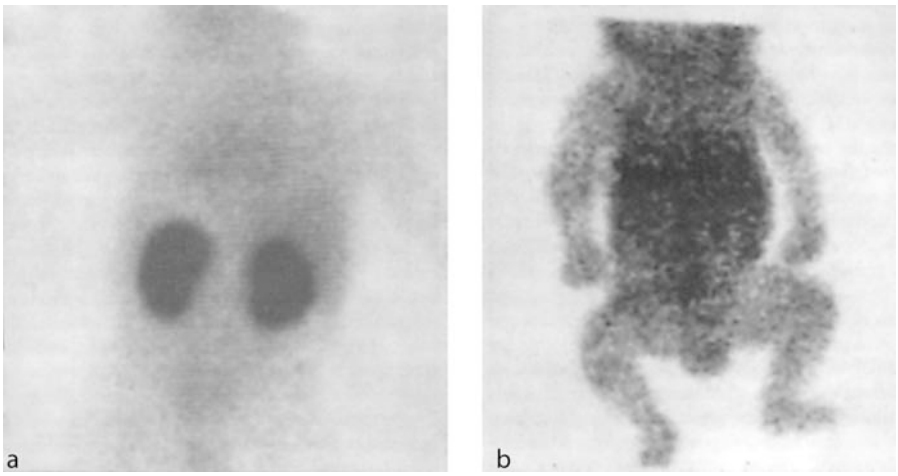
In the past it was thought that the prognosis of ATN was good except in cases when the insult is of sufficient severity to lead to vasculature injury and microthrombi formation with the subsequent development of cortical necrosis. However, it is now recognized that AKI from any cause including hypoxic ischemic can lead to chronic kidney disease (1–4, 50). The recovery of the child and the recovery of renal function depend upon the underlying events which precipitated the ischemic/hypoxic insults. The mortality and morbidity of children with acute kidney injury is much worse in children and neonates with multiorgan failure. Typically, the child does not succumb of renal injury but rather the child succumbs with renal injury and the associated conditions that precipitated the multi-organ injury (51–54). In children who recover from ATN, renal function generally returns but the length of time before recovery is quite variable. Some children will begin to recover renal function within days of the onset of renal injury while recovery may not occur for several weeks in other children. Return of renal function may be accompanied by a diuretic phase with excessive urine output at a time when the tubules have begun to recover from the insult but have not recovered sufficiently to appropriately reabsorb solute and water. When the diuretic phase occurs during recovery, close attention to fluid and electrolyte balance is very important to ensure adequate fluid management to promote recovery from acute tubular necrosis and prevent additional renal injury.

Nephrotoxic Acute Renal Failure

Many different drugs and agents may result in nephrotoxic acute kidney injury (55). Nephrotoxic acute renal

Figure 65-1

Mag-3 renal scan in a one year old with ATN (1a) and in a newborn with cortical necrosis (1b). Each scan is at 4 h after injection of isotope. Figure 1a shows delayed uptake of isotope with parenchymal accumulation of isotope with little to no excretion of isotope into the collecting system. In contrast, 1b demonstrates no renal parenchymal uptake of isotope in a neonate with cortical necrosis.



injury may result from the administration of a number of different medications as well as the ingestion of poisons or due to a nephrotoxic insult from indigenous compounds such as hemoglobinuria or myoglobinuria.

Drug Induced Acute Kidney Failure

Medications which are associated with acute kidney injury at least in part due to toxic tubular injury include aminoglycoside antibiotics, intravascular contrast media, amphotericin B, chemotherapeutic agents such as ifosfamide and cisplatin, acyclovir, and acetaminophen while other medications have been implicated less commonly (55). Aminoglycoside nephrotoxicity usually presents with nonoliguric acute kidney injury with a urinalysis showing minimal urinary abnormalities. The incidence of aminoglycoside antibiotic nephrotoxicity is related to the dose and duration of the antibiotic therapy as well as the level of renal function prior to the initiation of aminoglycoside therapy. The etiology of aminoglycoside nephrotoxicity is thought to be related to the lysosomal dysfunction of proximal tubules and is reversible once the aminoglycoside antibiotics have been discontinued. However, after the aminoglycoside is discontinued, the serum creatinine may continue to increase for several days due to ongoing tubular injury from continued high parenchymal levels of the aminoglycoside. Studies have demonstrated that once daily dosing of aminoglycoside antibiotics is associated with a slightly decreased incidence of nephrotoxicity without compromising efficacy (56).

Intravascular contrast media may also precipitate acute kidney injury particularly in patients who are at higher risk for contrast nephropathy including patients with dehydration, diabetes, or pre-existing renal disease and patients with hypergammaglobulinemia. The incidence of contrast nephropathy can be reduced with the use of nonionic, low osmolality contrast media. The prophylactic administration of N-acetylcysteine may decrease the decline in glomerular filtration rate associated with the administration of intravascular contrast in adults at risk for contrast nephropathy (57, 58). Similar studies have not been performed in pediatric patients and the potential risks and benefits of N-acetylcysteine in pediatric patients at risk for contrast nephropathy are unknown.

Non-steroidal anti-inflammatory drugs may also precipitate acute kidney injury by their effect on intrarenal hemodynamics. Cisplatin, ifosfamide, acyclovir, amphotericin B, and acetaminophen are also nephrotoxic and may precipitate acute kidney injury (55). The combination of a cephalosporin and acyclovir may be particularly nephrotoxic (59). Several other drugs have also been associated with acute kidney failure, but the incidence of acute kidney injury with other drugs is less common.

Exogenous Toxin Induced Acute Kidney Failure

The ingestion of toxic compounds either in their native state or once they are metabolized may precipitate acute kidney failure. Ethylene glycol and methanol are not toxic until they are metabolized by alcohol dehydrogenase to organic acids and oxalate for ethylene glycol while methanol is metabolized to formic acid and formaldehyde. Once sufficient quantities of the metabolites have been generated, the patient will present with severe metabolic acidosis with a large anion gap. Ethylene glycol intoxication may also be characterized by calcium oxalate crystalluria and hematuria on a urinalysis; other laboratory findings may include hypocalcemia. Both ethylene glycol and methanol intoxications can result from the accidental ingestion of antifreeze or transmission fluid or as a suicide attempt in older children. In the past ethylene glycol and methanol intoxication was best treated with an ethanol drip to competitively inhibit ethanol dehydrogenase and decrease the metabolism of ethylene glycol and methanol to their toxic metabolites. In addition, for patients for moderate to severe intoxication as indicated by a substantial metabolic acidosis and/or an initial ethylene glycol or methanol level greater than 50 mg/dl, the patient should be urgently treated with bicarbonate hemodialysis. This will allow for removal of ethylene glycol and methanol before it is metabolized to its toxic compounds and will also treat the acidosis as well. Fomepizole (4-methylpyrazole), a recently developed inhibitor of alcohol dehydrogenase, has been shown to be effective in the therapy of ethylene glycol intoxication in adults and in pediatric patients (60, 61). Femopizole does not have the adverse effects of ethanol infusion in pediatric patients and has been successfully used in pediatric patients (60, 61).

Endogenous Toxin Induced AKI

Hemolysis and rhabdomyolysis from any cause can result in sufficient hemoglobinuria or myoglobinuria to induce tubular injury and precipitate acute kidney injury (62, 63). Risk factors for acute kidney injury during an episode of rhabdomyolysis n children include dehydration, the serum concentration of myoglobin, the presence of other organ failure, and the presence of the systemic

inflammatory response syndrome (61). The mechanisms of injury is complex but may be related to vasoconstriction, precipitation of the pigments in the tubular lumen and/or heme-protein induced oxidant stress (62, 63). If the rhabdomyolysis is a result of tissue injury with substantial third space fluid losses as occurs in a crush injury, rapid fluid resuscitation can prevent or limit the renal injury. Once intravascular volume has been established, diuretic therapy with mannitol or other loop diuretics to promote flow and prevent precipitation of the heme proteins in the tubules and alkalization of the urine to potentially increase the solubility of hemoglobin and myoglobin have been proposed to decrease tubular injury (62, 63). Hyperkalemia, acidosis and other electrolyte abnormalities may develop rapidly and requires prompt therapy (*Table 65-3*). In children with AKI secondary to rhabdomyolysis, risk factors for the need for renal replacement therapy were a history of fever, persistent oliguria, and the degree of renal insufficiency at the time of admission (63).

Uric Acid Nephropathy and Tumor Lysis Syndrome

Acute kidney injury may be detected at the time of diagnosis of leukemia or lymphoma due to infiltration of tumor cells in the kidney or due to uric acid nephropathy. Radiographic studies demonstrate either mass lesions in the kidney in the case of lymphoma or diffuse infiltrative disease in leukemia. Children with acute lymphocytic leukemia and B-cell lymphoma are at the highest risk for uric acid nephropathy and tumor lysis syndrome (64, 65). Although the pathogenesis of uric acid nephropathy is complex, a potential important mechanism of injury is related to the precipitation of uric acid crystals in the tubules to obstruct urine flow or in the renal microvasculature to obstruct renal blood flow (64–68). A more common cause of acute kidney injury in leukemia is the development of the tumor lysis syndrome during chemotherapy (64–68). Therapy with allopurinol will limit the increased excretion of uric acid with chemotherapy but allopurinol therapy will result in a markedly increased excretion of uric acid precursors including hypoxanthine and xanthine (65, 68). Xanthine is less soluble than uric acid and precipitation of hypoxanthine and xanthine may play a role in the development of acute kidney injury during tumor lysis syndrome. Tumor lysis syndrome results in rapid increases in the serum potassium, BUN, purine metabolite products and phosphorus with a reciprocal decrease in the serum calcium as tumor cells are lysed. Rasburicase is a recombinant form of urate oxidase that catalyzes uric acid to allantion which is five times more soluble than uric acid (69). Rasburicase has been shown to be effective and well tolerated in pediatric patient with tumor lysis syndrome to prevent renal failure (69). AKI during tumor lysis syndrome can also result from extreme hyperphosphatemia from rapid breakdown of tumor cells and the precipitation of calcium phosphate crystals (70). Acute kidney injury due to tumor lysis syndrome is transient and the patient will eventually recover renal function once tumor lysis is complete. However, frequent hemodialysis may be necessary to control hyperkalemia and other metabolic abnormalities resulting

Table 65-3

Treatment of Hyperkalemia

Agent	Mechanism	Dose	Onset	Complications
Sodium bicarbonate	Shifts K+ into cells	1 meq/kg IV over 10–30 min	15–30 min	Hypernatremia, change in ionized calcium
Albuterol	Shifts K+ into cells	400 μg by nebulizer	30 min	Tachycardia, Hypertension
Glucose and insulin	Shilfts K+ into cells	Glucose 0.5 gm/kg	30–120 min	Hypoglycemia
		Insulin 0.1 U/kg IV over 30 min		
Calcium gluconate 10%	Stabilizes membrane potential	0.5–1 ml/kg IV over 5–15 min	Immediate	Bradycardia, arrhythymias, hypercalcemia
Kayexalate	Exchanges Na+ for K+ across the colonic mucosa	1 g/kg PO or PR in sorbitiol	30–60 min	Hypernatremia, constipation colonic membrane irritation if given PR

from the rapid lysis of tumor cells (66). Alternatively, continuous hemofiltration has been used to successfully control metabolic derangement associated with tumor lysis syndrome (66).

Acute Interstitial Nephritis

Acute interstitial nephritis may cause acute kidney injury as a result of a reaction to a drug or due to idiopathic acute interstitial nephritis. Children with acute interstitial nephritis may have rash, fever, arthralgias, eosinophilia and pyuria with or without eosinophiluria (71, 72). Uveitis has been reported in several cases of acute interstitial nephritis is important to recognize as therapy with tropical steroids may be indicated for the uveitis (71, 72). Radiographic studies demonstrate large echogenic kidneys while a kidney biopsy demonstrates interstitial infiltrate with many eosinophils. The pathogenesis of drug induced acute interstitial nephritis is thought to be related to a hypersensitivity reaction with the development of antitubular basement membrane antibodies in some cases (71, 72). Medications commonly associated with acute interstitial nephritis include methicillin and other penicillin analogues, cimetidine, sulfonamides, rifampin, non-steroidal anti-inflammatory drugs while other drugs have been associated with acute interstitial nephritis less commonly. Acute interstitial nephritis associated with non-steroidal anti−inflammatory drugs may also present with high grade proteinuria and nephrotic syndrome. Specific therapy for acute interstitial nephritis includes withdrawal of the drug implicated in causing the acute interstitial nephritis. In addition, corticosteroids may aid in the resolution of the renal failure (73).

Rapidly Progressive Glomerulonephritis

Any form of glomerulonephritis in its most severe degree can present with acute kidney injury and rapidly progressive glomerulonephritis. The clinical features include hypertension, edema, hematuria which is frequently gross, and a rapidly rising BUN and creatinine. The characteristic pathologic finding in rapidly progressive glomerulonephritis is extensive crescent formation. Some glomerulonephritides such as post-infectious glomerulonephritis, membranoproliferative glomerulonephritis, HSP nephritis and lupus nephritis present with a rapidly progressive course in a minority of cases. However, other glomerulonephritis such as anti-neutrophil cytoplasmic antibody (ANCA) positive glomerulonephritis, Goodpasture's syndrome, and idiopathic rapidly progressive glomerulonephritis typically presents with acute kidney injury (74–76). Serologic tests including an ANA, ANCA, anti-GBM titers and complement studies are required to evaluate the etiology of the rapidly progressive glomerulonephritis. Active lesions on biopsy are associated with recovery of renal function and may be reversible with immunosuppressive therapy (74–76). Since specific therapy will depend upon the pathologic findings, a biopsy should be preformed quite promptly when a child presents with clinical characteristics suggestive of rapidly progressive glomerulonephritis so that specific therapy can be initiated promptly.

Vascular Insults

Large vessel insults such as renal artery thrombosis and renal vein thrombosis will present with acute kidney injury only if bilateral or if they occur in a solitary kidney. Microvascular insults occur in cortical necrosis, in typical (diarrhea positive) and atypical (diarrhea negative) hemolytic uremic syndrome (HUS), and in HUS following bone marrow transplantation.

Renal Artery Thrombosis and Renal Venous Thrombosis

Renal artery thrombosis, cortical necrosis and renal venous thrombosis occur much more commonly in newborns and small children. Renal artery thrombosis is strongly associated with an umbilical artery line and a patent ductus arteriosus (77, 78). In addition to acute kidney failure, children may demonstrate hypertension, gross or microscopic hematuria, thrombocytopenia and oliguria. In renal artery thrombosis, the initial ultrasound may appear normal or demonstrate minor abnormalities while a renal scan will demonstrate little to no blood flow. Renal vein thrombosis begins in the intrarenal venous circulation and may spread to the main renal vein and the inferior vena cava. Thrombus formation is mediated by endothelial cell injury resulting from hypoxia, sepsis, or other insults (79, 80). The early findings in renal vein thrombosis include either no renal enlargement or minimal renal enlargement with perivascular streaks while later ultrasound findings demonstrates an enlarged, swollen kidney. The renal scan typically demonstrates decreased blood flow and function in the involved kidney(s). Risk factors for renal vein thrombosis include dehydration, asphyxia, maternal diabetes, low renal blood

flow, polycythemia, cyanotic heart disease, and low Apgar scores (80). Therapy for renal artery thrombosis and renal vein thrombosis should be aimed at limiting extension of the clot by treating the underlying risk factor such as removal of the umbilical arterial catheter for renal artery thrombosis. Anticoagulant or fibrinolytic therapy has been used in cases where the clot was particularly large (78).

Cortical Necrosis

Cortical necrosis as a cause of acute kidney injury is much more common in young children, particularly in the neonate. Cortical necrosis is associated with hypoxic/ischemic insults due to perinatal anoxia, placenta abruption and twin-twin or twin-maternal transfusions with resultant activation of the coagulation cascade. Children and newborns with cortical necrosis usually have gross or microscopic hematuria, oliguria and may have hypertension as well (5). In addition to laboratory features of an elevated BUN and creatinine, thrombocytopenia may also be present due to the microvascular injury. Radiographic features include a normal renal ultrasound in the early phase while ultrasound in the later phases may show that the kidney has undergone atrophy and has substantially decreased in size. A radionucleotide renal scan will show decreased to no perfusion with delayed or no function (*Fig. 65-1b*) in contrast to delayed uptake of the radioisotope which is observed in ATN (*Fig. 65-1a*). The prognosis of cortical necrosis is much worse than that of acute tubular necrosis. Children with cortical necrosis may have partial recovery or no recovery at all. Typically, children with cortical necrosis will need short or long-term dialysis therapy but children who do recover sufficient renal function are at risk for the late development of chronic kidney disease.

Hemolytic Uremic Syndrome

HUS is a common cause of intrinsic acute kidney injury in children and leads to substantial morbidity and mortality as well as long term complications which may not become apparent until adulthood (81–90). Typical HUS usually follows a gastrointestinal illness characterized by hemorrhagic colitis associated with verotoxin producing E. coli infection of which 0157:H7 is the most common serotype. Shiga toxin-producing *E. coli* (STEC) were first linked to human disease in the early 1980s (81–84). Several well-publicized outbreaks of hemorrhagic colitis and HUS have highlighted the morbidity and mortality of infection with verocytotoxin producing E. coli (81–90). While undercooked hamburger is the most common vector for 0157:H7 infection, apple juice, radish sprouts, sausages as well as other food sources have been implicated in the spread of verocytotoxin producing E. coli infections. In the US and Europe, the O157:H7 serotype is the serotype most commonly implicated in verocytotoxin producing infection but in other areas of the world non-0157:H7 strains are emerging as important pathogens (90, 91). E. coli O111:H^- caused a large outbreak of hemorrhagic colitis and HUS in Australia and other serotypes have been associated with hemorrhagic colitis and HUS as well (90, 91).

Once a person is infected with verocytotoxin producing E. coli, the percentage of patients who progress to HUS ranges from approximately 5 to 5%. In children under 5 years of age, the attack rate of hemolytic anemia or HUS is 12.9% compared to an attack rate of 6.8% and 8% for children aged 5–9.9 years and over 10 years of age, respectively (92). In another separate study, children with a white blood cell count greater than 13,000/mm^3 during the initial 3 days of illness with verocytotoxin producing E. coli infection, had a sevenfold increase in the risk of developing HUS compared to age matched children with a white blood cell count less than 13,000/mm^3. The use of anti-motility agents was also associated with a higher risk for the development of HUS (92). A recent study demonstrated that children with hemorrhagic colitis associated with shiga toxin producing E. coli who received antibiotic therapy were much more likely to progress to HUS (93). Environmental or genetic factors that might predispose to the progression of hemorrhagic colitis associated with shiga toxin producing E. coli to HUS are currently unknown. It has been suggested that alterations in the gene for Factor H recently described in patients with atypical HUS may also be relevant to epidemic diarrhea positive HUS (88).

At the time the diarrhea is subsiding, the child appears pale and lethargic due to the hemolytic anemia. Irritability is very common while some children may also develop petechia due to the thrombocytopenia. Oligo/anuria occurs in about 30–50% of children and about 40–75% of children will need dialysis therapy. The kidney and gastrointestinal tract are the organs most commonly affected in HUS while evidence of central nervous system, pancreatic, skeletal, and myocardial involvement may also be present (94–97). Gastrointestinal involvement may lead to rectal prolapse, ischemic colitis and transmural colonic necrosis requiring surgical intervention. Pancreatic involvement manifested as elevated pancreatic enzymes

occurs in 10–20% of children while glucose intolerance due to pancreatic islet cell involvement occurs in less than 10% of children (95). Central nervous system disease may present as seizures, coma, lethargy, and irritability.

Management of HUS requires appropriate attention to fluid and electrolyte balance, nutritional status and careful attention to the extrarenal complications of HUS. Hemolysis may be brisk and require frequent transfusions of packed red blood cells. Platelet transfusions are reserved for patients with active bleeding or for children with severe thrombocytopenia who need to undergo a surgical procedure such as placement of a dialysis catheter or a central line. In general, antibiotic therapy is not indicated since antibiotic therapy has the theoretical potential to alter the bacteria production of toxin resulting in the increased release of toxin. Similarly, anti-motility agents are also not indicated as they may increase the systemic absorption of toxin due the slower gastrointestinal transit time. Some studies have demonstrated a harmful effect of antibiotic therapy in hemorrhagic colitis. Children with hemorrhagic colitis associated with shiga toxin producing E. coli who received antibiotic therapy were more likely to develop HUS compared to children who did not receive antibiotic therapy (93). Other studies have not demonstrated such an association and a recent meta analysis concluded that administration of antibiotics in people infected with shiga toxin producing E. coli was not associated with the development of HUS (98, 99). While there is not a consensus on the benefit versus harm of antibiotic therapy for shiga toxin producing Escherichia coli infection, in vitro studies have shown that sub-inhibitory concentrations of several antimicrobial agents promotes and increases the release of verotoxins (100). Plasmapheresis has not been definitively shown to alter the natural history of diarrhea associated HUS (101).

A diatomaceous silicon diamide compound linked to an oligosaccharide chain (Synsorb Pk) has been developed and has been shown to avidly bind and neutralizes shiga toxin. A clinical trial has recently been completed to determine if oral administration of Synsorb Pk can decrease the rate of progression of hemorrhagic colitis to HUS or if it can decrease the need for dialysis or extrarenal complications in children who have developed HUS (102, 103). Unfortunately, the Synsorb Pk was not found to be beneficial in preventing extra renal complications or in decreasing the duration of dialysis in children with new onset HUS (103). Starfish is a new compound that has recently been developed and has been shown to bind to shiga toxin 1,000 times more efficiently than Synsorb Pk. Starfish is a pentamader that bind shiga toxin and has the potential to be administered intravenously (104). Other studies have demonstrated that vaccination with a plant lased oral vaccine protected mice against a lethal systemic intoxication with shiga toxin-2 (105).

Atypical HUS is not associated with diarrhea and is much less frequent in children. Atypical HUS may be associated with a number of immunologic diseases including systemic lupus erythematosus, with infections such as Streptococcus pneumonia or with the administration of medications such as cyclosporin, birth control pills, or chemotherapeutic agents such as mitomycin (83, 106). Acute kidney injury may also occur following bone marrow transplantation and the etiology is likely to be multifactorial but a high incidence of microvascular disease resembling HUS has been observed usually in association with veno-occlusive disease (106). Acute kidney injury following bone marrow transplantation in children is usually multifactorial and may be associated with long term renal insufficiently in some children (106).

Obstructive Uropathy and Lower Tract Lesions

Obstruction of the urinary tract can cause acute kidney injury if the obstruction occurs in a solitary kidney, if it involves the ureters bilaterally or if there is urethral obstruction. Obstruction can result from congenital malformations such as posterior urethral valves, bilateral ureteropelvic junction obstruction or bilateral obstructive ureteroceles. Acquired urinary tract obstruction can result from passage of kidney stones or rarely tumors. It is important to evaluate for obstruction since the management is to promptly relieve the obstruction. An unusual cause of acute kidney injury associated with ascites is bladder rupture; bladder rupture in children is typically due to trauma while other causes include infection, chemotherapy or post radiation therapy (107, 108).

Potential Strategies to Decrease Injury

Therapies for specific causes of AKI such as solumedrol for rapidly progressive glomerulonephritis, rasburicase for tumor lysis syndrome and preventive measures have been previously described. Therapies to prevent, limit or promote recovery for hypoxic/ischemic AKI have been the study of intense investigation for many years. Advances in understanding the cellular and molecular events that precipitate acute kidney injury has allowed for the development of strategies to potentially decrease injury and/or promote recovery from acute kidney injury. While there is

not a current specific therapy to prevent renal injury or promote recovery in human hypoxic/ischemic AKI, several potential therapies are being studied and future management of AKI may also include antioxidant, anti-adhesion molecule therapy, the administration of vascular mediators or mesenchymal stem cells to prevent injury and/or promote recovery (109–116).

Several different therapies have been shown to prevent, decrease or promote recovery in animal models of AKI. Melanocyte stimulating hormone (MSH) has anti-inflammatory activity and has been shown to protect renal tubules from injury (116). Recently, an analogue of alpha-MSA was found to ameliorate sepsis induced acute kidney injury and mortality in an animal model (115). Scavengers of free radicals and reactive oxygen and nitrogen molecules as well as anti-adhesion molecules have been shown to decrease the degree of injury in animal models of AKI (112). Recently very interesting studies have also demonstrated that multipotent mesenchymal stem cells (MSC) may have a role in promoting recovery in animal models of AKI (113, 114).

In children in the intensive care unit with multi-organ dysfunction, the systemic inflammatory response is thought to contribute to renal injury and multi-organ failure by activation of the inflammatory cascade with the participation of cytokines, reactive oxygen species, proteolytic enzymes and adhesion molecules. Ischemic injury stimulates the activation of neutrophils and the expression of adhesion molecules with resultant tissue injury. Activated neutrophils generate reactive oxygen molecules including superoxide anion, hydrogen peroxide, hydroxyl radical hypochlorus acid, and peroxyntirate. In addition, activated neutrophils release proteolytic enzymes that can results in substantial tissue injury (36, 117, 118). Pediatric patients with acute renal failure have been found to have biochemical evidence of oxidant stress as demonstrated by elevated levels of lipid peroxidation products (119). Infusion of the membrane permeable scavenger of reactive oxygen molecules resulted in significantly reduced BUN, creatinine, kidney myeloperoxidase activity and decreased lipid peroxidation in rats undergoing bilateral clamping of the renal pedicle (120). Other studies suggest that inhibition of the inflammatory cascade with adenosine 2A agonist will ameliorate ischemic/hypoxic AKI (121). While multiple interventions decrease the severity and promote recovery of acute kidney injury in animal models, interventional studies in patients with acute kidney injury have been disappointing (122, 123). As previously discussed, the development and testing of interventions for AKI will require the development of early biomarker(s) of injury that are much more sensitive than serum creatinine so that therapeutic interventions can be initiated in a timely manner.

Outcome and Prognosis

The prognosis of AKI is highly dependent on the underlying etiology of the AKI. Children who have AKI as a component of multisystem failure have a much higher mortality rate than children with intrinsic renal disease such as HUS, RPGN, and AIN. Recovery from intrinsic renal disease is also highly dependent on the underlying etiology of the AKI. Children with nephrotoxic AKI and hypoxic/ischemic AKI usually recover normal renal function. In the past it has been thought that such patients are low risk for late complications but several recent studies have demonstrated that chronic kidney disease can evolve from AKI (1–4, 124–130). Children who have suffered substantial loss of nephrons, as in the HUS or RPGN, are at risk for late development of renal failure long after the initial insult. Several studies in animal models have documented that hyperfiltration of the remnant nephron may eventually lead to progressive glomerulosclerosis of the remaining nephrons. Thus, children who have had cortical necrosis during the neonatal period and recovered renal function or children with an episode of severe Henoch-Schönlein purpura or HUS are clearly at risk for the late development of renal complications. Such children need life-long monitoring of their renal function, blood pressure, and urinalysis.

As described above, it has been thought that acute kidney injury due to hypoxic/ischemic and nephrotoxic insults were reversible with return of renal function to normal. However, recent studies have demonstrated that hypoxic/ischemic and nephrotoxic insults can lead to physiologic and morphologic alterations in the kidney that may lead to kidney disease at a later time (50, 131). Studies in adults demonstrate that CKD may evolve from AKI [12662 (128)]. Thus, acute kidney injury from any cause can be a concern for later kidney disease. Importantly, acute kidney injury is likely to be especially deleterious when the kidney is not yet grown to adult size and/or before the full complement of nephrons have been developed. Since nephrogenesis is not complete until approximately 34 weeks gestation, acute kidney injury during this interval might lead to a decreased nephron number and indeed studies have suggested that acute kidney injury during nephrogenesis results in decreased nephron number and subsequent glomerulomegaly (127). Acute kidney injury in the full term neonate is also associated with later kidney disease (129). In one study

of 6 older children with a history of AKI not requiring dialysis in the neonatal period, only two were normal, three had chronic renal failure and one was on dialysis. Studies in older children have also shown that AKI leads to CKD in a higher percentage of children than previously appreciated (126). In a prospective study of renal insufficiency in children undergoing bone marrow transplantation, the incidence acute renal insufficiency was high and was predictive of chronic renal insufficiency (130). Of those who survived 11% developed chronic kidney disease and AKI was the sole predictor of chronic kidney disease (130). Thus, children with a history of AKI from any cause need long-term follow-up.

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66 Management of Acute Kidney Failure

Michael Zappitelli · Stuart L. Goldstein

Introduction

No uniform acute kidney injury (AKI) treatment strategies exist currently. Lack of consensus results from the inability to detect AKI early enough to prevent the cascade of cellular events leading to renal tubular cell death, since current diagnosis relies on serum creatinine changes. The recent discovery of early biomarkers of AKI may allow for detection prior to serum creatinine changes and interventional trials to prevent AKI development. Until then, AKI management, summarized in *Table 66-1*, remains supportive, aimed at maintaining fluid and electrolyte homeostasis, preventing life-threatening complications, avoiding further kidney injury, providing appropriate nutrition and renal replacement therapy (RRT) in the most severe forms of AKI. The current chapter outlines these management concepts with a focus on RRT – in particular continuous renal replacement therapy (CRRT).

Non-Dialytic Management

Prevention

In the past, AKI etiology most often resulted from primary renal disease (1). Today, most common causes are secondary AKI due to systemic illnesses or their treatments, such as renal ischemia (associated with sepsis, multiple organ dysfunction syndrome, and post-operative complications) or nephrotoxic medication use (2). Measures to prevent further kidney injury by these mechanisms are also important and include avoidance of (1) hypotension by providing appropriate inotropic support and (2) nephrotoxic medication use, with re-evaluation of the need for nephrotoxins and closely following serum concentrations. Careful evaluation of intravascular volume status is important to determine if AKI is reversible by fluid administration. Maintaining intravascular volume status can prevent progression of "pre-renal AKI" to acute tubular necrosis (ATN).

Fluid Management

Careful attention to fluid management is critical to mitigate AKI complications and is dependent on an accurate assessment of the underlying cause of AKI. Patients with pre-renal azotemia will often respond to fluid resuscitation, whereas patients with acute tubular necrosis should be treated with volume restriction, to prevent development of worsening fluid overload. Patients with AKI secondary to nephrotoxic medications or interstitial nephritis often demonstrate polyuria, placing them at risk for severe fluid and electrolyte losses.

Fluid prescription for children with AKI is directed by individual clinical situation. Generally, a safe starting point is to provide insensible losses (400 ml/m^2 of body surface area in patients with normal basal metabolic rate, higher in febrile patients and lower for ventilated patients who have lower respiratory losses), plus replacement of ongoing losses (urinary, gastrointestinal, chest tubes). Such fluid prescription will generally be appropriate in either patients who require fluid restriction or those with polyuria, but may not be appropriate in the setting of acute volume depletion. To promote achievement of negative balance in patients exhibiting fluid overload, less than the total urine output may be replaced.

While fluid resuscitation is essential for patients with acute hypovolemia and septic shock (3), growing evidence suggests that fluid overload development contributes to poor outcome in critically ill patients. Adult surgical patients with fluid retention had increased blood product requirements, prolonged dependency on pressors and a two-fold increase in death (4). Fluid overload has also been associated with decreased survival in adults with ARDS (5, 6) and more conservative fluid management in adults with acute lung injury was associated with improved lung function (7). Recent data from adults with septic shock demonstrates that early goal-directed fluid therapy, referring to fluid resuscitation targeted to achieve specific physiologic endpoints, may significantly improve patient survival (8, 9).

There are increasing number of studies in children supporting the negative effects of fluid overload development.

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Table 66-1
General management of acute kidney injury

Treatment of underlying disease
Correct electrolyte abnormalities
Avoid/manage fluid overload
Avoid further renal injury
Provision of adequate nutrition
Drug dosing for renal function
Renal replacement therapy

Single center data (10–12) and a multicenter effort, the Prospective Pediatric Continuous Renal Replacement Therapy Registry Group (13, 14), demonstrate that worse fluid overload is an independent risk factor for mortality, irrespective of severity of illness, in patients who receive CRRT. Aggressive fluid control with early initiation of diuretics and CRRT to prevent >10% fluid overload may lead to improved survival in children with stem cell transplantation and AKI (15), a pediatric population where outcome is particularly poor once mechanical ventilation is initiated. The predilection for early multi-organ system failure and death in critically ill children with AKI (16, 17), may argue for early and aggressive initiation of RRT in association with an early goal-directed fluid repletion strategy in critically ill children. A very recent study in children with septic shock applied such goal-directed therapy using superior vena cava oxygen saturation as the main physiologic end-point, and found reduced need for blood transfusions and inotrope use and decreased mortality (18).

Different methods for fluid overload assessment based on calculating changes of daily weights and fluid balance as a percentage of estimated dry weight have been proposed (12, 13, 19). For critically ill children, the most commonly reported formula uses the difference in fluid output from fluid intake since PICU admission,as a percentage of the patient's estimated dry weight, using the following formula to quantify the degree of fluid overload:

$$\text{Fluid overload}(\%) = (\text{fluid in liters} - \text{fluid out liters}) / \text{weight in kg} \times 100\% \quad (12)$$

Data from the ppCRRT Registry demonstrates significant better patient survival when CRRT is initiated at <20% fluid overload versus >20% fluid overload (59% vs 40%) using this calculation in critically ill children with multi-organ dysfunction syndrome. Thus, CRRT should be considered for critically ill patients with AKI with >10% fluid overload and who are not expected to recover kidney function expeditiously to allow for appropriate nutrition, blood product and medication administration without development of worsening fluid overload status.

Electrolyte Management

Electrolyte management in AKI includes both acute and subacute management and is also dependent on the cause of AKI. Patients with oligo-aneuric kidney failure or acute tubular necrosis should not receive potassium or phosphorus unless they exhibit hypokalemia or hypophosphatemia. Even then, they must be administered with great caution. Sodium intake should be restricted to 2–3 mEq/kg body weight per day, together with fluid restriction, to prevent sodium and fluid retention with resultant hypertension. Conversely, patients with AKI secondary to nephrotoxic medications or interstitial nephritis often demonstrate polyuria, placing them at risk for severe fluid and electrolyte losses. Polyuria in this setting may lead to wastage of salt or other electrolytes, and may require higher administration than normal. In order to safely achieve these goals, it is ideal to request insertion of a urinary catheter and obtain weights and serum electrolyte levels at least daily.

Acute non-dialytic management of electrolyte problems due to AKI includes management of severe acute hyperkalemia which can lead to potentially fatal cardiac arrhythmias. Intravenous calcium may mitigate these effects, and measures to increase potassium entry into the cells, such as sodium bicarbonate (if the patient is acidotic), beta-2 agonists or insulin with dextrose, are effective in the short-term. Severe hypocalcemia may also cause cardiac conduction defects and should be corrected judiciously, especially in the setting of severe hyperphosphatemia, to avoid crystal formation. Moreover, administration of sodium bicarbonate for correction of hyperkalemia may exacerbate ionized hypocalcemia. Hyperphosphatemia, if not severe and not associated with severe hypocalcemia, may be managed with phosphate binders and restriction.

Pharmacologic Therapy

There is no proven specific pharmacologic treatment for AKI. However, preservation of renal perfusion with appropriate inotropic agents is essential in critically ill patients not responsive to volume repletion (20). The effects of dopamine, a commonly used inotropic agent in children, are varied and complex, leading to controversy with respect to its utility in the setting of AKI. At low or so-called "renal doses" (0.5–2 mcg/kg/min), dopamine

increases renal plasma flow and sodium excretion. However, these effects are short-lived (21). At increasing doses, dopamine binds beta- and then alpha-adrenergic receptors, rendering it difficult to ascertain whether any observed renal benefit from dopamine occurs as a result of its dopaminergic or intropic effect. Well-designed prospective randomized studies of adult patients at risk of acute tubular necrosis have seriously questioned the utility of "renal-dose" dopamine in reversing oliguria (22, 23), and many centers have abandoned its use for this purpose (22, 24).

The benefits of dobutamine and norepinephrine for patients with AKI reside in the ability to increase cardiac output, leading to an increase in renal blood flow (25). Vasopressin increases systemic vascular resistance by direct action on the vascular smooth muscle cells. Vasopressin has been shown to be especially effective in maintaining renal perfusion in patients with septic shock who were unresponsive to catecholamines (26).

Prospective randomized studies of adult patients at risk for acute tubular necrosis have shown that intravenous furosemide is unlikely to reverse oliguria (27, 28). However, the practice of providing furosemide, either as an intermittent bolus or as a continuous infusion (0.1–0.3 mg/kg/h) in combination with a thiazide diuretic has potential to maintain urine output in patients at risk of developing anuria, and allow for appropriate nutrition delivery.

Other pharmacologic agents, such as calcium channel-blockers and N-acetyl cysteine have been studied in adults but have shown no substantial benefit on mortality or renal outcomes (29, 30). Selective dopamine A-1 antagonist fenoldapam may improve urine output in children undergoing cardiopulmonary bypass surgery (31). Some studies suggest that nesiritide (a human natriuretic peptide) may have favorable renal hemodynamic effects and increase urine output after cardiac surgery (32–34). However, randomized controlled trials are lacking in children for each of these medications. Several other potential therapies such as growth factors (35), erythropoeitin (36), and free radical scavengers (37, 38) are being investigated in animal models from which AKI pathophysiologic mechanisms were originally delineated.

Renal Replacement Therapy

When fluid or electrolyte abnormalities are severe, RRT becomes the main supportive treatment of choice for AKI. Traditional indications for RRT in AKI are hyperkalemia, severe hyperphosphatemia (especially if accompanied by hypocalcemia), severe metabolic acidosis or fluid overload, and symptoms of uremia. Non-renal indications include prevention or treatment of tumor lysis syndrome, and removal of toxins (ingestions or inborn errors of metabolism). While little has changed in terms of acute indications, prevention or early treatment of fluid using RRT has become more prevalent, as discussed above. Acute RRT can be provided as intermittent hemodialysis (IHD), peritoneal dialysis (PD), or continuous renal replacement therapy (CRRT). There are advantages and disadvantages to each of these modalities.

Modality Choice

Several factors including dialysis indication and factors related to the patient's clinical status determine optimal RRT modality. These factors have been recently reviewed by North American and European authors (39, 40). In general, factors determining optimal modality include patient size, hemodynamic stability, and institutional expertise. *Table 66-2* outlines various advantages and disadvantages of different acute RRT modalities. PD and CRRT are better suited for patients with hemodynamic instability, since daily total ultrafiltration goals can be achieved over a 24 h period instead of a 3–4 h IHD treatment. In patients with disrupted or severely scarred peritoneal membrane, PD may not be possible. Acute drug intoxications and hyperammonemia secondary to inborn errors of metabolism are treated most efficiently with IHD, since rapid drug removal is important to prevent morbidity and IHD is the most efficient RRT modality (41, 42). However, recent studies demonstrate CRRT to be very effective in protein bound drug removal and hyperammonemia (43–45). Finally, the particular center's expertise and nursing support availability should play a major role in determining the optimal RRT modality.

Polls of US pediatric nephrologists demonstrate increased CRRT use over peritoneal dialysis as the preferred modality for treating pediatric AKI. In 1995, 45% of pediatric centers ranked PD and 18% ranked CRRT as the most common modality used for initial AKI treatment. In 1999, 31% of centers chose PD versus 36% of centers who reported CRRT as their primary initial modality for AKI treatment (46).

Acute Intermittent Hemodialysis

IHD is only briefly discussed here, as most details on materials, procedures, and prescription are discussed in the chapter covering maintenance chronic HD. Vascular access issues are similar to what is discussed below in the section on CRRT. Acute IHD can be performed using similar machinery and dialysis solutions as in chronic HD, which

Table 66-2

Advantages and disadvantages of different acute renal replacement therapy modalities

Modality	Advantages	Disadvantages
Intermittent HD	Short treatment times	Vascular access necessary
		Hemodynamic instability
	Accurate UF	Heparin anticoagulation
Peritoneal dialysis	No need for vascular access	Less efficient than HD/CRRT
	Minimal equipment needs	Variable UF dependent on BP, catheter placement and function.
	Minimal training needs	
	Feasible in small infants	
	Continuous treatment	
CRRT	Accurate UF that can be altered to account for changes in intake/patient BP	Vascular access necessary
	Smaller circuit volumes	
	Citrate anticoagulation	

means the need for a portable reverse osmosis device, purified water, and nurses specialized in performing HD. In hemodynamically stable patients with primary renal disease (e.g., acute glomerulonephritis, hemolytic-uremic syndrome), or in patients with inborn errors of metabolism or intoxications, IHD is preferred because very efficient small solute clearance can be obtained within a short period and these patients will generally tolerate the procedure well. In patients with severe uremia, who are being treated with IHD for the first time, a relatively low clearance (aiming at a urea reduction ration $\approx$ 30%) should be prescribed, in order to avoid the dialysis disequilibrium syndrome. If blood urea nitrogen is very high, intravenous mannitol or ultrafiltration profiling during dialysis may be helpful to avoid this complication. Heparin anticoagulation is generally used in IHD, which may complicate the clinical picture for patients with coagulopathy. Heparin-free IHD can be achieved by performing frequent (approximately every 20–30 min) flushes with normal saline to the hemodialysis circuit. Hemodynamically unstable patients will often not tolerate a session of IHD, and very strict fluid control will be necessary in between dialysis sessions, which may impair ability to provide adequate amounts of nutrition. In these types of patients, continuous modalities are preferable.

Acute Peritoneal Dialysis

Acute peritoneal dialysis is a continuous dialytic therapy which requires much less technical expertise, expense, and equipment compared to IHD and CRRT. PD catheters can be placed quickly and easily. In centers with expertise, a non-tunneled PD catheter can be placed percutaneously at the bedside, but these have a high tendency to leak. Placing catheters surgically is preferable, similar to chronic PD catheter placement, and may avoid problems with peritoneal leak and resultant risk of infection (47).

A recent trend toward providing PD therapy earlier in the post-cardiopulmonary bypass course has been reported, with one study of 20 patients demonstrating 80% patient survival (48). While improved survival with early PD initiation may result from prevention of fluid overload, some posit improved survival with early PD initiation results from increased clearance of cardiopulmonary bypass-induced pro-inflammatory cytokines. Further study however is required to support this hypothesis (49).

Standard PD equipment (including cyclers, or manual setups and solutions) can be used to perform dwells. Initial dwell volumes should be limited to 10 cc/kg of patient body weight in order to minimize intra-abdominal pressure, especially in patients with severe pulmonary dysfunction, and potential for fluid leakage along the catheter tunnel. Perioperative prophylactic antibiotics (cefazolin 15–20 mg/kg) are advisable, and post insertion intradialysate cefazolin and heparin are typically instilled to prevent infection and fibrin formation, respectively for 48–72 h (40). In patients for whom higher clearance is desired, increasing cycle frequency dwell volumes or dextrose concentration will help. However, in patients who cannot tolerate increasing volumes, increasing cycle frequency and dextrose may be the only

options. Although PD may deliver less efficient solute removal than hemodialysis or CRRT, its relative simplicity and minimal associated side effects allow for RRT provision in settings lacking pediatric dialysis specific support and personnel. Complications include peritonitis, leakage around the tunnel or poor catheter "function", referring to blockage or inability to drain.

Continuous Renal Replacement Therapy

Various CRRT aspects including dose, dialysis/hemofiltration fluid composition, and anticoagulation methods have been studied in recent years.

The term CRRT generally refers to one of three treatment modalities: continuous venovenous hemodialysis (CVVHD), continuous venovenous hemofiltration (CVVH), or continuous venovenous hemodiafiltration (CVVHDF), but applies to any modality which is continuous (such as PD). In CVVHD, just as with IHD, physiologic dialysis solution is run countercurrent to blood flow through the hemofilter. The mode of solute removal is by diffusion and small solutes (like urea) are removed effectively. With CVVH, replacement solution, which is typically the same or similar solution as that used for dialysis, is infused into the CRRT circuit either pre-hemofilter ("pre-dilution") or post-hemofilter ("post-dilution"). Simultaneous to the infusion of replacement solution, the same amount of fluid is removed by ultrafiltration through the hemofilter in order to achieve solute removal by convection (solute drag). Small solute removal is similar to CVVHD, but larger "middle molecules" are removed. In CVVHDF, both dialysis and replacement solutions are used to achieve solute removal. In all forms of CRRT, additional fluid is removed by ultrafiltration with the goal of achieving a negative balance.

Vascular Access

Well-functioning vascular access is absolutely essential to the success of IHD or CRRT. The most common sites for acute catheter placement are the internal jugular, subclavian and femoral veins. Avoiding the subclavian vein is preferable to prevent subclavian vein stenosis in patients who may need permanent vascular access in the ipsilateral upper extremity. The femoral vein provides advantage of relatively easy and prompt access. However, with the availability of skilled surgeons or intensivists, internal jugular venous placement is also easily attainable. Patient characteristics may also influence the choice of catheter placement. For example, in patients with severe ascites or with suspected need for a future kidney transplant, internal jugular placement may be preferable, whereas in patients receiving high-frequency oscillatory ventilation, femoral placement will be technically easier and reduce the risk of pneumothorax associated with internal jugular venous access. *Table 66-3* displays suggested options for catheter size and location by patient size.

A study of adult patients reported increased recirculation and worse performance in catheters placed in the femoral vein versus subclavian or jugular veins (50). Substantial pediatric data suggests that internal jugular access is associated with longer access survival than femoral or subclavian access, independent of anticoagulation method (51) even though the femoral vein was most commonly used (69%), followed by subclavian and internal jugular access (51). Catheter size should also be matched to patient size. However, smaller catheters (5 French) are associated with worse access survival due to clotting and poor blood flow (51). *Table 66-3* lists catheter configurations and patient size combinations. Ultimately, access placement will also be largely determined by technical expertise of the individual placing the catheter. Maintaining access

Table 66-3

Acute catheter configuration and patient size combination

Patient size	Catheter size & source	Site of insertion
NEONATE		
	Dual-Lumen 7.0 French (COOK/MEDCOMP)	Femoral or Internal Jugular vein
3–6 KG	Dual-Lumen 7.0 French (COOK/MEDCOMP)	Internal/External-Jugular, Subclavian or Femoral vein
	Triple-Lumen 7.0 Fr (MEDCOMP)	Internal/External-Jugular, Subclavian or Femoral vein
6–30 KG	Dual-Lumen 8.0 French (KENDALL, ARROW)	Internal/External-Jugular, Subclavian or Femoral vein
>15-KG	Dual-Lumen 9.0 French (MEDCOMP)	Internal/External-Jugular, Subclavian or Femoral vein
>30 KG	Dual-Lumen 10.0 French (ARROW, KENDALL)	Internal/External-Jugular, Subclavian or Femoral vein
>30 KG	Triple-Lumen 12.5 French (ARROW, KENDALL)	Internal/External-Jugular, Subclavian or Femoral vein

patency between dialysis sessions using heparin or citrate catheter locks is essential to prevent access clotting.

Machine and Hemofilter

Modern CRRT machines are generally user-friendly and consist of computer modules from which physicians may choose the CRRT modality. Accurate ultrafiltration with volumetric/gravimetric control incorporated into machines and disposable lines, circuits and dialyzers, sized for the pediatric weight spectrum have made CRRT safer in children (52). Accurate UF and blood flow rates are crucial for pediatric RRT since the extracorporeal circuit volume can comprise more than 15% of a small pediatric patients' total blood volume, and small UF inaccuracies may represent a large percentage of a small pediatric patient's total body water. All these machines also contain blood air leak detectors, a blood pump, dialysis fluid, and replacement fluid pumps.

Membranes or hemofilters come in different sizes, as measured by surface area – from as low as approximately 0.1 m^2 to over 1 m^2 – ranging from 15 to over 100 ml of blood. Some machines have specific hemofilters associated with them, whereas others are more versatile and can accommodate different hemofilters. CRRT hemofilters are made of polysulfone or acrilonitrile (such as the AN69). Thus, membranes are chosen based on the type of machine being used and the size of the patient.

Despite the smaller sizes of some hemofilters, delivery of IHD or CRRT to infants or small patients entails a significant portion of their blood volume to be pumped through the extracorporeal circuit. Therefore, extracorporeal circuit volumes which comprise more than 10–15% of patient blood volume (or even less, if the patient is hemodynamically unstable) should be primed with whole blood to prevent hypotension and anemia. Since the prime volume is not discarded, it is important to not re-infuse the blood into the patient at the end of the treatment in order to prevent volume overload and hypertension.

Solutions

Dialysis and replacement solutions must contain an acid buffer. Until recently, most CRRT solutions used lactate as a buffer. A crossover study in adult patients receiving CRRT revealed that lactate based solutions could lead to rising serum lactate level in patients (53), a phenomenon that could lead to unnecessary investigation for tissue ischemia. Bicarbonate buffered solutions can be made by hospital pharmacies, but are also now available from industry sources (54). A recent pediatric study has highlighted the potential adverse patient safety implications with pharmacy prepared solutions that can arise from compounding errors (55). Thus, a solution composition validation program should exist in centers that opt to use pharmacy-made solutions for CRRT.

Anti-Coagulation

Anticoagulation of the CRRT circuit is essential to provide therapy. Heparin and citrate are the two most common forms of anticoagulation. Heparin anticoagulation is achieved by continuous infusion of heparin to the CRRT machine circuit. Side effects of heparin include systemic anticoagulation and rare occurrence of induction of heparin induced thrombocytopenia. Regional (restricted to the CRRT circuit) citrate anticoagulation is performed by continuous infusion of citrate solution through a stopcock at the access ("arterial") portion of the dialysis catheter. Anticoagulation is achieved by binding to and decreasing ionized calcium in the blood required for the clotting cascade. Calcium is infused into a separate systemic central venous line or at the return ("venous") portion of the dialysis catheter to maintain physiologic ionized calcium in the patient. Potential complications of regional citrate anticoagulation include metabolic alkalosis (especially if used in combination with bicarbonate buffered CRRT replacement/dialysis solutions) and citrate lock. Citrate lock is a phenomenon where the delivery of citrate exceeds the patient's hepatic clearance and the clearance of citrate through the hemofilter. As a result, citrate concentrations increase in the blood and bind calcium. Citrate lock is identified by decreasing serum ionized calcium in the presence of rising total calcium. The treatment for citrate lock is to discontinue citrate for one to four hours and then restart at a lower citrate delivery rate. Recent pediatric studies have reported practical and safe citrate anticoagulation protocols (56, 57).

Complications

Patients who receive CRRT with an AN-69 membrane are at risk of the bradykinin release syndrome (BRS) when circuits require blood priming. BRS leads to a range of mild to profound hypotension. Several methods of mitigating BRS in children have been published, including normalizing the blood pH and administering a calcium bolus to the patient to counter the citrate in the blood

unit (58) as well as performing pre-dialysis of blood used to prime the circuit in smaller children (59).

Another complication of CRRT is hypothermia, particularly in infants. This can be avoided by the use of heat lamps, dialysis fluid warming, and blood tube warmers. Electrolyte disturbances can occur – particularly hypophosphatemia, hypomagnesemia, and hypokalemia, – due to clearance and is avoided by frequent electrolyte monitoring and appropriate adjustment of dialysis and/or replacement of solution composition. Excessive ultrafiltration is a problem that may occur, but can be avoided by careful patient monitoring with hourly fluid balance, and frequent reassessment of the day's goals by the pediatric nephrologist. Nutritional losses also occur through the hemofilter, necessitating increase in intake.

Other Types of Continuous Therapies

With increase in the level of critical illness in children admitted to the critical care unit and with a widening of the spectrum of diseases seen today, the role of the pediatric nephrologist has extended greatly from not only providing RRT through IHD, CRRT, or PD.

A modification to CRRT is SLED or sustained low-efficiency daily dialysis, in which a standard hemodialysis machine is used for more prolonged periods than a standard IHD session (generally 8–12 h) (60). While there may be some potential advantages in the use of SLED in institutions that do not perform standard CRRT, this procedure requires staffing with highly specialized nephrology nurses. Moreover, SLED has not been adequately described in children.

The pediatric nephrologist may also need to combine CRRT with other continuous or intermittent extracorporeal therapies. The performance of CRRT in patients treated with extracorporeal membrane oxygenation (ECMO) is becoming more common. In this setting, a hemofilter can be placed in series along the ECMO circuit to perform either ultrafiltration alone or with countercurrent infusion of dialysis fluid to perform solute removal (dialysis). A CRRT machine can also be attached to the ECMO circuit to perform CVVHD, CVVH, or CVVHDF, which may offer more accurate ultrafiltration control (61).

Patients with AKI requiring RRT may also require other forms of extracorporeal therapy for non-primary renal diseases, using plasmapheresis or immunoadsoprtion procedures (62, 63). These may be performed simultaneous to CRRT therapy using a separate machine attached via stopcocks to the CRRT machine or intermittently between dialysis sessions. Clearly, these procedures require expertise and good overall knowledge of extracorporeal therapy and the role of pediatric nephrologists in performing these treatments is likely to increase.

Nutritional Management

Adequate nutrition in critically ill children with AKI is a crucial component of their treatment, as they are at increased risk of developing protein-energy malnutrition (64). Presence of renal replacement therapy (RRT) contributes to nutritional losses, especially amino acids and water-soluble vitamins. Similar to what has been found in adults, amino acid losses by continuous venovenous hemofiltration (CVVH) (65, 66), continuous venovenous hemodiafiltration, (CVVHDF) (66) and continuous venovenous hemodialysis (CVVHD (67)) can be anywhere from 10 to 20% of the amount provided in the parenteral nutrition (PN), which is associated with the development of negative nitrogen balance (66). Given the age-associated baseline elevated protein needs of children and the presence of abnormal amino acid synthesis and increased protein catabolism in critical illness and AKI, daily protein intake should be in the order of at least 2–3 g/kg/day in children with AKI. During RRT, commensurate adjustment of protein intake must be made to account for losses (10–20% of amino acid intake). Some programs aim at serum urea nitrogen levels in the range of 40–80 mg/dL as a guide to determining whether protein alimentation intake is sufficient during RRT (68).

Drug Dosing

Drug dosing must often be altered for patients with AKI, not so much for avoiding kidney injury, but to avoid toxic accumulation of drugs and their metabolites which should be excreted by the kidney. When glomerular filtration rate is estimated to be less than 50% of normal, most drugs excreted by the kidney will require modifications in scheduled dosing. For patients receiving CRRT, drug dosing can be quite challenging. However, understanding the size, volume of distribution, and protein binding of the drug (for each of which, if high, leads to less clearance through the hemofilter), can lead to a fairly good estimate and appropriate drug dosing can be made. Several recent excellent review articles discuss how to specifically dose different drugs during CRRT or with AKI, based on these biochemical properties (69, 70).

Outcome

The mortality of children with AKI who receive acute RRT is high, ranging from about 40–70% (71–73) and unchanged from the past. Despite advances in technology, children who now receive AKI supportive therapy have concomitant increasing severity of illness, so the impact leading to improved survival is not readily apparent. Survival rates stratified by RRT modality have also been stable; survival rates for patients receiving IHD (73–89%) are higher than those receiving PD (49–64%) or CRRT (34–42%) (72). Better survival in patients who receive IHD likely results from improved hemodynamic stability, but no prospective pediatric study that controls for patients illness severity has compared survival across modalities. Children receiving CRRT with non-renal organ disease or multiple organ system dysfunction syndrome (MODS) have higher mortality rate (>50%) compared to other diagnoses (<30%) (74). Infants demonstrate even higher mortality when compared to older children (2, 74). Few studies have examined long-term outcome of children with AKI, but a substantial portion may not have normal renal function by discharge (2). Recent data demonstrates that long – term kidney injury is prevalent in survivors of an AKI episode (75, 76). In summary, hospital mortality of patients with AKI is related to the level of illness severity. In those who survive, there appears to be a definite risk of long term negative effects on renal function and potentially increased risk of chronic kidney disease.

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Chronic Renal Failure

67 Pathophysiology of Progressive Renal Disease

Allison Eddy

Clinical Overview

The high and increasing prevalence of end-stage kidney disease (ESRD) is consuming a major portion of healthcare resources for both pediatric and adult populations (*Fig. 67-1*). While the incidence rates for pediatric ESRD have remained relatively constant at 14 per million children under the age of 19 years, many adults with ERSD suffer from a kidney disease that began as chronic kidney disease (CKD) during childhood (*Fig. 67-2*) (1). Pediatricians and pediatric nephrologists are challenged to become more involved in the prevention, early diagnosis and optimal management of childhood-onset CKD. The rapidly increasing number of overweight and hypertensive children and adolescents highlights the challenges ahead. In addition to the obvious measures, including changes in diet, exercise and lifestyle, new medical interventions are also needed. An understanding of the basic cellular, molecular, genetic, and environmental pathogenetic mechanisms that will be reviewed in this chapter will provide an essential framework for developing and testing new therapies. For patients with established CKD that is predicted to progress, a therapeutic protocol comprising multiple agents that target different pathogenetic pathways will likely become standard treatment in the future. Currently available therapies can be effective but in general they have only a modest effect on the rate of renal functional decline. An important advance in recent years has been the recognition that most patients with CKD (now estimated to be one in eight adults) never reach ESRD and the need for renal replacement therapy (2). This is because CKD patients are at risk for accelerated cardiovascular disease and are more likely to die prematurely due to cardiovascular or cerebrovascular disease. Thus clinical management of all patients with CKD must consider measures to reduce cardiovascular risk factors, even in the pediatric population.

The underlying renal diseases that lead to ESRD in childhood (*Fig. 67-2a*) are dominated by congenital structural disorders (especially dysplasia with/without reflux or obstruction) during the first decade, while acquired diseases, especially glomerular disorders such as FSGS, become more important in the second decade (3). Diabetic and hypertensive nephropathy dominate in adults, but may begin in childhood (*Fig. 67-2b*).

Crossing the GFR Threshold

Individuals with normal kidneys have considerable renal functional reserve that may be compromised in congenital and developmental anomalies and in acquired disorders that are not completely reversible. There is a critical set-point beyond which progressive renal functional decline is inevitable, even after the primary disease process becomes quiescent. For many, the threshold appears to be in the GFR range of 60 ml/min/1.73 m^2, but there is considerable variability. Relevant factors are the age of the patient and the ability of their kidneys to grow in parallel with postural growth. For this reason the risk of progressive renal failure may not be clear until after the adolescent growth spurt. Another important variable is nephron number. Once estimated to average 1×10^6 nephrons per kidney, it is now evident that for normal individuals this number may vary between 0.2 and 2.0×10^6 per kidney (4). Even if the primary disease process is no longer a factor, secondary factors reviewed in the next section may modify the natural history of the disease (*Fig. 67-3*).

The underlying process shared in common by all chronic progressive kidney diseases is one of kidney scarring or fibrosis. This process leads to glomerular obsolesce as a consequence of glomerulosclerosis and tubular atrophy (often accompanied by atubular glomeruli formation) due to interstitial fibrosis. Because the tubulointerstitium accounts for the largest kidney compartment (approximately 85% by volume), progressive interstitial fibrosis has become synonymous with kidney disease progression; interstitial fibrosis is considered the final common pathway to renal failure shared by all primary kidney disorders. This fact also explains why the degree of interstitial fibrosis is the best histological

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Figure 67-1

End-stage kidney disease (ESRD): Prevalence and Incidence. While the incidence of ESRD has remained constant in the pediatric age group and has not increased in adults since the early 2000s (not shown) the cumulative ESRD prevalence continues to increase (1).

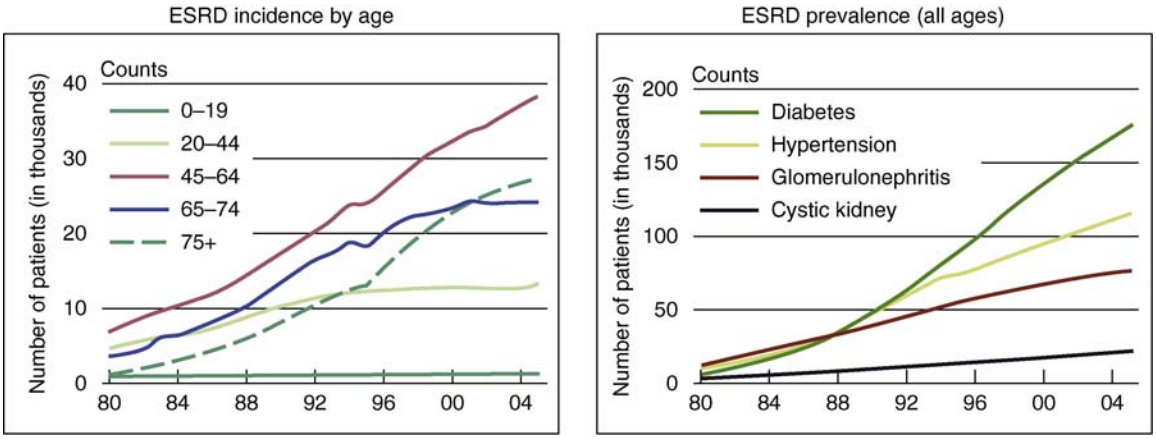
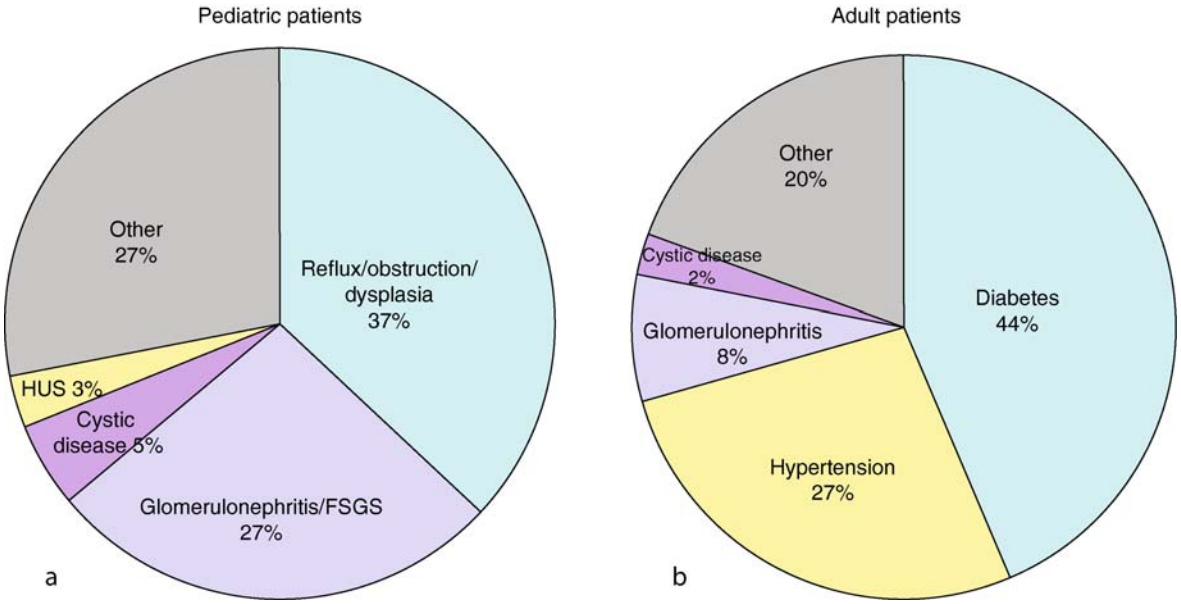


Figure 67-2

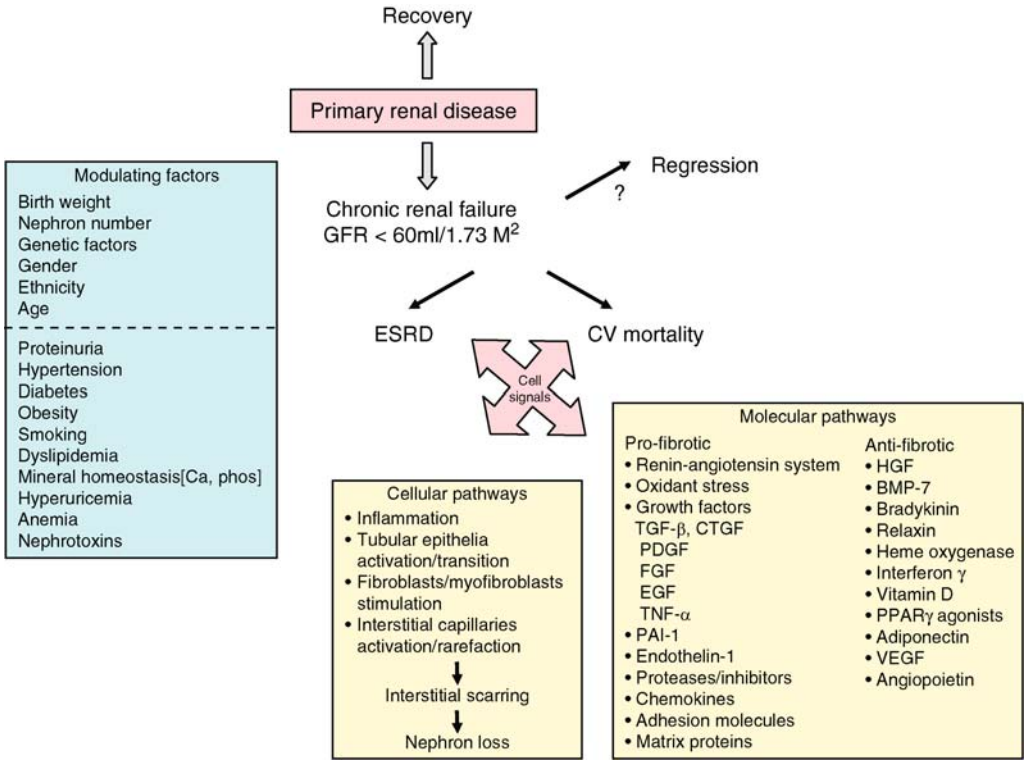
Primary cause of ESRD. The etiology of the primary kidney disease differs between adult and children. Pediatric data are from the North American Pediatric Renal Transplant Cooperative Study (https://web.emmes.com/study/ped/index.htm); adult data are from the 2008 USRDS report (1).



correlate of renal functional decline (*Fig. 67-4*) and long-term prognosis. In general, the pathogenetic mechanisms that lead to glomerulosclerosis and interstitial fibrosis share many similarities, but there are certain unique features that will be considered in the sections that follow. The fundamental process of CKD is essentially a process of accelerated aging as a consequence of an injurious process that diminished renal functional reserve. In the absence of known renal pathological events, renal function declines progressively with advancing age due progressive glomerulosclerosis and interstitial fibrosis (*Fig. 67-5*).

Figure 67-3

Chronic kidney disease (CKD) mechanisms schematic summary. Patients with glomerular filtration rates less than 60 ml/min/1.73 m^2 have CKD and a significantly increased risk of premature cardiovascular death and end-stage kidney disease. Several factors modify these risks. The pathophysiological mechanisms of CKD progression involve an integrated network of cellular events and molecular pathways. Early renal scarring characteristic of CKD may be reversible. The figure is modified from (5).



Factors that Influence Renal Failure Progression Rates

Chronic Kidney Disease Progression in Patients

Non-Modifiable Risk Factors

Fetal Programming

Low birth weight (even after correction for gestational age) is associated with an increased risk of chronic and end-stage kidney disease. Early work by Brenner, Barker, Law and others proposed an association with birth weight, renal filtration surface area and the risk of hypertension. Although the mechanisms have not been fully elucidated, a congenital defect in nephron number appears to be important. Careful stereological studies such as those performed by Hughson et al. (*Fig. 67-6*) determined a mean of 923,377 ± 256,391 nephrons in adult white patients while the mean was reduced to 754,320 ± 329,506 when the data for white hypertensive patients were examined (9). These data are similar to the findings of others. Proposed mechanisms linking nephron number and CKD risk revolve around the increased hypertension risk due to associated changes in natriuresis and glomerulosclerosis caused by intraglomerular hyperfiltration. Systemic hypertension and/or microalbuminuria may be an early indicator of low nephron number.

Ethnicity

For reasons that may pertain to genetic, environmental and/or socioeconomic factors, certain races are more likely to develop end-stage kidney disease (ESRD). Risks may be due to a higher incidence of certain diseases or a more aggressive clinical course for specific diseases entities. African Americans have a higher incidence of

Figure 67-4

Renal interstitial fibrosis: the CKD histological hallmark. Quantitative histological studies of human kidney biopsies demonstrate an inverse correlation between the scarred interstitium and renal function. The photomicrographs illustrate interstitial inflammation and fibrosis and tubular atrophy, the histological features of CKD that are not present in a normal kidney. The graph is from (6). (See color plate 42)

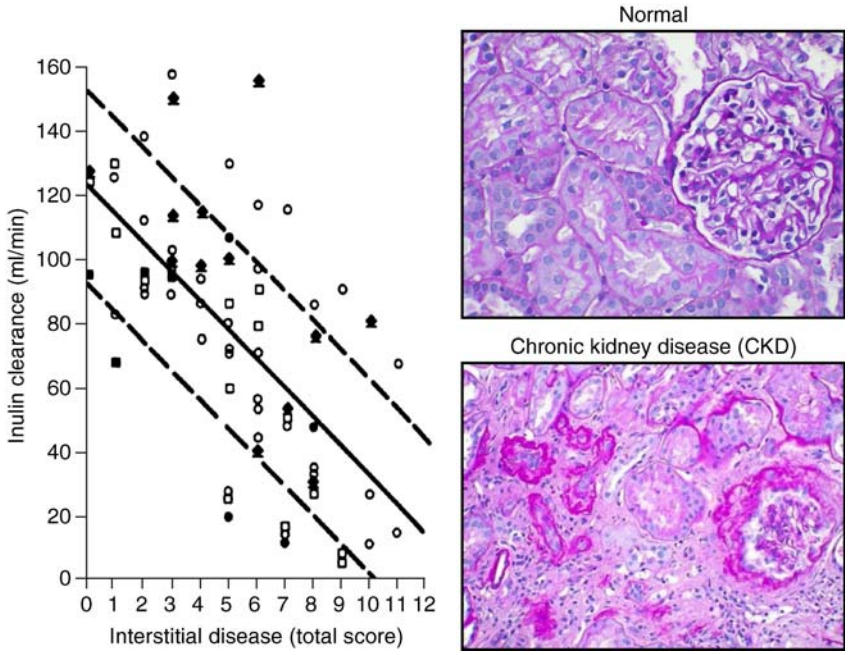
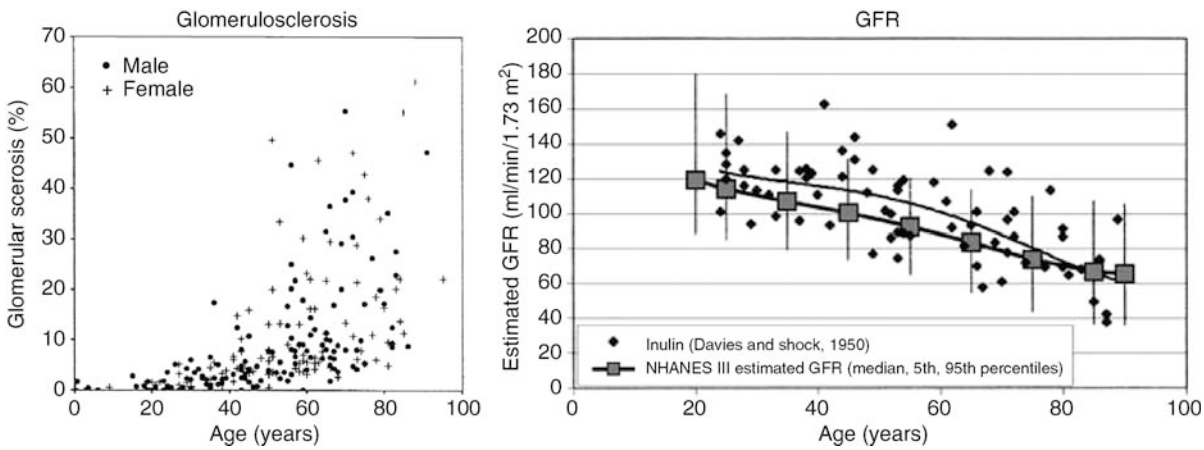


Figure 67-5

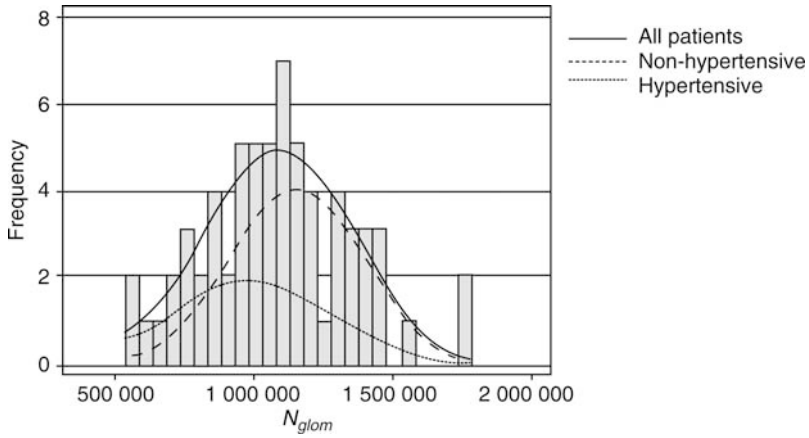
Progressive glomerulosclerosis and declining GFR characterize aging kidneys. Normal human aging is characterized by renal scarring and a progressive decline in kidney function. These figures are reproduced from (7, 8).



hypertensive nephrosclerosis and idiopathic focal segmental glomerulosclerosis. These diseases, together with lupus nephritis and HIV nephropathy, are also more aggressive in African Americans (10). Hispanic patients also have a worse prognosis than Caucasians. In a study of lupus nephritis by Contreras et al. (11), the end point of a doubling of serum creatinine or ESRD was reached by 31% of African Americans, 18% of Hispanics and 10% of

Figure 67-6

Low nephron number is a risk for hypertension. Increasing evidence supports an association between low nephron numbers (Nglom) and the risk of developing hypertension as shown by this study of white females. Data are from (9).



Caucasians. The escalating incidence of obesity and the metabolic syndrome also has significant racial differences. African Americans with the metabolic syndrome have a 31% increased risk of developing proteinuria. Certain indigenous populations, such as the Zuni Indians of New Mexico and the Australian Aborigines, have a significantly increased risk of chronic kidney disease, both diabetic and non-diabetic (12, 13). The metabolic syndrome is highly prevalent in these populations.

Age

It is not clear that age per se is an independent risk factor for CKD progression rates in the pediatric population, although it is commonly perceived that renal function deteriorates more rapidly around the time of puberty. Gonzalez et al. (14) investigated the natural history of CKD in a group of children with renal hypodysplasia and suggested three discrete phases: (1) initial improvement in GFR after birth until a median age of 3.2 years; (2) a second pre-pubertal phase characterized by stable renal function for several years in half of the patients, while the remainder had progressively declining renal function (−1.7 ml/min/1.73 m^2/year median); (3) the pubertal period when 43% with previously stable renal function began to deteriorate (−4.0 ml/min/1.73 m^2/year median) beginning at a median age of 11.4 years. Increased body mass at puberty was thought to be associated with systemic and intrarenal hemodynamics that accelerated glomerular and interstitial fibrosis. Baseline GFR, albuminuria, hypertension and recurrent febrile urinary tract infections were predictors of more rapid renal functional decline.

Other Genetic Risk Factors

Naturally occurring polymorphisms in several genes have been associated with differences in biological activity of the proteins that they encode. A field of active investigation seeks to determine if polymorphic genetic variants can be used to predict progression rates in patients with CKD (15). Most extensively investigated is the angiotensin converting enzyme (ACE) gene, known to have insertion (I) and deletion (D) variants; D is associated with increased ACE activity. The homozygous ACE D/D genotype has been associated with more aggressive renal disease in some but not all studies of patients with IgA nephropathy and diabetic nephropathy. Other polymorphisms of interest include additional genes of the renin-angiotensin system such as angiotensinogen; pro-inflammatory molecules: monocyte chemoattractant protein-1, interleukin-1, interleukin-10; pro-fibrotic molecules: transforming growth factor beta; angiogenic factors: vascular endothelial growth factor; and others: vitamin D receptor, endothelial nitric acid synthase. This list will undoubtedly expand.

Modifiable Risk Factors

Hypertension and proteinuria have been identified in numerous studies as independent determinants of the rate of renal functional deterioration that can be modified with therapy. Other factors play a role, although it is not always clear if their effects are direct or indirect, ultimately mediated through their influence on blood pressure and urinary protein excretion rates.

Hypertension

Although CKD as a consequence of primary hypertension is extremely rare in children, hypertension is a common manifestation of many pediatric chronic kidney diseases. In the 2004 annual report of the North American Pediatric Renal Transplant Cooperative Study (NAPRTCS), 38% of children with CKD were on antihypertensive medication (16). Stratified by primary kidney disease rates ranged from 83% for polycystic kidney disease to 19% for patients with structural kidney disease. Hypertension is one of the most critical determinants of progression rates in children as well as adults (*Fig. 67-7*) (17). The pathophysiology is complex but alterations in the activity of the renin-angiotensin system play a central role while impaired renal arteriolar autoregulation makes the kidney an especially vulnerable target organ. The ensuing glomerular hyperfiltration is implicated in the genesis of glomerulosclerosis and proteinuria which is thought to induce inflammation and fibrosis within the interstitial compartment. Whether hypertension directly causes interstitial damage via effects on interstitial arterioles and peritubular capillaries is unclear. Current treatment guidelines recommend treating CKD patients with ACE and/or ARB drugs to achieve blood pressure targets of less than the 90th percentile for age, gender and height (18). Whether lower blood pressure goals will reduce progression rates is unclear. Optimal blood pressure control is also critical for cardiovascular protection, noting that cardiac disease is the second most common cause of death in children with ESRD.

Proteinuria

In virtually every epidemiological study the degree of proteinuria emerges as a significant independent predictor of CKD progression rate. This correlation is even true for non-glomerular diseases such as reflex nephropathy as shown in the ItalKid project cohort of 343 patients where urinary protein/creatinine ratios greater than 0.8 (0.2 upper normal) were associated with faster deterioration (*Fig. 67-8*) (19). There is increasing evidence that treatment designed to lower urinary protein excretion rates is beneficial. In an analysis of 542 patients with primary IgA nephropathy in the Toronto Glomerulonephritis Registry, multivariate analysis determined that proteinuria during follow-up was the most important predictor of the rate of GFR decline (*Fig. 67-9*) (20). Reducing proteinuria is especially beneficial in patients with nephrotic syndrome. Achieving proteinuria levels less than 300 mg/day has been proposed as the ideal goal for adults. Even in normotensive adults with renal disease, the use of ACE drugs to reduce albuminuria has been shown to be renoprotective (21, 22). Proteinuria is also a strong predictor of cardiovascular disease (23).

Proteinuria may be a marker of glomerular damage. While pediatric evidence is still evolving, three large adult studies have shown that controlling hypertension

Figure 67-7

Hypertension increases the rate of CKD progression. In a study of 3,834 children with chronic kidney disease, hypertension was shown to increase the rate of progression to end-stage kidney disease. Data are from (17).

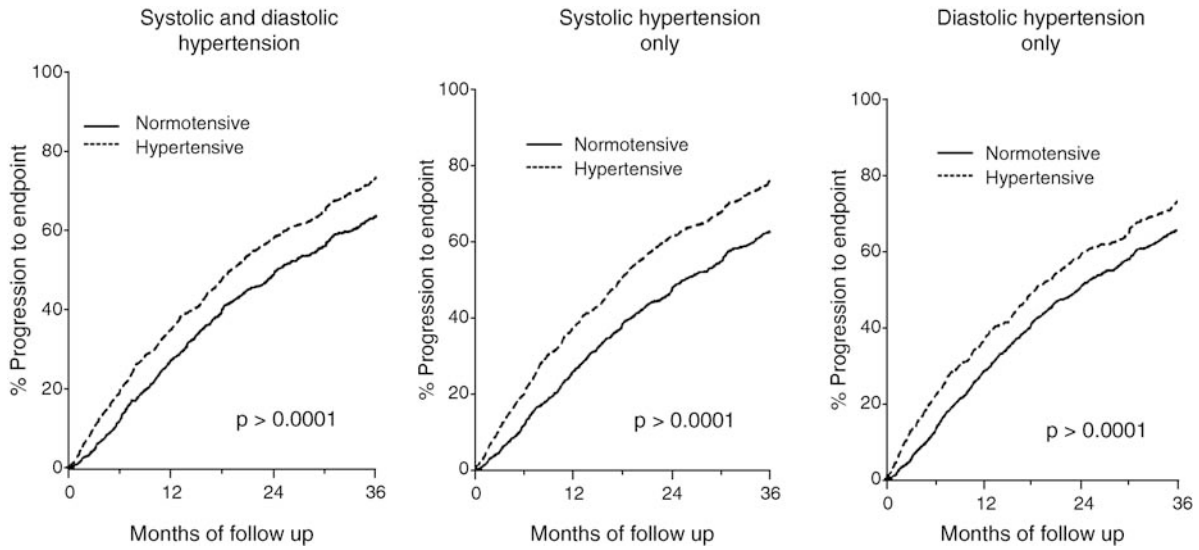
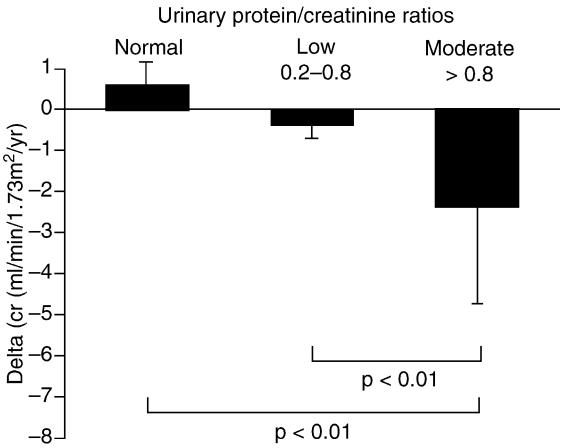


Figure 67-8

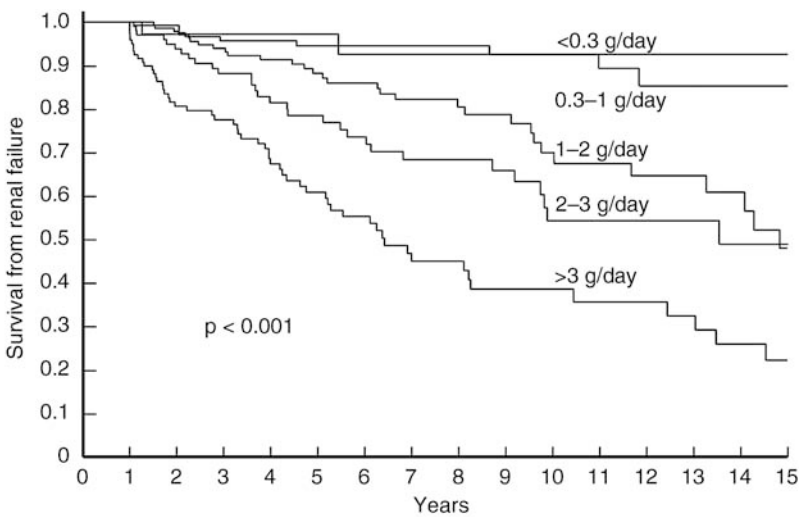
Proteinuria is an outcome predictor even in primary non-glomerular diseases. In a study of 343 children with vesicoureteral reflux, moderately elevated urinary protein/creatinine ratios showed a faster rate of decline in renal function. Data are from (19).



had important proteinuria-reducing effects (24). In the ESCAPE trial (Effect of Strict Blood Pressure Control and ACE Inhibition on CRF Progression in Pediatric Patients) use of ramipril to control hypertension was shown to reduced proteinuria in children (25). Data from experimental studies support the hypothesis that proteinuria has direct damaging effects on the tubulointerstitium (26). Through breaks in Bowman's capsule or disruptions in glomerulo-tubular junctions, biologically active proteins within the glomerular ultrafiltrate may gain access to the interstitial compartment and cause damage. Similar molecules reaching peritubular capillaries via the post-glomerular arterioles may increase permeability and/or promote inflammatory cell recruitment. In addition, direct effects on proximal tubules have been increasingly documented (*Fig. 67-10*). Interactions with the endocytic receptor complex megalin-cubulin-amnionless are likely to be involved, but the receptor-dependent pathways have not been fully elucidated. Similarly, the molecular composition of the offensive urinary proteins remains unclear. Albumin modified by lipids, transferrin

Figure 67-9

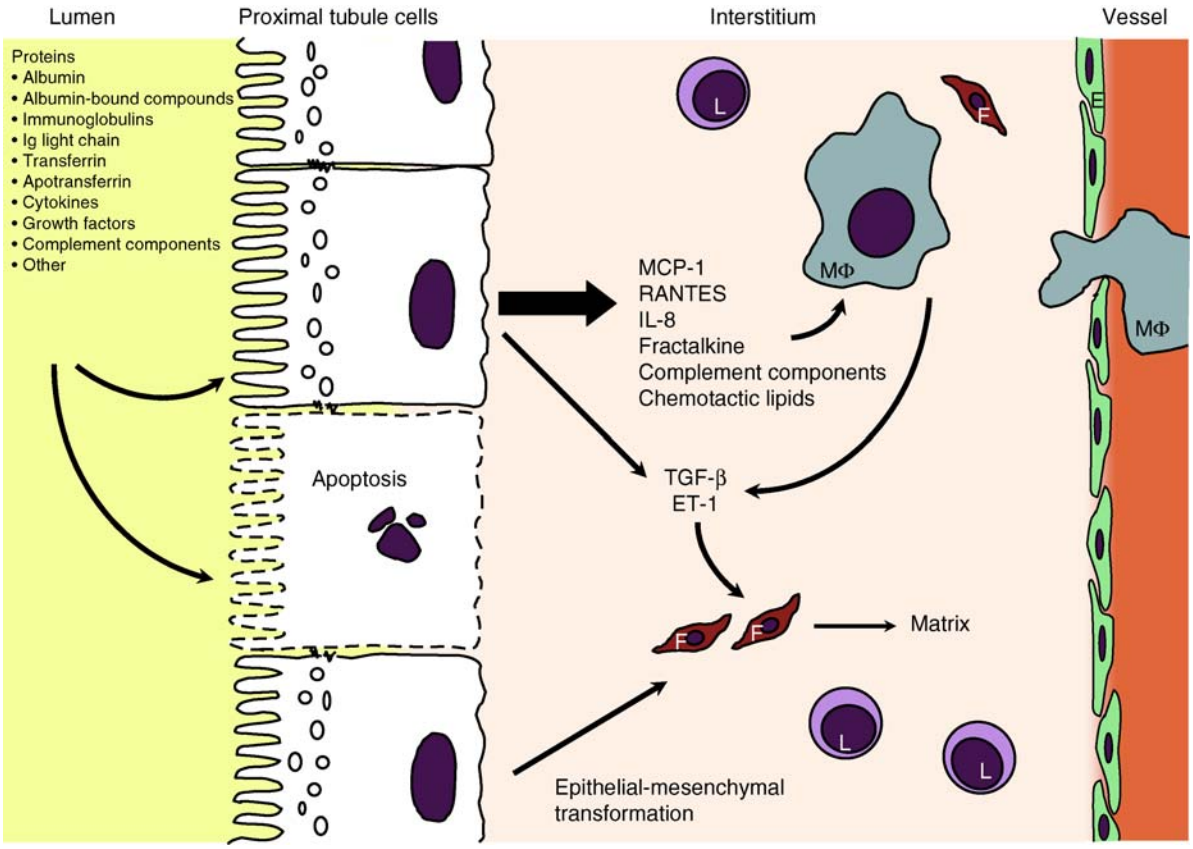
Proteinuria severity in glomerular disease predicts renal outcome. In most glomerular diseases except steroid-responsive nephrotic syndrome, the risk of renal failure increases with urinary protein excretion rates as shown in this study of patients with IgA nephropathy. Data are from (20).



<0.3 g/day	37	22	8	1
0.3–1 g/day	134	79	35	11
1–2 g/day	145	79	28	10
2–3 g/day	105	50	18	4
>3 g/day	120	44	13	6

Figure 67-10

Mechanistic links between proteinuria and tubulointerstitial disease. Various proteins that are filtered into the urine during glomerular proteinuria may interact with tubular epithelial cells to trigger the release of proinflammatory and profibrotic molecules, to initiate epithelial-to-mesenchymal transdifferentiation and/or apoptosis. The figure is reproduced from (26).



and complement proteins have been of particular interest. These interactions may induce profound changes in tubular epithelial cells, including synthesis of pro-fibrotic factors such as TGF-β, endothelin-1, angiotensin II; proinflammatory chemokines and cytokines such as MCP-1 and interleukin-8; and they may promote oxidative stress, tubular transdifferentiation and tubular apoptosis.

Obesity

Morbid obesity itself is associated with a significant risk of glomerulomegaly and glomerular pathological changes. Based on pre-operative studies in patients undergoing bariatric surgery, focal segmental glomerulosclerosis may even be present without proteinuria (27). Epidemiological studies have reported a strong positive correlation between excess body fat and relative risk of ESRD, ranging from 1.9 for overweight individuals to 7.1 for those who are morbidly obese (28). The pathogenesis of the renal disease appears to involve hemodynamic and other factors that develop as a consequence of associated features of the metabolic even in the absence of overt diabetes.

Dyslipidemia

Lipoprotein abnormalities are common in patients with CKD; the specific profile varies depending upon the nature of the primary kidney disease and the use of certain medications. In animal models, hypercholesterolemia is associated with accelerated CKD progression. Human epidemiological data support an association between dyslipidemia, cardiovascular disease and faster rates of renal functional decline (29). A recent study identified hyperlipidemia as a predictor of more rapid renal deterioration after lung transplantation (30). In experimental studies, statin drugs have been shown to confer significant

renoprotection that appears to extend beyond its cholesterol-lowering effects. Amongst their recognized pleotrophic effects, inhibitors of inflammatory pathway signaling molecules are important. Evidence that statins delay CKD progression in humans is less compelling. A meta-analysis by Sandhu concluded that the intrinsic anti-proteinuria and renoprotective effects of statins reached statistical significance but differences were quantitatively small (29). A more recent meta-analysis by Strippoli et al. (31) concluded that statins reduced cholesterol levels and proteinuria but did not improve GFR or all cause mortality. After initial concerns about their safety for use in children, several statin drugs are now approved for use in children over 10 years of age, but data are not yet available to address their renoprotective efficacy and safety in this age group.

Mineral Homeostasis

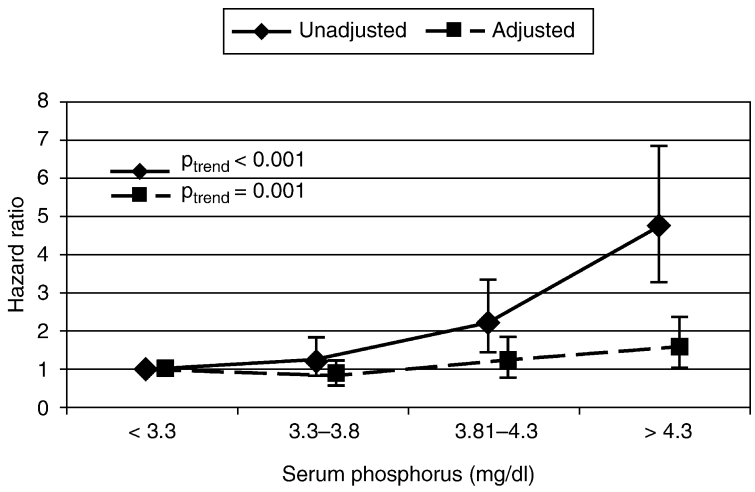
Dystrophic calcification is an important pathogenetic mechanism leading to vascular and bone disease associated and significant morbidity and mortality in CKD patients. There is reasonable evidence from both animal models and human epidemiological studies that hyperphosphatemia is an independent risk factor for more rapid renal functional decline. In a study of adult male veterans, every 1 mg/dl increase in serum phosphorous above normal was associated with an adjusted hazard ratio of 1.29 for the composite renal end-point of dialysis or serum creatinine doubling (*Fig. 67-11*) (32). Suggested mechanisms include metastatic calcification due to calcium phosphorous crystal deposition in tubular mitochondria and the renal interstitium. Recent studies by Giachelli et al. (33) have shown that hyperphosphatemia directly induces smooth muscle cell mineralization via a process that involves the type III sodium-dependent phosphate co-transporter Pit-1. These effects of phosphorous are believed to contribute to uremic vascular calcification and accelerated atherosclerosis. It is possible that similar effects on renal parenchymal cells also contribute to CKD progression.

Hyperuricemia

Elevated uric acid levels may be present in patients with CKD, but whether this is a primary or secondary event is unclear. Recent studies suggest a potential pathogenetic link between hyperuricemia and essential hypertension. Although studies in animal models support an association between hyperuricemia and renal structural damage, human data are conflicting. In a study of IgA nephropathy patients, hyperuricemia at the time of biopsy was identified as a risk factor for disease progression (34). In a cohort of Japanese patients, hyperuricemia was an independent predictor of ESRD in females (adjust hazard ratio 5.8) but not males (35). Large adults studies have reported a significant but weak association between hyperuricemia and CKD prevalence (36, 37) while a recent study of 177 adults with non-diabetic CKD by Sturm

Figure 67-11

Hyperphosphatemia increases the risk of renal functional decline. When serum phosphorus levels were divided into quartiles in a group of males with CKD, higher phosphorus trends (P_{trend}) were associated with a greater risk of renal functional deterioration (ESRD or serum creatinine doubling) even after adjusting for 15 known progression risks factors (Adjusted). The data are reproduced from (32).



et al. (38) did not identify uric acid levels as an independent predictor for CKD progression. Experimental studies suggest that in addition to urate nephropathy due to intratubular crystal deposition, uric acid may have damaging effects on smooth muscle and vascular endothelial cells that contribute to renal dysfunction (39).

Smoking

Limited experimental evidence suggests that smoking may be an additional risk factor for functional deterioration. Smoke exposure induces glomerulosclerosis in aging estrogen-deficient mice (40). Mesangial cells are known to express nicotinic acetylcholine receptors and to undergo mitosis and increased fibronectin synthesis in response to nicotine (41). Clinical data support a potential association between smoking and declining renal function, but it is unclear if smoking is an independent risk factor or if its effects are mediated indirectly via vascular or oxygen-dependent effects (42).

Anemia

Hypoxia and oxidant stress are recognized as fundamental pathways contributing to renal fibrosis, as discussed further below. It therefore seems reasonable to suggest the degree of anemia may influence these pathways. Clinical trials with erythropoietin have begun to address the relative importance of anemia as an independent progression factor. Comparing outcomes in patients with mild to moderate CKD, recombinant erythropoietin has been found to significantly slow renal disease progression in some but not all studies. Data from pediatric clinical trials are not yet available. Interpretation of the results is complicated by the fact that in addition to erythroid progenitor cells, erythropoietin receptors are expressed in several tissues including the kidney (43). Erythropoietin is now known to have cytoprotective effects in several organs suggesting a potential for renal protection that may extend beyond anemia correction.

Mechanisms of Renal Fibrosis: Insights from experimental studies

Overview

The fundamental process of renal fibrosis recapitulates wound healing at the cellular and molecular levels. Like superficial cutaneous wounds, minor kidney injury can completely resolve if the inciting event is short-lived; while severe and/or sustained damage typically leaves a scar. Recent evidence supports the view that the loose scaffold of extracellular matrix proteins that constitutes the early scar may be remodeled allowing fibrosis to regress. Evidence of at least partial reversible renal fibrosis comes from experimental studies of the tubulointerstitium in acute tubular necrosis, puromycin-induced reversible nephrotic syndrome and reversible ureteral obstruction; and glomerular studies of human and experimental diabetic nephropathy and pharmacological inhibition of the renin-angiotensin-albosterone system, especially in the remnant kidney model (44–47). Well-organized advanced scars may contract but there is no evidence that they can be degraded. Preservation of intact and functional nephrons to maintain glomerular filtration is more relevant than fibrosis reversibility. An advanced understanding of pathways of renal inflammation, fibrogenesis and tissue regeneration will most certainly lead to innovative and more effective therapies for CKD in the future.

Mechanisms of Renal Fibrosis: Cellular Pathways

Macrophages All chronic kidney diseases are characterized by an interstitial infiltrate of mononuclear cells. Although there is a population of resident interstitial macrophages in normal kidneys, these cells are considered terminally differentiated; their expansion by proliferation has been reported but is considered unusual. Interstitial inflammation associated with CKD is primarily a consequence of monocyte recruitment from the circulation, entering via the peritubular capillary microcirculation. There is also a well-developed and continuous network of dendritic cells that process antigens and regulate local immune responses (48, 49). Whether dendritic cells are mediators of progressive kidney damage in unclear. Although exposure of "neoantigens" during the primary disease process could conceivably activate an adaptive immune response and perpetrate injury, such a pathway has not yet been clearly identified during the common phase of progressive chronic damage.

Two classes of molecules have important roles during active interstitial inflammation: endothelial-cell associated leucocyte adhesion molecules (integrins and selectins) and a variety of soluble molecules that serve as chemical attractants (cytokines, chemokines, other chemoattractants). Each functions through monocyte membrane receptors that serve to direct inflammatory cell migration. In addition, these ligand-receptor interactions may initiate intracellular signals that regulate other mononuclear cell functions. Since interstitial macrophages in particular are thought to contribute to renal fibrosis, several experimental interventions have been devised to block monocyte recruitment. Targeting various adhesion molecules (ICAM-1, VCAM-1, VLA-1), chemokines or their

receptors (MCP-1, RANTES, MIP-1, fractalkine) and chemoattractants (complement proteins, osteopontin, lipids) has been shown to reduce renal fibrosis.

Macrophages produce several soluble secreted products that may directly promote fibrosis (fibrogenic growth factors such as transforming growth factor-beta [TGF-β], connective tissue growth factor [CTGF], platelet-derived growth factor [PDGF], fibroblast growth factor [FGF], tumor necrosis factor alpha [TNFα]) and certain matrix proteins such as collagen, fibronectin and thrombospondin. They also have the potential to synthesize proteins that may amplify and propagate renal injury such as plasminogen activator inhibitor-1 [PAI-1], angiotensin converting-enzyme inhibitor [ACE], matrix metalloproteinases [MMPs], endothelin-1, complement proteins and reactive oxygen species. Many of these products are also produced by other cells participating in the fibrogenic response.

It has long been known that macrophages also serve alternative roles with the potential to limit the degree of tissue damage and promote healing. These effects often pertain to their ability to serve as scavengers due to their expression of certain membrane receptors that bind, internalize and degrade participants in tissue injury. For example, macrophages are involved in eliminating apoptotic cells. The angiotensin I receptor (ATR1) and the urokinase receptor (uPAR) are examples of scavenging receptors expressed by macrophages that have been shown to attenuate renal fibrosis in mice (50, 51).

The molecular basis of macrophage functional heterogeneity is beginning to be deciphered with the recognition of two distinct activation pathways that are associated with unique functions. These have been labeled as classically activated or M1 macrophages and alternatively activated or M2 macrophages (*Fig. 67-12*) (53, 54). In vitro M1 macrophages can be generated by exposure to interferon gamma or tumor necrosis factor alpha while M2 macrophages are generated by exposure to interleukins -4 and -13. M1 macrophages are typically present during acute tissue damage while M2 macrophages appear during stages of tissue repair and fibrosis. The phenotype of interstitial macrophages associated with renal fibrosis has not been carefully examined, but the recent identification of cell surface markers unique to the M1 and M2 subsets in mice should help to address this question. It is not yet clear if M1 and M2 macrophages derive from a common precursor cell or if one phenotype can transform into another. The differential function of macrophages has been established in a study of reversible liver injury performed by Duffield et al. (55). When macrophages were depleted during the acute injury phase, liver fibrosis was attenuated. If depletion was delayed until the recovery phase, liver fibrosis was more severe. In a crescentic glomerulonephritis model these authors also demonstrated that conditional macrophage ablation on days 15–20 prevented disease progression (56). Differential macrophage function has also been established in a model of persistent nephrotic syndrome in immunodeficient mice. Following ex vivo priming, infusion of M1 macrophages exacerbated while M2 macrophages attenuated disease severity including the degree of interstitial fibrosis (57).

Immunosuppressive medications already in clinical use may help to preserve renal function through macrophage inhibition, even when the primary kidney disease is not immunologically mediated. For example methylprednisone has been shown to reduce interstitial inflammation and preserve renal function in rodent models of polycystic kidney disease while mycophenolate mofetil attenuated interstitial inflammation and fibrosis and lowered serum creatinine levels in the remnant kidney model (58, 59).

Fibroblasts/Myofibroblasts A key event in progressive kidney disease is fibrosis or the progressive accumulation of extracellular matrix (ECM) proteins, especially in the renal interstitium. The primary source of the ECM is a unique population of activated mesenchymal cells that appear to be fibroblasts which also express contractile proteins typically associated with myocytes such as alpha smooth muscle actin (αSMA). The de novo appearance of these interstitial "myofibroblasts" is an essential prerequisite for interstitial fibrosis and has been used clinically as a prognostic indicator in diseases such as membranous nephropathy where renal outcome is highly variable (60). The myofibroblasts produce not only ECM that constitutes the normal interstitial matrix scaffold (such as the fibrillar collagens I and III and fibronectin), but they may also synthesize a variety of proteins not normally present, including glycoproteins, proteoglycans and various matrix proteins more typically found in basement membranes. Transformed mesangial cells appear to play a similar role during glomerulosclerosis.

The origin of these interstitial myofibroblasts is of great interest given their central role in kidney scarring. It appears that they may derive from several different sources (*Fig. 67-13*). Also unknown is whether their origin determines functional diversity. These questions have been difficult to answer due to the lack of specific cell markers to differentiate unique subsets of interstitial myofibroblasts. In normal kidneys, αSMA+ pericytes are associated with arterial and capillary vessels. It is possible that these cells migrate into the interstitium in response to injury. In addition the population of αSMA-negative

Figure 67-12

Macrophage polarization and functional heterogeneity in CKD pathogenesis. Circulating monocytes that enter the kidney in response to inflammatory cues undergo distinctive pathways of differentiation into classically activated M1 macrophages or an alternative M2 phenotype. Activation of M1 inflammatory macrophages by classical immune pathways may lead to the expression of MHC class II antigens and release of pro-inflammatory cytokines. In response to ongoing injury, M1 macrophages propagate inflammation and ultimately the development of fibrosis. Dependant on microenvironmental cues, M2 macrophages may be recruited from the circulation or activated in situ as a result of a M1 to M2 phenotype switch. M2 anti-inflammatory macrophages secrete regenerative trophic factors that promote cell proliferation and reduce apoptosis and stimulate angiogenesis. Macrophages derived from engrafting bone marrow myeloid progenitors may contribute to the repopulation of injured tubular epithelial and glomerular cells by a process of transdifferentiation or cell fusion leading to replacement of damaged cells. Ex vivo modulation of macrophages to a M2 phenotype for transplantation may be used therapeutically to suppress the immune response and promote tissue remodeling leading to structural repair and functional recovery. Reproduced from (52).

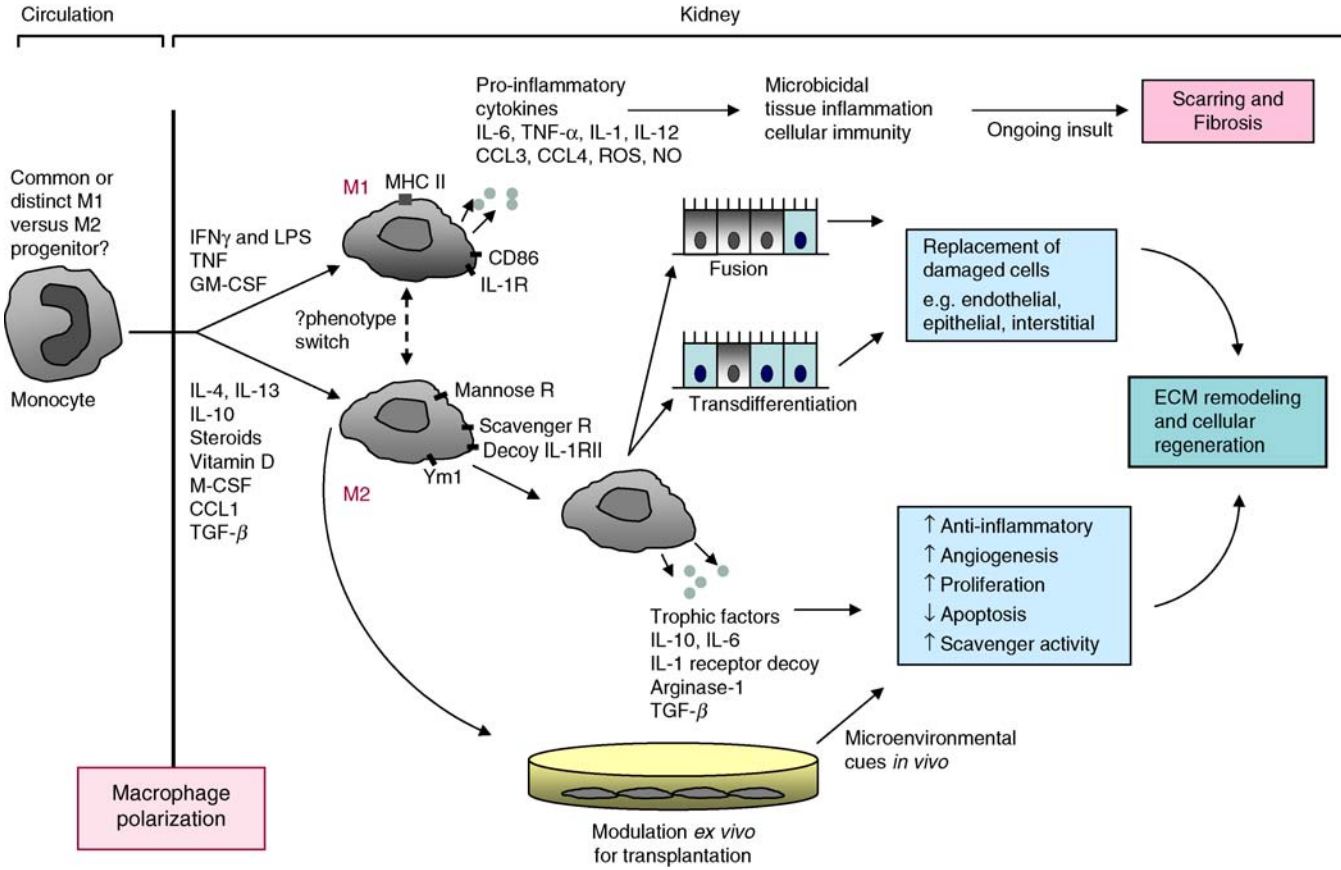
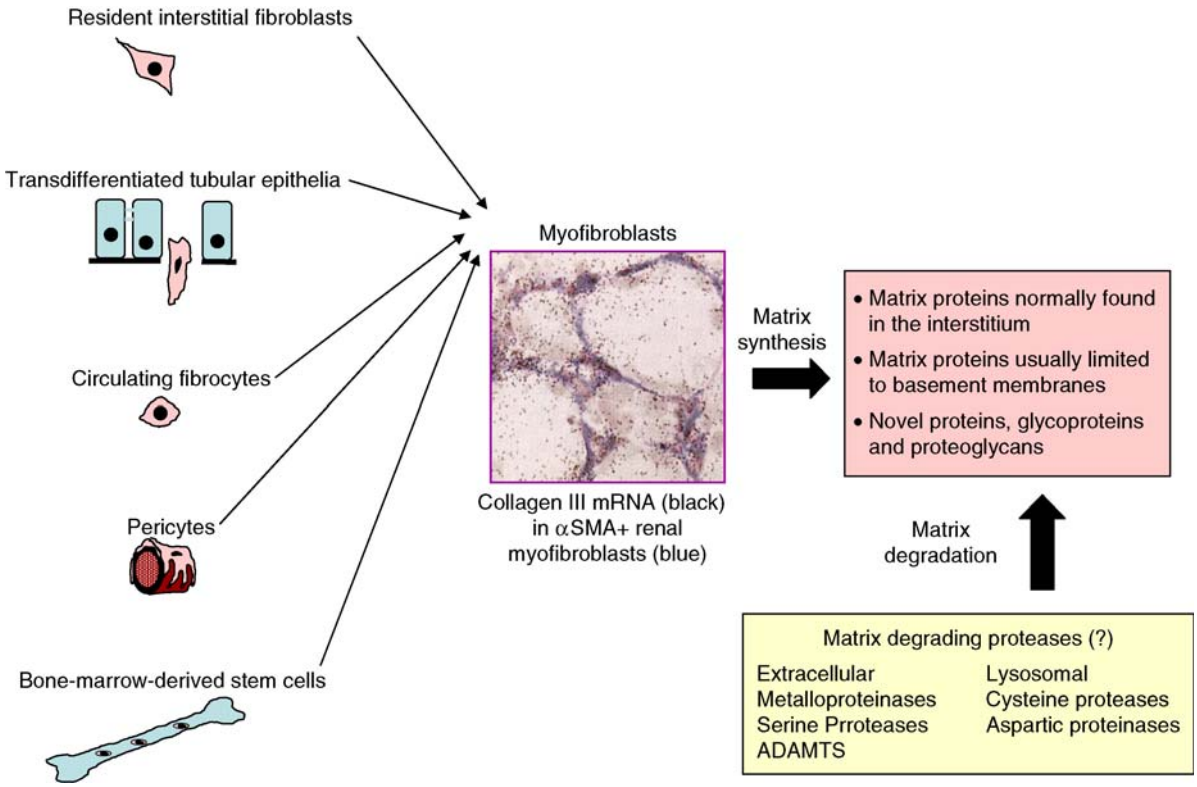


Figure 67-13

Renal interstitial myofibroblast origins in CKD. Although resident interstitial fibroblasts are considered the primary source of the interstitial myofibroblasts that an important source of scar-forming extracellular matrix (ECM) proteins, four alternative sources have been reported. In addition to increased ECM synthesis, impaired activity of matrix-degrading pathways is also thought to contribute to fibrosis, although the primary protease(s) that perform this function in vivo in the kidney is still unknown. The photomicrograph is from (61). (See color plate 43)



interstitial fibroblasts that reside in the normal interstitium are an alternative source of the population of interstitial myofibroblasts that expand early in the course of chronic kidney disease through processes of activation and proliferation (62). Numerous factors released by damaged kidneys have been shown to function as fibroblasts mitogens in in vitro studies. TGF-β is an especially potent inducer of fibroblasts αSMA expression and matrix protein synthesis.

As renal disease progresses, tubular cells that have undergone a process of transdifferentiation or epithelial-to-mesenchymal transition (EMT) may make their way into the interstitium, where they may be characterized by a myofibroblast-like phenotype and are presumed to contribute to the expanding pool of interstitial matrix proteins (63, 64). The relative contribution of tubular epithelia and resident interstitial fibroblasts and pericytes to the pool of interstitial myofibroblasts is unclear, although it is possible that the latter predominates in early disease and the former in more advanced disease as tubules degenerate. A study in an obstructive uropathy model using of genetically tagged tubular cells provides the most compelling evidence for the EMT process in CKD (65).

A fourth origin of interstitial myofibroblasts is from a blood-borne pool of fibrocytes. It has been established in a study of obstructive uropathy that CCR7+ fibrocytes migrate into the kidney in response the chemokine SLC/CCL21 and contribute to renal fibrosis (66).

Myofibroblasts are presumed to have a finite lifespan. Therapeutic interventions that reduce myofibroblasts numbers by blocking their appearance or hastening their demise should in theory be an effective anti-fibrotic strategy. Blocking the EMT process through the use of hepatocyte growth factor (HGF) or bone morphogenic protein-7 (BMP-7) has been shown to reduce the severity of experimental chronic kidney disease (67, 68).

Tissue-type plasminogen activator (tPA) acting through its receptor low density lipoprotein receptor related protein 1 (LRP1) has been shown in in vitro experiments to block fibroblast apoptosis, while in vivo studies comparing tPA wild-type and knockout mice found that genetic tPA deficiency reduced αSMA^+-cell numbers and fibrosis severity after ureteral obstruction (69, 70). The use of tPA-specific inhibitors may be a therapeutic possibility, although its classical endogenous inhibitor plasminogen activator inhibitor-1 (PAI-1) has several other biological effects that lead to impressive pro-fibrotic effects (71).

Within damaged glomeruli, mesangial cells may also transform into myofibroblast-like cells (72). The transition from a normal to a sclerotic glomerulus is characterized by important changes in the composition of glomerular ECM that have not yet been fully explored; that is, mesangial matrix expansion and segmental/global glomerulosclerosis have distinct molecular compositions. Glomerulosclerosis is characterized by the accumulation of novel ECM proteins not normally present within the mesangium such as fibrillar collagens I and III. An important distinction is the ease of reversal of an expanded mesangial matrix as seen in acute glomerular proliferative disorders, while sclerotic glomerular lesions are more resilient, although the potential for regression has been reported. In addition to transformed mesangial cells, there are a few studies reporting that inflammatory macrophages and even endothelial cells may co-express smooth muscle cell markers during certain pathological states but the role of these cells as important sources of extracellular matrix proteins during chronic kidney disease is unclear (73, 74).

Microvascular Endothelial Cells Due to active transport and other metabolic activities that take place within a relatively hypoxic milieu, renal tubular health is highly dependent upon an adequate oxygen supply delivered via an extensive peritubular capillary network (75, 76). Although this is a low pressure microcirculation, these cells – together with tubular epithelial cells and the tubular basement membranes – can be considered as a tubulointerstitial barrier unit analogous to the glomerular capillary wall. One of the earliest responses to kidney damage is an increase in permeability that leads to interstitial edema composed of fluid and plasma-derived proteins. Exactly how this plasma exudate contributes to the ensuing fibrogenic response in the kidney is not clear. The presence of fibrinogen and its cleavage to fibrin have been implicated in pulmonary fibrosis. Fibrin not only forms an early provisional matrix upon which additional ECM can expand, but it is also a powerful monocyte chemoattractant. Despite many strong associations between fibrin accumulation and pulmonary fibrosis, its fundamental role has recently been questioned by studies in genetically engineered mice which found that experimental pulmonary fibrosis was similar between fibrinogen wild-type and knockout mice (77). The specific role of fibrinogen and other plasma proteins such as oxidatively modified albumin that leak into the interstitial space during chronic kidney disease has not been determined. Another fundamental unanswered question is what mediates changes in the permeability of the interstitial microcirculation. In other inflammatory models, changes in the expression and/or function of endothelial adherens junction proteins (such as VE-cadherin) and triggers such as thromboxane A2 and the Galphai-coupled receptors CXCR2 and Galphai2 are known to be important (78, 79).

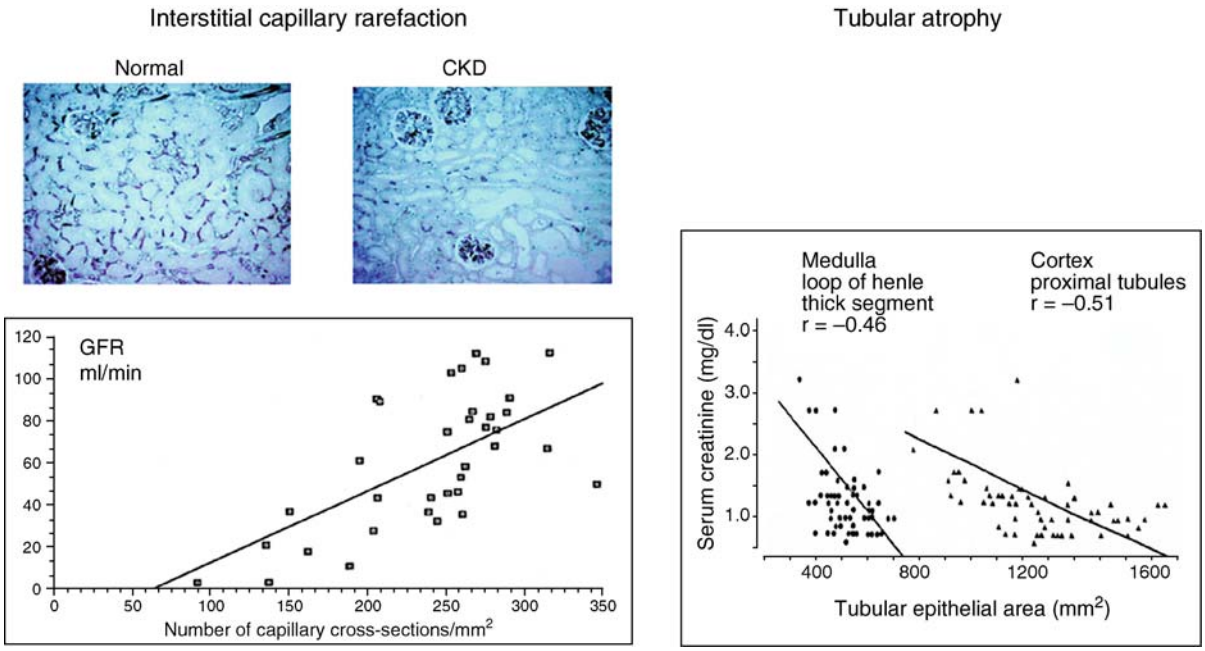
Peritubular capillaries also serve as the conduit for blood-borne inflammatory cells that migrate into the interstitium in response to injury. Locally generated signals may induce endothelial cells to express certain transmembrane proteins that enable them to facilitate this process. In particular, the family of endothelial selectin proteins serve to slow leukocyte flow through capillaries, while specific leukocyte adhesion molecules – such as intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) – in turn form tighter associations between leukocytes and the endothelium, facilitating their transmigration into the interstitium. ICAM-1 and VCAM-1 interacting with their leukocyte counter-receptors (CD11/CD18 and α_4 β_1 leukocyte integrins) also activate intracellular signaling pathways that regulate endothelial cell function.

A perplexing and unique aspect of the renal interstitial microcirculation is its resilience to angiogenesis. Many pathological processes are characterized by excessive angiogenesis, but this is not so in the renal interstitium, where lack of angiogenesis leads to capillary rarefaction in association with renal disease progression as shown when capillary density is mapped using endothelial cell proteins such as the von Willebrand factor, rat endothelial cell antigen RECA-1 or endothelial antigen platelet-endothelial cell adhesion molecule (PECAM) (*Fig. 67-14*) (83). Functional studies suggest that prior to capillary loss vasoconstrictor molecules reduce perfusion leading to tubular ischemia (84).

An inverse correlation between interstitial capillary density and glomerular filtration rate has been reported (80). These findings suggest that therapeutic administration of angiogenic factors to help preserve the interstitial capillary network and tubular oxygenation and to reduce oxidant stress might reduce chronic kidney disease severity. When a long-acting form of the angiogenic factor angiopoietin-1 was administered in an experimental

Figure 67-14

Loss of peritubular capillaries and renal tubules correlates with renal functional decline. Quantitative histological studies of human kidney biopsies demonstrate an inverse correlation between interstitial capillary density, tubular epithelial cell area and renal function. The photomicrographs are kidney sections stained with the endothelial cell antibody JG-12 and demonstrate a striking reduction in the number of peritubular capillaries in the rat remnant kidney model, reproduced from (80). The capillary density graph is reproduced from (81) and tubular epithelial cell graph from (82). (See color plate 44)



model of chronic kidney disease, it bound to the endothelial cell receptor Tie-2, increased interstitial capillary density and reduced the degree of fibrosis (85). Rats with remnant kidneys treated with vascular endothelial growth factor (VEGF) showed improved renal function (80). Placenta-derived cell growth factor (Plgf), an interesting angiogenic factor that is only produced during pathological states including chronic kidney injury (unpublished data) appears to be another potential angiogenic factor worth investigating. As a consequence of tubulointerstitial hypoperfusion, hypoxia and oxidant stress develop and perpetuate renal injury as discussed below in section C.

It has recently been appreciated using histological techniques that important changes in lymphatic vessels are a feature of chronic kidney disease. In normal kidneys, lymphatic vessels are limited to regions around arteries and arterioles. With the recognition that lymphatic but not endothelial capillaries express lymphatic vessel endothelial hyaluronan receptor-1 (LYVE-1) and podoplanin, it has become possible to map the fate of lymphatic capillaries using imaging techniques. In both human and experimental chronic kidney disease, a new network of interstitial lymphatic vessels has been identified (86, 87). The origin and significance of these neocapillaries in CKD pathogenesis awaits exploration.

Renal Epithelial Cells Renal tubules are estimated to account for approximately 80% of the kidney by volume and thus play a central role in chronic kidney disease. Renal tubular atrophy is probably the single most important factor that determines the rate of decline in renal function and final outcome (*Fig. 67-14*) (88). Quantitative evaluation of kidney biopsies from patients with chronic kidney disease demonstrates a strong correlation between glomerular filtration rates and tubular area, both cortical and medullary. The presence of atubular glomeruli is an histological feature of CKD (89). Irreversible degeneration of renal tubules occurs primarily through a process of programmed cell death or apoptosis.

While the tubular epithelia are viable they play multiple roles in the renal response to chronic injury and disease progression. Recent studies have focused on

these cells as active participants in inflammation and fibrosis, but it is also obvious that preservation of normal tubular structure and biological function is key to kidney health and interventions that protect renal tubules should have significant therapeutic benefit for CKD patients.

In addition to their important role as transporters of water and several solutes present within the glomerular ultrafiltrate, renal tubular epithelial cells produce a vast repertoire of molecules that may be modulated by injury and many more that are expressed de novo in response to injury. Renal tubular cells are thought to promote interstitial inflammation via their ability to synthesize chemokines and chemoattractants such as activated complement components. This function has been most extensively investigated in the context of chronic proteinuric kidney diseases (*Fig. 67-10*) (26, 90). Following studies in animal models showing an association between proteinuria and interstitial inflammation, numerous studies have reported that cultured tubular cells exposed to high concentrations of albumin (especially lipidated forms) and other proteins lost by glomerular filtration in glomerular disease states such as transferrin can stimulate expression of chemokines such a monocyte chemoattractant protein-1, interleukin-8 and RANTES. Proximal tubular receptors megalin, cubulin and amnionless are thought to be involved, although the specific signaling pathways and possible role of other receptors has not yet been fully explored (91). It is assumed that these small peptides are able to cross the TBM and find their way into the interstitium, although this has not been definitely shown. Tubular epithelia may also be directly involved in signaling pathways that are activated in response to inflammation by virtue of their ability to express receptors such as toll-like receptor and receptors for members of the interleukin-6 family of receptors, such as gp130 (92, 93). Normally thought to form a watertight barrier that blocks urinary backflow, it is clear that renal injury that disrupts epithelia cell tight junctions and/or glomerulotubular junctions allows urinary products (including those generated by inflamed and diseased glomeruli) to leak into the interstitium and become involved in inflammatory processes. Breaks in Bowman's capsule in association with glomerular crescents is another pathway of communication with the interstitium. Interleukin-1 has been associated with periglomerular inflammation in experimental crescentic interstitial nephritis (94). Increased intratubular pressure due to obstruction may cause early disturbances in tubular cell tight junctions. Leakage of Tamm-Horsfall proteins produced by the thick ascending limb of the loop of Henle into the interstitium has been demonstrated histologically.

As chronic kidney disease transitions from an acute inflammatory to a chronic fibrotic phase, tubular epithelia may take on new roles. They have been shown to produce profibrotic factors such as TGF-β, endothelin-1, angiotensinogen, FGF, CTGF, and PAI-1. They have the capacity to synthesize specific ECM proteins. An important contribution of tubular epithelial cells results from their expression of receptors that may induce EMT upon ligand engagement; these include receptors for transforming growth factor β, angiotensin II, MMP-9, and plasmin. As these transdifferentiated cells find their way into the interstitium through gaps in the TBM they may function as matrix-producing cells that accentuate fibrosis (*Fig. 67-13*).

A recent observation derived from genetic studies of polycystic kidney disease is that normal tubular epithelia cells express a prominent single cilium that is clearly involved in the maintenance of epithelial cell health given the striking phenotypic change that occurs when genetic mutations generate abnormal ciliary-associated proteins (95). The role and fate of tubular cilia in chronic kidney disease is a new area of investigation that is likely to be important.

The resident glomerular epithelial cell, the podocyte, plays a central role in the preservation of normal glomerular capillary wall function. Abnormalities in podocyte function underlie pathologic proteinuria in glomerular disease states (96). There is emerging evidence that proteinuria may in turn modify the function of podocytes in ways that may contribute to glomerulosclerosis (97). Podocytes also produce factors such as vascular endothelial growth factor (VEGF) that appears to be able to travel upstream against glomerular ultrafiltration to reach glomerular endothelial cells (98). This pathway appears critical to preserve the endothelial cell barrier as mice genetically deficient in podocyte VEGF develop chronic kidney disease due to a thrombotic microangiopathic process.

Like tubular epithilia, loss of glomerular podocytes ("podocytopenia") is the common final pathway leading to glomerulosclerosis (99, 100). When podocytes were selectively depleted in a transgenic rat strain in which the human diphtheria toxin receptor was specifically expressed in podocytes, a correlation was found between podocyte number and the number of sclerosed glomeruli (101). However, in contrast to tubular epithelia, glomerular podocytes lack the ability to proliferate except under rare circumstances, making early podocyte loss of considerable concern.

Mechanisms of Renal Fibrosis: Matrix Deposition and Turnover

In normal kidneys, the extracellular interstitial space is barely visible histologically, especially in the renal cortex

where tubules align "back-to-back" with little intervening space. However using antibody-based immunohistochemical staining techniques, small amounts of interstitial collagens and fibronectin can be detected within the normal interstitium. Expansion of interstitial ECM is the fundamental process of kidney fibrogenesis that characterizes progressive kidney disease. An important pathogenetic concept is that the ECM components that constitute the scar participate in a dynamic process. The specific molecular components change with time and those that persist undergo significant biochemical modifications such as forming cross-links between themselves and neighboring proteins. Such alterations make the matricial scar more resistant to protease-based degradation. In addition to forming a scaffold within the interstitium that becomes increasingly dense and rigid over time, this new multi-molecular network has a profound effect on the behavior of adjacent cells and proteins. Hypoxia occurs as a consequence of impaired oxygen diffusion. Inflammatory cells, (myo)fibroblasts, and epithelial cells express cell surface receptors such as integrins that bind to specific ECM proteins and activate intracellular signaling pathways. More recently, other cellular receptors have been identified as matrix-binding, such as the low density lipoprotein receptor-related protein (LRP) and CD36 that bind to thrombospondin and biglycan that interacts with toll-like receptors (102). The term "dynamic reciprocity" has been used to describe this interaction between matrix-synthesizing cells and the modification of the behavior of these same cells in response to their extracellular matrix milieu (103).

Many of the interstitial matrix components that appear de novo in scarring kidneys have unique biological activities that may modulate the fibrogenic response. The profibrotic growth factor TGF-β can be sequestered in an inactive form by binding to the small proteoglycans decorin and biglycan. Alternatively, thrombospondin is known to active TGF-β. Fibroblast growth factor can also be sequestered due to interactions with proteoglycans. PAI-1 binds avidly to vitronectin. Hyaluronan has complex functions that influence inflammatory processes and TGF-β activation (104). PDGF and VEGF bind to secreted protein acidic and rich in cysteine (SPARC).

While this network of ECM develops, a competing enterprise dependent upon the activity of matrix-degrading proteases attempts to remodel and degrade it. Their task becomes more difficult when cross-linking molecular bridges are generated by enzymes such as lysyl oxidase and transglutaminase. Use of transglutaminase inhibitors or genetic transglutaminase 2 deficiency has been shown experimentally to attenuate fibrosis (105, 106). Virtually all of the cells involved in the renal fibrogenic process are involved in the production of these proteases.

There are five primary families of matrix-degrading proteases: the metalloproteinases (MMP) and the serine proteases have been most extensively investigated in chronic kidney disease, but others include lysosomal cysteine proteases and aspartic proteinases and the ADAMTS (a disintegrin and metalloproteinase with thrombospondin motifs) family. The MMPs are a large family with each member having specific preferred matrix substrates. The most abundant kidney MMPs are the gelatinases that effectively degrade basement membrane collagen IV: MMP-2 (inducible) and MMP-9 (constitutive). CKD is associated with collagen IV accumulation in the interstitium. Published research with MMP inhibitors as antifibrotic agents are limited and generally unimpressive, suggesting that endogenous MMP-2 and -9 may not function as interstitial fibrosis inhibitors. In a mouse model of Alport syndrome MMP inhibitors were beneficial if administered before the onset of proteinuria; disease was accelerated if they were started once proteinuria was present (107). This is further supported by the observation that genetic deficiency of endogenous tissue inhibitors of MMPs such as TIMP-1 has no impact on interstitial fibrosis severity despite the fact that TIMP-1 is highly induced by chronic injury (108). In contrast, in the liver TIMP-1 expression has been closely correlated with fibrosis (109).

There are still several studies that need to be performed before the role of the entire MMP family is understood in renal fibrogenesis, although the prospects that MMP activity might be therapeutically manipulated does not appear promising as an approach to CKD. The reason for this may be that MMPs have additional functions that can promote fibrosis (110). The activity of the gelatinases in degrading collagen IV-rich basement membranes has been implicated in glomerular injury and in the tubular EMT process. Several MMPs are involved in regulating chemokine activity during inflammation. Some MMPs serve as ligands for specific cellular receptors (LRP1, integrins) to activate cellular signaling. These alternative activities likely explain why mice genetically engineered to over-express MMP-2 in renal tubules spontaneously develop interstitial fibrosis as they age (111). MMP-7, also known as matrilysin, is expressed by damaged tubular epithelia where it may promote interstitial fibrosis via its ability to cleave E-cadherin and facilitate EMT (112).

Recent studies on the role of the serine protease family in renal fibrogenesis have also led to some surprising findings, including the observation that their roles appear to be organ-specific (113). PAI-1 is expressed by and is

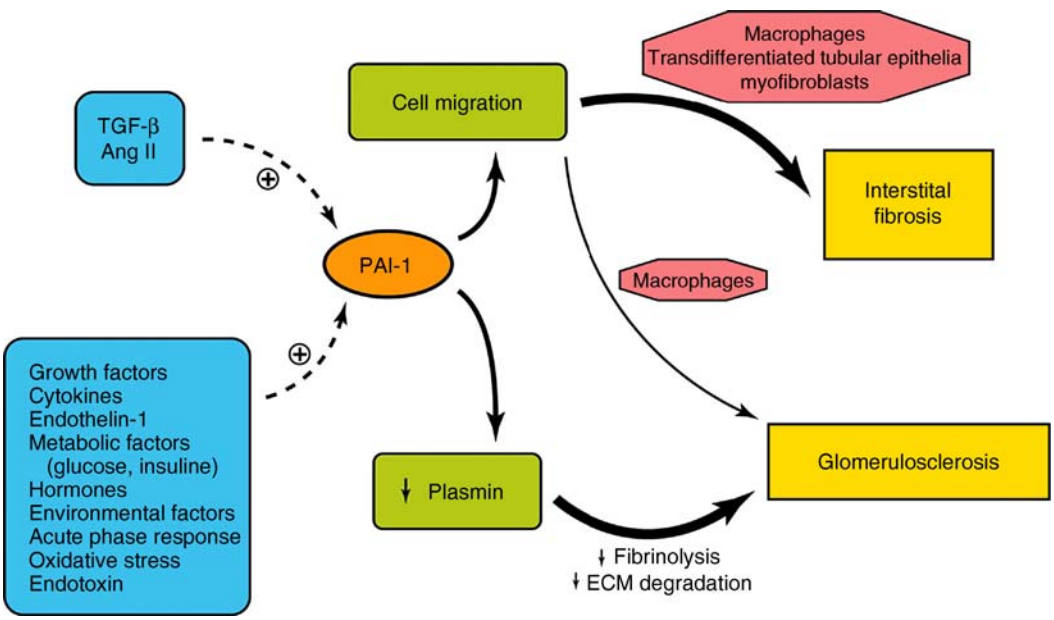
fibrosis-promoting in many solid organs (kidney, liver, lung) but current evidence suggests that in the renal interstitium, this effect is not due to serine protease inhibition (*Fig. 67-15*) (71). Urokinase-type plasminogen activator (uPA) is produced in large quantities by renal tubules but it is secreted across the apical membrane into the urinary space. Chronic injury induced experimentally by ureteral obstruction is associated with a further increase in renal uPA activity. The potential for uPA to mediate anti-fibrotic functions has been ascribed to its ability to degrade certain matrix proteins such as fibronectin, to activate plasminogen and to activate latent hepatocytes growth factor (HGF), an important anti-fibrotic growth factor. The latter action is thought to account for the ability of uPA to reduce pulmonary fibrosis (114). uPA can also serve as a fibroblast mitogen acting through classical (uPAR) and non-classical uPA receptors (LRP1, acetylcholine receptor, for example) (115). However, the observation that uPA deficiency has no effect on fibrosis severity after ureteral obstruction indicates that it plays a non-essential fibrosis-regulating role in the kidney (116). These findings do not negate the possibility that uPA might still be beneficial as a therapeutic agent. It is interesting that uPA has been used in Japan as a treatment for IgA nephropathy (117).

In the tubulointerstitial compartment, plasmin activity has been of interest as a possible anti-fibrotic pathway, especially given its ability to activate certain latent MMPs and to directly degrade matrix proteins, including fibronectin, laminin, tenascin, and perlecan Chronic kidney disease is associated with an increase in renal plasmin activity. However, when the obstructive uropathy model was compared in plasminogen wild-type and knockout mice, interstitial fibrosis was more severe in the wild-type mice (118). This difference was at least in part due to plasmin's ability to activate latent TGF-β and to promote tubular EMT via a process that involved the protease-activated receptor 1 (PAR1) and ERK signaling.

The other classical serine protease, tissue-type plasminogen activator (tPA), is traditionally viewed as an intravascular protease primarily involved in fibrinolysis. However, recent studies indicate that tPA may promote fibrosis in the kidney via at least two distinct mechanisms. tPA can bind to LRP1; when this interaction occurs on fibroblasts, their apoptotic death is inhibited (69). In the extracellular space adjacent to TBMs, tPA activity may

Figure 67-15

Plasminogen activator inhibitor-1 (PAI-1) profibrotic effects in CKD. Renal PAI-1 expression can be induced by a number of factors involved in disease pathogenesis. The ability of PAI-1 to reduce plasmin activity appears to promote thrombotic and necrotizing glomerular lesions, many of which progress to sclerosis. However, within the tubulointerstitium the pro-fibrotic effects of PAI-1 align more closely with its ability to promote cell migration, of monocytes/macrophages, transdifferentiated tubular epithelia and (myo)fibroblasts in particular. The figure is reproduced from (71).



increase MMP-9 dependent-TBM destruction to facilitate EMT and worsen fibrosis (70).

At this juncture it is not at all clear how matrix remodeling occurs normally and in pathological states. However, it is clear that matrix turnover does occur and that even early interstitial fibrosis can be reversed. This has been shown in models of self-limited kidney injury such as acute tubular necrosis and puromycin aminonucleoside nephrosis when mild interstitial fibrosis disappeared with resolution of the primary disease process (44). Recent studies in a model of reversible ureteral obstruction demonstrate that even more advanced fibrosis can partially regress with time (119) (*Fig. 67-16*). Regression of glomerulosclerosis has also been reported (120).

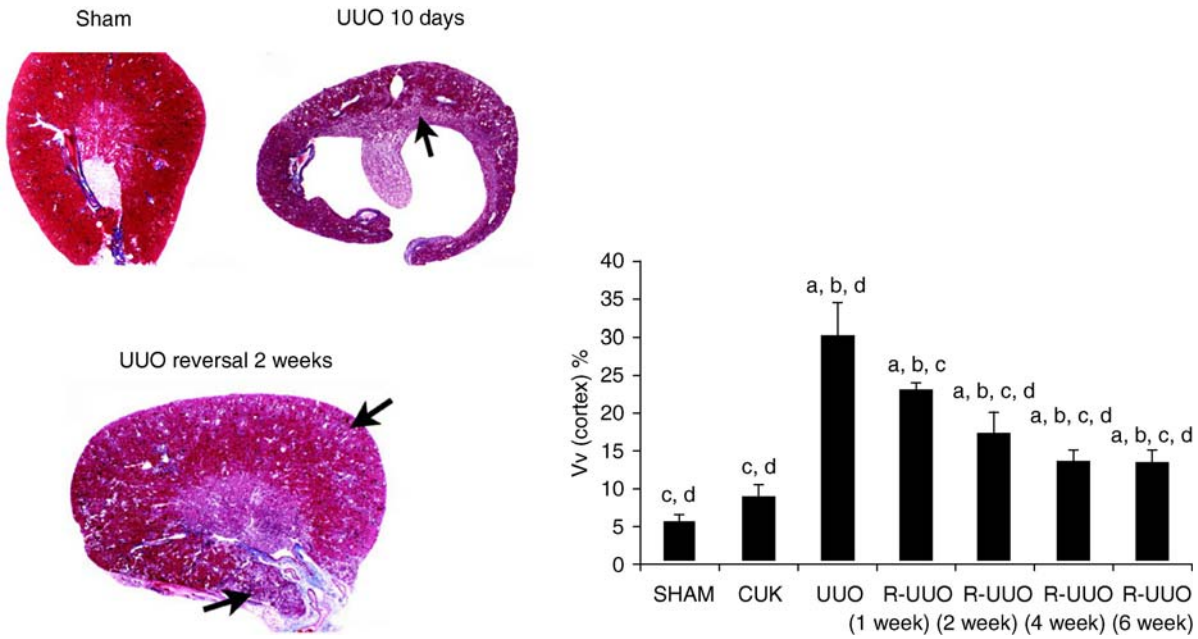
With the recent recognition that the endocytosis receptor Endo180 (also known as uPAR-associated protein) binds to several collagen proteins, it raises the possibility that lysosomal proteases and intracellular degradation pathways may play a significant role in renal fibrogenesis (121). Indeed, we have recently found that renal fibrosis is significantly worse in Endo180 knockout mice (122).

The effects of matrix-degrading proteases may differ in the glomerulus, especially in diseases such as thrombotic microangiopathies where significant fibrin deposition is a factor. In these situations, the serine proteases may play a significant role in reducing glomerulosclerosis. Glomerular PAI-1 expression is also an important harbinger of glomerulosclerosis, though the extent to which its role in glomeruli is dependent on protease inhibition or other biological effects is unclear (*Fig. 67-15*) (71). Certain members of the MMP family, such as MMP-2, have been shown to interact directly with mesangial cells, inducing proliferation and activation pathways (123). Thus, much more needs to be learned about the mechanisms involved in remodeling mesangial matrix in areas of glomerular fibrosis. Megsin is a unique serine protease inhibitor that has been identified in human glomerulopathies associated with mesangial cell proliferation and matrix expansion. Megsin transgenic mice develop glomerular lesions (124). Although it inhibits plasmin, it is not clear if this or other biological actions of megsin underlie mesangial matrix expansion in the transgenic mice. Its role in glomerulosclerosis remains unclear.

An emerging story of interest is that several small peptides derived from ECM proteins have significant effects on angiogenesis. These include the inhibitors arresten, canstatin and tumstatin derived from collagen

Figure 67-16

Renal fibrosis regression. Studies in a mouse model of reversible unilateral ureteral obstruction (UUO) illustrate the potential for fibrosis regression (as shown in the interstitial cortical volume [Vv cortex] graph) and reconstitution of renal tubules (shown in the photomicrographs) after UUO was released (R-UUO). The figures are reproduced from (119). (See color plate 45)



IV (125). Angiostatin, an internal fragment of plasminogen, and thrombospondin also inhibit angiogenesis. These observations suggest that there may be a close interplay between matrix turnover and angiogenesis in the renal interstitium. Pathways that preserve the interstitial microcirculation should help to attenuate fibrosis severity.

Mechanisms of Renal Fibrosis: Oxidant Stress

Reactive oxygen species have long been implicated in the pathogenesis of acute ischemic renal diseases such as acute tubular necrosis. The best clinical translation of this information is the use of the antioxidant N-acetylcysteine to prevent contrast-induce nephropathy (126). Beginning with studies in diabetic nephropathy, the importance of oxidant stress as a participant in kidney disease progression has been increasingly recognized. However the metabolic, cellular and molecular pathways involved are complex and thus the specific pathogenetic mechanisms in CKD are far from clear.

Oxidant stress is usually triggered by hypoxia and characterized by the production of reactive oxygen species (ROS)* due to the presence of an unshared electron pair: oxygen (O_2) → superoxide (O_2-)* → hydrogen peroxide (H_2O_2) → hydroxyl radical ($OH^{\cdot}$)* → water. CKD is characterized as an "oxidant stress" state systemically as well as locally within the kidney (127). ROS may oxidize DNA, proteins or lipids with important functional consequences. It is increasing recognized that ROS may also modulate intracellular signaling responses to potentiate tissue injury (128). Despite its high blood flow, tissue oxygen tension is rather low in the kidney (30 mm Hg in the cortex; 10 mm Hg in the medulla) making it especially vulnerable to hypoxic injury.

Summarized in *Fig. 67-17* are the four primary pathways that can induce oxidant stress: the classical pathway activated by mitochondrial dysfunction; the chlorinated pathway dependent on myeloperoxidase activity typically associated with inflammation; nitrosative stress that occurs when nitric oxide is available to produce reactive nitrogen species such as peroxynitrate (a situation that also depletes nitric oxide, a potentially beneficial product); and, the carbonyl stress that occurs when glucoxidation generates advanced glycosylation end-products (AGE). Iron catalyzes $OH^{\cdot}$ production via the Haber-Weiss reaction. There is experimental evidence that tubular iron accumulation (as a consequence of transferrin filtration during proteinuric states for example) enhances tubular damage and renal dysfunction (127).

Tissues have developed an array of antioxidant defense mechanisms that include catalase, glutathione peroxidase and superoxide dismutase. Thus intrarenal oxidant stress may also occur as a consequence of reduced activity of endogenous antioxidants.

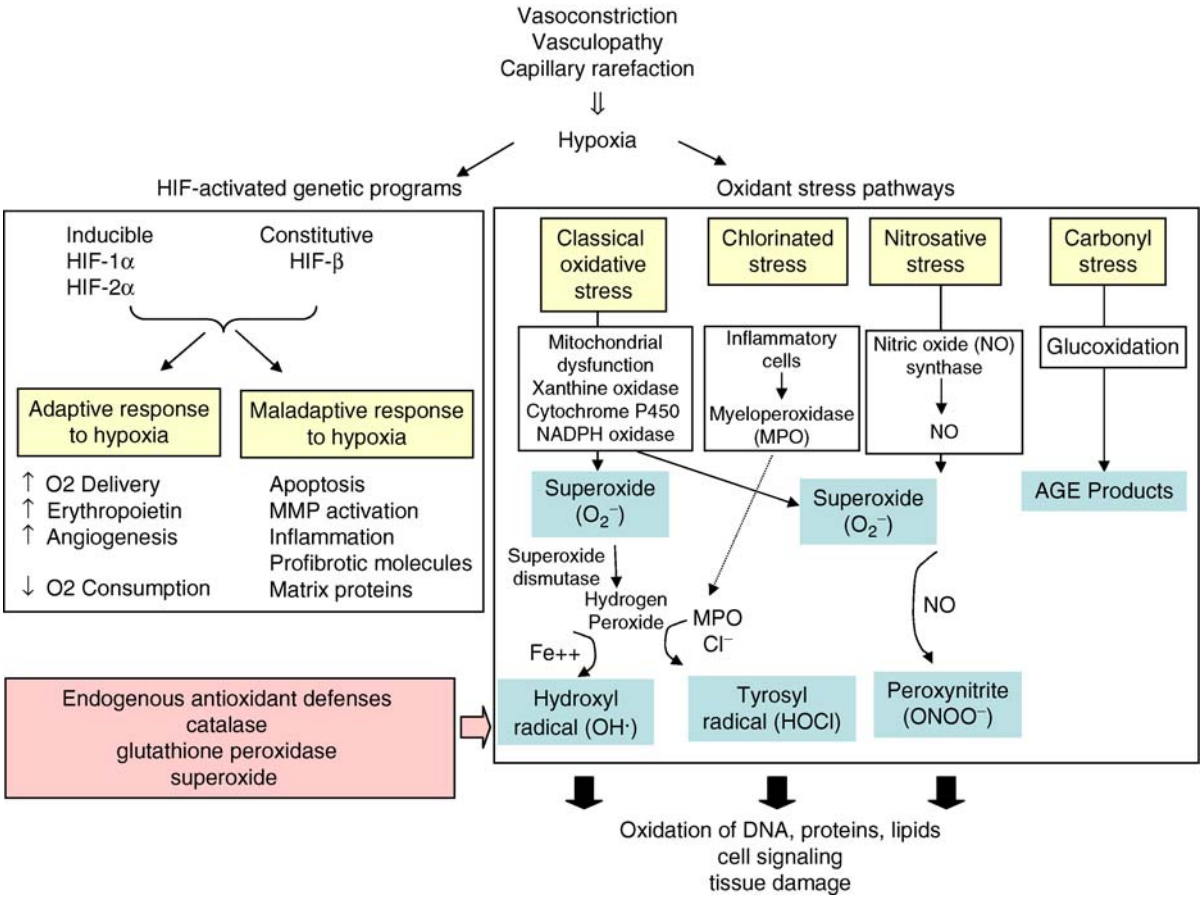
Another important consequence of renal hypoxia that may contribute directly to fibrosis is the activation of the hypoxia inducible factor (HIF) transcription factors (*Fig. 67-17*). HIF-β is constitutively and ubiquitously expressed; HIF-α expression (3 isoforms) is induced by hydroxylation in response to hypoxia. Numerous genes express HIF-responsive elements. Many of them encode proteins that allow cells to survive in a low oxygen environment such as erythropoietin, angiogenic factors, vasoactive molecules and proteins that promote anaerobic energy metabolism. However other HIF-dependent responses can lead to inflammation, EMT and fibrosis (129). The HIF-α isoforms are differentially expressed; in a model of acute renal ischemia HIF-1α was detected in tubules while HIF-2α was associated with erythropoietin-producing interstitial fibroblasts (130). Genetic deletion of renal tubular HIF-1α inhibited interstitial fibrosis (131). More work is needed to determine if these differential expression patterns occur in CKD and activate distinct genetic programs.

Much remains to be learned about the cellular and molecular basis of oxidant stress-induced kidney injury. Protein and lipoprotein modifications by reactive oxygen species is presumed to have significant functional consequences, yet most of the specific molecular targets remain to be identified. One known reaction produces oxidized low density lipoprotein (oxLDL). While hyperlipidemia is common in CKD patients, oxLDL may be generated within damaged kidneys even when serum lipoprotein levels are normal, due to an imbalance in the endogenous kidney pro-oxidant and antioxidant systems. Unlike native LDL that binds to LDL receptors primarily expressed in the liver, oxLDL bind instead to a unique family of scavenger receptors that are expressed by inflammatory and endothelial cells. These interactions play an essential role in atherogenesis and similar events within the kidney contribute to CKD progression. Renal tubular epithelial cells have recently been identified as an important source of scavenger receptors that are activated by oxLDL (132). Scavenger receptors (SR) are currently divided into 7 families (A – G), only some of which bind oxLDL. The later include receptors in class A (SR-AI, SR-AII), class B (CD36), class D (CD68), class E (LOX-1) and class G (SR-PSOX/CXCL16).

The classical macrophage scavenger receptor (SRA) and CD36 are the primarily oxLDL receptors implicated in atherogenesis; both are expressed by macrophages and renal tubular cells. Studies comparing renal outcomes in

Figure 67-17

Functional consequences of renal hypoxia in CKD progression. Schematic summary of the hypoxia-response pathways that are activated in chronically damaged kidneys and thought to perpetuate renal damage. The hypoxia-inducible factors (HIF) are a family of transcription factors that regulation the expression of a large number of genes, many of which are detrimental to the kidney. In addition, four distinct pathways synthesize several reactive oxygen species that contribute to ongoing kidney injury.



wild-type and CD36-deficient mice have identified CD36 as an important profibrotic pathway. Obstructed kidneys of CD36-deficient mice are characterized by lower levels of the pro-inflammatory transcription factor nuclear factor kappa B (NFκB) and lower levels of cytochrome C and hypochlorous acid halides, indicating reduced activity of pro-oxidant pathways and by significantly less interstitial fibrosis compared to wild-type mice (133).

Elucidating the expression and function of other scavenger receptors in chronic kidney disease is incomplete. Lox-1 is normally expressed by endothelial cells but tubular expression has been observed in response to obstruction and diabetes (132, 134). Dyslipidemia is also thought to contribute to the genesis of glomerular inflammation and fibrosis via an activating effect on macrophages and mesangial cells although little is known about the cellular pathways involved (135, 136). There is considerable experimental evidence that inhibition of 3-hydroxy-3-methylglutaryl CoA reductase by statins attenuates CKD but it is not clear if this occurs as a consequence of cholesterol reduction or other effects since these drugs have also been shown to blunt inflammation and/or fibrosis via other mechanisms.

Mechanisms of Fibrosis: Pro-Fibrotic Molecules

Clearly, there are a multitude of molecules that are activated in order to execute a full fibrogenic response (*Fig. 67-3*). A key question is which molecules initiate this cascade of events? At present, most pathways incriminate TGF-β. This growth factor plays a vital role

in wound healing responses and immune surveillance. Due to transplacental transport and secretion into breast milk, TGF-β genetically deficient mice are normal until weaned. Thereafter, they die within a few weeks due to a systemic inflammatory disease and cardiopulmonary failure (137). In the absence or TGF-β, advanced fibrosis may not occur although experience with the mice illustrates the dangers inherent in systemic TGF-β inhibition. Understanding mechanisms of TGF-β activity thus becomes critically important if novel therapeutics are to be developed (138, 139). TGF-β (tissue fibrosis is primarily associated with the TGF-β1 isoform) is produced as a latent molecule that can be activated by different agents, including the proteases (cathepsins, plasmin, furin, MMP), thrombospondin and αvβ6 integrin. Monocytes and macrophages are a major cellular source; some intrinsic renal cells may also produce TGF-β and they all express its receptors. Binding to decorin, biglycan (accumulating in the interstitium) or soluble TGF-β receptors inhibits TGF-β activation. This information has been used successfully as therapeutic interventions in experimental CKD models to inhibit TGF-β activity and reduce fibrosis severity.

The active form of the growth factor requires a dimeric receptor complex: the type II receptor captures the ligand and interacts with receptor I to initiate phosphorylation and intracellular signaling (140). Several intracellular pathways appear to be involved, activated by phosphorylation of serine threonine kinase activity of the TGF-β RI. The Smad 2/3 signaling pathway is typically implicated in profibrotic responses. Mice with genetic Smad3 deficiency develop significantly less fibrosis after ureteral obstruction (141). An important endogenous inhibitor of the Smad 2/3 pathway is Smad7. Interventions that increase renal Smad7 levels reduce fibrosis severity (142). There are additional endogenous pathways that block TGF-β-dependent fibrosis including Smurf, SnoN and Ski. Numerous target genes that promote fibrosis are activated by this pathway, including matrix genes, integrin receptors, tubular cell transdifferentiation pathways and fibroblast activation. Some effects of TGF-β are dose-dependent and cell and tissue specific. These include cell proliferation and apoptosis. In particular, fibroblasts may proliferate while epithelial cells undergo apoptosis – both with negative consequences in the tubulointerstitium of a damaged kidney.

Two TGF-β target genes deserve further mention due to their own intrinsic fibrosis-promoting effects that may amplify the fibrogenic cascade. The first is connective tissue growth factor (CTGF), also known as CCN2, a protein induced by TGF-β that promotes fibrosis via interaction with its receptor LRP1. CTGF has been reported as increased in a variety of human and animal models of chronic kidney disease and implicated in renal fibrosis (143).

The other TGF-β-regulated protein is PAI-1, which is often induced when TGF-β is active. Not detected in normal kidneys, PAI-1 is typically present in glomeruli undergoing sclerosis and within scarring interstitial regions. In addition to TGF-β, numerous other PAI-1 agonists are known (*Fig. 67-15*) (144). Another serine protease inhibitor, protease nexin-1 is often up-regulated together with PAI-1. Expression of protease nexin-1 has also been reported in mice with cryoglobulinemic nephropathy but its functional role has yet to be determined (145). That PAI-1 plays a significant role in CKD has been shown in studies of obstructive nephropathy in genetically engineered mice: PAI-1 deficiency reduces fibrosis while PAI-1 over-expression exacerbates fibrosis (71). In experimental models of glomerulonephritis and diabetic nephropathy, mesangial matrix expansion is blocked by a mutant PAI-1 peptide that functions as an antagonist of endogenous PAI-1 (146).

Many cells can produce PAI-1. Hepatic production occurs as an acute phase response. Adipose tissue is another important source of PAI-1 (147). PAI-1 mRNA is not detected in normal kidneys but in response to injury inflammatory cells, glomerular cells, tubular cells and myofibroblast may produce it (71, 144). Exactly how PAI-1 promotes fibrosis is still under investigation, but recent data suggests that in the tubulointerstitium its ability to facilitate the recruitment of myofibroblasts and macrophages is most important. This is a complex process that involves PAI-1 interactions with vitronectin, the urokinase receptor and some of its co-receptors (integrins and LRP1 in particular). The classical urokinase receptor (uPAR or CD87) is a complex receptor that does not signal alone. Working in collaboration with LRP1, it serves as the only currently known endocytic degradation pathway for PAI-1 which may explain in part why renal fibrosis after ureteral obstruction is more severe in uPAR-deficient mice (113).

Two molecular pathways are of particular interest as potent TGF-β agonists, as they are highly expressed in the kidney during fibrotic reactions and orally active inhibitors are already available for clinical use in humans: the renin-angiotensin system and the endothelin system. In addition to its important role in blood pressure regulation, all components of the renin-angiotensin-aldosterone system (RAAS) are expressed locally within the kidney and are often activated in CKD. Coupled with the local expression of the angiotensin II (ATII) receptor, aldosterone and renin receptors this local system makes an

important contribution to initial as well as ongoing kidney injury (148). ATII receptor signaling is known to induce the expression of several inflammatory and fibrogenic signals, especially TGF-β. There is also evidence that it can directly stimulate matrix protein synthesis. These effects likely explain why beyond their important antihypertensive effects, angiotensin converting enzyme inhibitors (ACE) and angiotensin receptor blockers (ARB) have impressive renoprotective effects. Numerous experimental studies have documented the ability of ACE and/or ARB therapy to reduce TGF-β activity and renal fibrosis severity. These observations are supported in mice with genetically-regulated differences in the activity of the RAAS system. The RAAS also regulates rates of urinary protein excretion which are closely associated with the severity of tubulointerstitial inflammation and fibrosis. Since polymorphisms in ACE-encoding gene are associated with enzyme activity levels (highest with the deletion [D] and lower with the insertion [I] variants), there has been considerable interest in ACE genotyping as a determinant of CKD outcomes. Results have been highly variable, perhaps due to ethnicity differences (148).

In addition to its role in regulating angiotensin II levels, the ACE degrades bradykinin. Some of the renoprotective effects of ACE inhibitors may be attributed to increased bradykinin levels. Mice with impaired bradykinin responses due to a genetic bradykinin B2 receptor deficiency or treatment with a receptor blocker develop severe fibrosis in response to ureteral obstruction (149).

In addition to its vasoconstrictor properties endothelium-1 (ET-1) is known to promote inflammation and fibrosis in damaged kidneys (150). ET-1 transgenic mice are not hypertensive yet they develop progressive renal fibrosis. Tubular ET-1 production in response to proteinuria has been suggested as one mechanistic link between glomerular with progressive tubulointerstitial disease (26). Orally active ET-1 receptor (ETA and ETB) blockers have been reported to reduce renal fibrosis although ETB blockade appears to accelerate renal cystic disease (151).

Platelet-derived growth factor (PDGF) is known to exist in four isoforms (A to D). PDGF is a potent mitogen for mesangial cells, which likely account for its role in proliferative glomerular diseases (152). In chronic tubulointerstitial disease, tubular and interstitial cells produce PDGF-B, -C and –D and express both receptors (PDGFR-α and -β). Genetic ablation of several members of the PDGF family causes embryonic death. Renal interstitial abnormalities were observed in the face of combined PDGF-A and -C or PDGFR-α deficiency. Up-regulated PDGF expression has been reported in several human kidney diseases as well as animal models. Infusing rats with high doses of PDGF-B rapidly induces the appearance of interstitial myofibroblasts. Evidence that PDGF contributes to interstitial fibrosis comes from studies in experimental models using neutralizing antibodies to PDGF-AB or –C or –D or a PDGF-B-specific oligonucleotide aptamer (152). The PDGF receptor kinase blocker imatinib has been protective in several models of CKD but not in renal ischemia/reperfusion injury. PDGF-B is also known to be a potent mesangial cell mitogen that is up-regulated in several proliferative glomerular diseases. Its interaction with mesangial cells also induces the synthesis of several pro-inflammatory and pro-fibrotic proteins.

Additional cytokines, growth factors and hormones have been implicated in fibrogenesis. Basic fibroblast growth factor (FGF-2) expression has been associated with podocyte injury and glomerulosclerosis; and with fibroblast proliferation, EMT and interstitial fibrosis (153, 154). Increased expression acidic of FGF (FGF-1) by inflammatory cells and its receptor FGFR-1 by renal tubules has been reported in human chronic kidney diseases. Interleukin-13 (IL-13) is fibrogenic in the liver and the lung due to its ability to induce TGF-β expression and activation. The soluble decoy receptor IL-13Rα2 can attenuate fibrosis progression in these organs. However, in the kidney IL-13 has been shown to have renoprotective effects in acute models of injury associated with inflammation such as ischemia-reperfusion, transplantation and crescentic glomerulonephritis while infusion of an IL-13-expressing expression vector induced minimal-change-like nephropathy (155–157). Further studies are needed to delineate the role on IL-13 in CKD. Epidermal growth factor (EGF) can induce cell proliferation, migration and collagen synthesis. Interaction with its receptor EGF-R, which also binds transforming growth factor (TGF)-α, has been shown to play a role in the development of renal cysts (158). Mice lacking TGF-α or over-expressing a dominant-negative EGF-R develop less fibrosis in experimental kidney disease models (159, 160). The pro-inflammatory cytokine tumor necrosis factor (TNF)-α is of current interest due to the availability of biological neutralizing agents available for use in humans. Mice with a genetic deficiency of TNF-α receptors (TNFR1 or TNFR2) developed less inflammation and fibrosis after ureteral obstruction while TNF-α blockade with a neutralizing antibody or soluble receptor was reported to reduce glomerulosclerosis and interstitial fibrosis respectively (161–163). It may be that the pro-fibrotic effects of TGF-α occur as a secondary consequence of persistent inflammation. Tubular expression of parathyroid hormone-related protein (PTHrP) has been detected in

both and chronic models of renal injury. Evidence that it promotes renal fibrosis comes from studies in transgenic rats that over-express PTHrP in renal tubules and develop more severe inflammation and fibrosis following renal injury (164).

Deciphering intracellular signaling pathways has identified specific molecular targets that might be accessible to therapeutic agents that are able to penetrate cell membranes. Smad 2/3 inhibition or deficiency reduces the fibrogenic effects of TGF-β. Drug inhibitors of p38 mitogen-activated protein kinases (MAPK), Ras-Raf-Mek-Erk and the Rho kinase ROCK have reduced renal fibrosis in experimental models (165). Protein kinase C inhibitors have reduced interstitial fibrosis in experimental diabetic nephropathy. Recently the Notch-1 pathway has been shown to play a role in proteinuria and glomerulosclerosis (166). Toll-like receptors (TLR) can activate fibroblasts into collagen-producing myofibroblasts suggesting that TLR signaling pathways may contribute to renal fibrosis (167).

Anti-Fibrotic Factors

Several endogenous pathways have been shown to attenuate renal fibrosis severity. Some of these are expressed within normal kidneys and chronic kidney injury is associated with activity down-regulation. Others may not have significant renal expression but systemic administration has been shown to be protective. Hepatocyte growth factor (HGF) activity has impressive anti-fibrotic effects as shown in studies of HGF-deficient mice or by therapeutic HGF administration (67). HGF can be synthesized by a variety of cells. Latent HGF requires proteolytic activation and how this is accomplished in the kidney is still unclear. In the lung but not the kidney uPA appears to be an important activation pathway (114). Endogenous HGF activator inhibitors (HAI-1 and -2) are known to be key regulators of HGF activity, but whether this pathway is important in the kidney is unknown. Relevant to its ability to prevent fibrosis, HGF blocks TGF-β-induced tubular EMT and it has significant anti-inflammatory properties.

Bone morphogenic protein-7 (BMP-7) is produced by normal renal medullary tubules. Kidneys of mice with genetic BMP-7 deficiency fail to develop normally, leading to death in the neonatal period. Recombinant BMP-7 therapy has been shown to attenuate renal fibrosis through activities closely associated with tubular EMT blockade (168). Kidneys also express molecules that modify endogenous BMP-7 activity such the inhibitor uterine sensitization-associated gene-1 (USAG-1) (169). USAG-1 knockout mice developed less fibrosis after ureteral obstruction.

Relaxin is an endogenous hormone of the insulin-growth factor family that is produced in several organs including the kidney. It inhibits TGF-β-induced matrix production and promotes fibronectin degradation (170, 171). Relaxin-deficient mice spontaneously develop renal fibrosis with aging and manifest worse fibrosis after ureteral obstruction (172, 173) while exogenous relaxin therapy had renoprotective effects in several experimental models. Heme oxygenase-1 (HO-1) is an ubiquitous intracellular enzyme that is induced by a wide variety of stimuli (174). HO-1 has important cytoprotective actions that relate in part to its role in heme degradation and to the effects of its reaction products (carbon monoxide, biliverdin, bilirubin). Antioxidant, anti-inflammatory, anti-proliferative, anti-apoptotic and vasoactive properties have been reported that may be relevant to renoprotection in CKD. Interferon gamma (IFNγ) has been associated with anti-fibrotic properties in a variety of organs but there are less data available for CKD. In a remnant kidney model IFNγ therapy was reported to reduce glomerular and interstitial fibrosis Other agents that have been associated with anti-fibrotic effects in experimental models include vitamin D (175), peroxisome proliferator-activated receptor (PPAR)-γ agonists (176) and adiponectin (177).

Fibrosis Regression and Renal Regeneration

Until excessive renal extracellular matrix deposits become highly organized, they may be degraded by specific proteolytic pathways that have not yet been clearly identified. Whether this process of fibrosis regression is associated with an improvement in renal function depends upon the status of adjacent nephrons. If intact nephrons are preserved and/or regenerated, renal function should be restored. Therefore, it is a great importance to determine how renal tubules can be preserved and what goes wrong when they degenerate and atrophy. Although still not fully understood, current evidence suggests that tubular regeneration is a self-renewal process as surviving tubular epithelial cells proliferate and repopulate regions of cell loss. Bone marrow-derived stem cells may contribute via a process of cell fusion, but their primary role may be an indirect one dependent upon the release of soluble survival factors that facilitate tubular epithelial cell regeneration or they may fuse with resident kidney cells (178–180). Most of the available data come from detailed studies of nephron recovery following acute ischemic injury, observations that require confirmation in chronic progressive models of renal injury.

Tubular regeneration is characterized by the reactivation of a set of developmentally programmed genes that

may determine cell fate. In recent years there has been considerable interest in tubular cell transdifferentiation from an epithelial to a mesenchymal cell phenotype as a pathway to matrix-producing interstitial cells. However, it also appears that this might be a reversible process (168). As long as these mesenchymally transformed cells remain confined by intact tubular basement membranes, phenotypic reversal should preserve nephron structure.

Preservation of the endothelial aspect of the tubulointerstitial barrier is also essential if surviving nephrons are to remain functional. In chronic kidney disease, the histological features of tubular atrophy and peritubular capillary rarefaction are tightly correlated but the nature of their co-dependency in survival and revival is unclear.

In summary, great advances have been made in our understanding of the cellular and molecular pathways that lead to nephron destruction and renal functional decline as a consequence of extracellular matrix accumulation. Several candidate targets for new therapeutics have emerged and captured the interest of research and development enterprises. It is clear that the process of renal fibrosis is complex and progresses in a temporally, spatially, and at times an organ-specific manner. By analogy with the lessons learned from cancer biology and therapeutics, a revolutionary new approach to CKD treatment now seems within the realm of possibility but will require the use of regimens that employ several agents that target different critical elements. Since there appears to be a common progression pathway shared by most chronic kidney diseases, such an approach should have broad clinical application. One of the greatest future challenges is the need to distinguish progressors from non-progressors amongst the population of chronic kidney disease patients.

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68 Management of Chronic Kidney Disease

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Introduction

Chronic kidney disease (CKD), although uncommon in children, can be a devastating disorder with many long-term consequences. Despite similarities to adult disease, CKD in children has many unique features that are not seen in older patients. Its manifestations can affect multiple body systems (cardiovascular, endocrine, hematopoietic, gastrointestinal, central nervous) during particularly vulnerable times of development in children with the potential for permanent sequelae. It is noteworthy that children with CKD, like adults, may often be asymptomatic in the initial stages of the disease and its complications may go undiagnosed and untreated early in its course. However, as renal function gradually declines, various signs and symptoms (e.g., volume overload, hypertension, fatigability, and failure to grow) become more evident. In most cases, the dysfunction is progressive and shares a common pathway of injury, whether the etiology of the CKD state is developmental, genetic, immunologic, metabolic, traumatic, or infectious in origin. Thus, early recognition and intervention is of significant importance as only then might the progressive loss of kidney function, and the associated morbidity, be prevented or delayed (1).

Historically, the terms chronic renal failure (CRF) and chronic renal insufficiency (CRI) have been used to describe varying degrees of renal dysfunction. In this chapter, we will use the term chronic kidney disease (CKD) as this is now the accepted terminology in the adult and pediatric nephrology communities in North America and around the globe. The use of the term chronic kidney disease allows for both consistency when discussing degrees of injury and also highlights the fact that renal dysfunction occurs along a continuum rather than as discrete steps of declining function. Also, the primary focus of this chapter is on the management of the earlier stages of CKD and does not address the management of patients with end stage renal disease (ESRD).

Estimation of GFR

The glomerular filtration rate (GFR) is the sum of the filtration rates in all functioning nephrons and an estimate of GFR provides a rough measure of the number of functioning nephrons. The normal GFR varies with age, gender, and body size with children generally achieving size-adjusted adult values of GFR by age two (2). Whereas the GFR may be measured by inulin or iohexol clearance (3), the estimation of GFR may be predicted by the measured creatinine clearance in a 24 h urine collection. Unfortunately, the accuracy of a 24 h urine collection is often compromised by incomplete collections (4) and the tubular secretion of creatinine when the GFR is decreased (5). Urine collections preceded by oral cimetidine, which blocks tubular secretion of creatinine, have also proven to be a fairly accurate means of estimating the GFR (6), although this approach is rarely used clinically. As a result, multiple prediction equations have been developed to more simply estimate GFR by using the serum creatinine concentration, with recognition of the fact that the GFR is overestimated when kidney function is decreased because of creatinine secretion. The MDRD equation has been developed for use in adults with CKD, but it has not proven to be applicable to the pediatric population (7). The Schwartz (8) and Counahan-Barratt equations (9) have been the most commonly used GFR estimating equations used in children. The Schwartz equation, which was developed based on creatinine determinations using the Jaffe methodology, estimates GFR by utilizing the subject's height in centimeters, plasma creatinine, and a constant, K, based on the subject's age and gender (see *Table 68-1*). Despite its ease of use, the Schwartz equation is imprecise in most instances, as a result of the current widespread use of enzymatic methods for creatinine measurement (10). A new and more accurate estimating equation has been developed by the investigators of the Chronic Kidney Disease in Children (CKiD) study

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Table 68-1

Different constant values (K) for the Schwartz formula for the estimation of GFR

$GFR = \frac{K \times Height\ (in\ cm)}{P_{cr}\ (mg/dl)}$	
Table 1- Values of K for different age groups	
Low birth weight infants (<2.5 kg)	0.33
Infants (0–18 months)	0.45
Children 2–13	0.55
Adolescent girls (13–16)	0.55
Adolescent boys (13–16)	0.70

Table 68-2

K/DOQI stages of chronic kidney disease

Stage	Description	GFR (ml/min/1.73 m^2)
1	Kidney damage with normal or increased GFR	≥ 90
2	Kidney damage with mild decrease in GFR	60–89
3	Moderate decrease in GFR	30–59
4	Severe decrease in GFR	15–29
5	Kidney failure	< 15 (or dialysis)

using measured GFR values based on the plasma disappearance of iohexol. The formula, which is applicable to children between the ages 1–16 and with GFRs ranging from 15 to 75 ml/min/1.73m^2, incorporates the patient's height, plasma creatinine (P_{cr}), serum cystatin C level, blood urea nitrogen (BUN), and gender into the following:

$$\text{Estimated GFR} = 39.1 \times [\text{Height}/P_{cr}]^{0.516} \times [1.8/\text{CysC}]^{0.294} \times [30/\text{BUN}]^{0.169} \times [1.099^{\text{male}}] \times [\text{Height}/1.4]^{0.188}$$

A "bedside" version of the equation that can easily be used in clinical practice for patients with CKD uses the similar ratio of height (cm) divided by plasma creatinine (mg/dL) but multiplied by the set constant of 0.413, irrespective of age or gender (10).

Serum cystatin C, a protease inhibitor which is produced by all nucleated cells, has also been closely investigated as a potentially more accurate biomarker of renal function than serum creatinine. Cystatin C is freely filtered at the glomerulus but is reabsorbed and catabolized by the proximal tubule and, therefore, is not a classic marker of glomerular filtration. However, cystatin C has several advantages over creatinine. Its inverse has been shown to correlate with renal function independent of age, gender, height, and body composition in patients over 2 years of age (11) and GFR-estimating formulas using cystatin C have been developed (12, 13). All but one study comparing serum cystatin C to creatinine have shown superiority or equivalence of cystatin C as a marker of GFR in pediatric patients (14). Early criticisms of cystatin C were based upon its variability in measurement; however, with the consistent use of immunonephelometry, serum cystatin C measures have been shown to have lower intrapatient variability than serum creatinine (15). Serum cystatin C has not been endorsed to estimate renal function in CKD, mainly because the studies prior to this were characterized by limited sample size and lack of consistency in terms of the assay method (16). More recently, and as noted before, the CKiD study has incorporated the measure of cystatin C as determined by the turbidimetric assay along with blood urea nitrogen in generating the new GFR estimating equation for children with CKD (10).

Classification of CKD

In 2002, the National Kidney Foundation's Kidney Disease Outcomes Quality Initiative (K/DOQI) published their CKD classification scheme that is based on estimated GFR and is applicable to adults and children above 2 years of age (16). CKD is categorized into five different stages, from Stage 1 (mild disease) to Stage 5 (kidney failure) (see *Table 68-2*). Characterization of these different stages has allowed nephrologists and general health care providers, both adult and pediatric, to have a common nomenclature when discussing CKD with respect to the anticipation of co-morbidities and treatment plans. Although this system has been criticized, as it is argued that earlier disease stages may be better defined by the associated abnormalities (e.g., proteinuria, structural anomalies) while more advanced stages would be better characterized by the severity of renal solute clearance (17), the scheme has proven to be clinically useful and has been incorporated into everyday care.

Epidemiology of CKD

Information on the epidemiology of CKD in the pediatric population is somewhat limited, especially for less advanced stages of the disease. As mentioned previously, this is partly because of the asymptomatic nature of CKD early in its course which has resulted in it often

being underdiagnosed and underreported in children. Historical epidemiologic data have also used a variety of criteria to define chronic impairment of kidney function prior to K/DOQI. The term, chronic renal insufficiency (CRI) has traditionally been used to refer to the condition associated with an estimated GFR less than 75 ml/min/1.73 m^2. Other traditionally used terms such as mild (50–80% of normal GFR), moderate (25–50%), and severe (<25%) kidney disease do not directly correlate with the same GFRs as classified by K/DOQI, making direct comparisons of the data difficult.

Perhaps the most comprehensive pediatric epidemiologic data comes from the ItalKid Project, a prospective population-based registry which includes all incident and prevalent cases of CKD (defined as GFR <75 ml/min/1.73 m^2) in children below the age of 20 throughout Italy. This registry has reported an incidence of CKD of 12.1 cases per million of the age-related population and a prevalence of 74.7 cases per million (18). Other sources of epidemiologic data have included national surveys such as in Chile (19) and Sweden (20) which have reported incidence rates from 5.7 to 7.7 cases per million population; however, most of this data has focused on children with severe kidney disease (GFR <30 ml/min/1.73m^2). Reports from major tertiary hospitals in Jordan (21) and Nigeria (22) have reported the incidence of severe CKD to be 10.7 and 3.0 cases per million population, respectively; however these data likely reflect the accessibility to hospital care in these nations. Finally, pediatric data on CKD in North America primarily arises from the registry of the North American Pediatric Renal Trials and Collaborative Studies (NAPRTCS). It began as a voluntary transplant registry in 1987 (23) and expanded to include data on patients with CRI (GFR <75 ml/min/1.73 m^2) in 1994. Its data is gathered voluntarily from pediatric nephrologists in Canada, Costa Rica, Mexico, and the United States. While this registry contains a wealth of information on more than 6,000 children with CKD, no incidence or prevalence information is available.

Unlike adults in whom the primary etiologies of CKD are diabetes and hypertension, the greatest percentage of pediatric CKD cases are congenital in origin, with some variability among nations. In the CRI/CKD registry of NAPRTCS, nearly half of the cases have congenital causes of renal disease such as obstructive uropathy (21.1%), renal dysplasia (17.5%), reflux nephropathy (8.4%), and Prune Belly syndrome (2.8%). These disorders make up four of the six most prevalent diagnoses (see *Table 68-3*). Data from Italy has also revealed renal hypoplasia to account for the majority (nearly 58%) of all pediatric CKD cases (18). In contrast, data from the Japanese registry has revealed that 34% of their pediatric CKD cases were secondary to glomerulonephritis, primarily focal segmental glomerulosclerosis and immunoglobulin A (IgA) nephropathy (24). Heritable causes of

Table 68-3

2007 NAPRTCS report, primary diagnosis of CRI

Primary Diagnosis	Number of patients (Total N = 6,794)	Percentage
Obstructive uropathy	1,436	21.1
Aplastic/Hypoplastic/ Dysplastic Kidney	1,187	17.5
Focal segmental glomerulosclerosis	589	8.7
Reflux nephropathy	568	8.4
Polycystic disease	271	4.0
Prune Belly	192	2.8
Renal infarct	157	2.3
Hemolytic Uremic Syndrome	138	2.0
Systemic Lupus Erythematosus nephritis	108	1.6
Familial Nephritis	108	1.6
Membranoproliferative Glomerulonephritis, Types I and II	102	1.5
Cystinosis	100	1.5
Pyelo/interstitial nephritis	95	1.4
Medullary cystic disease	86	1.3
Chronic Glomerulonephritis	81	1.2
Congenital Nephrotic Syndrome	74	1.1
IgA (Berger's) Nephropathy	66	1.0
Idiopathic crescentic glomerulonephritis	46	0.7
Henoch-Schonlein nephritis	42	0.6
Membranous nephropathy	35	0.5
Wilms tumor	31	0.5
Other systemic immunologic disorders	25	0.4
Wegener's granulomatosis	21	0.3
Sickle cell nephropathy	14	0.2
Diabetic glomerulopathy	11	0.2
Oxalosis	7	0.1
Drash syndrome	6	0.1
Other	1,020	15.0
Unknown	178	2.6

CKD such as cystic kidney disease, primary hyperoxaluria, cystinosis, Alport syndrome, and congenital nephrotic syndrome have been reported to represent a greater percentage of the cases of CKD in Jordan (21) and Iran (25) where consanguinity is more common. In less developed nations, acquired etiologies of CKD seem to predominate. This may reflect differences in the ability to detect CKD in many of these nations, where patients often present in the later stages of the disease. It may also reflect the burden of infectious diseases, with subsequent infection-related glomerulonephritis, in these countries. Publications from Nigeria have reported various infections glomerulopathies as the cause of CKD in half of its pediatric patients (22), while Familial Mediterranean Fever and resultant amyloidosis was responsible for 10% of CKD cases in Turkish children (26). Human Immunodeficiency Virus (HIV)-associated nephropathy, which causes ESRD in only a small number of children in the United States (27), is also likely an underreported source of nephropathy in children, as an increasing incidence of pediatric HIV is evident in the underdeveloped regions of South America, Africa, and Asia (28), where pediatric CKD data is poorly collected or non-existent.

Characterization of the pediatric patient population having CKD shows some similarities worldwide with regards to gender. Incidence and prevalence rates are universally greater for boys than girls (18, 22, 23, 25, 29). Sixty-four percent of the patients in the NAPRTCS CRI registry and 67% of patients in the ItalKid registry are male (18, 23). This gender distribution reflects the higher incidence of congenital causes of CKD seen in boys.

Prevention of CKD Progression

Although information about children is limited, there appears to be certain modifiable and non-modifiable risk factors associated with CKD progression. Most available data shows that acquired glomerular diseases tend to progress more rapidly than congenital disorders, evident in a relatively higher proportion of glomerular diseases that are characterized by the more advanced stages of CKD (30). Renal hypoplasia and dysplasia appear to have a pattern of disease progression characterized by an initial period of improving renal function, followed by a prolonged period of stabilized function, and then ultimately a period of declining function (31). This deteriorating phase is often seen during periods of rapid growth, such as puberty, and its later appearance may account for the slower progression seen in congenital renal disorders.

Other non-modifiable factors for CKD progression include race and genetics. An increased racial susceptibility may be present in African Americans as an increased concordance of kidney disease (32) and a faster rate of disease progression have been noted in this population compared to their adult Caucasian counterparts (33). Similarly, the incidence rate of pediatric ESRD is nearly doubled in African American children compared to Caucasians (34) and is significantly higher during late adolescence, although this may reflect, in part, different incidence rates of acquired glomerulopathies such as focal segmental glomerulosclerosis. Certain genetic polymorphisms of the angiotensin-converting enzyme (ACE) gene have been associated with an increased susceptibility to disease progression in IgA nephropathy (35) and congenital uropathies (36, 37) in some, but not in all (38), studies and may also play a role.

There is clear and consistent evidence from adult studies that hypertension is a significant and modifiable mediator of CKD progression (39, 40). Numerous studies have shown that anti-hypertensive therapy slows the rate of ESRD development in adults, with a linear relationship existing between the achieved median blood pressures on therapy and the rates of renal failure progression (41). This has resulted in a target blood pressure range in adults of less than 120/80 mmHg within the guidelines from both the American (42) and European (43) expert panels. Mitsnefes and colleagues reviewed the NAPRTCS database and demonstrated that hypertensive children with CKD had a more rapid decrease in their estimated GFR or progression to renal replacement therapy than normotensive children with CKD (44). Systolic hypertension was found to be a significant, independent predictor of disease progression along with the patient's age, acquired etiology of CKD, and African-American ethnicity. A large prospective study of European children with CKD has, in fact, found that a systolic blood pressure greater than 120 mmHg was associated with a faster decline of GFR (45). However, the optimal blood pressure goal for children with CKD is not known and no published studies in pediatrics have demonstrated a slowing of CKD progression following the prospective introduction of improved blood pressure management. Presently, the recommendation from K/DOQI for children with CKD is a targeted systolic and diastolic blood pressure less than the 90th percentile for age, height, and gender or less than 130/80 mmHg, whichever is lower (46). This mirrors the recommendations of the National High Blood Pressure Education Program (NHBPEP) Working Group on Children and Adolescents (47) for children with concurrent diseases. The Effect of Strict Blood Pressure

Control and ACE Inhibition on Progression of Chronic Renal Failure in Pediatric Patients (ESCAPE) trial, a large-scale prospective study in Europe, is addressing the question of whether even lower mean blood pressure targets, such as the 50th percentile for age, provides added reno-protection in children with CKD (48).

Proteinuria has also been demonstrated to be a significant, modifiable risk factor for CKD progression, especially in adults (49, 50). In an Italian study on the efficacy of ramipril in adults (REIN study), the degree of proteinuria was the only baseline variable which correlated with a decline of kidney function towards ESRD (51). This same group also showed the efficacy of anti-proteinuric therapy with ACE inhibitors as a means of slowing GFR decline, as the reduction of proteinuria by one gram daily resulted in a decreased decline in GFR of 2 ml/min per year (52). Similarly, the European Study Group for Nutritional Treatment of Chronic Renal Failure in Children first showed that proteinuria was a significant risk factor for pediatric CKD progression from Stage 3–4 to ESRD (45). The ItalKid project also demonstrated that the degree of proteinuria predicted disease progression in patients with renal dysplasia (53). Finally, initial evidence from the ESCAPE trial has shown that the degree of residual proteinuria, irrespective of ACE inhibition, is also associated with the rapidity of CKD progression (54).

There is evidence that decreasing blood pressure alone will reduce proteinuria to some degree (39, 55), and therefore any anti-hypertensive agent may prove to be anti-proteinuric. However, while several classes of anti-hypertensive agents are often comparable in their blood pressure-lowering efficacy, there are significant differences in their ability to reduce proteinuria (55, 56). ACE inhibitors and angiotensin II type 1 receptor blockers (ARBs) are most effective in this respect, with both primary anti-hypertensive and anti-proteinuric effects. These agents, in turn, have been found to be most reno-protective. Studies of adults have shown a 30–40% reduction of proteinuria in non-diabetic patients with CKD who receive an ACE inhibitor compared to placebo (56), while ARBs have been associated with similar results in patients with diabetic nephropathy (57). The renal protection afforded by both classes of medication are likely mediated through a combination of reduced proteinuria, lowered intra-glomerular pressure, reduced sympathetic hyperactivity, and anti-fibrotic effects. ACE inhibitors may potentially have greater reno-protective effects because of the accumulation of bradykinin, with its vasodilatory and anti-fibrotic effects, that accompanies their use but is not seen with ARBs. Nevertheless, an equivalent clinical effect was seen in a comparative clinical trial between the two drug classes (58). There is limited data on the role of ACE inhibitor or ARB therapy for renoprotection in children with CKD. Small studies have shown stability of kidney function with ACE inhibitor use in patients with a history of hemolytic uremic syndrome (59) and combined ACE inhibitor and ARB use have been associated with slowing the histopathologic progression in IgA nephropathy (60). However, the ItalKid study did not show a significant change in CKD progression with the use of ACE inhibitors in children with renal dysplasia (61). The aforementioned ESCAPE trial is also evaluating the impact of ramipril therapy on CKD progression in children (48).

Other anti-hypertensive medication classes have been associated with effective reduction in GFR decline in patients with CKD but without effective anti-proteinuric effects, with two exceptions. The beta-blocker metoprolol was shown to have anti-proteinuric effects comparable to ramipril in the African American Study of Kidney Disease and Hypertension trial (55) and the newer beta-blocker carvedilol has also been shown to have improved anti-proteinuric effects compared to other beta-blockers (54). Perhaps direct adrenergic stimulation of the renal sympathetic nervous system is as important in the progression of CKD as the other theorized etiologies that ACE inhibition targets and accounts for these results.

Other methods have been proposed as means by which CKD progression might be slowed, purportedly by targeting other mechanisms, such as oxidative stress and inflammatory pathways. Proposed methods have included treatment of dyslipidemia, early erythropoietin therapy, phosphorus control, and dietary protein restriction. Some of these interventions compliment data collected from the NAPRTCS database which identified low values for serum albumin (<4.0 gm/dl), serum calcium (<9.5 mg/dl) and hematocrit (<33%) and elevated values of serum phosphorus (>5.5 gm/dl) and BUN (>20 mg/dl) as risk factors for progression to ESRD (62). Lipid-lowering therapy is commonly prescribed for adults with CKD for the primary prevention of cardiovascular morbidity and mortality. However, evidence also suggests that statin therapy may slow renal disease progression as well through its anti-inflammatory and oxidative stress reducing properties (63). Additionally, there has been evidence that statins may possess the ability to limit proteinuria in adults (64). However, the utility of statins to slow CKD progression in children has not been proven.

Although erythropoietin therapy has not been shown to influence the progression of CKD in children, adult studies have provided evidence that early therapy of mild to moderate anemia in patients with CKD may slow the progression of kidney disease (65) by reducing oxidative

stress in the renal interstitial and tubular cells and/or by the mobilization of progenitor cells for renal tissue injury repair (66).

Treatment with active vitamin D has been associated with attenuation of CKD progression in animal models (67), but it is not yet clear if the same effect exists in humans. Studies in adult CKD have suggested that dietary phosphorus restriction may help reduce progression of kidney disease (68) and oral paricalcitol has been shown to have an anti-proteinuric effect in adults (69).

Lastly, there is no evidence that a low-protein diet will affect the progression of CKD in children. For decades, it had been hypothesized that dietary protein restriction would diminish renal injury as high protein diets have been associated with increased renal scarring in animal models (70). In adults, the Modification of Diet in Renal Disease (MDRD) trial did not demonstrate that a restricted protein intake could modify the progression of CKD in adults with non-diabetic nephropathies (71). Likewise, Wingen et al. showed no efficacy of a low protein diet on the decline in GFR in children with CKD after 1 year of treatment (72). Not surprisingly, there is also concern that caloric intake and growth may be compromised in pediatric patients on a restricted diet.

Anemia in CKD

Anemia is one of the most common complications of CKD during childhood and its near universal presence in patients with advanced disease has prompted the development of anemia evaluation and treatment guidelines in both Europe and North America (73, 74). The treatment of CKD was revolutionized in 1986 with the introduction of recombinant human erythropoietin (rHuEPO or EPO) therapy (75). Despite the proven benefits of correcting anemia in children with CKD and the availability of consensus recommendations, there remain several unresolved issues about anemia management in pediatric CKD, including target goals of treatment and factors contributing to the frequent presence and persistence of anemia in patients with early stages of CKD.

Definition of Anemia

The 2000 K/DOQI Anemia Management guidelines defined anemia as a hemoglobin value less than 11 gm/dl in pre-pubertal patients with CKD (76). This, in turn, was translated into the threshold goal for therapy. However, the guidelines did not take into account the fact that hemoglobin values in children vary greatly by age and gender. The NHANES III Study (see *Table 68-4*) showed that the fifth percentile for hemoglobin may vary by as much as 2.8 gm/dl from younger to older boys and by 2 gm/dl for girls and boys of a similar age group (77). This wide variation in hemoglobin values was studied in 350 children with CKD by Wong et al. (78). There were significant differences in the prevalence of anemia using the age- and gender-specific reference intervals compared to the uniform level originally recommended by the guidelines committee, as 90 subjects met the age- and gender-variation definition versus only 54 subjects who did so by the K/DOQI criteria ($p<0.001$). More recently, Staples et al. reviewed the NAPRTCS database and found a 25% increase in the prevalence of anemia in pediatric CKD subjects when using the age and gender appropriate norms for hemoglobin compared to the age-independent definition of anemia previously used by K/DOQI (79). The recent revision of the K/DOQI anemia guidelines now states that anemia is present in the pediatric CKD patient "when the observed hemoglobin is less than the fifth percentile of the normal, adjusted for age and sex" (80).

Pathophysiology of Anemia

Multiple factors may contribute to the development of anemia in pediatric patients with CKD (see *Table 68-5*). The principal cause is the diminished production of erythropoietin by the interstitial cells of the renal cortex. As GFR declines, there is a decrease in the fractional reabsorption of sodium by the kidney and a decrease in oxygen utility. This, in turn, leads to an increase in kidney tissue oxygen pressure and a subsequent decrease in

Table 68-4

Hemoglobin levels from NHANES III for boys and girls of all race/ethnic groups according to age

Age (years)	5th percentile hemoglobin level for boys (gm/dL)	5th percentile hemoglobin level for girls (gm/dL)
1–2	10.7	10.8
3–5	11.2	11.1
6–8	11.5	11.5
9–11	12.0	11.9
12–14	12.4	11.7
15–19	13.5	11.5

Table 68-5

Common causes of anemia in chronic kidney disease

Erythropoietin deficiency
Iron deficiency
- Dietary iron deficiency
- Gastrointestinal loss, Phlebotomy, Menses
- Poor absorption of enteral iron
- Iron depletion from ESA use
Chronic inflammation
- Complement activation from dialysis
- Systemic inflammatory diseases (systemic lupus erythematosus, Wegener's granulomatosis,. . .)
- Surgical procedures
Bone marrow suppression
- Inhibitory factors
- Hyperparathyroidism
- Medications (immunosuppressive drugs)
Increased red cell turnover
- Carnitine deficiency
- Primary renal disease (Hemolytic Uremic Syndrome)
Malnutrition
- B12 or Folate deficiency
- Carnitine deficiency
Aluminum toxicity

erythropoietin production (81). Decreased erythropoietin leads to an increase in apoptosis of erythroid progenitor cells (82) and decreased red cell maturation. Children with CKD have been shown to have erythropoietin levels that are inappropriately low for their degree of anemia (83), which was generally thought to occur when the GFR decreases below 35 ml/min/1.73 m^2, with a linear correlation between hematocrit and creatinine clearance noted (83, 84). Most recently, results from the CKiD study showed that hemoglobin levels decreased by 0.1 gm/dL for every 5 ml/min decrease in measured GFR until the GFR fell below 43 ml/min/1.73 m^2 (85). Below that level, the decline in hemoglobin was 0.3 gm/dL for every 5 ml/min drop in GFR. However, the prevalence of anemia in children with Stage 2 CKD has been estimated at 19–30% (78, 79, 85) highlighting the fact that there is not an "absolute threshold" for anemia in CKD.

Iron deficiency is another factor that frequently contributes to the development and/or persistence of the anemia associated with CKD. The etiology of iron deficiency is multi-factorial. Patients with CKD may experience greater than normal blood loss, estimated at 6 ml/m^2 daily from gastrointestinal losses (86) as well as from the repeated phlebotomy necessary for routine serum laboratory tests. Low dietary intake of iron may occur as a result of anorexia related to advanced stages of CKD and there may be poor adherence with oral iron supplementation secondary to gastrointestinal side effects (87).

Recently, hepcidin, a hepatically-activated peptide first discovered because of its anti-bacterial properties (88, 89), has been found to be a key regulator of systemic iron homeostasis in patients with CKD (90). Hepcidin affects the turnover of the iron exporter, ferroportin, on the cell surface of enterocytes and attenuates the uptake of iron by the intestine. Taes et al. have shown that serum hepcidin correlates inversely with creatinine clearance (91) and its serum accumulation may have a direct negative effect on enteral iron absorption (92).

Hepcidin accumulation may also be one of the main mechanisms by which inflammation contributes to the development of the anemia of chronic disease. Inflammatory markers are often increased in patients with CKD, either secondary to uremic impairment of the immune system, decreased clearance of inflammatory factors (93), or directly from systemic diseases such as vasculitides. The cytokine interleukin-6 (IL-6) is a key mediator in the inflammatory-anemia effect as IL-6 has been shown to increase the liver production of hepcidin (94). Hepcidin also affects ferroportin on the cell membrane of macrophages in addition to enterocytes, preventing iron release from the reticulo-endothelial system and limiting iron bioavailability. Thus, hepcidin may be the key mediator in the inflammatory "block" to the utilization of total body iron stores in patients with CKD.

The erythrocyte life span is also shortened in children with CKD (86) and may contribute to the development of anemia. Erythrocyte survival in CKD patients has been shown to increase through the use of rHuEPO (95) although the direct mechanism is not known. Carnitine deficiency may also reduce the strength of the red cell membrane (96) and contribute to decreased red cell survival, an effect primarily noted in hemodialysis patients associated with dialysis-related carnitine removal. In addition to decreased erythropoietin production and red cell survival, bone marrow suppression may also occur in patients with CKD. In vitro assays have shown erythropoiesis to be suppressed with the addition of serum from children with CKD (83). The inhibitory substance in the serum of patients has not yet been identified, but it is thought to be effectively removed by hemodialysis, as rHuEPO doses often decrease after initiation of that dialysis modality (97). Hyperparathyroidism has also been

shown to cause suppression of erythropoiesis (98) and may also contribute to the anemia seen in CKD.

Finally, malnutrition may contribute to the anemia seen in the later stages of renal dysfunction. A low serum albumin level has been associated with the anemia of CKD (99). However, a low serum albumin may merely be a surrogate marker of either CKD severity or inflammation, rather than having a direct contribution to anemia. In addition to deficiencies of iron or carnitine, deficiencies of other water-soluble vitamins, such as B12 and folate, may also contribute to anemia. Routine folate supplementation has been shown to improve the response to rHuEPO even without frank serum folate deficiency (100).

Evaluation of Anemia

All children with CKD, regardless of the disease etiology or stage, should have their serum hemoglobin checked at least annually (80). In younger children, more frequent laboratory monitoring for CKD stage and hemoglobin level is often performed because of expected changes in values during growth, although no specific recommendations for an age-related frequency of laboratory evaluation have been made (80). The serum hemoglobin level rather than the hematocrit should be used as the standard assessment for anemia, as the hematocrit may vary from changes in volume status, body temperature, and blood sugar (101).

Once the diagnosis of anemia is made, the initial evaluation of anemia in patients with CKD should include a complete blood count with red blood cell indices, reticulocyte count, serum ferritin and iron levels, and a total iron binding capacity. Erythropoietin deficiency typically causes a normocytic anemia with an inappropriately decreased reticulocyte count, although a microcytosis may be seen when the anemia is secondary to iron deficiency or chronic disease. Other contributing factors to anemia should be considered if abnormalities of the other cell lines or red cell indices are found (see *Table 68-6*). Decreased white blood cell or platelet counts should elicit inquiry as to other factors possibly contributing to bone marrow depression such as transient viral infections, malignancy, medication side effects, or an auto-immune disorder. A high mean corpuscular volume (MCV) suggests a folate or vitamin B12 deficiency while a low MCV may be seen in thalassemia and other hemoglobinopathies, in addition to iron deficiency and anemia of chronic disease. An elevated reticulocyte count should prompt consideration of either blood loss or hemolysis.

Iron status is evaluated by both the serum ferritin and transferrin saturation levels. While serum ferritin levels reflect total iron body stores, it is also an acute phase reactant and may be elevated in the face of systemic

Table 68-6

Differentiation of types of anemia based on mean corpuscular volume (MCV) and red blood cell distribution width (RDW)

	Low MCV (microcytosis)	Normal MCV	High MCV (macrocytosis)
High RDW	Iron deficiency	Early iron deficiency	Folate deficiency
	Hb S-β thalassemia	Hemoglobinopathy (SS, SC)	Vitamin B12 deficiency
	Hemoglobin H	Myelofibrosis	Hemolytic anemia
	Erythrocyte fragmentation	Sideroblastic anemia	Immune hemolytic anemia
			Cold agglutinin
Normal RDW	Heterozygous thalassemia	Normal	Aplastic anemia
	Chronic disease	Chronic disease	Pre-leukemia
		Chronic renal failure	
		Chronic liver disease	
		Hemoglobinopathy (AS, AC)	
		Transfusion	
		Chemotherapy	
		Hemorrhage	
		Chronic myelocytic leukemia	
		Hereditary spherocytosis	

Reproduced from Management of Renal Anemia in *Pediatric Dialysis* by Warady B, et al., Springer, 2004

inflammation or malnutrition, compromising its ability to serve as a reliable measure of iron status when its value is elevated. The target serum ferritin level in the absence of inflammation is >100 ng/ml and a low serum ferritin level has been shown to be a specific predictor of iron deficiency in pediatric CKD (102). Transferrin saturation is a measure of iron immediately available for hemoglobin synthesis. It is typically expressed as a percentage and is calculated as the serum iron divided by total iron binding capacity multiplied by 100%, with a therapeutic target of greater than 20% in patients with CKD (76). Although it too is a highly specific predictor of iron deficiency in pediatric CKD patients (103), this measure also has limitations as it loses its value for evaluating the availability of iron when the total iron binding capacity is low (< 200 mcg/dL), as may be seen in malnutrition or severe proteinuria (104). The percentage of circulating hypochromic red blood cells and the reticulocyte hemoglobin content have been proposed as additional methods to evaluate functional iron adequacy in adults (105, 106). In contrast, the experience with these laboratory tests is limited in children with CKD (107).

Treatment of Anemia

The use of erythropoietic stimulating agents (ESA) such as rHuEPO, in addition to iron supplementation, are the key elements of anemia management in patients with CKD related anemia. Prior to the availability of rHuEPO, virtually all pediatric ESRD patients required repeated blood transfusions with their incipient risks of iron overload, antigenic exposures, and infectious complications. Now, blood transfusions should be reserved for patients with symptomatic anemia, significant on-going hemolysis, or unresponsiveness to ESA therapy. Many of the symptoms of CKD, some of which were historically attributed to the presence of uremia, were actually secondary to anemia, as correction of the anemia with rHuEPO has regularly been associated with clinical improvement. Studies on the use of rHuEPO in pediatric CKD patients have documented improvements in appetite, exercise tolerance, oxygen consumption, intelligence testing scores, and quality of life (108–111) subsequent to the initiation of therapy. Severe left ventricular hypertrophy has also been associated with low hemoglobin values (112) and treatment of anemia with rHuEPO has produced a significant reduction in left ventricular mass index within a year of the introduction of therapy (113). Possibly most important is the fact that the persistence of anemia 1 month after dialysis initiation has been associated with a significant increase in morbidity (e.g., hospitalizations) and mortality in pediatric patients (114).

As mentioned above, rHuEPO (trade names- Epogen, Procrit, Eprex, NeoRecormon) is a component of the treatment of CKD related anemia and it has been shown, for more than 15 years, that it is efficacious in children with CKD (115, 116). The maintenance dosing of rHuEPO must be individualized and factors known to be associated with an increase in the required per kilogram dosing of rHuEPO include greater renal dysfunction, younger patient age (117), a fall in the remaining endogenous erythropoietin activity, and iron deficiency. Generally, an appropriate starting dose for subcutaneous rHuEPO, the route of therapy for most pre-dialysis CKD patients, is 100 units/kg/week divided into semi-weekly doses. Patients younger than 5 years of age, however, often require a 50–100% increase in these starting dose recommendations (150 units/kg/week per subcutaneous route) as there may be more non-hematopoietic binding sites for rHuEPO in younger children (118). Recently, adult CKD patients were found to maintain adequate hemoglobin levels using less frequent dosing of rHuEPO, every 2–4 weeks (119), but this decreased frequency has not been evaluated in children.

The hemoglobin level, MCV, and iron stores should routinely be monitored subsequent to the initiation of ESA therapy. The K/DOQI guidelines recommend that when ESA therapy is used, hemoglobin and iron status should initially be monitored monthly, while iron studies may be checked quarterly once the patient is receiving a stable ESA dose (80). The hemoglobin value should be evaluated every 2 weeks following a change in ESA dosage to make sure that there is not too rapid an increase in hemoglobin and the development of complications related to the rapid change. Typically, when patients respond to therapy, a mild increase in MCV may be seen as a result of the reticulocytosis. An initial poor response to ESA therapy is often secondary to iron depletion and is reflected by a decrease in transferrin saturation, as plasma iron stores are quickly depleted.

Dose adjustments are often needed with rHuEPO therapy as factors which affect rHuEPO dosing may change.The goal of the treatment is to achieve a monthly increase of hemoglobin by 1–2 gm/dL until reaching target levels. The rHuEPO dose should be increased 25% if the patient is still anemic and their hemoglobin value has not increased by at least 1 gm/dL over the past month. Similarly, the dose should be reduced by 25% if the hemoglobin exceeds target levels or the rate of increase in hemoglobin is greater than 2 gm/dL per month. Generally, the dose of rHuEPO in children should be

reduced and not held when the serum hemoglobin level is excessively high. This approach decreases the risk of "hemoglobin cycling" (120).

Complications from rHuEPO use are most commonly related to changes associated with the rate of rise in the serum hemoglobin. Increases in blood pressure can be the result of a rapid rise in hemoglobin (121) or secondary to a direct effect of rHuEPO on blood vessels (122). Rarely, the development of antibodies against erythropoietin, both endogenous and the recombinant exogenous forms, may occur causing pure red cell aplasia (123).

Darbepoetin-alfa (trade name Aranesp) is an effective alternative to rHuEPO with less frequent administration requirements (124, 125). Darbepoetin-alfa has one amino acid substitution and additional N-glycosylation sites which gives the molecule a longer half-life than rHuEPO. The half-life of darbepoetin-alfa given intravenously to children is approximately 22 h and given subcutaneously is nearly 43 h (126), almost three times longer than that of rHuEPO. In general, therapy with subcutaneous darbepoetin-alfa is initially provided on a weekly basis. However, in patients previously requiring rHuEPO only weekly, darbepoetin-alfa may be given every other week or even over longer intervals. Typical starting doses for an ESA-naïve patient is 0.5 mcg/kg/week; however, patients converting from rHuEPO should be prescribed approximately 0.42 mcg/kg/week darbepoetin-alfa for every 100 units/kg/week of rHuEPO. The side effect profile of darbepoetin-alfa is similar to that of rHuEPO with some reports of increased pain at the injection site with darbapoetin-alfa (124, 127).

As mentioned above, iron supplementation is typically also part of the therapeutic regimen in children with anemia from CKD, not only because of the high prevalence of iron deficiency as a result of poor intake or increased losses, but also because of the increased iron demands associated with the use of ESAs. Oral iron therapy (3–5 mg/kg of oral elemental iron daily) is usually adequate in patients with CKD, although intravenous iron can be provided to children unable or unwilling to take oral supplement or in those who are unable to meet the targets for iron therapy with oral supplement alone (128, 129). Oral absorption of iron is somewhat limited and may be adversely affected by its administration with food or the concomitant use of either calcium-containing binders or H2-receptor antagonists. Other adjunctive therapies for the anemia of CKD such as carnitine (130) or vitamin C (131) have not been shown to be associated with a significant improvement in outcomes and are not currently recommended by K/DOQI.

The target hemoglobin level continues to be somewhat controversial, especially as it applies to the pediatric patient. Currently, there are no pediatric studies which define the ideal target hemoglobin level. However, in 2007, the United States Food and Drug Administration (FDA) issued a warning stating that the use of ESA agents with targeted hemoglobins greater than 12 gm/dL increases the risk of cardiovascular events and death, all based upon the results of studies conducted in adults with CKD (132, 133). K/DOQI recommended target hemoglobin levels for children and adults were subsequently amended to generally fall in the range of between 11 and 12 gm/dL and not to exceed 13 gm/dL (134), while the FDA established a target range of 10–12 gm/dl. The lack of variability for target hemoglobin levels obviously does not take into account the age and gender-related variability of normal hemoglobin values in children (135). Additionally, Staples et al. recently reviewed NAPRTCS data on children with CKD and found that hospitalization rates decreased as hematocrits increased, even with hematocrits in excess of 42% (79).

The proven benefits of treating anemia in CKD are slowly being realized as ESAs are being prescribed more frequently in CKD. According to the 2007 United States Renal Data Systems Report, the percentage of children receiving ESAs prior to dialysis initiation increased from 34.5% over the years 1996–2000 to 39.1% during the span of 2001–2005 (34). During those same time periods, the mean hemoglobin at dialysis initiation had increased from 9.1 to 9.7 gm/dL. There remains some variability in terms of the population of children receiving an ESA as over 45% of children less than 15 years old received ESA therapy prior to dialysis compared to only 30% of those aged 15–19. Additionally, patients with cystic kidney disease were most likely to receive early treatment with an ESA in contrast to those with glomerulonephritides ($>$ 50% vs 37%, respectively). The immediate challenge is to optimize the management of anemia throughout all stages of CKD so that there is universal achievement of the target hemoglobin level.

Mineral and Bone Metabolism

Disorders of calcium and phosphorus homeostasis associated with pediatric CKD inevitably develop with worsening kidney function. The changes in calcium and phosphorus regulation can cause significant alterations in bone re-modeling and somatic growth. There is also increasing evidence that the presence of hyperphosphatemia and hypercalcemia in patients with CKD contribute

to cardiovascular morbidity even during childhood. Thus, early astute management of mineral and bone disorders of CKD during childhood is of utmost importance.

Pathophysiology of Mineral and Bone Disorders

The intricacies of the renal regulation of calcium, phosphorus, and bone metabolism are covered in greater detail in other chapters of this book. However the two basic causes for much of the pathology of the bone and mineral disorders associated with CKD are decreased calcitriol (1,25-dihydroxy vitamin D3) synthesis and decreased phosphate secretion by the kidney. The kidney regulates calcium metabolism through the actions of 1α-hydroxylase, which converts 25-hydroxy (OH) vitamin D3 to its active form, 1,25-dihydroxy vitamin D3 (calcitriol). Calcitriol, in turn, helps regulate intestinal calcium absorption and, thus, has a significant impact on serum calcium levels. The loss of functioning renal mass in CKD leads to suppression of calcitriol synthesis, often very early in the disease before changes in serum calcium, phosphorus, or parathyroid hormone (PTH) can be detected (136). Also, the phosphaturic hormone fibroblastic growth factor (FGF)-23 may also have a role, as it is known to suppress calcitriol synthesis (137) and it too accumulates in CKD (138).

The decrease in intestinal calcium absorption that occurs with CKD leads to decreased serum calcium levels which are quickly mitigated by an increase in PTH synthesis and secretion. In order to maintain normocalcemia, PTH stimulates the resorption of calcium and phosphorus from the bone matrix. PTH also has secondary phosphaturic effects (139) and, in early CKD, elevated PTH levels are often associated with a normal or slightly decreased serum phosphrous level. However, as CKD advances and there is a progressive loss of glomerular filtration, urinary phosphorus excretion decreases and serum phosphorus levels increase. Hyperphosphatemia in children with CKD is not typically seen until Stage 4 or 5 CKD (GFR <30 ml/min/1.73 m^2), although impaired phosphate clearance may first occur when the GFR decreases below 40 ml/min/1.73 m^2 (140).

The combination of deficient calcitriol synthesis and impaired phosphorus excretion results in the changes of bone and mineral metabolism. Hyperphosphatemia potentiates PTH secretion in and of itself (141), while the lack of calcitriol causes decreased suppression of PTH transcription (142). With time, the stimuli of low calcium, elevated phosphorus, and decreased calcitriol levels lead to secondary hyperparathyroidism and subsequent parathyroid gland hyperplasia and possibly even chromosomal changes (143, 144). Elevated PTH levels cause increased activation of the PTH receptor protein on osteoclasts and osteoblasts, increasing their cellular activity. This, in turn, results in high bone turnover which, if persistent, may lead to fibrous changes of the bone, referred to histologically as osteitis fibrosa cystica. Increased skeletal resistance to PTH is also seen in CKD, secondary to either low calcitriol levels or the accumulation of uremic toxins or PTH fragments from a decreasing GFR (145, 146).

More recent work has focused on the pathology of vascular calcifications seen in patients with CKD, since their presence is closely linked with cardiovascular mortality (147). Unlike the calcification of atherosclerotic plaques which are found in the intimal layer, vascular calcifications in CKD are primarily found in the medial layer (148). It is unclear why these calcifications develop in CKD, but the hypotheses have centered around three potential etiologies- the common mesenchymal origin of vascular smooth muscle cells and osteoblasts (149), the increase of mineralization factors seen in uremia (150, 151), and the suppression of inhibitors of calcification (152). Although the pathophysiologic mechanisms causing the calcifications have not been fully elucidated, their presence has been associated with elevated serum phosphorus levels, elevated calcium-phosphorus product, elevated PTH levels, and increased doses of vitamin D and calcium-containing phosphorus binders (153–156). Rising awareness that altered mineral metabolism contributes to the increase in cardiovascular pathology seen in CKD has led to the reclassification of mineral, skeletal, and vascular diseases together under the term CKD mineral and bone disorder (CKD-MBD) (157), encompassing the more systemic nature of the disorder.

Evaluation of Mineral and Bone Disorder

As the bone remodeling of renal osteodystrophy is preventable, patients with CKD should have their serum calcium, phosphorus, CO2, alkaline phosphatase and PTH levels checked regularly. Secondary hyperparathyroidism has been reported in children with Stage 2 CKD (158) and thus annual monitoring is recommended even at this early stage (159). The pediatric K/DOQI bone guidelines have also addressed the frequency of monitoring (see *Table 68-7*) with a recommendation for increased vigilance with more advanced kidney disease and for more frequent monitoring if treatment of renal osteodystrophy has already been initiated. Recommendations from the

Table 68-7

Frequency of measurement of bone and mineral factors and target ranges of serum PTH by stage of CKD

CKD Stage	Frequency of calcium, phosphorus, and CO2	Frequency of PTH and alkaline phosphatase	Target serum PTH (pg/ml)
2	annually	annually	35–70[a]
3	every 6 months	every 6 months	35–70[a]
4	every 3 months	every 3 months	70–110[a]
5	every month	every 3 months	200–300

[a]based on expert opinion

European Pediatric Dialysis Working Group concur with these guidelines for monitoring frequency, except for delaying routine monitoring until Stage 3 CKD and monthly monitoring of all five parameters in Stage 5 CKD (160). Newer recommendations from the Kidney Disease: Improving Global Outcomes (KDIGO) initiative will be forthcoming (157).

Intact serum PTH is routinely monitored as a less-invasive surrogate biomarker for osteodystrophy and may be used to discern between high and low bone turnover states. PTH levels should be checked using a first generation immunometric assay (159), which recognizes an epitope of the intact PTH (1–84) molecule between residues 7 and 34. However, these assays have been found to react with PTH fragments lacking the N-terminus of the molecule (161) and may over-estimate biologically active PTH. Therefore, second generation assays were developed with antibodies binding to the N-terminus and measuring intact PTH more specifically (162), with values often 50–60% of those measured with first generation assays. The difference in the measures of the two assays represents the N-terminus truncated fragments of the molecule. However, studies comparing the two assay types in children have shown similar predictive values in determining the specific bone histology (163, 164). Therefore, because of the limited experience and availability of second generation assays, first generation assays are still used. In mild to moderate CKD (Stages 2 and 3), normal PTH levels are desired as this correlates with normal bone formation rates (165). In more advanced stages of the disease, increased PTH levels are desired (159, 160) because of the increased skeletal resistance to PTH and the increased risk of adynamic bone disease associated with low/normal PTH values (see *Table 68-7*).

Therapeutic target levels of serum phosphorus and calcium are the age-appropriate norms in CKD Stages 2–4 (159) with increased stringency in CKD Stage 5. Target levels for serum calcium in Stage 5 CKD is towards the lower end of the normal range, 8.8–9.5 mg/dL [2.20–2.37 mmol/L] with interventions to lower serum calcium recommended if levels exceed 10.2 mg/dL [2.54 mmol/L] (159).

Serum alkaline phosphatase is a measure of the osteoblastic activity of bone. It is assessed in conjunction with serum PTH as it increases the predictive power of PTH for determining high versus low bone turnover (166). In patients with hepatic disease, total alkaline phosphatase may be elevated and the determination of the skeletal fraction of the enzyme may be determined by separately measuring the heat-stabile and labile fractions. Alkaline phosphatase levels may be useful in monitoring skeletal response to vitamin D therapy with decreasing levels indicating improved histologic bone appearance (167). Other bone markers (osteocalcin, collagen C-terminal telopeptide) have not been studied widely in children with CKD.

Serum levels of 25-OH vitamin D3 provide an estimate of vitamin D body stores, with a value of less than 30 ng/ml indicative of deficiency. Monitoring of 25-OH vitamin D3 is often practiced in pediatric CKD patients once they have elevated PTH levels (159). K/DOQI recommends that the 25-OH vitamin D3 level should be checked annually if it remains in the normal range and more frequently, every 6 months, if patients are vitamin D deficient and receiving replacement therapy. With the recently reported high prevalence of vitamin D deficiency in pediatric CKD patients and the documented efficacy of vitamin D repletion therapy on bone mineralization in earlier stages of CKD (168), more regular monitoring may soon be recommended. Since serum calcitriol levels offer limited information and are expensive to check, they are not currently recommended as part of routine clinical monitoring in patients with CKD.

Routine skeletal radiography has little diagnostic yield in the early stages of CKD, but may be indicated if bone disease is already present. In severe secondary hyperparathyroidism, subperiosteal mineral resorption may be seen, especially in the phalanges of the hand. Therefore, annual hand and wrist X-rays are recommended in Stage 5 CKD (160), when high turnover bone disease is most often present. In contrast, no pathognomonic radiologic findings are present in adynamic bone disease. Other bone density imaging techniques, such as DEXA scans, are not routinely indicated at this time. Finally, the gold standard for the determination of bone histology is bone biopsy. Although it is a safe procedure in the hands of experienced practitioners, the cost and invasive nature of bone

biopsy precludes its inclusion in the care of most pediatric CKD patients; it is generally considered only in patients with pathologic fractures, suspected aluminum toxicity, or with persistent hypercalcemia despite normal PTH levels (159).

Treatment of Mineral and Bone Disorder

The main tenets of treatment of pediatric CKD bone and mineral disorder is the maintenance of normal mineral metabolism and prevention of high turnover bone disease. The close monitoring of serum calcium, phosphorus, and PTH are used to guide therapy and minimize the development of the secondary complications of impaired growth, adynamic bone disease, and extra-skeletal calcification. As hypophosphatemia can contribute to bone disease at earlier stages of CKD, the maintenance of serum phosphorus above the lower limits for age is recommended (159). Total CO_2 is also routinely monitored in CKD because of the effect metabolic acidosis has upon bone remodeling and its potential remediation with bicarbonate supplementation.

The development of high turnover bone disease associated with CKD may be optimally prevented by limiting the development of hyperphosphatemia. Hyperphosphatemia tends not to develop in the earlier stages of CKD, as the loss of the kidney's phosphate excretory ability typically does not occur until the GFR is less than 40 ml/min/1.73 m^2, as noted previously. In these early stages of CKD, elevated serum phosphorus levels and the accompanying elevated PTH value are often the result of an excessive dietary intake and may be ameliorated by dietary restriction (169) with recommended limits of 80% of the Dietary Reference Intake for age (170). However, as CKD progresses and phosphate excretory function decreases, the need for dietary phosphate binders, in addition to avoidance of high phosphate containing foods, becomes more apparent.

Calcium salts (calcium carbonate or acetate) are frequently first-line treatments as phosphate binders. They are inexpensive and are most often well-tolerated with gastrointestinal complaints as their most common side effect. The phosphate binding capacity of calcium salts are, however, limited and large doses are often required. The recommended total (diet and binders) intake of calcium for children with CKD is twice the daily dietary recommended average for age (159), which may easily be exceeded with large calcium-based binder dosages. The use of calcium acetate, which has less elemental calcium and is able to bind more phosphorus per unit of calcium than calcium carbonate, may result in a lower incidence of hypercalcemia than might be seen with the latter agent. Calcium citrate is as effective as acetate and carbonate in its phosphate binding capabilities, but has been shown to enhance aluminum resorption across the gastrointestinal tract and is not usually recommended. In most cases, hypercalcemia in CKD is secondary to renal osteodystrophy therapies and modification of calcium-containing phosphate binders or vitamin D supplementation is therapeutic.

Sevelamer hydrochloride is a metal-free phosphate binder which has been shown to effectively lower serum phosphorus levels (171) and may shortly be approved for adult use in non-dialysis CKD. Sevelamer is resistant to digestive degradation and, therefore, it is not absorbed from the intestinal tract and does not accumulate in tissues. Although not yet approved for use in children, it has been shown to be effective in lowering serum phosphorus levels in children requiring dialysis (172) with a lower risk of hypercalcemia in comparison to calcium-based binders (173). Sevelamer use has also been associated with a decreased progression of vascular calcifications (174) and lower overall mortality rates in adults (175). Although decreased serum bicarbonate levels may be seen with sevelamer hydrochloride use (173), the recently FDA approved agent, sevelamer bicarbonate, does not have that associated side effect when taken by adults; pediatric studies with this agent have not yet been completed. Sevelamer hydrochloride has also been shown to lower total and low-density lipoprotein (LDL) cholesterol levels in children (173), an action that may have secondary benefits on overall cardiovascular risk.

Metal-containing phosphate binders are of limited use in pediatric CKD. Aluminum-containing binders were an early mainstay of therapy but are no longer recommended, except for limited time periods in very rare circumstances (159) because of the risk of aluminum toxicity. Magnesium salts also have limited use in pediatric CKD because of their associated side effects of hypermagnesemia, hyperkalemia, and diarrhea. Lanthanum carbonate is a more potent binder of intestinal phosphate than other current binders. However, tissue accumulation of lanthanum has been shown in rats (176) and increased bone lanthanum levels have been seen in adult subjects 2 years after drug discontinuation (177). The long-term effects of lanthanum carbonate on growing bone and tissues are not known and, hence, its use is not recommended in pediatric CKD.

Vitamin D deficiency is a relatively common finding in patients with CKD (178, 179) and the current K/DOQI guidelines recommend replacement of vitamin D when

25-OH vitamin D3 levels are less than 30 ng/ml (159). Therapeutic Vitamin D is currently available in two forms, ergocalciferol (D2) and cholecalciferol (D3). Ergocalciferol is available as a component of most multivitamins, usually at 400 IU; however, multi-vitamin supplementation is not recommended because of the risk of vitamin A toxicity (180). Ergocalciferol is also available as an 8,000 IU/ml liquid and a 50,000 IU (1.25 mg) capsule. Current recommended upper limits of Vitamin D intake is 2,000 IU daily in patients with normal renal function (181) which has been safely prescribed for older children and adolescents (182). Recommendations for repletion therapy are correlated with the severity of Vitamin D deficiency (see *Table 68-8*). No controlled studies have directly compared the efficacy of ergocalciferol to cholecalciferol in children, although an improved response of serum 25 (OH) D3 levels has been seen with cholecalciferol when compared to ergocalciferol in healthy adults (183, 184). Recently, Menon et al. (168) showed that treatment of the vitamin D deficient pediatric CKD population (Stages 2 through 4) with ergocalciferol significantly decreased serum PTH levels within 3 months of treatment, consistent with findings seen in adults (185, 186).

If secondary hyperparathyroidism persists despite normal serum phosphorus and vitamin D levels, treatment with a vitamin D sterol or vitamin D analog is indicated. Calcitriol and its prohormone, alfacalcidol, are widely used in children and both are effective in suppressing PTH (187, 188) by increasing intestinal calcium absorption and suppressing PTH gene transcription. Alfacalcidol is not available for use in the United States currently, but it has been widely used in Europe. Calcitriol may be given orally or intravenously with initial doses from 5–10 ng/kg/day and may be administered daily or intermittently with equivalent effectiveness in controlling secondary hyperparathyroidism (187). Calcitriol does increase intestinal absorption of phosphorus by nearly 50% and therefore may worsen hyperphosphatemia. Additionally, there is an increased prevalence of hypercalcemia and an associated increase in the calcium-phosphorus product with its use, especially when it is co-administered with a calcium-based phosphate binder. Newer vitamin D analogs, such as paricalcitol and doxercalciferol, minimize the likelihood of hypercalcemia while continuing to suppress PTH transcription (189). In the pediatric dialysis population, intravenous paricalcitol has been shown to effectively lower PTH when compared to placebo (190) and has been shown to decrease the calcium-phosphorus product when compared to calcitriol (191). However, its use was not associated with a decreased prevalence of hypercalcemia. Oral paricalcitol has recently been approved for use in adults with CKD Stages 3–5, although studies on its use in pediatrics have not yet been reported.

Calcimimetics treat hyperparathyroidism by binding to the calcium sensing receptor of the parathyroid gland and, through allosteric modification of the receptor, increase its sensitivity to ionized calcium. Cinacalcet, the only currently available calcimimetic agent, has been shown to effectively lower serum PTH levels in adult ESRD and CKD patients (192, 193). Cinacalcet has not been approved for use in children; however a recent open-label study of its dosing in pediatric ESRD patients showed that it was well-tolerated and effective, with no treatment-related adverse events (194).

When refractory hyperparathyroidism develops despite aggressive pharmacotherapy, total parathyroidectomy with auto-transplantation to the forearm or abdomen may be considered a safe and effective alternative (195) in pediatric patients. This approach to management is usually considered a last option because of the resultant potential difficulties with calcium homeostasis following renal transplantation (159).

Table 68-8

Recommended supplementation for vitamin D deficiency in patients with CKD

Serum 25(OH) D (ng/ml)	Definition	Ergocalciferol (Vitamin D2) dose
<5	Severe Vitamin D deficiency	8,000 IU/day orally (or 50,000 IU per week) × 4 weeks, then 4,000 IU/day (or 50,000 IU twice per month) × 2 months*
5–15	Mild Vitamin D deficiency	4,000 IU/day orally (or 50,000 IU every other week) × 12 weeks*
16–30	Vitamin D insufficiency	2,000 IU/day (or 50,000 IU every 4 weeks)*

*Recommended therapy duration is 3 months then re-measure 25(OH)D3 levels to determine if further treatment needed

Cardiovascular Disease

Adults with CKD have significantly increased rates of cardiovascular morbidity and mortality compared to the

general population (196, 197). Much of this increased risk is secondary to the fact that many of the traditional risk factors for adult cardiovascular disease (hypertension, diabetes mellitus, hyperlipidemia) are also primary etiologies of adult kidney disease. However, this increased cardiovascular risk is not unique to adults with CKD. Estimates of cardiovascular mortality rates in children and young adults who developed end stage renal disease during childhood are 1,000 times greater than comparably aged healthy individuals (198).

The life expectancy of children with ESRD who remain on dialysis is shortened by as much as 40–60 years with 30–50% of all deaths in this population attributed to cardiovascular causes (34, 154, 199, 200). However, the cardiovascular causes of mortality are different in children with CKD than in adults. Adult cardiovascular deaths are frequently from coronary artery disease and congestive heart failure while the leading causes of cardiac death in children with CKD are arrhythmia, valvular disease, and cardiomyopathy (201). Therefore, many questions about cardiovascular risk factors in pediatric CKD exist and firm recommendations still cannot be made on whether primary prevention should focus upon traditional "adult" cardiac risk factors, which appear earlier and are more accelerated in children with kidney disease, or upon uremia-related risk factors, which are unique to the CKD population.

The mechanism by which cardiovascular disease develops in children with CKD is thought to occur by two, potentially concurrent, processes- cardiac remodeling and vascular injury. Remodeling of the left ventricle with resulting hypertrophy occurs from mechanical or hemodynamic overload of the muscle and subsequent sarcomere changes. The development of concentric left ventricular hypertrophy (LVH) is most often secondary to increased resistance from hypertension while eccentric LVH is often secondary to volume overload or anemia. Over time, worsening LVH changes may lead to decreased subendocardial perfusion and an increased risk of arrhythmia generation. Persistent hypoperfusion may lead to myocardial fibrosis and diastolic or systolic dysfunction.

Vascular injury may occur through either atherosclerotic or arteriosclerotic changes, or both. Atherosclerosis involves penetration of the intimal layer of the vasculature with lipid-containing macrophages, the accumulation of smooth muscle cells and collagen to form an atheroma, with subsequent luminal narrowing. Arteriosclerosis involves thickening of both the intimal and medial layer of the arteries and is often characterized by increased wall thickness, luminal enlargement, and loss of vessel elasticity. Calcification of vessels may occur with either atherosclerosis or arteriosclerosis, primarily involving the large to medium-sized vessels such as the aorta and brachial artery, but is also noted to involve the smaller coronary arteries in children with advanced uremia on occasion (155, 156).

Markers of vascular injury are commonly found in pediatric patients with CKD. The prevalence of dyslipidemia in pediatric dialysis patients ranges from 70 to 90% (202) and a study examining the internal iliac artery samples of pediatric patients at the time of renal transplant revealed that >40% of subjects had histopathologic evidence of atherosclerosis with one-third having intimal micro-calcification or atheromatous plaque formation (203). Asymptomatic atherosclerosis, as measured by abnormal carotid intimal medial thickness (IMT) and vessel calcification, has been noted in pediatric CKD patients prior to the initiation of dialysis as well (204). In a study by Litwin et al., arteriopathy correlated with systolic blood pressure and dyslipidemia in pediatric CKD subjects (204).

Endothelial function, as measured by flow-mediated dilation of the brachial artery, has also been shown to be impaired in children with CKD (205). Recently, Muschietes et al. compared carotid IMT values and flow-mediated dilation in pediatric CKD, dialysis, and transplant patients (206). A majority of CKD patients had reduced flow-mediated dilation, which correlated well with increased carotid IMT and was thought to precede the arteriopathic findings. Although not shown in the longitudinal studies of children with CKD, there may be a progression of vascular injury from initial endothelial dysfunction to vascular wall thickness changes to eventual calcification of vessels. Likewise, the risk factors for disease progression may also change depending on the degree of pathology, with blood pressure and dyslipidemia having greater effects during earlier stages of CKD and calcium, phosphorus, and PTH levels in latter stages.

Hypertension is a traditional cardiovascular risk factor which develops early in the course of CKD. In the Chronic Kidney Disease in Children (CKiD) study, the prevalence of systolic and diastolic hypertension at study initiation were 53% and 54%, respectively (207), consistent with an earlier report in which the prevalence of hypertension in pediatric CKD was 50% (44). Risk factors associated with the presence of an elevated blood pressure at the time of study initiation in the CKiD study included a shorter duration of CKD, indicating that hypertension was often present early in the disease process, but either went unrecognized or under-treated. A potential reason that hypertension may be missed in this population is that the early BP changes in CKD are often associated with alterations of the

circadian rhythm of blood pressure regulation. Though not explicitly recommended, K/DOQI guidelines suggest that the use of ambulatory blood pressure monitoring (ABPM) may be a valuable tool in patients with CKD (46) because its use may help detect changes in BP which would likely not be discovered with casual blood pressure checks alone. Recently, Dionne et al. found that ABPM detected abnormalities in nearly 50% of pediatric CKD patients that were otherwise not detected by casual clinic readings (208). Mitsnefes et al. has reported rates of nocturnal hypertension in pediatric CKD patients as high as 24% (209) with attenuated nocturnal dipping in nearly 60% of subjects. Blunted nocturnal dipping has been shown to correlate with decreasing GFR (208, 209). In adults with CKD, Andersen et al. found that ABPM detected masked hypertension in 26–29% of their subjects which was otherwise not detected by office BP readings alone (210). Additionally, ABPM readings have been found to correlate better than casual blood pressures with measures of LVH in children and adults with CKD (209, 211) and, therefore, it may serve as a better means of predicting end organ injury than casual blood pressures in this at risk population.

Hypertension has been clearly linked to LVH in adults with CKD (212); however, the direct link in children is still being investigated. One-third of children with mild to moderate CKD have been reported to have an increased left ventricular mass index (LVMI) (213–215). In a prospective longitudinal study of pediatric patients with CKD Stages 2 to 4, Mitsnefes et al. reported a 32% incidence of LVH development over 2 years (216). In that analysis, increased LVMI, defined by mass divided by height raised to a power of 2.7, was associated with an increased nocturnal systolic blood pressure load as well as increased serum PTH and decreased hemoglobin levels. However, in the larger ESCAPE trial, Matteucci et al. did not find any relationship between casual or ambulatory blood pressure readings and LVM in their cross-sectional analysis (215), questioning the significance that hypertension alone has in cardiac remodeling.

Alterations in left ventricular function are also different in children with CKD compared to adults. Systolic function does not appear to be compromised in children (213, 217) but the earlier-appearing changes of diastolic dysfunction may be found. Studies have shown impaired left ventricular relaxation in children requiring dialysis (213 218). The newer index of diastolic function, tissue Doppler imaging, has also been shown to be impaired in children with CKD and those on chronic dialysis (219). Whereas the clinical significance of diastolic dysfunction in children is still under study, the poor diastolic function in pediatric dialysis patients has been associated with factors such as anemia and hyperphosphatemia (219), and may offer some insight into the complex interactions contributing to cardiac remodeling in CKD patients. Over the past few decades, abnormalities of the left ventricle and arterial circulation have been identified as strong predictors of cardiac risk in the adult CKD population and recent investigations have shown that children with CKD may also have many of these same cardiovascular abnormalities (220).

Evaluation and Treatment of Cardiovascular Disease Risk Factors

Recommendations for monitoring and treatment of cardiovascular risk factors in children with CKD have been primarily derived from adult data and have focused upon the traditional cardiovascular risk factors of hypertension and dyslipidemia. Target blood pressure in children with CKD should be less than the 90th percentile for age, gender, and height or 120/80 mmHg, whichever is lower, as recommended by the Fourth Report on Blood Pressure in Children (47). Routine screening ambulatory blood pressure monitoring is not currently recommended by the K/DOQI guidelines, but many experts endorse its use in high-risk populations (221) and recommendations for a standardized approach have recently been published (222). Currently, echocardiography is recommended for children at the initiation of dialysis to screen for the presence of end-organ damage or valvular disease (46) which may have developed during earlier stages of CKD. However, its routine use to screen for end organ injury in children with persistent hypertension or those at high-risk for cardiovascular morbidity (i.e., patients with CKD) should also be considered (221).

The preferred antihypertensive agents to be used for the treatment of hypertension in CKD are ACE inhibitors or ARBs because of their effects on proteinuria and slowing of CKD progression, as discussed earlier. Their role in the reduction of renal sympathetic activity, and the possible secondary effects on cardiac remodeling are additional rationale for selecting these first-line agents. Recent cross-sectional analysis of CKiD study data showed that uncontrolled hypertension was associated with the absence of ACE inhibitor or ARB use in pediatric patients with CKD (207). Diuretics may also be considered in children with CKD, to address the sodium and fluid overload that may be present in patients who do not have salt-losing nephropathies. Thiazide diuretics may be used in patients with earlier stages of CKD, but they are not effective when the

GFR falls below 30 ml/min/1.73m(2). Loop diuretics may be considered in CKD Stages 4 and 5. Beta-blockers may be used as second-line anti-hypertensive agents as well. Calcium channel blockers are also potent anti-hypertensive medications and may be considered for adjunctive therapy; however, dihydropyridine (DHP) calcium channel blockers, such as nifedipine and amlodipine, increase intraglomerular pressure and proteinuria (223, 224) and may have less beneficial effects in terms of slowing CKD progression when compared to other anti-hypertensive options. Although non-DHP calcium channel blockers, verapamil and diltiazem, have been shown to have effects equivalent to ACE inhbitors in slowing of CKD progression in adult diabetic patients (225), there are no published safety data regarding their use in children with hypertension and they are known to cause prolongation of the PR interval in adults (224). Alpha-adrenergic agents (prazosin, clonidine) and other vasodilators (hydralazine, minoxidil) may also be considered for therapy with their selection and continued usage dependent on efficacy and side effect profile in the individual patient.

The treatment of dyslipidemia in pediatric CKD is not well-studied and, therefore, not addressed in depth in the K/DOQI guidelines. Adolescents with CKD should be evaluated for dyslipidemia with a fasting lipid profile for total cholesterol, LDL, HDL, and triglycerides (226). Treatment recommendations are, however, limited to adolescents with Stage 5 CKD. The National Cholesterol Education Panel initially recommended that the use of lipid-lowering drugs in children be restricted to those over age 10 years with a fasting LDL >190 mg/dl or >160 mg/dl and two other risk factors (227). However, the American Heart Association expert panel released an updated scientific statement addressing high-risk pediatric patients which considered pediatric CKD patients to be in the highest risk group (228). In children with CKD and a fasting LDL >100 mg/dl, therapeutic lifestyle changes, such as reduced dietary saturated fat and cholesterol intake and moderate exercise, are first recommended for the initial 6 months. If target LDL levels (<100 mg/dl) are not reached, initiation of statin therapy is indicated. There is no information on the efficacy of lipid-lowering therapies on cardiovascular morbidity or mortality of pediatric CKD patients. Therefore, the presumed benefit in children is extrapolated from adult studies. However, statin therapy has been proven to be effective in improving endothelial function in children with hypercholesterolemia (229).

Other management strategies for minimizing cardiovascular risk in pediatric CKD address uremia-related risk factors. Anemia should be treated to maintain serum hemoglobin levels in the recommended range to reduce any potential effects on cardiac remodeling. Serum PTH, calcium, and phosphorus should also be closely maintained to K/DOQI recommended levels because of the growing evidence of their contribution to vascular abnormalities. Other potential uremia-related risk factors, such as hyperhomocysteinemia (230), elevated C-reactive protein (231), elevated asymmetric dimethylarginine (232), and low adiponectin levels (233) have not been well-studied in pediatric patients.

Fluid, Electrolyte, and Acid-Base Balance

With the gradual decrease in GFR, the remnant functioning nephrons and tubules compensate by increasing their excretory function in an attempt to maintain solute homeostasis. Such compensation will generally allow for adequate fluid and electrolyte balance until greater than 75% of renal function is lost. However, these adaptive changes are gradual and may not compensate for sudden changes in volume or solute load.

Sodium balance in CKD is generally maintained by a progressive decrease of sodium reabsorption by the proximal and distal tubules (i.e., gradual increase in the fractional excretion of sodium). Children with CKD from obstructive uropathy or renal dysplasia have defective urinary concentrating abilities, from a decreased tubular responsiveness to vasopressin (234). Because of a limited capacity to reduce distal tubular fluid sodium concentration in these patients, sodium excretion often becomes dependent on urine flow (235). Thus, these patients are often polyuric with substantial urinary sodium losses despite advancing renal disease. Fluid balance is normally maintained because of an intact thirst mechanism. Acute problems may arise if fluid intake is not maintained in these patients because of gastrointestinal illness or iatrogenic limitations. On a chronic basis, sodium balance may not be normally maintained because of chronic urinary losses, with the resultant signs of sodium depletion often being subtle, such as impaired somatic growth. In fact, linear growth has been shown to improve in young children with CKD with sodium and water supplementation (236). Supplementation with sodium chloride up to 4–7 mEq/kg/day is often required.

Conversely, children with CKD from primary glomerular diseases or with associated oliguria often require sodium and fluid limitation to minimize the risk for the development of edema and hypertension. While the exact sodium restriction that is needed in this population is not known, limitation to less than 1,500 mg daily as

recommended for hypertensive individuals (237) or 25–70 mg/kg/day (1–3 mmol/kg/day) in younger children seems reasonable, but can be sometimes difficult to achieve practically. In-depth education by a renal dietician about the sodium content of foods and the need to avoid processed foods is highly recommended.

Potassium homeostasis is usually preserved until the GFR is less than 15 ml/min/1.73 m^2. This is because aldosterone-stimulated distal tubular potassium secretion is maintained. Aldosterone also stimulates increased potassium secretion within the colon with as much as 35% of dietary potassium able to be excreted in the stool (238). However, children with renal dysplasia, obstructive uropathy, reflux nephropathy, or interstitial nephritis may have tubular resistance to the aldosterone activity and can develop hyperkalemia with relatively higher creatinine clearances (239). This hyperkalemia may be exacerbated by volume contraction and, in turn, responds to sodium and fluid repletion. Additionally, potassium-sparing diuretics, ACE inhibitors, and ARBs contribute to the hyperkalemic risk in these patients. For patients with persistent hyperkalemia, dietary potassium intake should be limited. As food labels frequently lack potassium content, patient and family education by a renal dietician about potassium-rich foods (chocolate, potatoes, green leafy vegetables, etc...) and alternate food preparation methods, such as soaking vegetables before cooking, should be reviewed. In infants and young children, the use of low-solute formulas or the incorporation of sodium polystyrene sulfonate (Kayexalate) resin with the formula should also be considered (240). In hypertensive children, calcium polystyrene sulfonate may also effectively bind potassium (241) with much less of a sodium load provided to the patient. Lastly, any constipation should be treated to improve the gastrointestinal elimination of potassium.

Unlike sodium and potassium disorders which develop late in the progression of CKD, metabolic acidosis may occur when the GFR declines below 50% of normal. As glomerular filtration decreases, the relative ammonia production of the remaining tubules increases (242) while titratable acid excretion is initially preserved. However, with progressive renal dysfunction, absolute ammonia, phosphate and titratable acid excretion decreases while bicarbonate resorption decreases (243, 244), leading to metabolic acidosis. In infants and children, the endogenous hydrogen ion load is actually increased compared to adults. Chronic acidosis has been associated with increased protein degradation (245) and decreased albumin synthesis (246). Additionally, chronic acidosis causes bone demineralization (247) and may suppress native growth hormone secretion (248). Therefore, a serum bicarbonate value of greater than 22 mEq/L should be maintained in order to maximize growth in children with CKD (249). Oral bicarbonate therapy is the standard treatment for metabolic acidosis as citrate preparations should be avoided because of the aforementioned increase in aluminum absorption seen with their use (250).

Growth Failure and Nutrition

Growth failure or failure to grow is a common and the most visible complication of CKD in children. Patients often fail to achieve final adult heights consistent with their genetic potential or within population norms. The cause of growth failure in these children is most often multi-factorial, based on a combination of nutritional, metabolic, and endocrine abnormalities. Affected children may have secondary physical and psychological complications associated with their poor growth. However, aggressive management of secondary causes of poor growth and the prudent use of recombinant growth hormone (rhGH) can minimize the prevalence of growth failure and its secondary sequelae.

According to the most recent NAPRTCS registry data, the average height of children with CKD is nearly 1.5 standard deviations below the age- and gender-specific norms upon entrance to the registry (23). More than 35% of children with CKD have significant growth failure, defined as a height less than the third percentile or a height standard deviation score (SDS) more negative than −1.88 (23). Although there is some correlation between kidney function and growth impairment, significant growth failure was seen at all levels of kidney function in the NAPRTCS registry, including 19% of subjects with the highest estimated GFR values (50–75 ml/min/1.73 m^2). A more significant correlation was found between growth failure and young age at the time of registry entry. The average height SDS in infants (age 0–1) and young children were −2.33 and −1.65, respectively, while it was only −0.93 for adolescents. Additionally, significant short stature was seen in 58% and 41% of all infants and young children, respectively, while it was present in only 22% of adolescents. This is important as one-third of overall postnatal linear growth is attained in the first 2 years of life, such that any early growth insults may have a profound long-term impact on final height.

Although other factors (acidosis, secondary hyperparathyroidism, and electrolyte imbalance) may contribute to growth failure in all children with CKD, malnutrition has the most marked effect in infants and

young children with CKD (251). Energy intake is the principal determinant of growth at this age (252) and maximizing caloric intake to at least 80% of requirements has been found to effectively improve height velocity in children who developed CKD as infants (252, 253). Inadequate protein intake may also contribute to insufficient growth in infants and experiments in young animals have shown that decreased protein intake during normally rapid periods of growth can adversely affect overall growth (254). Uauy et al. showed that even a modest restriction in protein intake had a negative impact on the growth of infants with CKD (255). It is equally important to note again that dietary protein restriction has no discernable effect on the progression of CKD when studied in children (45).

Outside of infancy and early childhood, perturbations of the growth hormone/insulin-like growth factor (GH/IGF) axis are the predominant influences on growth. In children with CKD, there is increased pituitary release and decreased renal clearance of GH, resulting in increased serum GH levels. However, down regulation of GH receptors by the liver and changes in post-receptor signal transduction results in decreased synthesis of IGF-1. Additionally, increased concentrations of IGF binding proteins, whose concentration is inversely correlated to GFR, lead to decreased levels of the bio-active or "free" IGF, which mediates many of the biological effects of GH such as longitudinal bone growth. Thus, CKD is associated with a state of dysregulation or resistance, and not deficiency, of the GH/IGF/binding protein system which alters somatic growth. Additionally, disturbances in the gonadotropic hormone axis, namely decreased pituitary secretion of lutenizing hormone (256) and reduced free testosterone levels (257), may be seen in adolescents with CKD. This is associated with both a delayed and shortened pubescence and contributes to a suboptimal peak height velocity during this period, estimated at only 50% of that experienced by children without CKD (258). All of this may often result in children with a final adult height that is far below their genetic potential. A retrospective analysis of children who were transplanted before age 15 showed that the median height of subjects was below the third percentile for age at the time of dialysis initiation and did not improve significantly following transplantation. Significant growth failure was present in 77% of adult male subjects and 71% of females (259). More recent clinical trials also support the observation that pre-existing height deficits in children with CKD are still associated with decreased final adult heights (260, 261).

The impact of growth retardation in the CKD population is most evident in a number of studies that have addressed quality of life. In a study of adults with short stature, 60% of patients who had kidney failure as a child reported wanting to be taller and 33% stated that they would sacrifice years of life expectancy to do so (262). A questionnaire given to 240 adults who received a kidney transplant in childhood showed that final height was significantly associated with marital status and level of education, while inversely related to level of employment (263). A survey of young adults on dialysis or with a functioning transplant revealed that 36% were dissatisfied with their final height and this dissatisfaction correlated with patient perceptions of quality of life (264).

The negative effect of growth impairment in children with CKD may extend beyond psychosocial issues to an increased risk of morbidity and mortality. Utilizing data from the U.S. Renal Data System on over 1,000 children on dialysis, Furth et al. showed that children with more severe growth failure, as defined by height velocity, had higher deaph rates than those with a normal growth rate (265). Additionally, growth failure was associated with an increased incidence of hospitalization, primarily for infectious complications. In a second study of 2,300 children in the NAPRTCS registry, Furth et al. demonstrated that children with CKD and a lower height SDS (more negative than −2.5) at the time of dialysis initiation had an increased risk of hospitalization and a two-fold higher risk of death (266). Although the cause of increased mortality in these studies was not clear, the results suggest that short stature is associated with a more complex clinical course of CKD and may be a reflection of other clinical risk factors, such as a poor nutritional status.

The current K/DOQI pediatric nutrition guidelines recommend correction of nutritional deficiencies and metabolic abnormalities prior to the use of growth hormone therapy for growth failure (249). This is especially true in infants and young children, whose growth is primarily dependent on caloric intake. Current experience suggests that the caloric intake of children with CKD should be equal to the recommended daily allowance (RDA) (267) as compromised growth is seen when caloric intake is less than 80% of the RDA, as noted above. A prospective study in children with CKD between 1.5 and 10 years of age showed that more than 50% of patients had less than the recommended energy intake, with the poorest results reported in the older patients (268). In order to achieve the necessary caloric intake, caloric supplementation through added density to formula should be considered in young children with fluid restrictions.

Nasogastric, gastrostomy, and gastrojejunostomy tubes, with daytime intermittent bolus or continuous nightly feeds, may also be used successfully in patients unable to maintain oral caloric needs secondary to anorexia, an approach that has been associated with improved height and weight SDS in young children with CKD (269). Caloric sources should be divided so that 50% of total calories are provided by primary complex carbohydrates and the remaining non-protein calories should be divided in a 2:1 ratio between polyunsaturated and saturated fat.

Protein energy malnutrition may also contribute to the poor growth of children with CKD. However, a study reviewing the nutritional intake of children with CKD by 4 day food records revealed a mean protein intake greater than 150% of the RDA (268), hinting that protein malnutrition is not as prevalent in this population as once thought. In the previously mentioned European study which examined the impact of a modest reduction in dietary protein (0.8–1.1 gm/kg/day) in children with CKD over 3 years, there was no impairment of subject growth, however there was also no benefit in terms of the prevention of disease progression (45). Therefore, it is recommended that children with CKD receive 100% of the age-recommended RDA for protein and that 50% of that protein come from high biologic sources such as milk, eggs, and meats. Protein intake should not be excessive and must be repeatedly evaluated with respect to the CKD stage because of its potential contribution to hyperfiltration and excess metabolite generation (270).

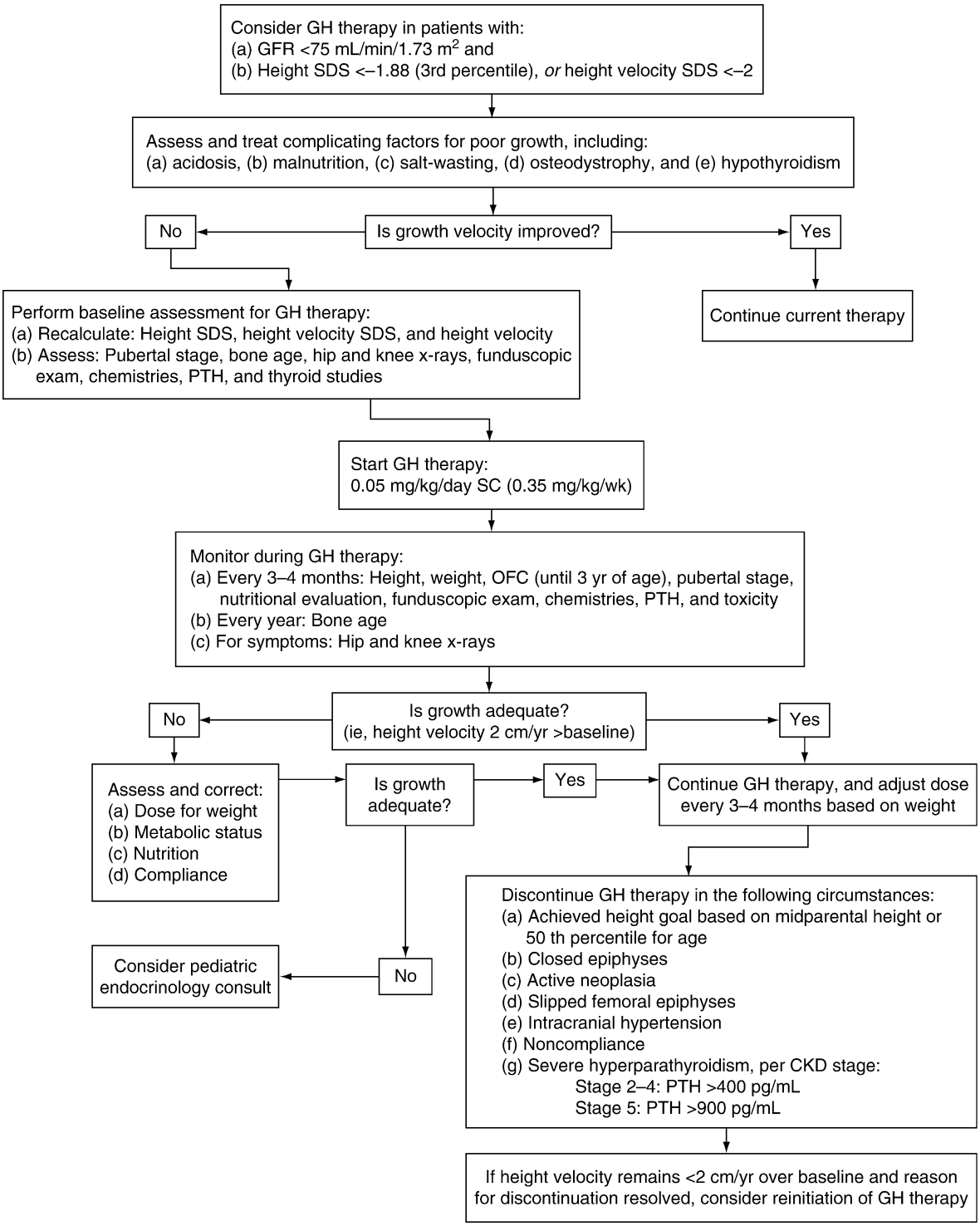
Despite optimization of nutritional management and correction of metabolic abnormalities, normal growth may not be obtained by these measures alone. A review of NAPRTCS data showed that catch-up growth without growth hormone treatment was primarily observed in children with CKD younger than age five (271). Therefore, the use of recombinant human growth hormone (rhGH) likely will be needed in order to achieve height norms in a substantial number of children with CKD and growth retardation. Multiple clinical trials have demonstrated the efficacy of rhGH to improve height velocity and height SDS in children with CKD (272). Recombinant GH has been approved for the treatment of growth failure in children with CKD at an optimal subcutaneous dosage of 0.35 mg/kg/week (28–30 IU/m2/week) administered daily. Children who are Tanner stage I-III and have either a height SDS of −1.88 or worse and/or a height velocity SDS of −2 or worse are candidates for -therapy (249). The safety profile of rhGH use in children with CKD is excellent, as its use has not been associated with a significant increase in adverse effects when compared to children not receiving rhGH (273, 274). Nevertheless, close monitoring of patients for the safety and efficacy of the therapy is routinely indicated. Increased insulin levels have been seen during the first year of rhGH use (275, 276), however irreversible diabetes mellitus has not been observed (274) and a return to baseline insulin levels usually occurs with long-term treatment (275). rhGH use has been associated with increased PTH levels in children with CKD, a problem that seems to be most significant in pubertal age children (277), and at least temporary discontinuation of therapy may be necessary in the setting of severe secondary hyperparathyroidism. The development of intracranial hypertension has also been noted (278) and mandates a stoppage of therapy. An algorithm for the treatment of growth failure, including the monitoring of growth hormone therapy, is provided in *Fig. 68-1*.

Although children who receive rhGH while on dialysis or after kidney transplantation generally experience a beneficial growth response to rhGH therapy, children with CKD have been shown to have the best response (279). A Cochrane review of rhGH studies in pediatric CKD showed a better response in patients with Stage 3 or 4 CKD than in those with Stage 5 disease (273). In recent analyses of longitudinal use of rhGH in French children with kidney disease and of the Pfizer International Growth Database (KIGS), height SDS gain was significantly better in patients with CKD treated conservatively than in children receiving dialysis or in those who had received a kidney transplant (261, 280). Additionally, a better response was associated with younger age at therapy initiation (261) with pre-pubertal children responding better than pubertal children (273). Because of the improved response apparent in younger children and during earlier stages of CKD, rhGH therapy should be initiated early in the course of the disease in order to maximize growth potential (272, 280). Other factors that are associated with the best response to rhGH include the extent of height SDS gain during the 1st year of therapy (280), total duration of therapy (261, 280), renal dysplasia as the primary disease etiology (281), degree of bone age retardation (261), and the lack of pubertal delay (261). Sustained use of rhGH therapy in children with CKD can often lead to an adult height within norms. Results from two European studies have provided evidence that 60–65% of children with CKD on rhGH therapy will have a final adult height SDS greater than −2 (258, 280).

Despite the benefits of rhGH therapy, a substantial number of growth-impaired children with CKD do not receive treatment. The 2007 NAPRTCS Report showed that rhGH utilization in eligible children in the CRI registry was only 11% upon entry into the registry and increased to

Figure 68-1

Algorithm for evaluation and treatment of growth failure in children with CKD.



only 22% after 12 months (23). Even more alarming is the fact that rhGH utilization seems to be decreasing. From 2000 to 2002, rhGH was used by 28.7% of eligible CRI patients at 1 year after registry entry, but decreased to 21.9% from 2003 to 2006 (23). A recent multi-center study examining obstacles to rhGH use in children with CKD revealed that psychosocial reasons (family refusal or non-compliance) was cited in 30% of the patients. However, there was no identifiable reason precluding rhGH use in 25% of the patients, based on a review of the medical records (282). Additionally, this study noted that boys with growth failure were twice more likely to be treated than girls, implying that growth failure may still be viewed more as a cosmetic issue which may be more acceptable in girls.

Cognitive and Psychosocial Development

The impairment of growth evident in pediatric CKD is not limited to somatic growth, as neurocognitive development and psychosocial adjustment have been shown to be affected as well. The etiology of these impairments is not clear. It may be a direct effect of uremia or other accumulated metabolites on the growing brain or it could be secondary to the impact of other sequelae of kidney disease on brain development. Environmental influences, such as lost educational opportunities from parental occupation or school absenteeism, are also likely contributions. The evolution of these issues and their influence on long-term outcome has become better appreciated as the overall care of the pediatric CKD population has improved.

Original reports on the neurological and developmental outcomes of infants and young children with CKD showed devastating results. Rotundo et al. reported on 23 children who developed CKD at less than 1 year of age with a remarkable 87% of the patients having evidence of a progressive encephalopathy and 83% with developmental delay (283). McGraw and Haka-Ikse found that of their subjects diagnosed with CKD at less than 1 month of age, only 20% had a normal developmental quotient and 75% developed a seizure disorder (284). These early and poor neurologic outcomes have been at least partially attributed to aluminum toxicity from the aluminum-containing phosphate binders that were commonly used at that time. Indeed, aluminum-containing binders were used in all of McGraw's subjects and in all but four of Rotundo's subjects had neurological sequelae. Poor nutritional status was also a common manifestation of CKD in these patients and, therefore, probably played a role in the genesis of the poor neurologic outcome. Not surprisingly, all of Rotundo's and 67% of McGraw's study subjects had significant growth impairment. Nearly a decade ago, Warady et al. showed a much improved developmental outcome in patients who initiated dialysis during infancy (<3 months old) with the avoidance of aluminum binders and the regular use of supplemental feedings, in this case provided by the nasogastric route (285). Of 28 infants in this study who were observed for a long-term, nearly 80% had normal developmental scores and only 4% had significant developmental delay. Thus, the prevention of aluminum exposure and the use of aggressive nutrition with dialysis contributed to improved neurologic outcomes in this high-risk population of infants with severe CKD. Other more recent reports have provided similar data (286, 287).

While cognitive function has been shown to be impaired in children with ESRD in a number of studies (288–290), data addressing the cognitive status of patients with less severe CKD are limited. Hulstijn-Dirkmaat et al. compared patients with ESRD to those with CKD and showed improved developmental index scores in those not requiring dialysis (288). Duquette et al. compared children with CKD to control subjects in the areas of intellectual and academic functioning and found that children with CKD had significantly lower scores on intelligence quotient (IQ), reading, and mathematical testing (291). Kidney function was found to correlate with intellectual and academic scores; recent school absences correlated negatively with IQ and math scores, as well. In more specific areas of cognition, Fennell et al. compared 56 children with CKD to age-matched controls and found that children with CKD had deficits in verbal abstraction, visual-motor abilities, and memory (292) with worsening of memory skills 1 year later. More recently, Gipson et al. compared the neurocognitive status of 20 children with CKD Stages 2–5 with age-matched controls (293). They found that the children with CKD demonstrated significantly lower memory abilities, specifically short-term verbal and visual memory, as well as impairment of new learning capacity. In addition, they found that children with CKD had deficits in selective higher order executive functioning of the brain related to attention. In a separate study, Slickers et al. found that in children with CKD, lower IQ scores were associated with greater severity of the disease and onset of the disease at a younger age while decreased memory function was associated with longer duration of disease (294). Recommendations for a battery of tests to help identify children with CKD who possess neurocognitive deficits have been published (295).

At present, there is no identified threshold of renal dysfunction that has been shown to affect neurodevelopment in children with CKD, as many of the previously cited studies included patients on dialysis (291, 293). However, this is currently being investigated in the CKiD study (296). The contribution of other sequelae of CKD to cognitive dysfunction is also being investigated as anemia (297) and hypertension (298) have been associated with lower intelligence and academic scores in non-CKD populations.

Psychological well-being has also primarily been studied in the pediatric ESRD population (299–301) with limited studies in children with less advanced kidney disease. A study comparing children with kidney transplants, CKD, or nephrotic syndrome to healthy controls failed to find differences in the prevalence of behavioral problems between these groups (302). In the few studies that have been performed, social functioning and quality of life were impaired in pediatric CKD patients. Gerson et al. compared the health-related quality of life in pediatric CKD patients to controls and found overall poorer functional health status in children with CKD (303). McKenna et al. studied 20 Canadian children with CKD and found that they had significantly lower quality of life scores in the areas of physical health and school performance when compared to healthy controls (304). In adults, survivors of pediatric ESRD have been shown to have low self-esteem, low rates of living independently, and are less likely to establish intimate relationships (299, 305). Whereas it has been thought that the basis for the lower quality of life scores in children with CKD is often the physical limitations from CKD, poorer scores have also been associated with worsening GFR (306) and anemia (111). Impaired self-esteem and perceptions of others may also have a significant impact, as improved quality of life scores have been associated with height gain (306).

Although initial studies indicate that children with more protracted or more severe CKD are at particular risk for cognitive and psychosocial impairment, all children with CKD should be considered at risk. In turn, early participation of experts in behavioral and developmental pediatrics to assess the cognitive ability and emotional well-being of children with advanced CKD is prudent so that individualized education plans and counseling may take place and educational, emotional, and functional potential may be optimized. Currently, only 15% of school-aged children with CKD in the United States receive special education services (307) which may under-estimate the needs of these patients. Lastly, incorporation of a multi-disciplinary approach, including individuals with psychosocial expertise, into the transition programs of adolescents with CKD as they move to "adult care" may help to maximize the success of patients as they aim to develop social and functional autonomy (308).

Transition to ESRD

Although a goal of early and aggressive treatment of CKD is the abatement of disease progression, ESRD will inevitably develop in most pediatric CKD patients. Therefore, early preparation for the transition to ESRD is warranted to avoid any emergent procedures and potentially unnecessary associated morbidity. K/DOQI guidelines recommend that formal discussions regarding ESRD preparation should begin once patients reach Stage 4 CKD (GFR less than 30 ml/min/1.73 m^2) (16). In practice, the introduction of anticipatory measures to avoid unnecessary morbidity at an even earlier stage is often most effective.

In anticipation of the possible need for long-term dialysis, the limitation of vascular procedures using multiple blood vessels should be emphasized, especially with the growing use of peripherally inserted central catheters (PICC) in pediatrics. Early identification of the non-dominant arm and its protection from proximal intravenous access will help preserve that extremity for future hemodialysis vascular access. Early consultation with a vascular surgeon to address the development of a fistula prior to the need for dialysis is also recommended (309). Subclavian catheters, as opposed to internal jugular catheters, should only be used as a last option for central venous access in pediatric patients (310) as there are high rates of subclavian stenosis found in adults even with short-term subclavian access placement (311). Additionally, femoral access should be avoided except for emergent situations as there is an increased risk for the loss of inferior vena cava patency and a resultant inability to use those vessels for kidney transplant anastamosis (312).

Since congenital abnormalities of the urinary system are present in a significant percentage of children with CKD, surgical procedures (cystoplasty, vesicostomy) involving the lower urinary tract may be necessary prior to transplantation. Abdominal surgical procedures should also be completed well in advance of progression to ESRD not only to allow for proper healing but, in the case of peritoneal dialysis candidates, to minimize any disruption of the peritoneal membrane. These procedures include pre-emptive nephrectomy as well as gastrostomy tube placement, which may be needed for nutritional supplementation and medication administration.

Avoidance of unnecessary blood product exposure should also be prioritized in order to minimize antigen exposure prior to transplantation. Multiple red blood cell transfusions prior to transplant increase the risk of donor specific antibody formation and have been associated with an increased risk of acute rejection episodes post-transplant (313). Therefore, aggressive use of erythropoiesis stimulating agents and iron supplementation to achieve hemoglobin goals, especially prior to any surgical procedures, should be encouraged.

Children with CKD should receive their standard immunizations. However, certain vaccinations merit special consideration in anticipation of renal replacement therapy. Vaccination for measles, mumps, and rubella (MMR) should ideally be given prior to the initiation of dialysis as variable responses have been noted in dialyzed patients (314). The MMR vaccine is contraindicated in immunosuppressed children following kidney transplantation (315) and should be given more than 6 weeks prior to the transplant procedure (316). The varicella-zoster vaccine (VZV) should also be given in advance to sero-negative transplant candidates to minimize post-transplant morbidity risk, as it too is contra-indicated in immunosuppressed individuals. Although it is not contra-indicated in immunosuppressed patients, the hepatitis B vaccine series should be completed 6 weeks prior to transplantation as well, as the antibody response to the vaccine is better when given to children with CKD before initiation of dialysis (317) or receipt of a transplant (318). Children who will require dialysis are at increased risk for pneumococcal infections and should receive the 23-valent polysaccharide pneumococcal vaccine (319); dialysis patients less than age 6 should also receive the heptavalent vaccine (PCV-7) (320). Annual influenza vaccinations should also be given to children with CKD, including those with kidney transplants or requiring dialysis.

Formal discussions regarding planning of renal replacement therapy should be multi-disciplinary, including not just the family and nephrologist but also the surgeons, specially trained nurses, social workers, dieticians, and psychologists. Pre-emptive transplantation should be considered in patients who are without contra-indications to transplant, as this approach allows for an improved quality of life, an easier rehabilitation to normal activities (321), and possibly the prevention of the various morbidities associated with dialysis dependence. While pre-emptive transplant was previously only thought possible for children with living donors, the substantial preference currently provided to children on the deceased donor list in the United States may make it possible for these children to also receive a transplant before dialysis is necessary. For children who will require dialysis prior to transplantation, discussion of the available dialysis options including their advantages and disadvantages should be conducted and the choice of dialysis modality made with respect to both the medical and psychosocial considerations of the patient and family.

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69 Handling of Drugs in Children with Abnormal Renal Function

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Introduction

Determining the dosage and frequency of a particular drug in a child with kidney dysfunction can often seem daunting. Many therapies have not been fully tested in children, even in those children with normal kidney function. Despite laws instituted in the past two decades to encourage pharmacological studies in pediatric populations, drug dosing in children is often based on extrapolation from adult data. Yet, children often metabolize drugs differently than adults, depending on the drug category or age group. In addition, renal dysfunction adds a further level of complexity to drug dosing as absorption, distribution, metabolism, and secretion of agents can be altered as kidney function worsens. The extent to which derangements in drug handling alter dosage can depend on the GFR, the child's age or size, the precision of the GFR estimation, or, when in renal failure, the modality of the renal replacement therapy.

The overriding principle of this chapter is that drug dosing in the pediatric population with renal impairment requires consideration of many factors and persistent scrutiny. The chapter reviews general pharmacological principles of drug handling and presents specific tenets for how dialysis alters drug levels. It also offers a step-wise approach for nephrologists and other providers to consider when making decisions about drug dosage and intervals in children with impairment of renal function.

Drug Dosing in Renal Failure: General Principles

In order to provide a safe and effective treatment protocol for children, rational drug selection is required. It is necessary for pediatric health care providers to know the therapeutic benefits, indications, contraindications, adverse effects, drug interactions, and precise mechanism of drug used in pediatric patient. Although children experience adulthood diseases there are very limited clinical trials in the pediatric population. In one study over 90% of neonates in intensive care units (1) and 70% of pediatric patients were receiving a drug which was not approved in the pediatric population (2). Most drug dosing are based on "targeted effects" or "concentration effects." Pharmacotherapy is complicated in pediatric patients with kidney disease as very limited information exist involving drug dosing in this setting. It is very important to emphasize that children are not miniature adults. In fact, children are not "just children." The pharmacokinetic properties of most drugs are different from 24 week gestation to 18-year-old adolescent. Metabolic activities, total body water, gastrointestinal functions and structure, overall renal function, body surface, tissue perfusion, skin thickness and protein binding are continually changing during child development (3, 4) (› *Fig. 69-1*).

Drug actions are further different when compared with adult in patients with normal renal function. The addition of renal dysfunction makes appropriate drug management complex and unpredictable in children. Not only is it vital to understand the disease states, comorbid conditions, pharmacodynamic properties of the selected drug, and potential drug-drug interactions, it is also vital to recognize the pharmacokinetics of these agents in the pediatric population. Pharmacokinetics is defined by the individual patient's ability to absorb, distribute, metabolize, and eliminate the drug from the body. Pharmacodynamics is characterized by how drugs affect the body (› *Fig. 69-2*). The objective of pediatric pharmacotherapy is to provide empathic but effective and rapid drug delivery with safe therapeutic outcomes. To attain this aim, pediatric health care providers must understand basic pharmacokinetics and pharmacodynamics (5).

The pharmacodynamics of drug therapy and desirable outcomes are controlled by the concentration of a specific drug at the site of drug action. To some degree, therapeutic plasma concentration is controlled by drug absorption, distribution, metabolism and excretion. First, the drug must be partially or completely absorbed in order to enter into plasma concentration. Next the drug must be distributed to the primary site of action to exert its critical modulation or pharmacodynamic effects. Lastly, drug

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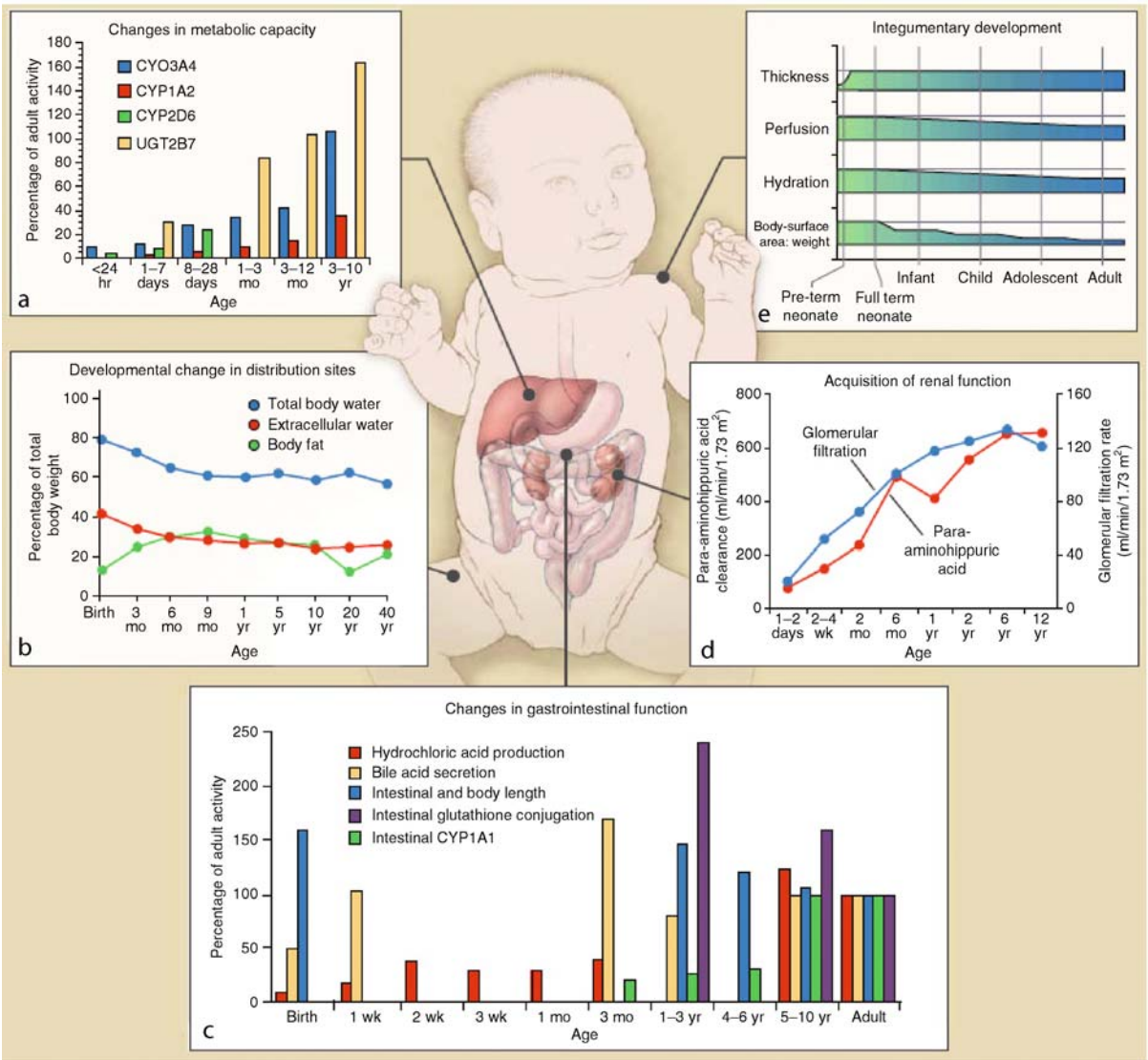
Figure 69-1

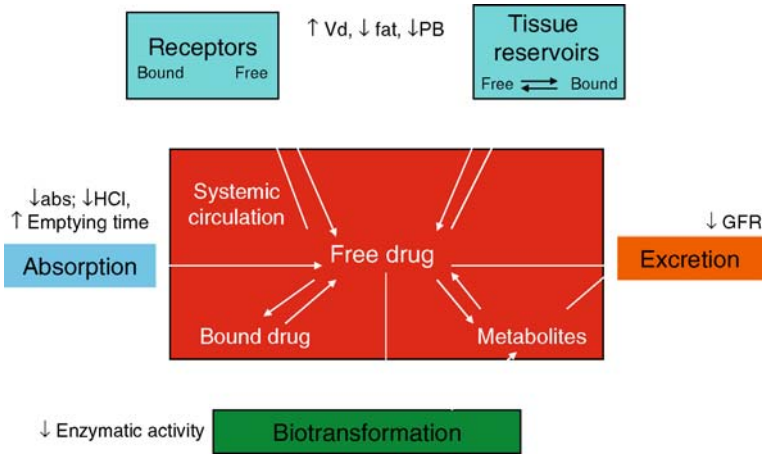
Human development and pharmacokinetic alteration in infant, children and adolescents. Many changes through human development may alter drug disposition. This figure emphasize on age-adjusted need for drug and dosage adjustment during the human development. Panel A shows the metabolic activity of phase I and II enzymatic reactions, Panel B reflect the changes in total body water at different time period, Panel C illustrate the gastrointestinal function and acid production, Panel D demonstrate active tubular secretion by clearance of para-aminohippuirc acid, and Panel E demonstrate age depended changes in skin structure, perfusion and body surface area. (Adapted from Kearns et al. (3)).



must be eliminated from body without causing unwanted adverse reactions. Drugs do not produce a new function, they only modify pathological processes. Pharmacologic agents act by affecting biochemical and physiological processes in the body. Most drugs bind to or interact with a specific receptor but may produce multiple effects because of the location of the receptors in different organs. Familiarity with these drug pharmacodynamic and pharmacokinetic properties helps pediatric health care providers to predict the behavior of a drug in the body. A complete presentation of these basic pharmacological principles is beyond the scope of this chapter. This chapter briefly reviews basic principles of pharmacokinetics in the pediatric population with renal dysfunction.

Figure 69-2

Pharmacokinetic activities; Differences between pediatric and adult patient. Abs: Absorption, HCL: hydrochloric acid, Vd: Volume of distribution, PB: protein binding.



Drugs or their active metabolites are eliminated changed or unchanged through the renal system. Thus, renal dysfunction from both acute kidney injury and chronic kidney disease may influence drug elimination. Assessment of renal function and drug behavior during kidney aids dysfunction ultimately help in appropriate choice of dose of drug and dosage interval (6). Due to the complexity of pharmocotherapy in pediatric patients with renal insufficiency, three important principles should be considered (1) identifying patients with acute kidney injury or chronic kidney disease, (2) accurately estimating level of renal function, and (3) adjusting drug dosages accordingly.

Pharmacokinetics

Pharmacokinetics defines and analyzes the time course of the drug in the body. Pharmacokinetic properties are altered in renal dysfunction. These include bioavailability, volume of distribution (Vd), protein binding, and biotransformation (7).

Drug Absorption

Following oral administration, the drug must be absorbed before reaching its site of action. Absorption is the first stage of pharmacokinetics. Bioavailability is a better pharmacokinetic phrase compared to drug absorption. Bioavailability defines the percent of the drug that reach the systemic circulation. In children with kidney disease, especially CAPD patients, vomiting out medications is a common problem; therefore, drug absorption would be variable and incomplete (8). Bioavailability is primarily determined by the rate and route of administration. For example, drugs administered intravenously are generally 100% bioavailable, because the entire dosage reaches the systemic circulation. When given orally, subcutaneously, or intramuscularly, bioavailability decreases. Uremia also alters drug absorptions or bioavailability. Most drugs require an acidic environment to be absorbed. At birth until age of 2–3 the gastric pH remain high approximately 6–8. For example, both phenytoin and phenobarbital require an acidic atmosphere to be absorbed completely. In addition, uremia may further affect the absorption of most drugs. Uremia-induced vomiting or gastroparesis may slow gastric emptying time leading to reduced drug absorption (9).

Children with kidney dysfunction commonly have bowel wall edema, which may impair drug absorption. Drugs that increase gastric pH, such as phosphate binders and H2-receptor blockers, may impair absorption of concomitantly administered drugs. Most pharmacological agents are absorbed by passive diffusion and changing ionization states may change drug diffusion through the gastrointestinal wall. Concomitant administration of some phosphate binders for the treatment of hyperphosphotemia (aluminum- or calcium-containing) with antibiotics or iron-containing supplements may result in the formation of insoluble complexes that both limit absorption and slow gastrointestinal motility. Parenteral drug

administration might provide a more reliable route of administration, but more painful and should be avoided as much as possible. In addition, because of a low muscle mass and poor blood flow to the muscle, drug bioavailablity is unpredictable following intramuscular administration. Finally, some intramuscular preparations are oil-based formulation and have a prolonged absorption period (6).

Drug Distribution

After reaching the systemic circulation, most drugs are distributed throughout body compartments in several phases. Initially, drugs are distributed to highly perfused organs such as the heart, liver, kidney, and brain. In the second phase, drugs are distributed to other areas with less or slower blood flow such as fat, bone, and skin. The rate of distribution and extent of drug concentration at the site of action determines the onset of drug action. Body composition, total body water, cardiac output, regional blood flow, and protein binding may affect drug distribution through the body (7). Compared to adults, pediatric patients population have significantly higher total body water and a larger volume of distribution (Vd). The volume of distribution is calculated by dividing the total amount of drug in the body by the concentration of the drug in the blood. The volume of distribution does not refer to a specific anatomic compartment. It is a mathematical model which is useful for calculating the appropriate dosage regimen needed to achieve a desired systemic plasma concentration. An inverse correlation exists between the serum concentration and the volume of distribution. For highly hydrophilic drugs such as aminoglycosides, both renal failure and higher total body water in children and neonates results in an increased volume of distribution. This rise in extracellular fluid volume results a lower serum concentration of aminoglycosides. Drug diffusion is also dependent on protein binding and lipid solubility. A highly water-soluble drug has a small volume of distribution and a high plasma concentration. A highly fat-soluble drug possesses a large volume of distribution and has a low plasma concentration. Pharmacologically, only unbound drugs are active and able to bind to specific receptors. The affinity of pharmacologic agents to receptors and degree of protein binding determines the pharmacodynamic properties of any agent. Drugs that are highly protein bound have a very large volume distribution. Any changes to protein binding due to decreased protein synthesis or proteinuria may alter therapeutic plasma concentration and potentially desired outcomes. In addition, drugs with high affinity to protein mostly distribute in the vascular compartment. Children with kidney dysfunction have decreased protein synthesis and increased protein elimination. For drugs such as phenytoin, which is highly protein bound, the free fraction concentration is elevated while total phenytoin plasma levels are subtherapeutic. In this setting only free, unbound phenytoin should be measured to avoid toxicities (10). Finally, in neonates with high bilirubin and kidney disease, most of the sites for drug binding are occupied with endogenous bilirubin or uremic molecules and results in increased plasma concentration of drugs that are highly protein bound. For example, about 97% of mycophenolate in the plasma is bound to albumin while 2–3% is free. Hypoalbuminemia (albumin less than 2.5 gm/dl) causes increased risk of toxicities due to an increased concentration of free mycophenolate in the plasma. Hypoalbuminemia may cause an increased from free fraction of mycophenolate from 3% to 6%. Although small increased in free fractions seems trivial, the amount of free mycophenolate available to exert immunosuppressive or adverse drug reactions is doubled, with possible serious consequences (11, 12).

Drug Metabolism

Most drugs are metabolized to more soluble compounds which are then removed from the circulation (13). In general, most drug metabolites are pharmacologically inactive, however, a number of drugs have active metabolites that are excreted through the kidney. Patients with chronic kidney disease are at a higher risk of drug accumulation and toxicities in this setting. For example, procainamide has an active metabolite; *N*-acetyl procainamide (NAPA). NAPA has pro arrhythmic properties and is excreted by the kidneys (14, 15).

There are two major metabolic pathways. In phase I reactions drugs are metabolized through oxidation, reduction, and hydrolysis. In oxidation reactions, oxygen atoms cause lipophilic pharmacologic agent to become more water soluble. The oxidative reactions typically involve the cytochrome P-450 enzymatic system (16–18). Cytochrome P-450 enzyme systems are subject to significant maturation through childhood development. In general, microsomal enzyme systems are immature in infants and neonates. It has been shown that drug metabolism is slower in the children than adult at this stage of life. However, drug metabolism is higher compared to adults during the toddler age and early stages of puberty. The mechanisms that account for this alteration of drug

metabolism are correlated to age-adjusted hormonal changes (19). Consequently for many drugs that are metabolized through the P-450 enzyme system a high dosage per kilogram body weight is required to achieve the same therapeutic plasma concentration compared to adult patients (20, 21) (*Figs. 69-3* and *69-4*). The second important pathway for drug metabolism is called synthetic or conjugation reactions. Phase II reactions involve the attachment of another chemical group to the drug resulting in greater water solubility and renal elimination. Most drugs undergo one or both of the phase I and II reactions in order to be eliminated from systemic circulation and the body. There is increasing evidence to suggest that there are significant amount of polymorphism exists on expression of both enzymatic systems in children and adolescents. Most patients with kidney disease take several drugs and these enzyme systems can be induced or inhibited by other pharmacotherapuetic agents (22). Drugs that stimulate this enzymatic system are considered inducers and may decrease the therapeutic benefit of other agent. For example, phenytoin may increase the metabolism of prednisone and cyclosporine and result in acute

Figure 69-3

Figs. 3 and 4 Illustrate that a higher dosage of drugs are needed for children than adults adjusted for body weight.

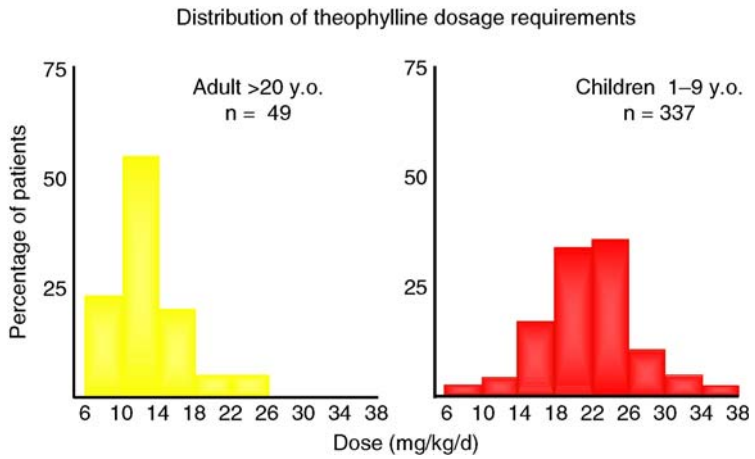


Figure 69-4

See Fig. 3.

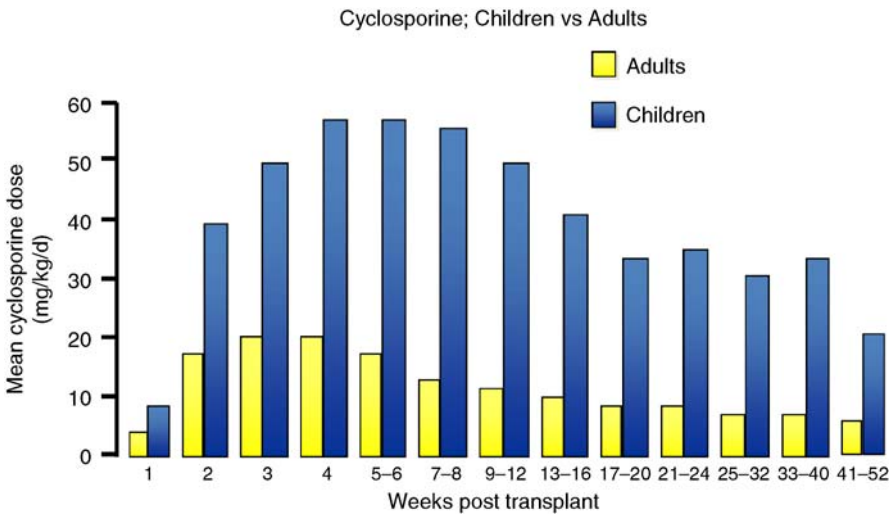


Table 69-1

Commonly used drugs with potential drug interaction in pediatric population with kidney disease. Modified form Lynch et al. (22)

Enzyme	Potent inhibitors	Potent inducers
CYP1A2	Amiodarone, cimetidine, ciprofloxacin, fluvoxamine	Carbamazepine, phenobarbital, rifampin, tobacco
CYP2C9	Amiodarone, fluconazole, fluoxetine, metronidazole, ritonavir, trimethoprim/sulfamethoxazole	Carbamazepine, phenobarbital, phenytoin, rifampin
CYP2C19	Fluvoxamine, isoniazid,ritonavir	Carbamazepine, phenytoin, rifampin
CYP2D6	Amiodarone, cimetidine, diphenhydramine (Benadryl), fluoxetine, paroxetine, quinidine, ritonavir, terbinafine	No significant inducers
CYP3A4 and CYP3A5	Clarithromycin, diltiazem, erythromycin, grapefruit juice, itraconazole, ketoconazole, nefazodone, ritonavir, telithromycin, verapamil	Carbamazepine, Hypericum perforatum, St. John's wort, phenobarbital, phenytoin, rifampin

rejection or allograft loss after kidney transplantation. Other drugs may inhibit the metabolism of another drug and increase the risk of toxicity. Erythromycin may decrease the metabolism of cyclosporine and cause in cyclosporine toxicity and drug induced-allograft injury (11, 23). Commonly used drugs in the pediatric population which can cause drug interactions through their effect on metabolism are listed in *Table 69-1*.

Dialysis and Drug Dosing

There is a paucity of published studies pertaining to drug pharmacokinetics and clearance in children undergoing various dialytic modalities. Therefore, most of the data are extrapolated form the adult literature. In general, drugs that are eliminated primarily via renal excretion are those which are significantly affected by dialysis, whereas drugs that undergo extrarenal elimination are not significantly altered by dialysis (24, 25). Drug removal during hemodialysis is a function of the dialysis dose, (Kt/V), "K" being the dialyzer clearance, "t" the time on dialysis, and "V" the urea volume of distribution which is practically equivalent to total body water (approximately 60% of body weight) (24). Since total body water is smaller in children, some authors suggested that dialytic drug clearance should be higher in children than in adults, if "K" and "t" are the same (24). This may be especially true for continuous renal replacement therapies (CRRT), where the same dialyzers used for adults are also used for children (24). The dialyzability of a drug is dependent on a number of variables that can be related to drug and/or dialytic properties. This section will discuss those variables in detail, and will outline the principles of drug dosing in children receiving various forms of dialytic therapies.

Drug Properties that Affect Dialyzability

The following drug characteristics affect drug dialyzability: molecular weight and size, protein binding, volume of distribution and electrostatic charge (24, 26, 27). In general, drugs with small molecular size or volume (<500 D) are more readily dialyzable than larger molecular-sized drugs (27). However, the molecular weight as a determinant of dialyzability is most relevant when low-flux dialysis membranes are used. In regions of the world where the majority of dialysis membranes used are high-flux such as in the U.S.A. and some European countries (28, 29), molecular size of the drug has become less relevant, since high-flux membranes can still remove drugs with molecular weights of more than 30,000–40,000 D (25). For instance, vancomycin (molecular weight 1,450 D) clearance with low-flux membranes is minimal, whereas a significant amount is cleared by high-flux membranes (30).

Protein binding affects drug clearance during dialysis. Drugs that are highly protein bound (>80%), in general, are poorly dialyzable, whereas unbound drugs and those with low protein binding capacity are more readily dialyzable (31). In patients with renal failure, there is a tendency for an increase in the unbound fraction of some drugs due to changes in the metabolic milieu or a reduction in plasma albumin, rendering them more dialyzable (32–34).

The volume of distribution of drugs also affects dialyzability. Drugs with a low volume of distribution (e.g., hydrophilic drugs, such as β–lactam antibiotics, aminoglycosides and glycopeptides), usually are confined to the intravascular and extracellular spaces, and therefore, are effectively removed by dialysis. Drugs with a large volume of distribution are usually highly tissue bound and

distribute throughout the extravascular and intracellular compartments such as adipose tissue (e.g., lipophylic drugs, such as the macrolides, tetracyclines, fluoroquinolones and linezolid), and are not effectively removed by dialysis even if the extraction across the dialysis membrane is 100% (25). In general, drugs with a volume of distribution less than 0.7 L/kg are well dialyzed, whereas drugs with a volume if distribution greater than 2 L/kg are not. Drugs whose volume of distribution is between 0.7 and 2 L/Kg have variable but low dialyzability (31, 34–36).

The effect of intercompartmental equilibration can also affect drug removal and blood concentration during high-flux intermittent hemodialysis, particularly with respect to drugs with a middle or large molecular weight (4). Although dialysis may rapidly clear a drug from the intravascular compartment thus causing a significant reduction in blood concentration immediately post-dialysis, slow equilibration of intracellular drug concentration with the extracellular fluid may cause a rebound effect with a gradual increase in intravascular drug concentration lasting several hours after dialysis (35). For instance, vancomycin blood concentration falls significantly immediately following high-flux dialysis, but it rebounds to as high as 90% of the predialysis blood concentration several hours post-dialysis (37, 38). This effect should be considered when dosing drugs and interpreting drug concentration in patients undergoing hemodialysis.

Drug molecular charge may affect dialytic clearance (39, 40). Cationic drugs may be retained by anionic protein charges in the blood, and therefore may not cross the dialysis membrane to the dialysate compartment, even if the molecular weight and other drug properties favor its dialyzability. Conversely, the movement of anionic drugs from the blood to the dialysate compartment across the dialysis membrane may become enhanced. For example, the anionic drug clavulinic acid is cleared much more rapidly by high-flux membranes than is simply predicted by its protein binding and volume of distribution characteristics, likely owing to its negative charge (41, 42).

Dialysis Characteristics that Affect Drug Dialyzability

Dialysis-related variables that affect drug dialyzability can be related to the dialysis procedure (blood and dialysate flow rates, ultrafiltration rate, dialysate pH) or the dialysis membrane (dialyzer pore size or flux, dialyzer surface area, membrane charge and membrane binding characteristics) (24, 26). Among those variables, dialyzer flux, surface area, ultrafiltration rate and blood flow rate are probably the most important.

Drug clearance during dialysis occurs via two mechanisms: diffusion and/or convection (43). Diffusion of a drug from the blood to the dialysate compartment occurs because of a favorable concentration gradient. This gradient is facilitated by the countercurrent of blood and dialysate which run in opposite directions during dialysis, thus maximizing the blood-to-dialysate drug concentration gradient. Diffusion of drugs to the dialysate compartment increases as the molecular weight of the drug decreases. It also increases with increased surface area of the dialyzer and increased blood and dialysate flow rates (24–26). Convection is the process of drug removal during ultrafiltration across the dialysis membrane, and is the result of the hydrostatic pressure generated across the dialysis membrane. Convection is important for clearance of drugs with middle or large molecular weights that cannot be removed by diffusion, and increases as the ultrafiltration rate increases. Convection is thus dependent on the surface area of the dialyzer, membrane pore size (flux), and the hydraulic pressure gradient across the membrane, and is independent of concentration gradient (24–26). In summary, drugs with small molecular weights are removed mainly by diffusion, whereas drugs with middle or large molecular weights are removed by convection. For example, gantamicin which has a relatively small molecular weight, is easily removed with low-flux cuprophane membranes (44). By contrast, vancomycin, a drug with a relatively large molecular weight, is only significantly removed by high-flux dialyzers with large pores but not with low-flux cuprophane dialyzers (3).

Membrane electrostatic charge and drug binding to the dialysis membrane may also affect clearance. For instance, positively charged drugs such as gentamicin may bind to negatively charged membranes such as polyacylonitrile, resulting in reduced clearance during dialysis (26, 45, 46). Furthermore, dialysis membranes that are reused may become protein coated over time, which may reduce the dialyzer surface area and therefore, drug clearance (47). These factors must be considered before determining appropriate drug dosing for patients on dialysis.

Drug Removal and Dosing During Intermittent Hemodialysis

A logical approach to drug dosing during intermittent hemodialysis in children has been outlined by Veltri et al. (24). The first step is to evaluate the residual renal function (RRF) of the patient. If the RRF is significant, and if the drug is primarily eliminated renally, dosing

requirement will increase. Unfortunately, most references assume that once patients are on dialysis, then RRF is negligible, and dosing recommendations are solely based on the effect of dialytic clearance. Therefore, therapeutic drug level monitoring may be necessary for drugs that can be accurately measured in plasma to guide dosing, especially for drugs with a narrow therapeutic index.

Drug and membrane characteristics should also be considered before appropriate dosing. If the drug is likely to be significantly removed during dialysis, a supplemental dose should be given following dialysis. Unfortunately, there is a paucity of data published on dosage regimens and supplemental dosing in children, and most data are extrapolated from adult dosing guidelines. It may be appropriate to consider a larger supplemental dose for small pediatric patients when the amount of drug removed may be larger than in an adult (24). Supplemental dosing may be calculated using the following formula (26):

$$\text{Supplemental dose} = (C_{desirable} - C_{obtained}) \times V_d$$

where $C_{desirable}$ is the desirable plasma concentration, $C_{obtained}$ is the obtained plasma concentration, and V_d is the volume of distribution (L/kg). Vancomycin is an example of a drug that can be significantly cleared renally if the RRF is significant and can also be significantly cleared by high-flux dialysis membranes (30, 37, 38). Therefore, therapeutic drug monitoring is necessary to guide dosing to avoid underdosing or toxicity. Drug doses due around the time of the dialysis session are usually given post-dialysis to avoid significant drug removal during dialysis. For some drugs with a narrow therapeutic-toxic window such as aminoglycosides, predialysis dosing may be more appropriate to maintain a high peak and avoid prolonged exposure that may cause toxicity (26). This approach, however, needs further validation in clinical studies before it can be implemented.

Drug Removal and Dosing During Continuous Renal Replacement Therapies

Continuous renal replacement therapy (CRRT) is increasingly being used in children in the critical care setting (27). As in adults, the major indication for CRRT use is acute renal failure in the setting of hemodynamic instability. CRRT modalities include continuous hemofiltration alone (high-rate ultrafiltration + replacement fluid, no dialysate), continuous hemodialysis (no or minimal ultrafiltration + dialysate) or continuous hemodiafiltration (high rate of ultrafiltration + replacement fluid + dialysis). Replacement fluid is added to compensate for the high-rate of ultrafiltration. This can be added predilution (before the dialysis filter) or post-dilution (after the dialysis filter) (40–43).

The major forms of CRRT currently used are continuous venovenous hemofiltration (CVVH), continuous venovenous hemodialysis (CVVHD) and continuous venovenous hemodiafiltration (CVVHDF), with the latter two modalities accounting for close to 80% of CRRT in the United States (28). Continuous arteriovenous hemofiltration (CAVH), hemodialysis (CAVHD) or hemodiafiltration (CAVHDF) are rarely used nowadays due to complications associated with arterial access. Slow continuous ultrafiltration (SCUF) is the process of slow isolated ultrafiltration without replacement fluid.

Dialysis filters used for CRRT are high-flux synthetic membranes, such as the polysulfone, polyamide, polyacrylonitrile or polymethylmethacrylate membranes (24). Drug removal during continuous hemofiltration occurs mainly via convection, whereas drug removal during continuous hemodialysis occurs mainly via diffusion. During hemodiafiltration, drug removal occurs via both convection and diffusion, and is usually greater than with either modality alone (24, 40). *Table 69-2* lists the clearance mechanisms and key parameters influencing drug removal during the different forms of CRRT and intermittent hemodialysis. It is important to remember that drugs with large volumes of distribution which are not significantly removed by intermittent hemodialysis may be significantly removed during CRRT since the length of therapy is prolonged over 24 h, which allows for greater removal of total body drug stores (24, 25).

Continuous Ultrafiltration and Hemofiltration

Drug removal during hemofiltration occurs via convection. Therefore, middle or large molecular weight drugs can be significantly removed by hemofiltration. Drug removal is proportional to the ultrafiltration rate and the sieving coefficient (S) of the drug and is not dependent on blood flow rate (24, 40, 49). The sieving coefficient is a mathematical term that reflects the extent of solute or drug removal during hemofiltration, and is expressed as the ratio of drug concentration in the ultrafiltrate to drug concentration in plasma (35, 49):

$$S = C_{uf}/C_a \quad (1)$$

where S is the sieving coefficient, C_{uf} the concentration of drug in the ultrafiltrate and C_a the drug concentration in plasma (or in the pre-filter plasma water concentration)

Table 69-2

Comparison of drug clearance with intermittent hemodialysis and different types of continuous renal replacement therapies (Adapted with permission from Joy et al. (48))

Technique	Clearance mechanism		Key parameter influencing drug removal
	Convection	Diffusion	
IHD	+	++++	Blood flow rate
SCUF	+		Ultrafiltration rate
CAVH/CVVH	++++		Ultrafiltration rate
CAVHD/CVVHD	+	++++	Dialysate flow rate
CAVVHDF/CVVHDF	+++	+++	Dialysate flow and ultrafiltration rate

IHD intermittent hemodialysis; *SCUF* slow continuous ultrafiltration; *CAVH* continuous arteriovenous hemofiltration; *CVVH* continuous venovenous hemofiltration; *CAVHD* continuous arteriovenous hemodialysis; *CVVHD* continuous venovenous hemodialysis; *CAVHDF* continuous arteriovenous hemodiafiltration; *CVVHDF* continuous venovenous hemodiafiltration
+ negligible; ++ some; +++ marked; ++++ major

(24–26, 40). A sieving coefficient close to one indicates that the drug freely crosses the dialysis membrane, while a sieving coefficient close to zero indicates no drug removal. Protein binding and drug-membrane interaction are the most important factors determining the sieving coefficient of a drug during hemofiltration (50, 51). Only the unbound fraction of the drug is removed by ultrafiltration (SCUF) or hemofiltration (CAVH or CVVH). The sieving coefficient of many drugs, but not all, therefore equates to the unbound fraction of plasma proteins (f_{up}). *Tables 69-3* and *69-4* list the sieving coefficients and the unbound fraction (f_{up}) of some commonly used drugs. The clearance of a drug ($Cl_{convection}$) during pure ultrafiltration (without dialysis) can then be calculated as (40):

$$Cl_{convection} = Q_f \times S \quad (2)$$

$$\text{Or, } Cl_{convection} = Q_f \times f_{up} \quad (3)$$

where Q_f is the ultrafiltration rate

Calculation of the clearance of a drug solely on the basis of the unbound fraction (3) should be approached with caution, because the unbound moiety may not always equate to the sieving coefficient (25). Vancomycin clearance is such an example. This may be especially true in critically ill patients due to factors such as hypoalbuminemia, alteration of protein binding to certain drugs, or drug binding to dialysis membranes (25, 47, 52). Therefore, frequent monitoring of plasma concentration of certain drugs during CRRT is necessary for dosage adjustment.

It is important to consider the effect of replacement fluid on drug clearance during CRRT (25, 43). If the fluid is administered in the post-dilution mode (post-filter), drug clearance can be calculated from the ultrafiltration rate as shown in (2). When the replacement fluid is added pre-filter, drug clearance is expected to be lower due to a dilution factor (DF) as (25, 53):

$$DF = Q_{bf}/Q_{bf} + Q_{rf} \quad (4)$$

where Q_{bf} is the blood flow rate and is Q_{rf} the replacement flow rate. Combining both (2) and (4), drug clearance in case of pre-dilution can be calculated as:

$$Cl_{convection\ (predilution)} = Q_f \times S \times Q_{bf}/Q_{bf} + Q_{rf} \quad (5)$$

Continuous Hemodialysis and Hemodiafiltration

In continuous hemodialysis, countercurrent dialysis is used during CRRT. There is minimal or no ultrafiltration. Therefore, drug clearance occurs mainly by diffusion, and is therefore dependent on the molecular weight of the drug, protein binding, volume of distribution and charge (24, 40). Drug clearance can be enhanced by increasing dialysate or blood flow rates, although the blood flow rate is usually limited to 50–180 mL/min. Diffusive drug clearance ($Cl_{diffusion}$) can be estimated as (40):

$$Cl_{diffusion} = Q_{dial} \times f_{up}$$

where Q_{dial} is the dialysate flow rate (mL/min)

In continuous hemodiafiltration (CAVHDF or CVVHDF), both a high rate of ultrafiltration that is replaced

Table 69-3

Sieving coefficient (S) and unbound fraction of selected antimicrobial agents (f_{up}). (Adapted with permission from Olyaei et al. (26) and from Pea et al. (25))

Drug	S	Unbound fraction (f_{up})
Amikacin	0.9	0.9
Amphotericin B	0.3	0.1
Ampicillin	0.7	0.8
Ceftazidime	0.9	0.9
Ceftriaxone	0.8	0.1
Cilastin	0.8	0.6
Ciprofloxacin	0.76	0.7
Clindamycin	1	0.4
Doxycycline	0.4	0.2
Erythromycin	0.4	0.3
Gentamicin	0.8	0.9
Imipenum	1	0.8
Meropenem	0.9	0.8
Metronidazole	0.8	0.8
Nafcillin	0.5	0.2
Penicillin	0.7	0.5
Piperacillin	0.8	0.8
Sulfamethoxazole	0.9	0.6
Tobramycin	0.8	0.9
Vancomycin	0.8	0.9

by IV fluids, and countercurrent dialysis are employed. Therefore, drug clearance occurs via both diffusion and convection. Total drug clearance during during hemodiafiltration (Cl_{CVVHDF}) can be calculated as (40):

$$Cl_{CVVHDF} = Cl_{convection} + Cl_{diffusion}$$

Drug clearance during any form of CRRT (Cl_{CRRT}) can be estimated from the following equation, by measuring drug concentration in the ultrafiltrate/dialysate effluent and in multiple plasma samples, and collecting the total volume of the ultrafiltrate/dialysate effluent (40):

$$Cl_{CRRT} = (C_{df} \times V_{df})/AUC_{0-t}$$

where C_{df} is the drug concentration in the ultrafiltrate/dialysate effluent, V_{df} is the volume of the effluent, and AUC $_{0\text{-}t}$ is the AUC during a timed effluent collection.

Table 69-4

Sieving Coefficient (S) and unbound fraction (f_{up}) of other selected drugs. (Adapted with permission from Olyaei et al. (26))

Drug	S	Unbound Fraction (f_{up})
Cisplatin	0.1	0.1
Cyclosporine	0.6	0.1
Diazepam	0.02	0.0
Digoxin	0.9	0.8
Famotidine	0.7	0.8
Lidocaine	0.2	0.4
Phenobarbitol	0.8	0.6
Phenytoin	0.4	0.2
Procainamide	0.9	0.9
Ranitidine	0.8	0.85

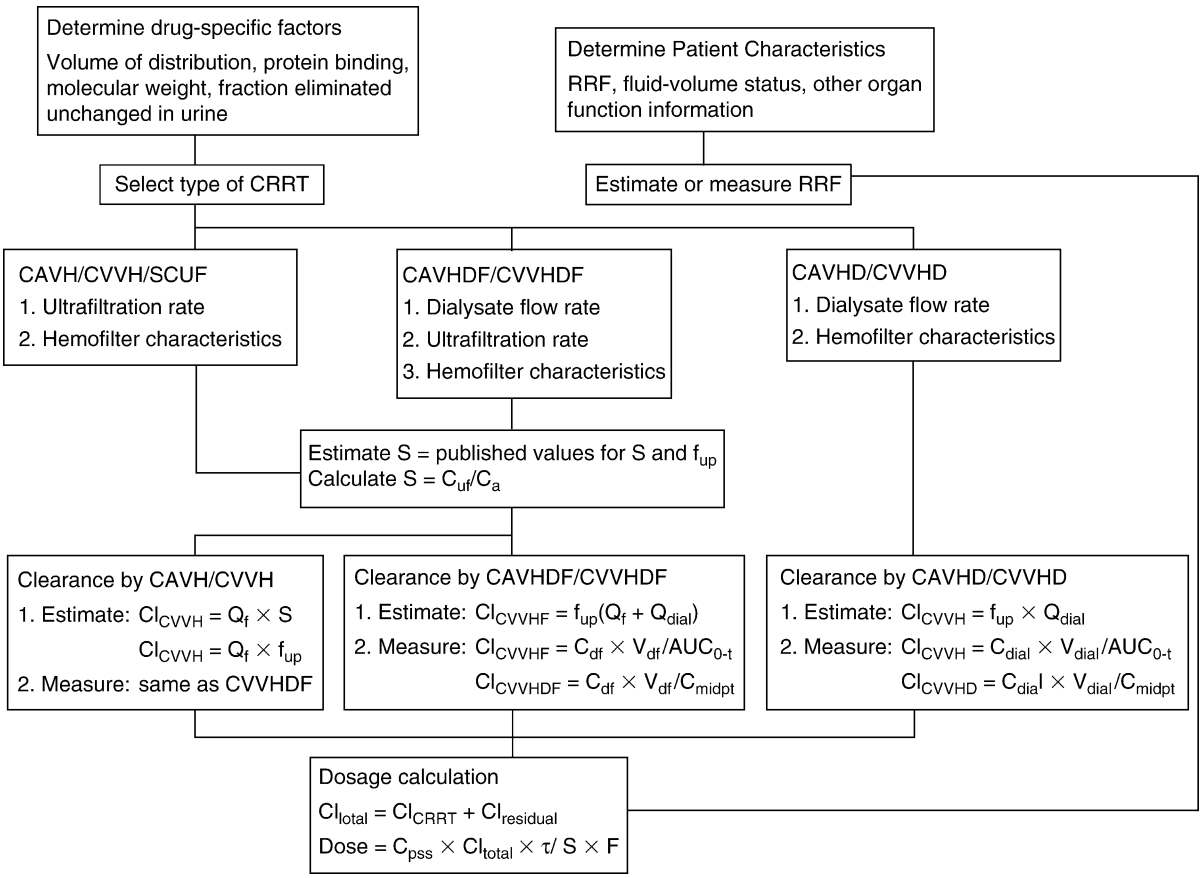
Principles of Drug Dosing During CRRT

Drug clearance during CRRT tends to be greater than during intermittent hemodialysis, therefore dosing usually needs to be greater. Increasing the amount of the drug per dose, the dosing interval or both may be necessary. In deciding on a dosing regimen, several factors must be considered: the severity of the illness, patient fluid-volume status, pathogen susceptibility to antibiotics, RRF, and the pharmacokinetic characteristics of the drug being administered (25). For instance, for time-dependent antimicrobials (e.g., β-lactams), increasing the individual dose without altering dosing frequency may be an appropriate approach to maintain a suitable minimum inhibitory concentrations for bacterial killing. Conversely, for concentration-dependent antimicrobials (e.g., aminoglycosides), it may be more appropriate to increase dosing interval. For life-threatening infections, it may be prudent to consider a high loading dose to ensure maximum efficacy (25). It is also important to consider the effect of RRF, since the renal clearance of some drugs may be significant and may affect the expected dialytic clearance. For instance, the total body clearance of meropenem is significantly increased, while its dialytic clearance is reduced during CRRT when the RRF is significant (54).

An algorithm for individualizing drug dosing during CRRT in adults has been developed by Joy et al. (40), and has also been recommended for use in pediatric patients (24) (*Fig. 69-5*). Although this algorithm provides an excellent framework to calculate drug dosages during CRRT, it has to be used with caution, since the kinetics of several drugs have not been rigorously studied in children,

Figure 69-5

Algorithm for individualization of drug dosing in patients receiving continuous renal replacement therapy (CRRT) (Adapted with permission from Joy et al. (40)). *Abbreviations: AUC $_{0\text{-}t}$* AUC during timed effluent collection; *C_a* prefilter plasma concentration; *CAVH/CVVH* continuous arteriovenous/venovenous hemofiltration; *CAVHD/CVVHD* continuous arteriovenous/venovenous hemodialysis; *CAVHDF/CVVHDF* continuous arteriovenous/venovenous hemodialfiltration; *C_{dial}* concentration in the dialysate; *C_{df}* concentration in the dialysate/ultrafiltrate effluent; *C_{midpt}* plasma concentration at the mid-point of effluent collection; *C_{uf}* concentration in the ultrafiltrate; *Cl* clearance; *Cl_{total}* total clearance; *Cp_{ss}* steady-state plasma concentration; *F* bioavailability; *f_{up}* fraction unbound to plasma protein; *Q_{dial}* dialysate flow rate; *Q_f* ultrafiltrate flow rate; *RRF* residual renal function; *S* salt from the drug; *S* sieving coefficient; dosing interval; *V_{dial}* volume of the dialysate; *V_{df}* volume of the effluent.



and extrapolation from the adult literature is necessary (6). In addition, drug clearance during CRRT can vary widely depending on changes in blood flow, ultrafiltration, and other CRRT parameters and on the patient's condition. This may further limit the accuracy of calculations. We therefore recommend rigorous patient and drug level monitoring when feasible to assess efficacy and avoid toxicity.

Drug Removal by Peritoneal Dialysis

Peritoneal dialysis is less efficient than hemodialysis at removing drugs. Usually, if the drug is not removed by hemodialysis, it will not be cleared by peritoneal dialysis. Drug removal is most effective for small molecular weight drugs with less protein binding and a small volume of distribution (55). The slow dialysate flow during continuous ambulatory peritoneal dialysis (CAPD) limits drug removal. Increasing dialysate flow via rapid exchanges such as during automated peritoneal dialysis (APD)

enhances peritoneal drug clearance. For instance, the removal of vancomycin and phenobarbital has been shown to be significantly enhanced with APD when compared to CAPD (56, 57). Therefore, APD may require different drug dosing regimens than CAPD. In addition, as in hemodialysis, the effect of RRF must be considered before dosing.

Pharmacokinetic studies for drugs administered during peritoneal dialysis in children are lacking, and much of the information has to be extrapolated from the literature in adults (58, 59). Recent observations in children suggest that current dosing guidelines for antimicrobial therapy in children with dialysis-related peritonitis, despite being mostly opinion-based, are effective (59). A recent study showed that vancomycin clearance by the pediatric peritoneal membrane was similar to that observed in adults, but the total body clearance was greater (60). The reasons for this are not clearly understood, and certainly more studies are needed to characterize drug pharmacokinetics in children receiving peritoneal dialysis.

Drug Dosing for Children with Renal Dysfunction: An Approach

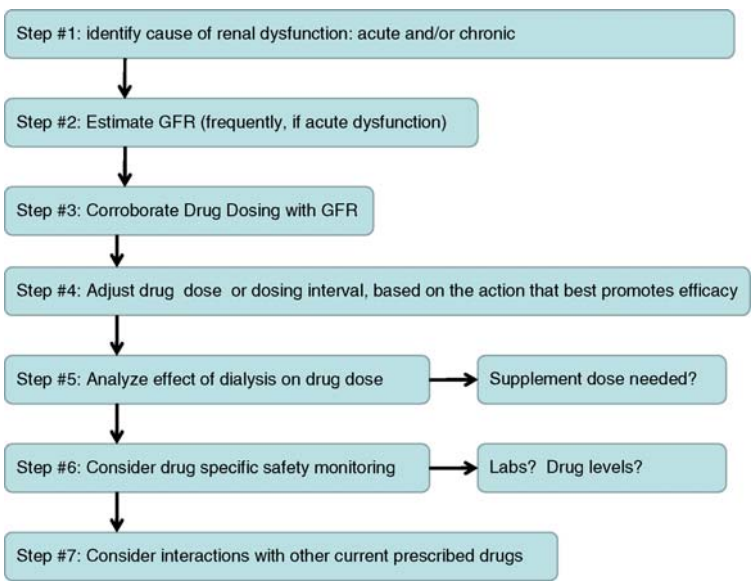
Optimizing the prescription of a drug in a child with some level of renal impairment is benefited by a thorough process of evaluation. We offer a step-wise format to aid in this scrutiny, in which the provider should arrive at answers to the following questions (*Fig. 69-6*). (1) What is the cause of the renal impairment? Is it acute kidney injury or chronic kidney disease? What is the level of residual renal function? (2) What is the estimated GFR in the patient? (3) How does this level of renal impairment affect the patient's current drug prescription(s)? For new drugs, how does renal impairment modify the loading dose of a drug? (4) How does renal impairment alter the maintenance dose, by the dosing interval or by the dosage itself? (5) How does the addition of a dialysis modality influence the drug's elimination? Is there an additional dose required, or more frequent interval dosing? (6) How is the drug action or renal function being monitored to promote safe usage of the drug? (7) What effect does a new drug, if any, have on the action or elimination of other currently prescribed drugs?

When renal impairment is due to acute kidney injury, the potential transient nature of the process means that there may be fluctuations in the GFR. Drugs that contribute to a reduction in renal function should be avoided, including angiotensin converting enzyme inhibitors, angiotensin receptor blockers, and non-steroidal anti-inflammatory agents. During the recovery phase of acute kidney injury, a drug's elimination properties could change drastically, necessitating more frequent monitoring of renal function as well as certain drug levels.

In chronic kidney disease, changes in renal function generally occur less frequently. Residual renal function, though, may be important in delineating a

Figure 69-6

Drug Dosing Strategy in Renal Dysfunction.



drug's dosage and interval, especially in patients undergoing complicated therapeutic regimens. As noted in previous chapters, methods to estimate GFR in children yield results that give a medical provider varying levels of confidence in that value. Radiolabeled tracers, such as DPTA, or studies using iohexol represent better methods of estimating GFR. Although more economical, creatinine clearance, or equations that utilize serum creatinine levels, are less reliable in their accuracy for GFR; in addition, the confidence of the calculated GFR from other biomarkers remains controversial. When feasible or when accuracy is critical (such as in chemotherapeutic regimens to treat malignancies), we recommend that the best available method be used to estimate the child's GFR and that reasonable follow-up monitoring occur. In addition, it should be recalled that renal tubules mature functionally at around 34 weeks gestation and that the GFR using the units ml/min/1.73 m^2 does not achieve comparable adult levels until the child reaches two years of age.

Guidance on changes to the loading or maintenance dose of a drug in the setting of renal impairment is available from multiple sources, including reference books and electronic sources. Loading doses can be affected by the changes that occur in renal failure when drug absorp-

Table 69-5

Recommendations for dosage adjustment in children with renal insufficiency. Modified form Daschner (6)

Drugs	Normal	Dose at GFR (ml/min/1.73 m^2)		
	Dose	50–30	30–10	Less than 10
Antibiotics				
Aminoglycosides				
Amikacin	15 mg/kg/day	40%	20%	10%
Gentamicin	3–5 mg/kg/day	60%	10%	5%
Netilmicin	6 mg/kg/day	60%	15%	10%
Tobramycin	6 mg/kg/day	60%	10%	5%
Carbapenems				
Imipenem + cilastatin	60 mg/kg/day	75%	25%	15%
Cephalosporins				
Cefaclor	40 mg/kg/day	Normal dose	Normal dose	Normal dose
Cefazolin	50–100 mg/kg/day	75%	30%	10%
Cefixime	8 mg/kg/day	Normal dose	75%	50%
Cefotaxime	50–100 mg/kg/day	Normal dose	60%	50%
Cefotiam	50–100 mg/kg/day	Normal dose	50%	20%
Ceftazidime	50–100 mg/kg/day	50%	15%	10%
Ceftriaxone	50–100 mg/kg/day	Normal dose	80%	50%
Cefuroxime	50–100 mg/kg/day	Normal dose	50%	15%
Cefuroxime axetil	25 mg/kg/day	Normal dose	33%	25%
Glycopeptides				
Teicoplanin	Loading dose 20 mg/kg	40%	10%	Loading dose followed per plasma levels
	Maintenance dose 6–10 mg/kg/day			
Vancomycin	20–40 mg/kg/day	30%	5%	Loading dose followed per plasma levels
Gyrase inhibitors				
Ciprofloxacin	PO 15 mg/kg/day	Normal dose	50%	25%
Ofloxacin	PO 7.5 mg/kg/day	50%	15%	15%
Macrolides				

Table 69-5 (Continued)

Drugs	Normal Dose	Dose at GFR (ml/min/1.73 m^2) 50–30	30–10	Less than 10
Azithromycin	10 mg/kg/day	Normal dose	Normal dose	Normal dose
Clarithromycin	10–20 mg/kg/day	Normal dose	50%	50%
Erythromycin	30–50 mg/kg/day	Normal dose	Normal dose	60%
Nitromidazoles				
Metronidazole	30 mg/kg/day	Normal dose	Normal dose	50%
Penicillins				
Amoxicillin	50 mg/kg/day	Normal dose	30%	15%
Amoxicillin + clavulanic acid	PO 40 mg/kg/day	Normal dose	25%	15%
Ampicillin	100 mg/kg/day	Normal dose	25%	15%
Other antibiotics				
Clindamycin	PO 20 mg/kg/day	Normal dose	Normal dose	Normal dose
Doxycycline	2–4 mg/kg/day	Normal dose	Normal dose	75%
TMP/SMZ	5 mg/kg/day TMP	Normal dose	50%	50%
Nitrofurantoin	3–5 mg/kg/day	contraindicated	contraindicated	contraindicated
Antifungal agents				
Amphotericin B		Normal dose	Normal dose	Normal dose
Fluconazole	5 mg/kg/day	50%	100% every 72 h	100% after HD
Itraconazole	5–10 mg/kg/day	Normal dose	No data	No data
Ketoconazole		Normal dose	Normal dose	Normal dose
Antituberculosis agents				
Ethambutol	PO 15 mg/kg/day	Normal dose	50%	50% after HD
Isoniazid	200 mg/m^2/day	Normal dose	Normal dose	Normal dose
Pyrazinamide	30 mg/kg/day	Normal dose	50%	Normal dose after HD
Rifampin	300 mg/m^2/day	Normal dose	Normal dose	Normal Dose
Antivirals				
Acyclovir	1,500 mg/m^2/day	60%	30%	10%
Foscarnet	100 mg/kg/day	50%	20%	Avoid
Ganciclovir	IV 10 1500 mg/kg/day	40%	15%	1.25 mg/kg after HD
Indinavir	1,500 mg/m^2/day	Normal dose	Normal dose	Avoid
Lamivudine	8 mg/kg/day	50%	15%	10%
Valacyclovir		50%	25%	15%
Valganciclovir	450 mg/m^2/day	50%	25%	25%
Zidovudine	450 mg/m^2/day	Normal dose	Normal dose	50%
Anticonvulsants				
Carbamazepine	10–30 mg/kg/day	Normal dose	Normal dose	Normal dose
Clonazepam	0.05–0.5 mg/kg/day	Normal dose	Normal dose	75%
Ethosuximide	15–20 mg/kg/day	Normal dose	Normal dose	75%
Phenobarbital	5 mg/kg/day	Normal dose	Normal dose	Per free level
Phenytoin	5 mg/kg/day	Normal dose	Normal dose	Per free level
Sodium valproate	10–100 mg/kg/day (plasma levels 50–100 mg/l)	Normal dose	75%	No data (plasma levels)

Table 69-5 (Continued)

Drugs	Normal Dose	Dose at GFR (ml/min/1.73 m^2) 50–30	30–10	Less than 10
Antihypertensive drugs				
ACE-inhibitors				
Captopril	0.3–3.15 mg/kg/day	40%	20%	10%
Enalapril	0.1–0.3 mg/kg/day	Normal dose	50%	25%
Ramipril	0.1–0.2 mg/kg/day	Normal dose	50%	30%
Sartans				
Losartan		Normal dose	Normal dose	Normal dose
β-blockers				
Atenolol	0.5–2 mg/kg/day	Normal dose	50%	25–50%
Bisoprolol	0.2 mg/kg/day	Normal dose	66%	50%
Propranolol	0.5–1 mg/kg/day	Normal dose	Normal dose	Normal dose
Ca-antagonists				
Amlodipine	0.05–0.15 mg/kg/day	Normal dose	Normal dose	Normal dose
Diltiazem	1 mg/kg/day	Normal dose	Normal dose	Normal dose
Nifedipine	0.5–2 mg/kg/day	Normal dose	Normal dose	Normal dose
Other drugs				
Clonidine	5–30 μg/kg/day	Normal dose	75%	50%
Doxazosin	0.5 mg/m^2/day	Normal dose	Normal dose	Normal dose
Nitroprusside sodium	0.5−5 μg/kg/min	Normal dose	Normal dose	Avoid
Prazosin	50−500 μg/kg/day	Normal dose	Normal dose	75%
Antiemetic drugs				
Ondansetron	0.1 mg/kg/dose maximum 3/day	Normal dose	75%	65%
Antigout drugs				
Allopurinol	10 mg/kg/day	50%	30%	25%
Colchicine		Normal dose	Normal dose	Avoid
Antiinflammatory drugs				
Acetylsalicylic acid		75%	50%	50%
Ibuprofen		Normal dose	Normal dose	Normal dose
Indomethacin		Normal dose	Avoid	50%
Antineoplastic agents				
Cisplatin	20 mg/m^2/day	Contraindicated	Contraindicated	Contraindicated
Ifosfamide	2,000 mg/m^2/day	Avoid	Contraindicated	Contraindicated
Immunosuppressive drugs				
Azathioprine	1–3 mg/kg/day	Normal dose	Normal dose	Normal dose
Cyclophosphamide	1–2 mg/kg/day	Normal dose	50%	25%
Cyclosporine	3–10 mg/kg/day	Normal dose	Normal dose	Normal dose
Mycophenolate mofetil	1,200 mg/m^2/day	Normal dose	Normal dose	Normal dose
Sirolimus		Normal dose	Normal dose	Normal dose

Table 69-5 (Continued)

Drugs	Normal Dose	Dose at GFR (ml/min/1.73 m^2) 50–30	30–10	Less than 10
Tacrolimus	0.15 mg/kg/day	Normal dose	Normal dose	Normal Dose
Diuretics				
Acetazolamide		50%	30%	25%
Spironolactone	1–5 mg/kg/day	Normal dose	Normal dose	50%
Gastrointestinal drugs				
Metoclopramide	PO 0.5 mg/kg/day	Normal dose	50%	50%
Omeprazole	0.5–1 mg/kg/day	Normal dose	Normal dose	Normal dose
Ranitidine	PO 4 mg/kg/day	Normal dose	50%	50%
Spasmolytics				
Oxybutynin	10 mg/kg/day	Normal dose	Normal dose	Normal dose
Scopolamine/ hyoscyamine	1–2 mg/kg/day	Normal dose	75%	50%
Miscelaneous				
Acetaminophan		Normal dose	Normal Dose	Normal dose
Codeine		Normal dose	Normal dose	Normal Dose
Digoxin		75%	25%	Per level
Dimethindene	0.1 mg/kg/day	Normal dose	Normal dose	Normal dose
Meperidine		Avoid	Avoid	Avoid
Methotrexate		Contraindicated	Contraindicated	Contraindicated
Morphine		Normal dose	Avoid	Avoid
Oxycodone		Normal dose	Normal dose	Normal Dose
Piritramide		Normal dose	Normal dose	Normal dose
Terbutaline		Normal dose	50%	Avoid
Terfenadine	2 mg/kg/day	Normal dose	Normal dose	50% (1 single dose)
Theophylline	10 mg/kg/day	Normal dose	50%	50%
Tramadol	4–8 mg/kg/day	Normal dose	50%	50%

tion and distribution properties are altered, as is the case for digoxin (61). In general, pharmacological agents in which 20% or more of the drug or its metabolites are eliminated through renal mechanisms may require dose adjustments in renal impairment (*Table 69-5*). The maintenance dose can be altered in dose or frequency of delivery. Drugs in which peak values predominate the therapeutic efficacy should have their dosing interval increased. Examples include antivirals like acyclovir (62) or aminoglycosides like gentamicin (63). Drugs in which therapeutic levels need to be maintained should have their doses reduced but the intervals maintained. Examples comprise the cephalosporins and some anticonvulsants like carbamazepine (64).

When a patient is receiving renal replacement therapy, the drug's elimination properties may be altered, as noted previously in this chapter. The extent of that elimination depends on the dialysis modality, but each potential modality may require a supplementation of the drug dose and a change in the interval of the dosing. Aminoglycosides illustrate the dilemma and the variability in dosing approach quite well. With adequate hemodialysis, gentamicin is eliminated effectively and is therefore redosed at the end of dialysis treatment. With peritoneal dialysis, gentamicin is dosed based on the volume of the dialysate prescription. Yet, even with therapeutic drug monitoring, the risk of acquiring vestibular toxicity is higher in peritoneal dialysis than in hemodialysis, likely due to the variability in absorption properties across the

peritoneum. For renal replacement therapy, drug monitoring is essential to determine proper dosing intervals.

Drug interactions can often be subtle and yield deleterious consequences. Children with acute kidney injury typically have co-morbid features that oblige multiple pharmacological agents. Children with chronic kidney disease require a compendium of medications to treat the etiology or offset its sequelae. Individualization of drug dosing is recommended to prevent complications with ongoing monitoring of renal function, blood pressure, and drug levels to assess for any evidence of renal toxicity. Drug monitoring should be directed at more than just known nephrotoxic agents, as careful attention should also be paid to drugs that may indirectly create renal impairment. Drugs that alter P450 enzyme activity are particularly notable as noted in *Table 69-1*. Antifungals like ketoconazole, macrolides like erythromycin, or non-hydropyridines like diltiazem all inhibit P450 enzymes. If these medications are prescribed for kidney transplant patient on a calcineurin inhibitor, renal function can worsen due to the toxic levels of the calcineurin inhibitor.

With the implementation of the electronic medical record, integrated pharmacy systems that recognize drug interactions ideally will reduce the number of mistakes leading to renal toxicity and other untoward outcomes, and there is some evidence to support that conjecture. It is additionally proposed that these systems will aid in individualization of therapy, allowing for safer treatments for children and adults with, or at risk for, renal impairment. Until such improvements are more widely available, vigilance by the medical providers and pharmacists will remain at the forefront for proper drug administration.

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70 Endocrine and Growth Disorders in Chronic Kidney Disease

Franz Schaefer

Endocrine Disorders in Chronic Kidney Disease

Pathophysiological Mechanisms

Uremia interferes with metabolism and regulation of hormones by various mechanisms. Disturbed endocrine function may arise either from inappropriate circulating hormone *concentrations* or from altered hormone *action* at the target tissue level. Both conditions may be present in the uremic state.

Increased Plasma Hormone Concentrations

Renal catabolism accounts for one to two thirds of the metabolic clearance rates of various polypeptide hormones (1). Most polypeptide hormones are almost freely filtered in the glomerulus, followed by either intratubular (brush border peptidases) or intracellular (cytosolic or lysosomal) degradation in tubular cells. Moreover, certain hormones are subject to receptor-mediated uptake across the basolateral tubular cell membrane. Hence, any reduction of renal mass will result in a decrease in the metabolic clearance of these hormones (*Fig. 70-1*). If catabolic mechanisms differ for different isoforms or subunits of a hormone, an imbalance of these constituents may arise, altering the relation between biologically active and inactive hormone fragments. In addition to renal clearance, extrarenal hormone elimination may also be reduced in renal failure. For example, degradation of insulin in skeletal muscle tissue is diminished (3), and hepatic catabolism of biologically active PTH is reduced in uremia (4). Finally, *hypersecretion* of various hormones or hormone binding proteins occurs in renal failure, either as an appropriate response to secretory stimuli (e.g., PTH) or without an apparent homeostatic signal (e.g., prolactin).

Decreased Plasma Hormone Concentrations

The reduction in functional renal mass is assumed to be the main cause for decreased levels of hormones produced by the *kidney* (erythropoietin, 1,25-OH_2-Vitamin D_3). In addition, the uremic milieu may suppress the production of these hormones by alterations of the internal milieu. For example, intracellular phosphate accumulation may inhibit 1-hydroxylase even before the reduction of renal mass becomes quantitatively important. Levels of *extrarenal hormones* may be decreased when the hormone-producing gland is the final effector organ of a complex hormonal axis (e.g., testis-testosterone, ovary-estradiol). In these cases, insufficient production of hormones may result either from direct toxic damage to the endocrine gland, from insufficient stimulatory input from the superior part of the hormonal axis, or from hyporesponsiveness of the gland.

Disorders of Hormone Action

Disturbed Activation of Prohormones

Concentrations of certain prohormones are elevated in chronic kidney disease (CKD), e.g., pro-IGF1A, a precursor of insulin-like growth factor 1 (IGF1) which is not detectable in normal serum (5), or proinsulin, which is not converted appropriately to insulin or C-peptide in patients with end-stage renal disease (6). Peripheral conversion of thyroxine (T4) to tissue-active tri-iodothyroxine (T3) is impaired (7). Some prohormones may block hormone action by competitively inhibating receptor binding at the tissue level.

Multimolecular Forms of Variable Bioactivity

Some polypeptide hormones circulate in plasma in multiple isoforms characterized by varying composition of their carbohydrate side chains. In CKD, altered glycosylation

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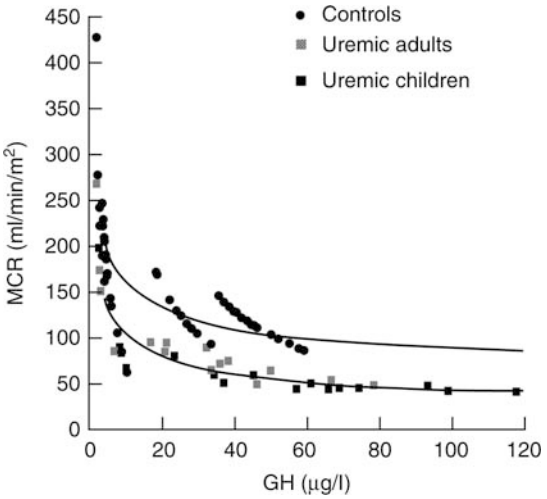
Figure 70-1

Total metabolic clearance rate (MCR) as a function of steady-state plasma GH concentrations in controls and CKD patients. MCR is reduced in children and adults with CKD at any prevailing plasma GH level. (From (2)).



and sialisation (8) may shift the isohormone spectrum towards less bioactive forms (e.g., LH (9)). This may be due to alterations of posttranslational processing or to differences in the renal clearance of individual isoforms.

Hormone Binding to Plasma Proteins

Excessive concentrations of several insulin-like growth factor (IGF)-binding proteins are found in CKD (10, 11). Binding proteins can compete for the hormone with target organ receptors, and this explains in part the reduced somatomedin bioactivity in the presence of normal total serum IGF. Abnormal concentrations of other polypeptide binding proteins may similarly be related to abnormal hormone action in uraemia.

Alterations of Target Tissue Sensitivity

Diminished target organ responsiveness is observed in various endocrine systems in uremia. Mechanisms for altered target tissue sensitivity include reduced cellular receptor activation due to the diminished receptor abundance, the presence of inhibitory substances, the accumulation of molecules inhibiting hormone-receptor interaction (e.g., insulin-like growth factor 1) and structural changes of either the hormone or its receptor (10). Moreover, defects of hormone–receptor complex dependent intracellular signalling may occur. Such postreceptor events seem to play a key role in the pathogenesis of insulin (12) and growth hormone (13) resistance in uraemia.

Gonadotropic Hormone Axis

Clinical Findings

The onset of puberty is usually delayed in adolescents with CKD. At least 50% of adolescents with end-stage renal disease (ESRD) enter puberty later than the normal range (14) and achieve the pubertal milestones beyond the normal age range (15–17). Late puberty is observed both in children on dialysis and after renal transplanation. In the Cooperative Study for Pubertal Development in CKD the onset of puberty was delayed by 2–2.5 years on average (18). The start of genital maturation (Tanner G2) was delayed by 1.8 years in uremic and 2.5 years in transplanted boys. Full genital maturation was achieved with a delay of 2.2 and 3.2 years, respectively. Thus, once started, puberty appears to proceed at a normal rate. However, in individual patients, particularly on long-term dialysis, pubertal maturation may arrest for years. Almost half of the girls treated by dialysis or renal transplantation fail to menstruate before the upper normal age limit of 15 years. Menarche even tends to occur later in transplanted than in dialyzed girls (15).

Unlike the development of secondary sexual characteristics, which is delayed but not permanently halted in CKD, reproductive function may be permanently impaired. In autopsy studies in boys with CKD, germ cell depletion in the testicular tubules has been described (19). These changes do not appear reversible after renal transplantation (20). Persistently reduced sperm counts were observed in 10 of 12 successfully transplanted young adults who had suffered from ESRD during childhood (20). Erectile dysfunction, decreased libido and fertility are primarily organic in nature and are due to uremia as well as to other comorbid conditions, fatigue and psychosocial factors (21). The frequency of conception is decreased in women with CKD and pregnancy is uncommon in adolescents with ESRD. The percentage of surviving infants ranges from 70% to 100% in women with CKD on conservative treatment or after renal transplantation (20) and from 50% to 80% in women on dialysis (22–24). Intrauterine growth retardation is frequent and the risk of delivering prematurely and/or a small-for-date baby is increased (17, 21, 22).

Gonadal Hormones

In adults with CKD plasma concentrations of *testosterone* (T) are usually low or low normal (25), due to reduced synthesis and, perhaps, increased metabolic clearance rate

(26, 27). In prepubertal children with predialytic renal failure, low total and free T and dihydrotestosterone (DHT) plasma concentrations have been reported (28). However, since the adrenal cortex is the major site of androgen production before puberty and specific adrenal androgens are also low in children with CKD (29), low prepubertal plasma androgen levels do not provide evidence for gonadal damage before puberty (30, 31). In pubertal patients, normal or slightly subnormal plasma T concentrations are observed (32–34). In late puberty, however, DHT concentrations are significantly reduced in children with CKD compared with healthy or post-transplant children (own unpublished observation). Impaired conversion of T to DHT due to decreased 5-reductase activity has been suggested (35, 36). Because this metabolite is responsible for many tissue actions of the androgens, reduced conversion of T to DHT may explain the frequently sparse development of secondary sexual characteristics in boys with advanced renal failure.

The testicular response to supraphysiological stimulation by human chorionic gonadotropin (HCG) is impaired in adult men (25) as well as in prepubertal and pubertal boys with CKD. Testicular insufficiency is most prominent in boys on hemodialysis (32). Leydig cell resistance is caused by a cAMP-dependent mechanism (37). A factor with LH-inhibitory activity has been demonstrated in uremic serum (38) (*Fig. 70-2*). The disorder is reversible after renal transplantation (32).

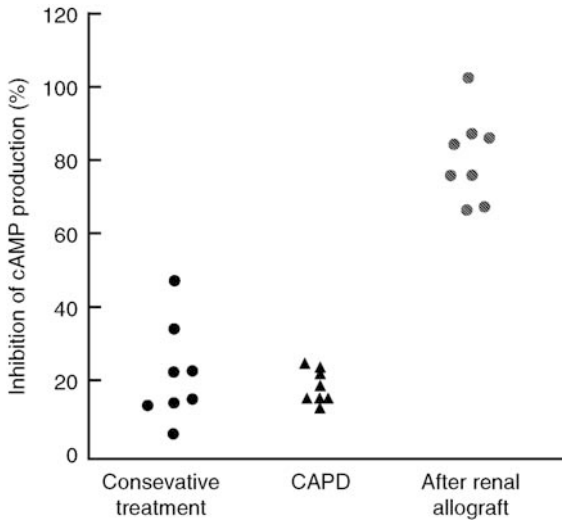
The physiological age-related decrease of sex-hormone binding globulin is conserved in prepubertal children on dialysis (33); however, at a given age sex-hormone binding globulin concentrations are higher and the unbound T fraction is lower than in normal children. The increase of sex-hormone binding globulin may be due to accumulation in end-stage renal failure; a normal fraction of free T has been reported in prepubertal children on conservative treatment (28).

The plasma concentration of *inhibin*, a polypeptide produced by the Sertoli and Granulosa cells, is elevated in peripubertal boys with CKD (39). It is unclear whether elevated plasma inhibin levels in uremia reflect alterations of Sertoli cell function, impaired feedback regulation of the pituitary-gonadal axis or decreased metabolic clearance of the hormone.

Estradiol plasma concentrations in the low normal range are observed in women with CKD (40). In pubertal girls with CKD, estradiol plasma levels were normal or low when related to pubertal stage (41, 42). An inverse correlation between serum creatinine levels and estradiol concentrations was found in patients with predialytic CKD. Longitudinal analysis revealed an insufficient increase in estradiol during puberty in those patients whose renal function deteriorated, whereas following renal transplantation, even after several years of dialysis, estradiol concentrations increased (42).

Figure 70-2

Evidence for circulating LH inhibitor in sera of boys with CKD. HCG-induced cAMP production by cell line expressing human LH/HCG receptor is suppressed in presence of serum of patients on conservative or CAPD treatment (upper panel). (Adapted from (38) with permission.)



Gonadotropins

Plasma luteinizing hormone (LH) levels are high normal or elevated in adult men, women, prepubertal and pubertal boys and girls with CKD (9, 25, 28, 40, 41, 43); follicle-stimulating hormone (FSH) concentrations are also usually elevated both in adults and children with CKD. After transplantation, LH levels usually return to normal, whereas plasma FSH frequently remains elevated.

The combination of elevated gonadotropins with decreased or low-normal gonadal hormone levels has been taken as evidence for a state of compensated hypergonadotropic hypogonadism (28, 44). However, the degree of hypergonadotropism in CKD is usually inadequate for the prevailing degree of hypogonadism, suggesting an additional defect of hypophyseal gonadotropin secretion.

An alteration at the pituitary level is suggested by the blunted increase of plasma LH and FSH following stimulation by a bolus of exogenous GnRH in men, women, boys and girls with CKD (25, 28, 41). These abnormalities appear even more marked when the diminished metabolic

clearance of gonadotropins is taken into account (45). The gonadotropin response to GnRH is normalized after successful transplantation (41).

LH is released from the pituitary in episodic (pulsatile) bursts occurring every 90–120 min. The plasma LH concentration peaks reflect intermittent secretion of hypothalamic GnRH into the hypophyseal-portal blood stream (46). Hence, the analysis of plasma LH pulses gives indirect information about the functional state of the hypothalamic GnRH "pacemaker". A differentiation of the secretion and elimination components underlying the fluctuating plasma LH concentration patterns by means of the deconvolution methodology (47) revealed that the elevation of basal plasma LH concentrations is entirely due to the diminished renal metabolic clearance of the hormone (45) both in humans (48) and in rats (49). Plasma half-life of LH is inversely correlated with GFR (48). In contrast, actual pituitary LH secretion rates are decreased in CKD; pubertal dialysis patients secrete 3 times less immunoreactive LH and 2.5 times less bioactive LH in episodic nocturnal peaks than normal adolescents (48). This abnormality, which has been reproduced in experimental uremia (50), gives strong evidence for a dysregulation of the gonadotropic axis at the hypothalamo-pituitary level. After transplantation, a regular pattern of LH pulses is reestablished (9, 51). As the onset of puberty is heralded by the emergence of a nocturnal pattern of pulsatile LH secretion, the observed disturbance of pulsatile LH secretion suggests that the delayed pubertal development in CKD is caused by a primary hypothalamic defect. Experimental evidence confirms that the subnormal pituitary gonadotropin secretion is caused by diminished release of gonadotropin-releasing hormone (GnRH) into the hypophyseal portal circulation (49, 52). In cultured GnRH producing neurons GnRH release is inhibited upon addition of a high-molecular weight fraction of uremic serum. The inhibitor is a hydrophilic protein suppressing GnRH exocytosis, but not synthesis (53). Moreover, using in vivo intracerebral microdialysis in experimentally uremic rats we observed an increased tone of the neuroinhibitory amino acid GABA in the extracellular fluid of the hypothalamic medial preoptic area, the region where the GnRH neurons reside (54). Hence, central nervous GABA accumulation may be another mechanism of downregulation of the gonadotropic hormone axis in uremia.

Clinical and experimental evidence indicates that the neuroendocrine control of pulsatile LH secretion is altered in CKD. Although overt hypogonadotropism is masked by a simultaneous reduction of metabolic clearance rates, the deficient physiological pulsatile GnRH-LH signal may be the key abnormality underlying the delayed onset of puberty in CKD. The observed disorders of LH secretion and metabolism appear to be reversible after successful renal transplantation.

Besides the quantitative insufficiency of the hypothalamo-pituitary unit, the biological *quality* of the circulating gonadotropins is also altered in uremia. LH bioactivity, measured by the potency of a plasma sample to induce testosterone in a Leydig cell culture, depends on the degree of glycosylation and sialisation of this glycoprotein hormone (55). During normal puberty, the relative bioactivity of LH gradually increases (56). In pubertal (57, 58) and adult patients (51) on dialysis, the ratio of bioactive to immunoreactive plasma LH is reduced, suggesting that the spectrum of circulating LH molecules is shifted towards bioinactive forms (9, 48, 51, 59). This may be attributed to altered glycosylation of plasma proteins in uremia (8). The physiological increase in hormone bioactivity during puberty is absent in dialysis patients (58). The recently characterized inhibitor of LH action circulating in serum of uremic boys may represent an accumulating LH fragment (38). After successful renal transplantation, LH biopotency tends to normalize.

Prolactin

Prolactin is a proteohormone secreted by the pituitary that is involved in the hormonal regulation of lactation. Its function in non-puerperal women, in men and in children is not clear. However, prolactin attenuates gonadotropin release. Plasma prolactin levels are elevated in men (60), and, more markedly, in women and pubertal girls (41, 60) with CKD. Uremic hyperprolactinemia appears to result from both decreased metabolic clearance rate and increased production rate of the hormone (61). Hyperprolactinemia may play a role in the pathogenesis of uremic hypogonadism, since elevated prolactin levels exert a suppressive effect on the GnRH-LH pulse generator (62). The physiological sleep-related nocturnal prolactin surge is absent (63), and the circadian rhythm of secretion is deranged (64). Prolactin secretion in CKD patients is insensitive to stimulation by TRH (65), chlorpromazine, metoclopramide, arginine or insulin-induced hypoglycemia (66). L-dopa and dopamine are not effective in suppressing prolactin secretion (66); however, hyperprolactinemia may be corrected by long-term treatment with dopaminergic agonists (67, 68). Uremic hyperprolactinemia may be related to other complications of CKD such as vitamin D deficiency and renal anemia. Substitution

of 1,25-Vitamin D_3 (69) and erythropoietin (70) leads to a partial normalization of plasma prolactin levels.

Various physiological studies and pharmacological tests reveal a partial disintegration of the gonadotropic hormone axis at the hypothalamo-pituitary level, in addition to alterations of gonadal function. The analysis of hormone secretory patterns has confirmed that the central nervous dysregulation is not restricted to the functional reserve capacity of the reproductive hormone system, but affects physiological spontaneous hormone secretion. The reversibility of the observed changes after successful renal transplantation gives further evidence that regulatory mechanisms, rather than toxic end-organ damage, affect gonadal function in uremia. It remains to be shown to what extent the apparent dysregulation of hormone secretion represents a "physiological" adaptation to an adverse metabolic environment.

Growth Hormone – Insulin-Like Growth Factor Axis

During normal childhood, the somatotrophic hormone axis plays a key role in the regulation of body growth. In addition, growth hormone is part of a complex system of counter-regulatory hormones maintaining the homeostasis of carbohydrate metabolism.

Growth Hormone (GH)

Serum Concentrations and Kinetics

Fasting GH concentrations are variably elevated in uraemic children and adults dependent on the extent of renal failure (71, 72). The kidney is a major site of GH degradation (73). In patients with endstage renal failure, the metabolic clearance rate of GH is reduced by approximately 50% (2, 74) (*Fig. 70-1*). Deconvolution analysis of GH plasma concentration profiles revealed that the increase in plasma GH concentrations is mainly due to an increased plasma half-life of the hormone, whereas the actual pituitary GH secretion rate varied between patients and studies. GH secretion rate was high-normal in prepubertal children with ESRD and increased in adult patients on hemodialysis, possibly as a result of attenuated bioactive IGF-I feedback of the hypothalamo-pituitary unit (75, 76). In pubertal patients with advanced CKD reduced GH secretion rates were observed, indicating an altered sensitivity of the somatotropic hormone axis to the stimulatory effect of sex steroids during this stage of development (77).

The variability of plasma GH levels in CKD may in part be due to associated conditions such as acidosis and malnutrition, which independently affect GH secretion. Metabolic acidosis suppresses GH release both in rodents and humans (78).

Neuroendocrine Control of GH Release

Dysregulation of GH secretion may be related to abnormalities of central neuroendocrine control mechanisms. Evidence for this is provided by several hypothalamo-pituitary function tests. The GH response to intravenous GH-releasing hormone (GHRH) is augmented and prolonged in children (79). Exogenous thyrotrophin-releasing hormone (TRH), which does not affect GH release in healthy subjects, markedly enhances GH secretion in subjects with renal failure (71, 80). Also, CKD patients respond to acute hyperglycaemia by a paradoxical increase of GH secretion (71, 81). Stimulation tests such as arginine infusion and insulin-induced hypoglycaemia lead to a sustained, exaggerated increase of GH (71, 82, 83). However, the altered metabolic clearance rates of GH as well as of the provocative agents in renal failure (84) make a meaningful clinical interpretation of such tests virtually impossible.

GH Receptor Signaling and Tissue Action

Growth failure despite elevated circulating GH concentrations suggests a state of GH resistance in children with CKD. Indeed, GH-induced hepatic IGF-I synthesis is markedly reduced in rats with CKD (13, 85). This GH insensitivity may in part be due to deficient GH receptor expression, although this is controversial. Reduced hepatic GH receptor mRNA and receptor binding has been reported in some but not all studies carried out with animals (13, 85–88). These discrepancies may be related to the effect of reduced nutritional intake. Indeed, controlling for the anorexia of chronic uremia by pair feeding control animals abolished the difference in GH receptor protein expression and binding to liver plasma membranes seen when ad-lib controls and uremic animals are compared (13, 87), although receptor mRNA levels remained subnormal (13). On the other hand, reduced GH receptor protein expression was observed in the growth cartilage of rats with CKD even though nutritional intake was controlled (89). In humans serum levels of GH binding protein, putatively reflecting hepatic GH receptor status, were decreased in some, but normal in other studies (74, 90–93).

Another mechanism accounting for the resistance to GH in uremia is provided by a marked *post-receptor* GH signaling defect recently observed in livers of chronically uremic rats (13). Despite unaltered GH receptor protein levels, phosphorylation of the GH receptor associated

tyrosine kinase janus kinase-2 (JAK2) was diminished by 75% (Fig. 70-3). This resulted in a similar suppression of GH/JAK2-dependent phosphorylation of specific downstream signaling molecules, namely signal transducer and activator of transcription (STAT)-1, -3 and -5. This defect was possibly caused by up-regulation of intracellular JAK2 inhibitors, namely the suppressors of cytokine signaling (SOCS)-2 and -3. Since these regulatory proteins are induced by inflammatory cytokines, it may be postulated that GH resistance in uremia is caused by the commonly associated with this condition.

Insulin-Like Growth Factors

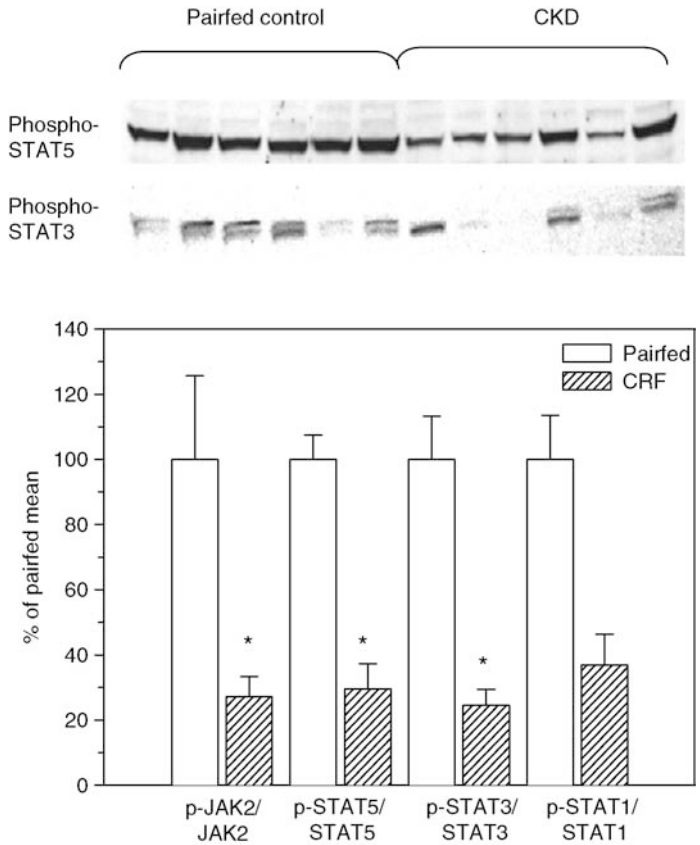
As most metabolic effects of GH are mediated by IGF-I, GH insensitivity in uremia may also be due to IGF resistance. Indeed, several studies in the rat as well as in humans demonstrated marked IGF-I resistance in CKD (94–97).

IGF Serum Concentrations

The effect of GH on longitudinal growth is partially mediated by stimulating the production of somatomedins, the two most important of which are the insulin-like growth factors (IGF) 1 and 2. Serum IGF-I and IGF-II levels in children with CKD are in the normal range, whereas in ESRD mean age-related serum IGF-I levels are slightly decreased and IGF-II levels moderately elevated (98). Hence, total immunoreactive IGF levels in CKD serum are normal. In contrast, IGF *bioactivity* is markedly reduced. Similarly, the level of free IGF-I is reduced by 50% in relation to the degree of renal dysfunction (99). This finding is one of the key abnormalities of the GH/IGF axis in children with CKD.

Figure 70-3

Impaired post-receptor GH signaling in rats with experimental uremia. Upper panel: Deficient nuclear accumulation of tyrosine-phosphorylated STAT5 and STAT3 protein in livers of rats with CKD compared to pair-fed controls. Lower panel: Reduced hepatic phosphorylation of JAK2, STAT-5, -3 and -1 in uremic rats. (Reproduced with permission from (13)).



IGF Plasma Binding and Tissue Action

The discrepancy between low somatomedin activity by bioassay and normal or elevated insulin-like growth factor by radioimmunossay or radioreceptor assay suggests the presence of circulating somatomedin inhibitors in uremia. An early study suggested the presence of a low-molecular weight IGF inhibitor (approximately 1 kDa), whose molecular structure has however not been characterized further (100).

The most likely explanation for the inhibition of somatomedin action in uraemia has emerged from the identification of six insulin-like growth factor-binding proteins (IGFBP-1 to -6) of which IGFBP-3 appears to be the most abundant in humans, constituting more than 95% of total circulating IGFBP. In children with CKD, the serum concentrations of IGFBP-1, -2, -4 and -6 are increased in a manner inversely related to glomerular filtration rate (10, 11, 98, 101–104) (*Fig. 70-4*). Ligand blotting shows that the elevation of radioimmunoassayable IGFBP-3 is due to an increase in low molecular weight forms mainly in the range of 14 and 19 kDa, whereas intact IGFBP3 (38 and 41 kDa) is markedly reduced (105, 106). IGFBP-1, -2 and -6 inhibit somatomedin bioactivity in vitro (107). Somatomedin bioactivity in uraemic serum can be returned to normal by removing unsaturated IGFBP (108). Experimental evidence suggests that the increase of IGFBP-1 and -2 is not only due to reduced renal metabolic clearance but also to increased hepatic synthesis (109). An important question is whether the imbalance between normal total IGF and the excess of unsaturated IGFBPs contributes to growth failure in children with CKD. Serum levels of IGFBP-1, -2 and -4 correlate inversely with standardized height in CKD children (98, 104, 110). While it is tempting to speculate that these IGFBPs could contribute to growth failure in children with CKD it is difficult to prove causality since IGFBP levels and height SDS are all correlated with GFR.

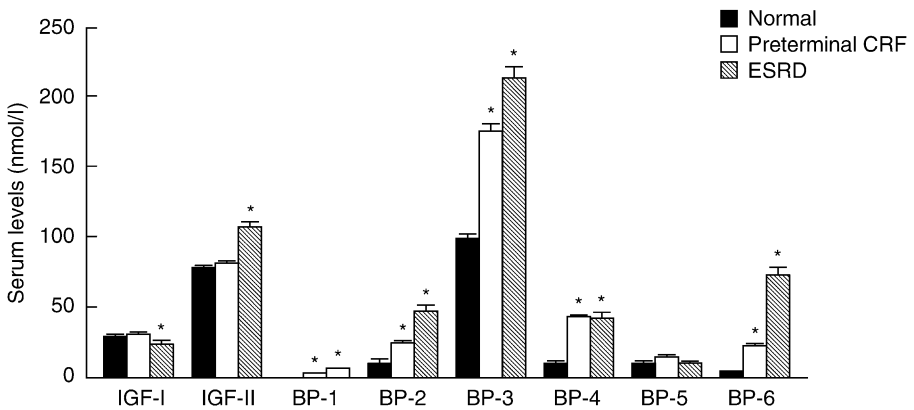
Apart from the increased plasma IGF-I binding capacity, a post-receptor IGF-I signaling defect may also contribute to IGF-1 resistance in CKD. In uremic rats the effect of IGF-I and various IGF-I analogs on protein turnover was suppressed (94). The observation that the inhibitory effect of IGF-I and its analogs were affected to a similar degree indicates that the resistance arises because of a defect at a cellular level and not because of changes in the IGFBP levels. Deficient autophosphorylation of the IGF-I receptor in skeletal muscle of uremic rats has been reported (94), but not confirmed in a later study (111).

GH-IGF-1 Homeostasis in Chronic Renal Failure

The pattern of elevated GH, normal total IGF and markedly elevated IGFBP plasma concentrations in uremia has interesting implications with respect to the estimated IGF production rate. In a functioning homeostatic system, the diminished free IGF-1 levels would be expected to stimulate IGF production in order to restore the steady-state between bound and unbound hormone at a higher level. In uremia, however, total IGF concentrations are normal rather than increased. Kinetic modeling suggests that the metabolic half-life of IGFs is markedly elevated, and the IGF production rate is decreased 10- to 100-fold in

Figure 70-4

Molar concentrations of IGFs and IGF binding protein concentrations in children with CKD and on dialysis in comparison to age-matched healthy children. (From (104) with permission.)



uremia (112). Taken together, the markedly deficient IGF1 synthesis and the modest elevation of plasma GH levels, which is mainly due to impaired metabolic clearance, in the presence of increased IGF binding capacity strongly support the notion of a multilevel homeostatic failure of the GH-IGF-1 system in uremia: Pituitary GH secretion is insufficiently feedback-stimulated by reduced free IGF-1 levels, and marked tissue GH resistance prevents an increase in total IGF-1 levels in the presence of elevated GH.

Glucocorticoids and GH-IGF Hormone Axis

Following renal transplantation, the use of glucocorticoids for immunosuppression interferes with the GH-IGF-1 axis on various levels. Endogenous GH secretion is reduced in pediatric renal allograft recipients, mainly by a reduction of amplitudes of the GH secretory bursts (34, 113). The physiological increase of GH burst amplitudes during puberty is blunted, and the normal correlation between sex steroid plasma levels and GH secretion rate is absent (34). GH release after insulin-induced hypoglycemia is inadequate (113). The insufficiency of spontaneous and stimulated GH secretion in post-transplant patients is most likely explained by a glucocorticoid-induced enhancement of hypothalamic somatostatin release (114). On the target tissue level, glucocorticoids suppress GH receptor and IGF-I gene transcription (115, 116). Nevertheless, basal IGF-I plasma levels in renal transplant recipients are in the normal range (117–119). This discrepancy between clinical and experimental findings suggests that glucocorticoids alter translation, synthesis and/or secretion of IGF-I in such a way that the IGF-I mRNA redundancy no longer reflects IGF-I protein synthesis. Whereas circulating immunoreactive IGF-I concentrations are not consistently reduced, IGF bioactivity is markedly diminished in patients on glucocorticoid treatment (117, 120). This may be due to the induction of IGF inhibitors of 12–20 kD molecular weight and/or to increased serum IGF-BP3 levels (117, 120). Moreover, increased IGFBP-2 concentrations are found in patients with Cushing's syndrome (121). IGFBP-2 may be another functional IGF-1 inhibitor in patients receiving chronic glucocorticoid treatment.

Glucocorticoids also interfere with chondrocyte growth and enchondral bone formation in various ways. They inhibit sulfate incorporation into cartilage matrix as well as mineralization and formation of new bone (122). In cultured epiphyseal chondrocytes, dexamethasone decreases DNA synthesis and cell proliferation, GH receptor expression and paracrine IGF-I secretion (123).

Thyroid Hormone Axis

Clinical Findings

The thyroid hormone axis plays an important role in the regulation of tissue metabolism. Throughout childhood, thyroid hormone is involved in growth and skeletal maturation, stimulating both cartilage proliferation and epiphyseal differentiation.

The prevalence of goiter is increased in patients with ESRD (124, 125). The prevalence of hypothyroidism ranges between 0% and 9.5% (124). Whereas primary hypothyroidism is observed 2.5 times more frequently in dialysis patients than in patients with other chronic non-renal disease, the prevalence of hyperthyroidism is not different (124). In children, the prevalence of hypothyroidism may be higher due to the greater proportion of patients treated for cystinosis and nephrotic syndrome. In cystinotic patients, deposition of cystine crystals in the thyroid can lead to destruction of the gland and frank hypothyroidism (126). Children with severe nephrotic syndrome, particularly with the congenital form (127), may become hypothyroid due to the renal loss of thyroid-hormone binding globulin.

As some manifestations of hypothyroidism, like hypothermia, pallor and dry skin also occur in uremia, the exclusion of the diagnosis of hypothyroidism on clinical grounds may be difficult in a uremic child. Therefore, exploration of the hormonal status of a patient is essential for the recognition of an accompanying thyroid disorder.

Thyroid Hormones

Inorganic iodine is physiologically excreted by the kidney, and plasma inorganic iodine levels increase as kidney function decreases (128).

The plasma levels of total T4 (thyroxine) and T3 (triiodothyronine) are decreased in patients with CKD (129). Significant depression of T4 and T3 levels usually occurs once the glomerular filtration rate falls below 50%. Thyroid hormone *production rates* are normal in patients with CKD (130, 131). Metabolic clearance rates of the hormones may (132) or may not (131) be increased. Due to impaired peripheral deiodination of T4 to T3 (7, 132), there is a more distinct suppression of T3 than of T4 levels. In ESRD, diminished T4 levels are found in a third, and diminished T3 levels in 50% of patients (125, 133–136) including children (137). Concentrations of reverse T3 (rT3), the inactive metabolite of T4 in plasma, are low (138) or normal (136). Production and metabolic

clearance rates of rT3 are normal (130) but extravascular binding of rT3 is increased (131).

The more pronounced decrease of plasma T3 compared to T4 levels in CKD resembles the thyroid hormone pattern observed in other states of chronic nonthyroid diseases ("sick euthyroid" or "low T3" syndrome). However, whereas in the sick euthyroid syndrome rT3 levels are elevated as a result of impaired peripheral conversion of T4 to T3, rT3 levels are in the low normal range in CKD. This constellation has been explained by a redistribution of rT3 into extravascular compartments in uremia (131).

Binding Proteins

Circulating thyroid hormones are bound to thyroid hormone-binding globulin (TBG), albumin and prealbumin. TBG levels usually are normal in hemodialysis (133, 139); they are frequently low in CAPD patients, who lose thyroid-hormone binding proteins via the dialysate (139, 140). Patients with severe nephrotic syndrome may have markedly low TBG plasma levels due to urinary protein loss. Only the unbound (free) T4 (fT4) and T3 (fT3) fractions are biologically active. Plasma fT4 and fT3 as measured by radioimmunoassay are low (141), and dissociation constants for specific T4 and T3 binding are normal (142).

Thyroid-Stimulating Hormone (TSH)

Despite low plasma total and free T4 and T3 levels, TSH concentrations are usually normal in adults (133, 141) and in children with CKD (137). Normal TSH in the face of low fT_3 and fT_4 points to altered regulation of the hypothalamo-pituitary–thyroid axis. Experimental evidence suggests that the sensitivity of the thyrotroph to feedback inhibition is increased in uremia. In addition, thyrotrophin-releasing hormone (TRH) administration causes a blunted, delayed rise of plasma TSH (7, 80, 142) The duration of the TSH response is prolonged due to decreased metabolic clearance and increased half-lives of both thyrotrophin-releasing hormone and TSH (84, 143). While basal iodine uptake is low (133), thyroid responsiveness to stimulation by thyroid-stimulating hormone is normal (144).

The most convincing evidence for a primary hypothalamic defect comes from studies on the temporal organization of TSH release. The physiological nocturnal TSH surge is frequently blunted in children and adults with ESRD (137, 145), and the pattern of pulsatile TSH secretion is altered towards low-amplitude, high-frequency pulses (145).

CKD is associated with a resetting of the central thyrostat towards lower levels of circulating thyroid hormones. The lacking upregulation of spontaneous TSH secretion despite low thyroid hormone levels may either indicate a failure of the thyrotroph to respond to the physiological stimulus of low thyroid hormone concentrations, or be interpreted as a "physiological" downregulation resulting from a reduced *demand* for thyroid hormone in the specific state of metabolism caused by uremia.

Children with nephropathic cystinosis are exceptional in that, even in advanced renal failure, they exibit an exaggerated TSH response to TRH stimulation. This reflects primary hypothyroidism due to destruction of the thyroid gland by deposition of cystine crystals (126). TSH may also be elevated in children with severe nephrotic syndrome (127), reflecting a hypothyroid state due to renal loss of TBG-bound thyroid hormones.

Thyroid Hormone Action

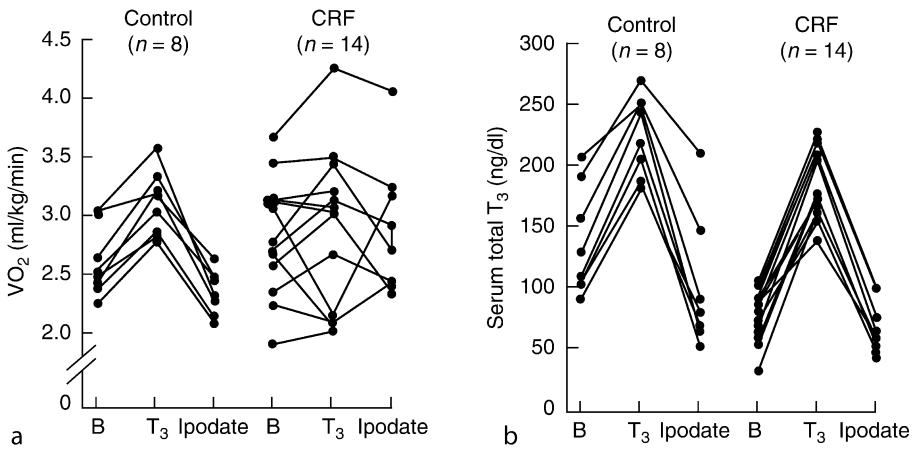
Clinically, patients with CKD usually appear euthyroid. Measurements of basal metabolic rate and rough clinical indices yield normal results (134, 146). Normal TSH concentrations suggest that euthyroidism is also present in pituitary thyrotrophs. In experimental uremia an upregulation of T3 receptor expression, possibly as a compensatory mechanism to low circulating T3, and normal expression of T_3-dependent hepatic proteins have been observed (147, 148). In contrast to apparently efficient endogenous hormone action, CKD patients show a marked resistance against exogenous thyroid hormone administration with regard to thermogenesis. Oxygen uptake is neither stimulated by administration of T3 nor suppressed by its antagonist sodium ipodate (149) (*Fig. 70-5*). In contrast to normal subjects, T3 supplementation in CKD patients results in exaggerated protein degradation and a negative nitrogen balance (138, 150, 151), similar what has been observed in patients with chronic illness or malnutrition (152). Hence, the "low T3 syndrome" of uremia may in part be interpreted as a physiological adaptation to conserve energy in an adverse metabolic environment, and supplementary thyroid hormone treatment might be not only useless but even harmful.

Diagnosis and Clinical Management of Thyroid Disorders in CKD

In a uremic patient hypothyroidism should only be diagnosed if total and free T4 levels are distinctly low, the TBG concentrations normal and basal TSH levels elevated

Figure 70-5

Failure of thyroxin administration to stimulate, and of thyroid suppression to reduce, oxygen consumption (VO_2, panel A) despite adequate changes in plasma T3 levels (panel B) in patients with CKD. (From (149) with permission.)



(>20 μU/mL). A normal plasma TSH is probably a valid indicator of tissue euthyroidism. Treatment with thyroid hormones should be limited to patients with clinical hypothyroidism and elevated plasma TSH. The increased risk for induction of tissue catabolism by thyroid hormone treatment needs to be recognized.

In hemodialysis patients heparin may interfere with the thyroid hormone status. Heparin competes with T4 at intra- and extravascular binding sites, thus increasing total and free T4 serum levels for at least 24 h post-dialysis (153). Therefore, strict standardization of the timing of investigations relative to dialysis is essential. Other substances can cause similar artifacts, e.g., high-dose furosemide (154).

Patients with CKD who undergo repeated radiologic investigations with iodinated contrast agents may be at increased risk of developing iodine-induced hyperthyroidism because of reduced iodine clearance.

Adrenal Hormone Axis

Clinical Findings

Analogous to thyroid disorders, dysfunction of the pituitary-adrenal axis may be difficult to diagnose in patients with CKD. Uremia shares certain clinical signs and symptoms with Cushing's syndrome, such as osteopenia, proximal muscle weakness with atrophy, glucose intolerance, negative nitrogen balance and hypertension (155); therefore, Cushing's syndrome may easily be missed if it occurs concomitantly with renal failure. Similarly, adrenal insufficiency may present with symptoms which are not uncommon in renal failure, e.g., hypotension, weakness, and hyperkalemia. To confirm or reject the diagnoses of Cushing's syndrome or adrenal failure, the clinician has to rely on the evaluation of the patient's hormonal status under basal and stimulated conditions. For a comprehensive interpretation of the endocrine status, the changes of the hypothalamo-pituitary-adrenal axis induced by CKD per se must be kept in mind.

Cortisol

Cortisol is conjugated in the liver to water-soluble metabolites, which are predominantly excreted by the kidney and accumulate in renal failure. While normal morning fasting cortisol levels are found in the majority of adult and pediatric patients with CKD (156–159), 24-h integrated mean total and free cortisol concentrations are consistently elevated (160). Basal hypercortisolism is particularly prevalent in patients on hemodialysis (159). The diurnal rhythm and the pulsatile mode of cortisol secretion is conserved in renal failure; however, the half-life of the endogenous secretory peaks is prolonged (156, 160, 161). In hemodialysis patients, the secretory activity is shifted towards the dialysis hours, whereas a normal pattern is observed on days off dialysis (160).

Stimulation of the zona fasciculata with exogenous ACTH in uremic patients yields a normal cortisol response, irrespective of whether supraphysiological

(159, 162, 163) or low doses of ACTH are used (163). Zona glomerulosa steroids (aldosterone, 18-OH-corticosterone) are stimulated normally in CAPD (163) but not in hemodialysis patients (164). Transient hyporesponsiveness to ACTH is observed in the majority of patients who return to dialysis after transplant failure (165).

Adrenal Androgens

Adrenarche marks an important milestone in endocrine maturation. Adrenarche occurs about 2 years before the initiation of puberty and is independent of it. Low plasma levels of dehydroepiandrosterone (DHEA) and DHEA-sulfate, the marker hormones of the zona reticularis, are observed in adult men as well as in pre- and midpubertal boys on hemodialysis, whereas normal levels are found in patients on conservative treatment (31, 166, 167). Conversely, androstendione, an adrenal androgen produced by the ACTH-dependent zona fasciculata, is elevated in patients on conservative treatment, and normal or elevated in hemodialysis patients (31, 159). A similar elevation of androstendione is observed in girls with CKD (42). In renal allograft recipients, glucocorticoid treatment invariably lowers adrenal androgen production to almost undetectable levels (30, 31, 159).

Adrenocorticotropic Hormone (ACTH)

Basal ACTH levels are normal (159) or elevated (157, 162) in patients with CKD. The functional status of pituitary corticotrophs in uremia is still under discussion. ACTH secretion is not suppressible by standard oral doses of dexamethasone (162, 168). Oral absorption of dexamethasone is, however, reduced in uremia (156), and suppression of ACTH can be achieved at higher doses (156, 160). After intravenous administration of dexamethasone, only incomplete suppression of plasma cortisol levels is observed; however, the metabolic clearance of dexamethasone is possibly increased in uremia (156, 169). The responsiveness of the corticotroph to stimulation by metapirone may (162) or may not (156) be reduced in uremia. The ACTH release after administration of corticotropin-releasing hormone (CRH) occurs early but is blunted (157). In normal subjects acute hypoglycemia elicits a counterregulatory stimulation of the CRH-ACTH-cortisol axis. In patients with CKD this stress reaction is markedly suppressed. The increase of ACTH and cortisol following insulin-induced hypoglycemia is blunted in patients on hemodialysis (156), providing further evidence of a disordered hypothalamo-pituitary regulation of the corticotropic axis in uremia.

Diagnosis and Management of Pituitary-Adrenal Disorders

The most frequent circumstance for a pediatric nephrologist to encounter adrenocortical failure is upon discontinuation of glucocorticoids in patients returning to dialysis after transplant failure. Also, accidental adrenalectomy can occur during nephrectomies particularly in young infants, and adrenal hemorrhage leading to functional disorders is not uncommon in the perinatal period, in children with coagulation disorders and as a side effect of therapeutic anticoagulation. Also, adrenal insufficiency is occasionally seen as a complication of amyloidosis also compromising renal function, as typically seen in patients with severe chronic vasculitis or familial Mediterranean fever. Demonstration of low cortisol levels and insufficient cortisol response to ACTH is required to confirm the diagnosis. In transplant recipients adrenal responsiveness is suppressed by steroid treatment (165). This poses the risk of acute adrenal insufficiency during severe stress, e.g., surgical procedures or after abrupt steroid withdrawal.

The diagnosis of Cushing's syndrome in a patient with CKD requires elevated plasma cortisol levels, measured by a radioimmunoassay in extracted serum. While a single measurement of cortisol may be misleading, loss of diurnal rhythm (24 h cortisol profile) is a characteristic of Cushing's syndrome not seen in uremia-related adrenal dysfunction. Failure of high-dose dexamethasone to suppress ACTH and cortisol levels is confirmatory evidence.

Hormones Involved in Carbohydrate Metabolism

Glucose intolerance is a common feature of CKD. The introduction of the euglycemic and hyperglycemic clamp techniques has been important in understanding insulin and glucose metabolism in patients with CKD (170). In the euglycemic insulin clamp technique, a given level of insulinemia is maintained and blood glucose is kept constant by infusing glucose at a continuously adjusted rate. Thus, the infusion rate equals tissue glucose uptake and metabolism. This allows to quantitate tissue sensitivity to insulin. In the hyperglycemic clamp technique, blood glucose levels are acutely raised by a priming infusion of

glucose, and then maintained constant at about twice the fasting level by a variable glucose infusion. Under these steady-state conditions, the glucose infusion rate is a measure of glucose uptake and metabolism by all cells of the body. The early plasma insulin response is an index of the ß cell responsiveness to the hyperglycemic stimulus, whereas the late insulin response is a measure of peripheral tissue sensitivity to insulin.

Insulin Secretion

Fasting serum insulin levels are usually normal or slightly elevated in patients with CKD. In contrast, serum levels of proinsulin and C-peptide are elevated. This discrepancy is explained by differences in the relative contribution of the kidney to the metabolic clearance rates of these peptides (171). Nevertheless, insulin half-life is increased two- to threefold in CKD. Taking this into account, a relative insulin hyposecretion is present under fasting conditions in CKD patients (172).

In hyperglycemic clamp studies the early insulin response, an indicator of the pancreatic ß-cell sensitivity to glucose, is variable: decreased (173), delayed (174), normal (175) or even increased (176, 177) responses have been reported. A decreased initial insulin release in response to high glucose concentrations is found in isolated pancreatic islets of uremic rats (178). The late insulin response is unvariably increased in uremic patients, indicating tissue resistance to insulin, and improves by dialysis (179).

Insulin is physiologically secreted in frequent, low-amplitude oscillations which are superimposed on slow, high-amplitude secretory pulsations (q. 75 min). In contrast to other states of insulin resistance, a characteristic slowing of both rhythms, and hyperrhythmicity and exaggerated width and amplitude of the low-frequency insulin pulses in response to meals was observed in patients with CKD, pointing to a specific abnormality in the neuroendocrine regulation of insulin secretion (172).

The variable ß-cell response may explain why only some patients develop overt glucose intolerance despite constant peripheral insulin resistance. Glucose intolerance becomes manifest only when the ß-cell insulin response to glucose is so impaired that it can no longer increase and overcome peripheral insulin resistance (81).

Some evidence suggests a role for parathyroid hormone (PTH) in the deranged ß-cell function of CKD. In children with CKD and severe secondary hyperparathyroidism, glucose intolerance resolves after parathyroidectomy by an improvement of the pancreatic insulin secretory capacity, whereas insulin insensitivity persists (180, 181). High PTH levels, with or without uremia, impair insulin secretion by a cAMP-independent mechanism (182). Chronic hyperparathyroidism might enhance calcium entry into the pancreatic islets, resulting in an accumulation of calcium that impairs insulin release. This hypothesis is supported by the prevention of glucose intolerance and the normalization of islet insulin secretion in uremic rats treated with the Ca-channel blocker verapamil (183).

Tissue Resistance to Insulin

Insulin resistance is a major independent risk factor for vascular disease both in healthy subjects (184) and in patients with CKD, in whom it has been found correlated with coronary artery calcifications (185).

Euglycemic insulin clamp studies in adults (186) and children (187) with CKD unanimously show marked decreases in tissue sensitivity to insulin, glucose uptake and metabolic clearance of insulin.

After 10 weeks of dialysis treatment all indices are markedly improved. The two major sites of carbohydrate metabolism are the liver and the muscle tissue. Impaired insulin action may be characterized by diminished splanchnic glucose extraction, increased hepatic gluconeogenesis, decreased peripheral glucose uptake or a combination of these. Most studies in uremic patients report normal basal and insulin- or glucose-suppressed hepatic glucose output (12, 186, 188). However, these observations may be valid only for supraphysiological insulin concentrations. When endogenous insulin secretion is blocked by somatostatin and insulin infused in physiological doses, suppression of hepatic gluconeogenesis is incomplete in uremic patients (189). Moreover, isotope studies suggest reduced glucose oxidation to CO_2 and increased glucose recycling (188). In contrast to these subtle changes of hepatic glucose turnover, glucose metabolism in the peripheral tissue is markedly impaired (186, 190, 191). Hence, the major site of resistance to insulin-mediated glucose uptake in uremia is the peripheral tissue, mainly skeletal muscle.

Studies in adipocytes, monocytes, hepatocytes or human muscle tissue showed a normal or even elevated expression and binding characteristics of the insulin receptor, normal insulin receptor kinase activity and normal receptor-mediated transmembranous hexose transport (12, 192–196). Insulin membrane receptors are expressed in abundance, and maximal hormone action occurs when only 10% of receptors are occupied. Consequently, insulin resistance at the receptor level can be

overcome by high insulin concentrations while a post-receptor defect cannot. Glucose uptake evaluated by the euglycemic clamp technique remains severely reduced in uremic patients even at the highest dose of insulin (12) (*Fig. 70-6*). This observation is direct evidence of a post-receptor defect.

Etiology and Mechanisms of Insulin Resistance

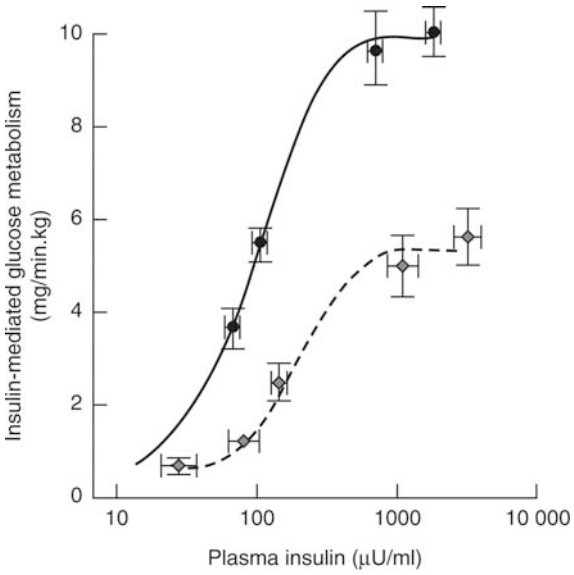
Glucose intolerance in patients with CKD is improved by dialysis (179, 197). This observation suggests the presence of dialyzable factors in uremic serum which compromise the biological actions of insulin. Several peptides possibly interfering with glucose metabolism have been isolated in uremic sera (198–200). Furthermore, 1,25-OH_2D_3 deficiency may play a role in the pathogenesis of insulin resistance in uremia (201). Also, insulin resistance improves in adolescents on dialysis after correction of anemia and amelioration of iron overload by recombinant erythropoietin therapy (202).

Recent research has linked CKD-associated insulin resistance to persistent inflammatory processes. Cytokine levels (e.g., IL-6, tumor necrosis factor, IL-1ß, and resistin) are increased in CKD (203). Inflammation is independently associated with insulin resistance in CKD patients. Inflammatory cytokines stimulate the synthesis of Suppressors of Cytokine Signaling (SOCS), a family of intracellular proteins regulating the sensitivity of cells to cytokines during acute inflammation. Since SOCS proteins also inhibit insulin signaling (204), they represent a potential molecular mechanism linking elevated cytokines with insulin resistance in CKD. Raj et al. recently demonstrated an association of the inflammatory response during hemodialysis with elevated SOCS-3 levels and insulin resistance (205).

While uremia and its sequelae entail specific mechanisms of insulin resistance, the growing global epidemic of obesity is also begining to take its toll in the CKD population. In a recent clinical study of adult patients with mild to moderate CKD, the body mass index (and alternatively percentage body fat) remained the only independent predictor of insulin resistance after controlling for age, race, sex, GFR and adiponectin levels and the prevalence of an abnormal HOMA index did not differ significantly between CKD patients (98%) and BMI-matched control subjects (94%) (206).

Figure 70-6

Dose–response relationship between the plasma insulin concentration and insulin-mediated glucose metabolism in patients with CKD (dashed line) and controls (solid line). Diminished maximal insulin-mediated glucose metabolism suggests insulin resistance by postreceptor defect. (From (12) with permission.)



Glucagon

Glucagon plasma levels are markedly increased in uremia. The biologically active 3.5 kD glucagon moiety is increased threefold. This increase is entirely due to decreased metabolic clearance; secretion is normal (207). Glucagon exerts its hyperglycemic action primarily by stimulating hepatic gluconeogenesis. Patients with CKD exhibit reduced endogenous glucose output after a glucagon challenge (189). Diminished glucagon binding with unchanged binding affinity by hepatic membranes was demonstrated in chronically uremic rats (208). Normal basal, but diminished stimulated adenylate cyclase activity was found. These results may be explained by receptor downregulation in response to chronic glucagon excess, since healthy rats treated with exogenous glucagon exhibit similar changes.

Carbohydrate Metabolism during Peritoneal Dialysis

Chronic peritoneal dialysis is characterized by continuous glucose absorption from the peritoneal fluid, which

amounts to 2–3 g/kg/day in children on an average PD regimen. In view of the known glucose intolerance of uremia, this has raised some concern. Basal glucose and insulin levels are normal or increased in CAPD patients (174, 209, 210). A transient increase of plasma glucose and insulin levels occurs during a CAPD cycle, which is correlated with the glucose content of the PD fluid (209). The area under the curve following an oral glucose load is increased in uremic patients on CAPD compared to non-dialysed and hemodialysed patients (193). Conflicting results have been reported with respect to the effect of CAPD on glucose tolerance and glucoregulatory hormones (192, 210). Peripheral insulin sensitivity improves after initiation of CAPD (211); the improvement is significantly better with peritoneal dialysis than with hemodialysis treatment (197). Insulin binding affinity and receptor numbers decreased on adipocytes of CAPD patients within the first 3 months of CAPD treatment, but no change in insulin sensitivity was observed as assessed by the effect of insulin on glucose uptake and lipogenesis (192).

The available information indicates that glucose intolerance is not exaggerated in patients on CAPD. Insulin resistance tends to improve after initiation of peritoneal dialysis, even more so than in newly hemodialyzed patients. This may be explained by removal of circulating inhibitors of insulin action. The partial substitution of dialysate glucose by icodextrin, an oligosaccharide mixture resorbed slowly and degraded to maltose, leads to a decrease of plasma insulin levels (212).

Adipokines

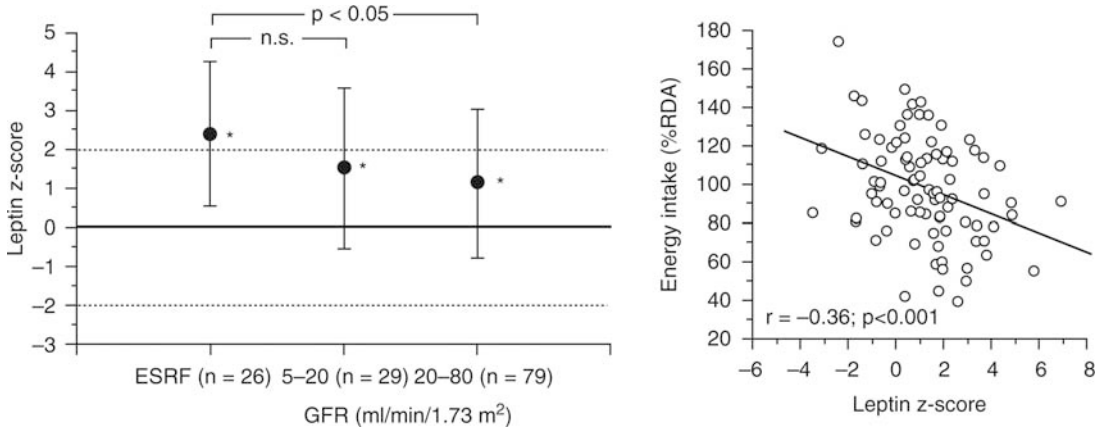
An increasing body of evidence suggests that the adipose tissue has complex pleiotropic functions far beyond the mere storage of energy. Fat tissue secretes various "adipokines", i.e., pluripotent signaling peptides with cytokine and endocrine properties affecting energy homeostasis, tissue metabolism, inflammatory processes and vascular functions (213).

Since the plasma concentrations of most adipokines are markedly elevated in CKD due to impaired renal metabolic clearance, this novel class of signaling molecules has recently been implicated in the pathogenesis of uremic anorexia, catabolism, inflammation, insulin resistance and cardiovascular morbidity (214).

Leptin was initially described as a modulator of feeding behavior, and thus of fat mass, in rodents, but has multiple additional functions. Plasma levels closely correlate with body fat mass, but are independently elevated in adults and children with reduced renal function (215, 216). Moreover, Daschner et al. found an inverse linear correlation of leptin levels normalized for body fat mass with spontaneous energy intake in children and adolescents with CKD, suggesting that leptin accumulation may contribute to uremic anorexia (*Fig. 70-7*) (216). Indeed, leptin signaling in the central nervous system is an important cause of anorexia in uremic mice (217). Experimental uremic cachexia can be ameliorated by blockade of leptin signaling through the hypothalamic melanocortin-4 receptor (218).

Figure 70-7

GFR-dependent elevation and inverse relationship with energy intake of serum leptin levels normalized for age, gender and BMI in 134 pediatric CKD patients. (From (216) with permission.)



Moreover, leptin levels are associated with those of inflammatory biomarkers in CKD (219, 220), suggesting that it may play a role in the malnutrition-inflammation-atherosclerosis (MIA) syndrome associated with uremia. Experimental findings in rodents suggest that circulating bioactive leptin is increased during acute inflammation (221). CRP may directly interact with leptin to attenuate its biological function (222). Furthermore, while leptin is capable of initiating the recruitment and activation of immunocompetent cells, leptin production may in turn be regulated by TNF (223). Notably, serum leptin levels predict epoetin requirements in CKD, even after adjustment for inflammation (224). While these data support the notion that leptin is an important mediator of uremic anorexia, catabolism and inflammation, the association of low leptin levels with poor outcome in dialysis patients (225) is likely explained by the fact that severe uremic wasting leads to a low fat mass with consequent leptin depletion.

Adiponectin is an endocrine peptide secreted exclusively by adipocytes (226). Adiponectin improves insulin sensitivity in the liver (227) and periphery (228), ameliorates endothelial dysfunction and counteracts pro-inflammatory signaling (229). The disruption of the specific adiponectin receptors Adipo-R1 and -R2 increases tissue inflammation and oxidative stress. In contrast to other adipokines, increasing adipose tissue mass is associated with low circulating levels of adiponectin (230). Circulating adiponectin is generally low in populations at enhanced risk of CVD (231, 232). Plasma adiponectin levels are generally markedly elevated in CKD patients (233, 234), and both adiponectin receptors are upregulated on circulating mononuclear cells in CKD patients. CKD patients with relatively lower adiponectin levels have an increased risk of cardiovascular events (234). Surprisingly however, large studies have associated high, rather than low, adiponectin levels with mortality in CKD (235). Hence, the role of upregulated adiponectin signaling in CKD is still unclear. It may be an adaptive, protective mechanism which is most activated in patients with the greatest vascular, metabolic and inflammatory stress (236).

Resistin, a peptide predominantly secreted by immunocompetent cells, is involved in obesity-induced insulin resistance (237). Increased circulating resistin levels in end-stage renal disease patients are not associated with fat mass, but rather correlate closely with GFR and inflammatory biomarkers (238, 239). Resistin is not a significant predictor of insulin resistance when correcting for GFR (238, 239).

Visfatin is a ubiquitous intracellular enzyme with insulin-mimetic effects which is selectively upregulated in the adipose tissue (240). Visfatin plasma levels correlate with endothelial damage in CKD 5 patients (241) and with insulin resistance and proteinuria in type-2 diabetes (242). The mechanistic link between the circulating levels of this cytosolic protein and albuminuria is unclear. In vitro overexpression of visfatin in human vascular smooth muscle cells lengthens cell lifespan and increases their resistance to oxidative stress (243); hence, increased visfatin expression may be a physiological cell response to elevated oxidative, or other, stress.

Growth and Developmental Disorders in Chronic Kidney Disease

Impact of Developmental Stage on Growth in CKD

The regulatory mechanisms of statural growth during childhood differ at the successive stages of development. During the first 1–2 years of life, growth is mainly driven by nutritional factors, particularly the intake of energy and protein. In later childhood, growth appears to depend mainly on the somatotropic hormone axis, with nutrition exerting a more permissive influence. In puberty, the growth process is dominated by the gonadotropic hormone axis, which stimulates and finally terminates body growth by direct action on the growth cartilage and by modulation of the somatotropic hormone axis. In view of these differences in growth regulation, the description of growth in renal disorders deserves separation for the periods of infancy, midchildhood, and puberty.

The first 2 years of life are the most dynamic period of growth. Some 30% of total postnatal statural growth normally is achieved during this period. Any disturbance of growth in infancy has a greater impact on growth potential than at later stages of development. Spontaneous growth in children with congenital CKD is characterized by a rapidly increasing height deficit during the first 2 years of life, followed by a rather percentile parallel growth pattern in the midchildhood years. In the late prepubertal period, height velocity again decreases disproportionately, resulting in a further deviation from the normal percentiles. A late pubertal growth spurt of diminished amplitude eventually results in an irreversible loss of growth potential, leading to a stunted adult height.

Infancy

Untreated CKD during early infancy is usually associated with severe growth retardation (244–247). The mean loss

in relative height in untreated patients is as high as 0.6 SD per month during the first year of life. A detailed analysis of the early infantile growth pattern observed in children with congenital CKD by means of the Infancy-Childhood-Puberty model revealed that the "Infancy" growth phase, starting in intrauterine life and vanishing during the second year of life, is affected in 50% of the patients (248). Height SDS was already reduced at birth, decreased further during the first 3 postnatal months, behaved normally between the 3rd and 9th month, and again decreased between the 10th and 12th month of life. The intrauterine period, the first 3 postnatal months and the period preceding the first birthday each contributed by about one-third to the overall reduction in height SDS observed in congenital CKD. The observed intrauterine growth retardation raises the question whether the prenatal accumulation of certain circulating substances not cleared by the placenta in children with severe renal hypoplasia may compromise fetal growth. Conversely, intrauterine malnutrition could be the primary cause not only of fetal growth retardation but also, if present in the early stages of pregnancy, of abnormal renal morphogenesis. With regard to early postnatal life, anorexia, water and electrolyte imbalances due to uremia, recurrent vomiting and catabolic responses to infections, metabolic acidosis and secondary hyperparathyroidism are the main factors compromising growth during this period. If appropriate management is instituted early enough, severe stunting can usually be prevented. Consequent forced enteral feeding using nasogastric tubes, gastrostomies and even fundoplication as required appears essential to prevent or reverse malnutrition (249, 250). Under optimal conditions it is possible to keep infants with CKD within 1–2 SD below the mean height for age (250, 251).

After a transient stabilization of growth rates, a variable further loss in relative height is commonly seen between 9 and 18 months of age. According to the Infancy-Childhood-Puberty model, this period reflects the transition from the "Infancy" to the "Childhood" growth phase. An irregular onset or maintenance of the Childhood growth component was observed in 60% of the patients and resulted in a further decrease in mean standardized height by 0.7 SDS (248). The reasons for this secondary deterioration of growth in infancy, which may occur despite adequate nutritional and medical supplementation, are poorly understood.

Midchildhood

After the period of rapid infantile growth, height velocity slows down to an almost constant increment per year. During this period endocrine mechanisms regulate growth.

In midchildhood, patients with hypoplastic renal diseases usually grow along the percentiles attained around the end of infancy (252). Patients who develop CKD after the 2nd year of life exhibit a loss of relative height early in the course of disease and follow the growth percentile after stabilization of the disease process. The degree of renal dysfunction is the principal determinant of the variability in growth during this period. Spontaneous midchildhood growth tends to be subnormal when glomerular filtration rate (GFR) is below 25 mL/min/1.73 m^2 (245, 252–254) (*Fig. 70-8*). Mid-childhood growth rates are consistently correlated with GFR, although only 10 – 15% of the variability in growth are actually accounted for by this parameter (252). The degree of anemia, metabolic acidosis and malnutrition contribute only marginally to annual growth rates.

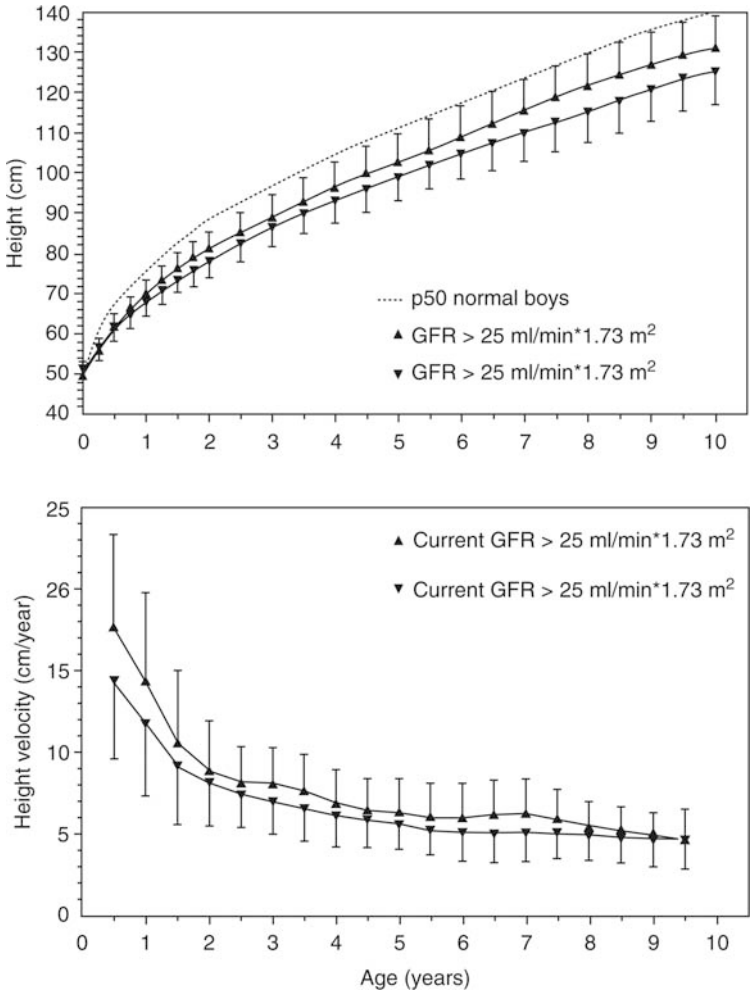
The more or less percentile-parallel growth of uremic children in mid-childhood has been interpreted as a "normal" growth pattern that could be expected after a loss of growth potential in early infancy. Such readjustment of the growth channel is seen in children with cardiac diseases after surgery or in children with adrenal insufficiency overtreated in infancy (255). However, the observation that complete catch-up growth, although not common, does occur in children in whom renal function is normalized by successful renal transplantation and glucocorticoid treatment can be minimized or withdrawn (256) suggests that catch-up growth is continuously suppressed in the uremic milieu. The percentile-parallel growth pattern during this period may therefore reflect a net balance between the growth-suppressive effect of uremia and the organism's inherent tendency for catch-up growth.

Puberty

The height gain achieved during the pubertal growth spurt is usually reduced (18, 118, 257, 258). In a longitudinal analysis of the growth curves of 29 adolescents with various degrees of CKD, the growth spurt started with an average delay of 2.5 years (18). The degree of the delay was correlated with the duration of uremia. Although a distinct acceleration of growth during puberty occurred, the total pubertal height gain was reduced in both sexes to approximately 50% of normal late-maturing children. This reduction was due to a marked suppression of the late prespurt height velocity, a subnormal peak height velocity, and a shortening of the pubertal growth period by 1 year in boys and 1.5 years in girls. Notably, the prolonged prepubertal growth phase, resulting from the delayed onset of the pubertal growth spurt, permitted the patients to grow up to an almost normal immediate prespurt height (−1 SDS in boys, +0.1 SDS in girls).

Figure 70-8

GFR-dependent growth pattern in children with chronic renal failure due to hypo-/dysplastic renal disorders. Approximately 100 children per age interval were evaluated. Upper panel: Mean ± SD of height in children with average GFR less or greater than 25 mL/min/1.73 m^2. Lower panel: Annual height velocity in children with current GFR greater or <25 mL/min/1.73 m^2 in the year of observation. (Adapted from (252) with permission.)



Subsequently, relative height was gradually lost during the pubertal growth spurt, ending up in an average relative height of −2.9 SDS in boys and −2.3 SDS in girls. This pattern of pubertal growth was confirmed in a control group of ESRD patients followed in the late 1990s who were not treated with recombinant GH (259).

In prepubertal children with long-standing renal failure, bone maturation is invariably retarded (260–263). In dialysis patients, skeletal maturation is increasingly retarded before the start of puberty and then accelerates dramatically. This observation and the fact that uremic boys respond to exogenous application of testosterone esters by an exaggerated increase in skeletal maturation (263) suggests that the sensitivity of the growth plate to sex steroids is at least conserved. Because proliferation, i.e., growth, cannot keep pace with differentiation, i.e., bone maturation, growth potential may irreversibly be lost during puberty in uremia.

In contrast, in many transplant patients an apparent standstill of bone maturation is observed even when the patient is growing and puberty is progressing. This phenomenon is thought to be related to direct interference of corticosteroids with the differentiation of the growth plate. Despite the delayed bone age, late growth is usually not observed (18, 264, 265). In fact, the successive stages of the pubertal growth spurt seem to occur at increasingly earlier bone ages than would be assumed in a normal population (18).

Final Height

Adult heights below the normal range are attained by 30% to 50% of children with CKD, although a trend of improving final heights has been noted during the past decade (264, 266–277). The mean final height in CKD patients stage 3–5 ranged between −0.6 and −3.5 SD in different studies. Consistent predictors of a low final height are young age at onset of ESRD, long duration of uremia, the presence of congenital nephropathies and male gender, whereas the use of recombinant growth hormone (rhGH) has been observed to improve adult height. It appears that the use of rhGH and the trend towards early transplantation are the major factors contributing to the gradual improvement of final height observed in developed countries.

Body Proportions

Conflicting information exists with respect to segmental growth in CKD. Graaff et al. investigated body proportions in 37 children with CKD. Body segments were related to height and expressed as "shape" values (278). All children had normal shape values, indicating unaltered body proportions. In contrast, a detailed and standardized prospective assessment of body morphology in 190 boys with CKD noted age-related disproportionate growth patterns in children with a long-term history of CKD and renal replacement therapy (279). Disproportionate growth failure was most obvious in early childhood. Sitting height was mostly preserved, whereas growth of the legs and arms was most severely affected.

Impact of Underlying Renal Disease on Statural Growth

Hypo-/Dysplastic Nephropathies

Congenital renal hypoplasia or dysplasia, with or without urinary tract obstruction, is the most common cause of ESRD during the first 5 years of life. Renal dysplasia is characterized by tubular dysfunction with electrolyte losses and polyuria, and a gradual decline of GFR. The time to ESRD is highly variable and depends on the degree of hypoplasia, tubular dysfunction and the incidence of infections. Growth failure usually develops during the first year of life. Thereafter, growth rates remain more or less stable, and are usually sufficient to keep the patient's height parallel to the centiles. In the care of these patients, it is important to compensate for tubular electrolyte losses and acidosis. Pharmacological renoprotective therapy with renin-angiotensin system antagonists may have a positive long-term impact on statural growth by stabilizing residual renal function.

Glomerulopathies

Progressive glomerular injury may appear as acute or chronic glomerulonephritis, or as nephrotic disease. Growth rates decline in patients with progressive glomerulonephritis as glomerular function deteriorates. Growth velocity is affected even in mild renal insufficiency (253). In patients with nephrotic syndrome, proteinuria per se impairs growth, both in congenital nephrosis and resistant nephrotic syndrome that appears later in childhood (280, 281). The dose and duration of glucocorticoid treatment in nephrotic syndrome and the evolution of renal function are predominant factors affecting growth. Prolonged high-dose corticosteroid administration suppresses growth rates (281–284). Although partial catch-up growth occurs after steroid withdrawal, final height is correlated with the cumulative glucocorticoid dose (281). Congenital nephrotic syndrome usually is associated with severe stunting during the first months of life while GFR still is in the normal range but massive edema and proteinuria are present. Growth failure in these infants may be secondary to edema, recurrent infections, protein-calorie malnutrition and endocrine alterations due to urinary loss of protein-bound and small peptide hormones. Bilateral nephrectomy, replacing proteinuria, edema and cachexia by ESRD, may be required to improve growth and nutritional status (285). In less severe cases of congenital nephrotic syndrome unilateral nephrectomy and medical treatment with prostaglandin synthesis inhibitors and RAS antagonists may reduce proteinuria sufficiently to permit satisfactory growth and weight gain (286, 287).

Tubular and Interstitial Nephropathies

Primary *tubular dysfunctions and interstitial disorders* may lead to severe growth impairment even in the absence of chronic renal insufficiency. Patients with either proximal or distal renal tubular acidosis may present with growth failure during the first years of life (288, 289). Growth impairment may be due to tissue catabolism, volume depletion, electrolyte disorders and/or to malnutrition. Catch-up growth is observed after correction of *acidosis* with alkaline therapy in distal RTA (288, 290).

Growth retardation occurs in about 50% of patients with nephrogenic *diabetes insipidus*; hypernatremia, volume contraction and malnutrition interfere with growth. Maintaining water balance and treatment with indomethacin are associated with catch-up growth (291). Bartter's syndrome and related disorders with chronic *potassium depletion* usually are characterized by a failure to thrive (292). Experimental potassium deficiency leads to growth failure characterized by both reduced endogenous GH secretion and complete resistance to exogenous GH (293, 294). In patients with hyperprostaglandin E syndrome, indomethacin medication leads to partial catch-up growth (295). In view of the various isolated tubular deficiencies that can cause growth impairment, it is conceivable that complex disorders of proximal and distal tubular function, such as idiopathic *Fanconi syndrome* (296, 297) or hereditary *fructose intolerance* develop severe growth failure. In these cases, only partial catch-up growth is possible even with rigorous electrolyte supplementation (288, 290, 292, 295).

Systemic metabolic disorders resulting in complex tubular dysfunction, progressive loss of renal function, and involvement of other vital organs usually lead to severe growth failure (298, 299). In children with *nephropathic cystinosis*, growth failure occurs already in infancy when glomerular function is typically not yet compromised, mainly due to water and electrolyte imbalances. In addition, dysfunction of the growth plate and multiple endocrine insufficiency (affecting the hypothalamus, pituitary, and thyroid) develops due to generalized cystine crystal deposition, adding further local and endocrine pathomechanisms of growth failure. Early initiation of treatment with the cystine depleting agent cysteamine results in improved growth and slows the progression of renal failure (300–302). As an additional option, rhGH effectively stimulates growth in cystinosis patients independently of renal function and cysteamine treatment (303). In patients with *primary hyperoxaluria* supplementary treatment with citrate and pyridoxine can delay the progression of nephrocalcinosis and renal failure, thereby contributing to maintained longitudinal growth (299). In patients with systemic oxalosis combined liver and kidney transplantation is a curative option; however real catch-up growth after combined transplantation is rarely observed even in prepubertal oxalosis patients (304).

In patients with chronic or recurrent interstitial disease, a minor degree of growth retardation may develop. Persistent or recurrent *urinary tract infections* may cause growth impairment by tubular dysfunction, by the catabolic effect of chronic disease, and/or progressive renal insufficiency. Growth rates may increase after medical and/or surgical intervention (305–307). In patients with vesico-ureteral reflux, moderate growth retardation has been demonstrated in case of bilateral disease and significant renal scarring (308). Whereas previous reports noted a growth spurt after successful anti-reflux surgery (306, 307), multiple regression analysis of 54 patients with obstructive urinary tract malformations suggested a positive relationship between the increase in relative height and the duration of antibiotic treatment, but no independent effect of surgical intervention (309).

Etiology of Growth Failure in CKD

The pathogenesis of impaired growth in CKD is complex. Although a particular cause can occasionally be found, a combination of pathophysiological mechanisms factors is usually responsible for growth impairment. Furthermore, the patient's age, the type, duration and severity of renal disease, the treatment modality, and the patient's social environment all play important roles.

Malnutrition and Inflammation

Protein-energy malnutrition frequently occurs in children suffering from CKD. Infants and young children are particularly prone to malnutrition because of their low nutritional stores and the high energy demands required allow high growth rates in this age group.

Malnutrition is a critical issue in children as it is in adults with CKD due to its close association with patient mortality (310, 311). Recently, malnutrition has been causally linked to inflammation and atherosclerosis as a distinct syndrome (MIA syndrome) peculiar to dialysis patients (312–314). It is thought to be caused by the combined effects of cytokine induction during dialysis, decreased clearance of inflammatory cytokines, oxidative and carbonyl stress, nutrient loss through dialysis, anorexia and low nutrient intake, uremic toxicity, volume overload and other dialysis-related factors. The MIA syndrome is considered the common cause of the high prevalence of early cardiovascular disease, excessive mortality, erythropoietin hyporesponsiveness, frequent hospitalizations and poor quality of life in dialysis patients. It is tempting to speculate that growth failure is a pediatric manifestation of the MIA syndrome. In support of this notion, adverse clinical outcomes including a two- to threefold elevated risk of death are associated with growth failure in children on dialysis (310, 315). Preliminary experimental and clinical evidence suggests an association

of both growth failure and malnutrition with elevated inflammatory cytokine levels.

Anorexia is one of the cardinal features of CKD, and universally observed in infants. Adequate energy intake is a primary prerequisite for tissue anabolism and growth. Energy malnutrition is particularly prevalent in uremic infants during the first year of life, when the metabolic rate in relation to body mass is high. Height SDS is correlated with body cell mass and serum transferrin or albumin in infants, emphasizing the importance of malnutrition for growth failure in this age group (244, 316, 317). Energy intake is inversely related to the degree of renal failure (318), and is correlated with growth rates if it is less than 80% of recommended dietary allowances (RDA) (319). However, further augmentation of energy intake above 100% RDA typically results in obesity rather than in a further stimulation of growth (319, 320). In later childhood, spontaneous food intake is usually low when related to the patient's age, but normal when adjusted for body mass (321, 322). Thus, it is difficult to differentiate whether low energy intake is the cause or the consequence of impaired growth.

In contrast to deficient calorie intake, spontaneous protein intake usually meets or even exceeds the RDA (321, 322). Dietary protein intake is not independently correlated with longitudinal growth in children with CKD. In a prospective study limiting protein intake to the safe levels of the WHO (e.g., 0.8–1.1 g/kg/day) but ensuring adequate calorie intake in a large cohort of children with CKD stage 2–4, no impairment of height or weight gain was seen during 3 years of observation (323).

Ample evidence suggests that the protein wasting observed in children with CKD is due to increased protein catabolism and/or impaired protein synthesis, independent of dietary intake. In experimental renal failure, the conversion of dietary to body protein at any given level of dietary intake is less efficient in uremic compared with pair-fed control animals (324). Resistance to the anabolic effects of insulin and IGF-1 and increased protein breakdown by activation of proteolytic ubiquitin-proteasome pathways may contribute to poor growth. The latter mechanism is caused to a large degree by metabolic acidosis (325).

Metabolic Acidosis

Metabolic acidosis usually develops when GFR is decreased by more than 50% due to the kidney's reduced ability to excrete ammonia. The severity of the acidosis is aggravated by nutritional protein and acid load, catabolism and altered electrolyte balance. Metabolic acidosis is associated with increased glucocorticoid production and increased protein degradation by activating branched chain ketoacid catabolism and the ubiquitin-proteasome pathway (326–328). In young children with CKD, the degree of protein wasting is tightly correlated with serum bicarbonate levels (329). Moreover, metabolic acidosis has profound suppressive effects on the somatotropic hormone axis by downregulating GH secretion (78), GH receptor and IGF-1 gene expression (330) and serum IGF-I levels (331). Hence, metabolic acidosis per se causes tissue catabolism and a state of GH-IGF1 insufficiency.

Disturbances of Water and Electrolyte Metabolism

Many congenital renal diseases that slowly progress towards renal insufficiency lead to a loss of electrolytes and a reduced ability of the kidney to concentrate urine. In particular, sodium chloride is lost in patients with obstructive uropathies and renal hypoplasia, and potassium is lost in patients with proximal tubular damage, most markedly in cystinosis. Polyuria, an expression of the reduced ability of the kidney to concentrate the urine, is seen in patients with Fanconi's syndrome and in nephronophthisis, but also in hypoplastic kidney disease.

It is not possible to independently assess the extent to which disturbances in water and electrolyte metabolism contribute to growth retardation in individual patients with CKD. The probability of these factors being significant has, however, been shown by analogous clinical and animal studies. In rats, sodium deficiency decreases protein synthesis and growth, which is only partially reversible by sodium repletion (332, 333). Part of the effects usually attributed to sodium deficiency are actually caused by concomitant depletion of chloride, which per se, if removed selectively from a sodium-replete diet, causes growth retardation and diminished muscle protein synthesis (334). The same applies to patients with a reduced chloride diet or with familial chloride diarrhea (335). Growth failure also occurs in children with diabetes insipidus, indicating that polyuria per se may contribute to growth retardation in CKD (336).

Anemia

Children with CKD develop increasing anemia as a result of erythropoietin deficiency. It is not certain if or to what extent chronic anemia leads to growth impairment.

Children with untreated chronic anemia of non-renal origin, e.g., thalassemia major, show retardation of growth and development. When treated with high-frequency transfusion regimens to keep hematocrits close to the normal range, growth rates may improve in these patients (337). Theoretically, anemia may interfere with growth via various mechanisms such as poor appetite, intercurrent infections, cardiac complications and poor oxygenation of the cartilage cells in the growth plate. The introduction of recombinant EPO for the treatment of renal anemia has offered the opportunity to study whether changes in growth are induced by the compensation of renal anemia. Correction of anemia in children with CKD leads to improved exercise capacity and decreased heart rate and resting oxygen consumption (338, 339). Although short-term stimulatory effects of erythropoietin-treatment on longitudinal growth have been reported anecdotally, no persistent catch-up growth could be demonstrated in several multicenter clinical trials.

Renal Osteodystrophy

Although gross skeletal deformities can contribute to the retardation of a child's growth, the appearance of renal osteodystrophy is not inevitably paralleled by alterations in epiphyseal growth of the long bones. Significant osteodystrophic changes are often detected radiologically in patients with relatively good growth rates. In such cases, osteopathy is unmasked by rapid growth. Growth is impaired only when severe secondary hyperparathyroidism results in destruction of the metaphyseal bone architecture.

Whereas treatment with vitamin D and $1,25(OH)_2D_3$ improves growth in uremic rats (340), an equivalent therapeutic success has not been achieved in children with CKD. Neither vitamin D_3 nor $1,25(OH)_2D_3$ treatment consistently affect growth in dialyzed children (341). This therapeutic failure contrasts with the remarkable growth improvement observed in patients treated for vitamin D deficient rickets, in whom a similar disorder of renal vitamin D metabolism without renal failure is present. Intermittent high-dose $1,25(OH)_2D_3$ administration, probably by inducing a low bone turnover state, can even impair growth in dialyzed children (342).

The extent to which secondary hyperparathyroidism contributes to growth impairment is unclear. Parathyroid hormone (PTH) is an anabolic hormone and an intrinsic growth factor, stimulating mitosis in osteoprogenitor cells and growth plate chondrocytes and upregulates the vitamin D receptor (343, 344). Intermittent PTH administration stimulates the skeletal growth of normal and uremic rats (345). Whereas two clinical studies reported weak positive associations between intact PTH levels and longitudinal growth in children with pre-dialytic CKD and on dialysis (342, 346), growth rates were neither correlated with complete 1,84-PTH nor with non-1,84-PTH fragments in recent studies using both whole- and intact PTH assays (347, 348). While there is no clear effect of mild to moderate hyperparathyroidism on growth, excessive PTH levels can lead to the destruction of growth plate architecture (349), epiphyseal displacement (350) and metaphyseal fractures (351).

Hormonal Factors

The multiple alterations of endocrine systems associated with CKD have been described above. Of particular relevance for statural growth is the complex state of GH and IGF-I resistance, which results in an impaired promotion of endochondral and appositional growth by IGF-1.

Treatment of Growth Failure in Chronic Kidney Disease

General Measures

Adequate *nutritional intake* is the most important prerequisite for early infantile growth. Growth rates in this period are correlated with energy intake (319). Consequently, forced feeding via nasogastric tube, gastrostomy or even fundoplication is an essential component in the management of infantile CKD (249, 250, 352). In later childhood, adequate nutrition is a permissive factor for growth; however, catch-up growth cannot be obtained by dietary manipulations alone. *Energy intake* should be targeted to achieve 80% to 100% of the Dietary Reference Intake (DRI) of healthy children. The caloric intake prescription should be related to height age rather than chronological age in patients whose height is below the third percentile. Increasing caloric intake in CKD patients above 100% of DRI is not useful since it usually leads to obesity rather than to additional catch-up growth. *Protein intake* should be 100% of RDA. In patients on peritoneal dialysis, a slightly higher intake (+0.2 to 0.4 g/kg/day) is recommended to compensate for dialytic protein losses. Higher protein intake should be avoided since anabolizing or growth promoting effects are not achieved by high-protein diets, which may even be detrimental in CKD by aggravating metabolic acidosis and augmenting the nitrogen and phosphorus load.

Supplementation of water and electrolytes is vital in patients presenting with polyuria and/ or salt losing nephropathies (251, 353, 354). Sodium losses are also common and require continuous replacement in anuric infants on peritoneal dialysis, who may lose up to 5 mmol/kg/day via the ultrafiltrate.

Metabolic acidosis should be vigorously treated by oral alkaline supplementation, aiming at serum bicarbonate levels of at least 22 mmol/L. *Water and electrolyte losses* must be consequently compensated (251, 353, 354). Prevention of renal osteodystrophy by *vitamin D* treatment is a further precondition for optimal growth rates. However, apart from the period of infancy, none of the above therapeutic procedures has been demonstrated to induce catch-up growth in short children with CKD.

Dialysis

Conventional dialysis does not improve growth in uremic children. Several large studies found mean annual losses of 0.2–0.8 SD standardized height (355–358).

More recently however, emerging clinical evidence indicates that catch-up growth can be induced in growth retarded children switched to intensified hemodialysis protocols with either short daily or extended thrice weekly sessions. (359, 360).

As with standard hemodialysis, catch-up growth is not commonly observed in children on *peritoneal dialysis*. Whereas early experience suggested continued significant losses of standardized height on CAPD/CCPD (247, 361–363), more recent studies suggest percentile-parallel growth patterns or slight losses of less than 0.5 SD per year (364, 365). Residual renal function is a more important predictor of growth on PD than dialytic clearance (365). In addition, a high peritoneal transporter state, a known morbidity and mortality risk factor in adults, predicted poor growth (−0.5 SD per year) in a prospective study of 51 children followed for 18 months (364). It is currently believed that apart from causing increased dialytic protein losses, the high transporter status may be an indicator of microinflammation, a putative cause of GH resistance in uremia.

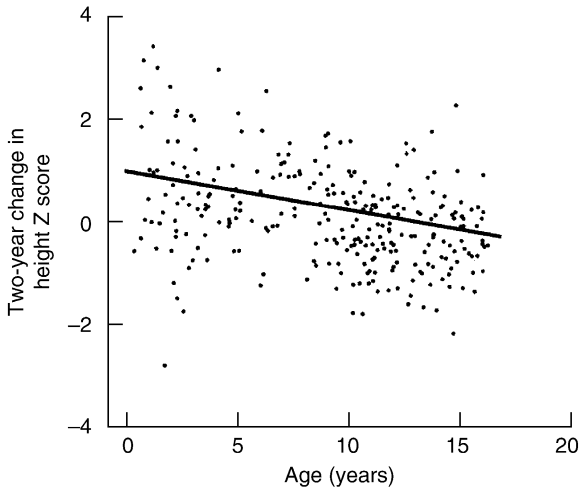
Transplantation

Only successful kidney transplantation is able to restore the conditions for normal growth compromised by the uremic state. However growth rates after transplantation vary widely, from further deterioration of standardized height to complete catch-up growth (118, 257, 276, 366–373). In an analysis of the North American Pediatric Renal Transplant Cooperative Study, the mean effect of transplantation before puberty on adult height was 0 SDS (269), confirming that the relative height achieved at time of transplantation is the most important predictor of final height (276, 372). Whereas pubertal patients tend to lose relative height following transplantation (374), a potential for post-transplant catch-up growth exists in patients younger than 6 years (277, 371, 373, 375) (*Fig. 70-9*). Infant allograft recipients typically exhibit excellent spontaneous growth rates, with a relative height gain of 1.5 SD within 2–7 years (366, 373, 377). Apart from the inverse relationship with age, the degree of growth retardapion positively predicts post-transplant growth rate (276, 277, 371, 373, 378). Furthermore, post-transplant growth critically depends on graft function (269, 273, 276, 277, 371, 373, 378). A marked deceleration in post-transplant growth is observed when GFR is below 60 mL/min/1.73 m^2, and in the French experience catch-up growth only occurred in children with a GFR above this value (379).

The daily and cumulative dose of glucocorticoids seem to be inversely related to the post-transplant growth rate (18, 380, 381). The hope that the prednisolone derivative deflazacort would interfere less with catch-up growth at equivalent immunosuppressive biopotency has not been substantiated (382, 383). Alternate-day corticosteroid

Figure 70-9

Change in standardized height during the first 2 years after transplantation. Improvement in mean height Z score is limited to children at pre-school age. (From (376) with permission.)

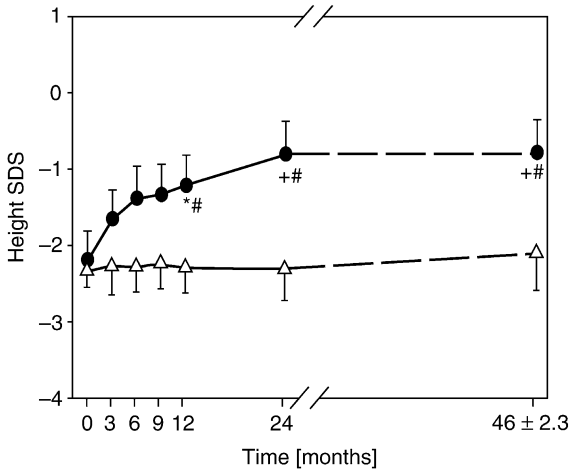


administration has been demonstrated in a controlled, randomized trial to improve growth growth by 0.25–0.5 SD of height per year (368, 384). The most impressive catch-up growth has been observed in patients in whom steroids could be completely withdrawn (256, 367, 385–388). The risk of early rejection and loss of renal function by steroid withdrawal, historically rated as 40–50% (367, 389, 390), has diminished considerably with the advent of more powerful and selective immunosuppressive agents. A cumulative height increment by 1.5 SD was observed within 3 years in children less than 5 years of age receiving tacrolimus monotherapy, and catch-up growth even occurred in pubertal patients (386). Glucocorticoids had to be reinstituted in 10% of patients. In a retrospective case control study glucocorticoids were weaned off in 20 selected patients on cyclosporine A and mycophenolate mofetil without acute allograft rejections and with stable graft function for at least 1 year (388). Standardized height increased in prepubertal patients off steroids by 1.5 SD, contrasting to no change in children on continued steroid medication (*Fig. 70-10*) (388) Even complete steroid avoidance seems to be possible in selected patients; in a study of 107 patients receiving induction therapy with IL-2R antibodies and maintenance tacrolimus combined with either steroids or mycophenolate mofetil, infants gained 1.5 SD (vs. −0.4 SD on steroids) and children aged 5–15 years 0.7 SD (vs. +0.2 SD) (391). Preliminary results from prospective controlled trials appear to confirm that steroids may be safely withdrawn or even entirely avoided with beneficial effects on growth and multiple metabolic features; however, subclinical rejection might still develop more commonly after steroid withdrawal and the risk of late acute or chronic rejection needs to be addressed in future long-term trials. Furthermore, it is currently unclear whether steroid withdrawal or avoidance will permit complete catch-up growth in severely growth retarded children; in clinical practice this is not seen consistently. Notably, incomplete catch-up growth is observed following transient local application of glucocorticoids to the tibial growth plate in rabbits, suggesting the induction of a permanent growth deficit (392).

An interesting observation is that living-related allografts appear to permit better long-term statural growth than grafts from deceased donors. In a restrospective analysis of children with similar standardized height at time of transplantation, recipients of living donor grafts developed superior catch up growth within 5 years of follow-up compared to recipients of deceased organs (393).

Figure 70-10

Longitudinal growth in 20 pediatric renal allograft recipients in whom maintenance glucocorticoid treatment was discontinued (black dots) as compared to matched controls continued on steroid medication. (From (388) with permission.)



Treatment of Renal Growth Failure with Recombinant Growth Hormone

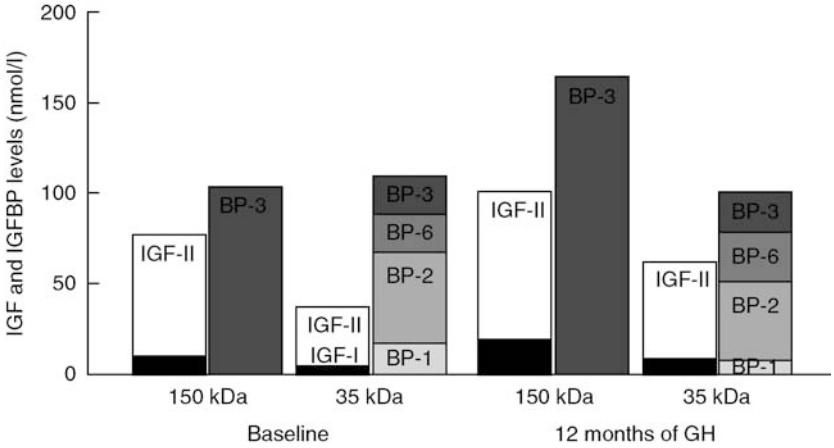
The GH resistance observed in uremia and during glucocorticoid treatment and the experimental proof that GH resistance can be overcome by supraphysiological doses of exogenous GH (394) have provided a rationale for treating children with CKD and after renal transplantation with recombinant human growth hormone (rhGH). Administration of rhGH increases the production of IGF-1 to a greater extent than that of IGF-binding proteins, thereby raising the availability of free IGF-1 at the tissue level (93) (*Fig. 70-11*).

rhGH Use in Prepubertal Children

In prepubertal children with CKD, numerous studies including two double-blind placebo-controlled trials rhGH demonstrated that rhGH induces a nearly twofold increase in height velocity during the first treatment year, with a diminishing but still significant effect on growth rate during the second year (396–403). Although the maximal height increment occurs in the first 3 treatment years, standardized height continues to increase slightly during extended treatment. After 5–6 years of rhGH administration, mean height SDS had increased from −2.6 at baseline to −0.7 in a North American study (402),

Figure 70-11

Balance between IGFBPs and IGFs in serum of CKD children before (baseline) and after 12 months of rhGH treatment. rhGH increases IGF/IGFBP ratio in the 35 kD complex. (Adapted from (395) with permission.)



from −3.4 to −1.9 in German children (401) and from −3 to −0.5 in Dutch patients (403). The remarkable prepubertal growth acceleration was not associated with a disproportionate advancement of bone age, resulting in a remarkable increase in predicted adult height at the end of the prepubertal phase (401).

There is limited data on the efficacy of rhGH therapy in *infants* with CKD. A placebo-controlled study in CKD patients younger than 2.5 years of age showed a mean increase in standardized height of 2 SD in the rhGH group versus −0.2 SD in controls (404). Along with similar findings in a previous uncontrolled study, these results provide support to the concept of early rhGH initiation in infants and young children with CKD if adequate energy intake fails to restore normal growth (405).

Prepubertal children on dialysis respond less well to rhGH than children with CKD on conservative treatment (401, 406). In the German multicenter study, 13 prepubertal dialysis patients gained only 0.8 SDS in height on average during the first two treatment years, as compared to an increment of 1.3 SDS in 41 CKD patients (401) (*Fig. 70-12*). These results were confirmed in a large cohort of French children on hemodialysis who exhibited only 0.5 SDS height gain during the first treatment year, with continued small annual increments for up to 5 years (407). The growth response to rhGH is similar in children on peritoneal and hemodialysis (408). In prepubertal children with nephropathic cystinosis, GH increased height by 0.8 SDS during the first year, and by 1.7 SDS within 5 treatment years (409). In prepubertal renal allograft patients, in whom alternate-day glucocorticoid administration does not induce catch-up growth and complete steroid withdrawal is not considered an option for safety reasons, a therapeutic trial with GH may be considered. Several studies have demonstrated a growth promoting effect of GH in prepubertal children with renal allografts over average treatment periods of 1–3 years (119, 410–415). Treatment efficacy was slightly inferior to that observed in CKD patients on conservative treatment, with mean cumulative height gains of 1–1.5 SD during the first 3 years.

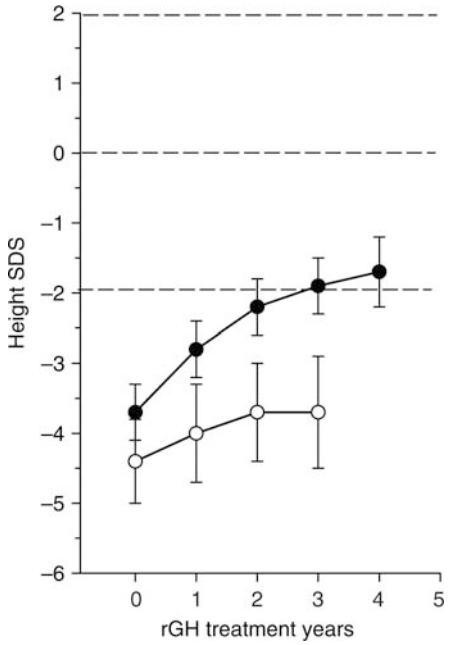
rhGH Use in Puberty

A systematic analysis of rhGH treatment efficacy in pubertal children is difficult due to methodological problems (416). These include delayed puberty with a lack of appropriate growth reference data, potential effects of rhGH on the onset and duration of the pubertal growth spurt, and frequent changes in treatment modalities with variable relative efficacy of rhGH. In addition, rhGH is usually discontinued at time of transplantation but is sometimes reinstituted if the growth rate remains low, introducing large variability in the duration of rhGH treatment during puberty. Hence, only controlled long-term observations, ideally recording total pubertal height gain in treated and untreated pataents, with comparable distributions of treatment modalities, permit a meaningful analysis of rhGH efficacy in puberty.

Haffner et al. followed 38 children with CKD in whom rhGH was initiated at prepubertal age until they reached

Figure 70-12

Superior efficacy of rhGH treatment in children with pre-endstage CKD (closed circles, n = 19) compared to children on dialysis (open circles, n = 6). (Adapted from (401) with permission.)



adult height (259) (*Figs. 70-13*, *70-14*). Since rhGH was stopped at time of transplantation, the patients received GH during 50% of the pubertal observation time. Fifty children, matched for age and degree of CKD, who did not receive rhGH due to still normal height, served as controls. The children receiving rhGH showed sustained catch-up growth, whereas the control children developed progressive growth failure (*Fig. 70-13*). Dissection of the prepubertal and the pubertal growth phases disclosed that the additional height gain relative to the control group was almost entirely limited to the prepubertal period, whereas pubertal height gain was insignificantly greater in the rhGH treated patients than in the controls (*Fig. 70-14*). The apparent inefficacy of rhGH in puberty may be explained in part by the incomplete continuation of treatment, as suggested by a positive correlation of the fractional duration of rhGH therapy with total pubertal height gain. In fact, Hokken-Koelega et al. demonstrated that allograft recipients may respond very well to rhGH even in late puberty (399), with an almost threefold greater 2-year height gain compared to untreated historical controls (417).

An advanced onset of puberty was not observed in the German study nor in the Dutch cohort (403), although a subtle acceleration of bone maturation and a

Figure 70-13

Favourable effect of long-term rhGH treatment on final adult height in children with CKD. Synchronized mean growth curves during GH treatment for 38 children (32 boys and 6 girls) with CKD, as compared with 50 control children with CKD not treated with GH. Normal values are indicated by the 3rd, 50th, and 97th percentiles. The circles indicate the time of the first observation (the start of GH treatment in the treated children) and the end of the pubertal growth spurt. (From (259) with permission.)

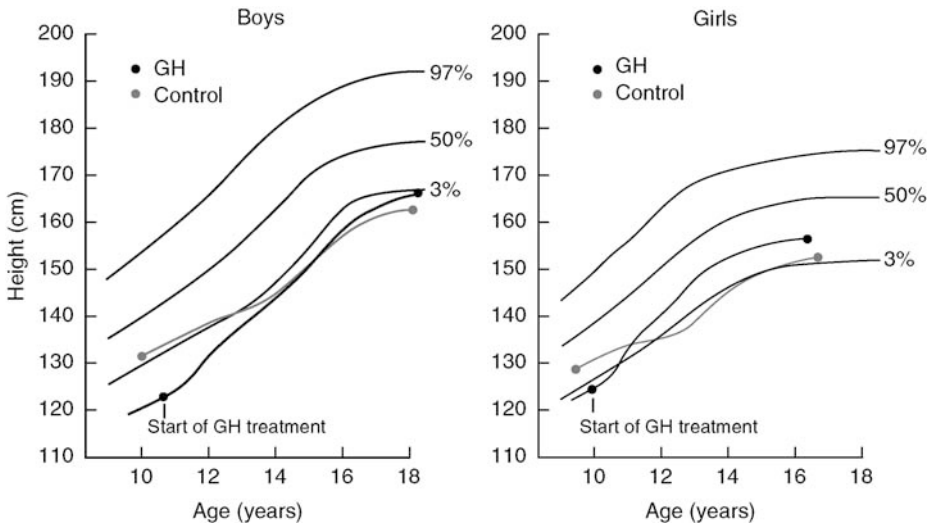
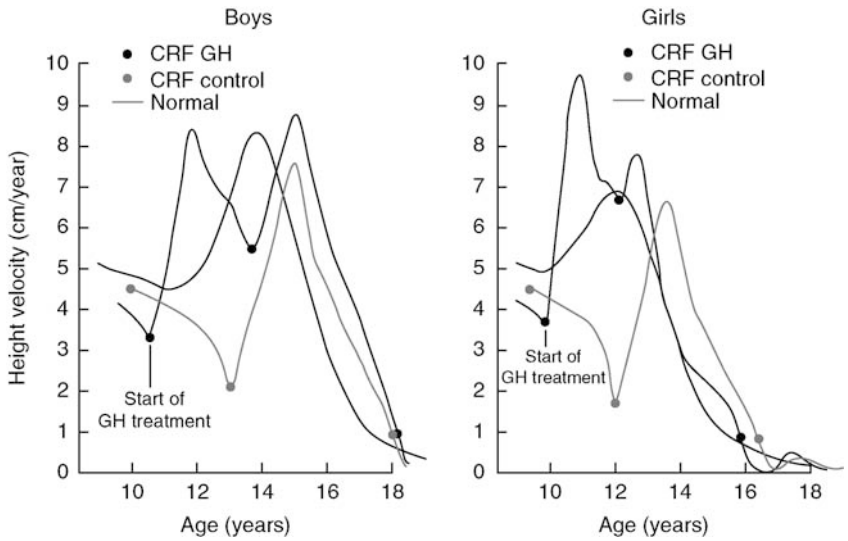


Figure 70-14

Synchronized mean height velocity curves during GH treatment for the same children as shown in *Fig. 70-13*. The circles indicate the time of the first observation (the start of GH treatment in the treated children), the time of minimal prespurt height velocity, and the end of the pubertal growth spurt. (From (259) with permission.)



slight shortening of the pubertal growth spurt was noted by Haffner et al. (259).

Adult Height Following rhGH Treatment

Final height outcome data are beginning to emerge which will permit to assess the ultimate treatment efficacy of rhGH therapy. In the German multicenter study, the mean adult height of 38 children who received rhGH for a mean of 5.3 years was −1.6 SDS, with two-thirds of patients ending up at a height above the 3rd percentile (259). The patients ultimately gained 1.4 SD when compared to their standardized height at baseline. Conversely, mean height SDS decreased in the untreated control group from −1.5 to −2.1 SDS at attainment of adult height (*Fig. 70-14*). Figures in the same range were reported from several other trials (*Table 70-1*). Of course, the variability of final height outcomes is large. The cumulative height gain appears to be positively associated with the initial target-height deficit and the duration of rhGH therapy, and negatively with the percentage of the observation period spent on dialysis (259, 403). The relative height gain attained during rhGH treatment appears to be maintained until final height in patients undergoing renal transplantation; neither spontaneous catch-up nor catch-down growth are common (420, 423).

Potential Adverse Effects of GH Treatment

The safety of long-term rhGH treatment in CKD has been monitored in numerous randomized clinical studies and registries. Both in the large cohort of the NAPRTCS registry and in a recent Cochrane meta-analysis of randomized clinical trials, adverse event rates in children with CKD patients on conservative treatment, on dialysis, and after renal transplantation, the use of rhGH was not associated with increased incidences of malignancy, slipped capital femoral epiphysis, avascular necrosis, glucose intolerance, pancreatitis, progressive deterioration of renal function, acute allograft rejection or fluid retention (424, 425).

Concern was raised initially that prolonged GH treatment might provoke *diabetes mellitus*, because patients with CKD already show impaired glucose tolerance due to peripheral insulin resistance. Insulin secretion increases persistently during rhGH treatment due to a direct inulin agogic effect of growth hormone. This increase is most pronounced in transplanted patients on concomitant glucocorticoid therapy. However, oral glucose tolerance did not change during up to 5 years of rhGH therapy in CKD patients on conservative treatment, dialysis, and after renal transplantation (426). The long-term consequences of increased insulin secretion are uncertain. Theoretically it may contribute to atherosclerosis or eventually induce diabetes mellitus by exhaustion of pancreatic ß cells.

Table 70-1

Synopsis of studies reporting adult height data after rhGH treatment of growth failure due to CKDMean values are given for age, time period and SDS values unless indicated otherwise

Study	Number of patients studied	CKD treatment modalities	Age at start of rhGH (years)	Pubertal status at start of rhGH	Duration of follow-up (years)	Duration of rhGH (years)	Initial height SDS	Final height SDS	Change in height SDS
Netherlands (418)	65	Cons Rx/dialysis	n.i.	Prepubertal	n.i.	5.8	−2.8	−1.4	+1.4
KIGS (419)	75	Cons Rx/dialysis	10.7	Prepubertal	n.i.	6.1	−3.5	−2.6	+0.9
UK (420)	2	Cons Rx	9.9*	Prepubertal	10.0*	0.4*	−2.2*	−1.1*	+1.1*
	5	Transplant	11.9	Prepubertal	>6.0	2.9	−3.3	−3.0	+0.3
	6	Transplant	15.6	Pubertal	>5.0	1.4	−3.4	−2.5	+0.9
Germany (259)	38	47% cons Rx, 24% dialysis, 29% post-transplant**	10.4	Prepubertal	7.6	5.3	−3.1	−1.6	+1.4
NAPRTCS (421)	9	Cons Rx	n.i.	n.i.	3.2	< 3.2	−3.0	−2.2	+0.7
	22	Dialysis	n.i.	n.i.	4.1	< 4.1	−3.6	−3.2	+0.4
	72	Transplant	n.i.	n.i.	3.7	< 3.7	−3.0	−2.5	+0.5
Belgium (414)	17	Transplant	n.i.	n.i.	n.i.	3.4	−3.0	−1.8	+1.2
Netherlands (412)	18	Transplant	15.5	Pubertal	n.i.	n.i.	n.i.	n.i.	Total height gain 19 cm
NAPRTCS (422)	71	Transplant	n.i.	n.i.	n.i.	n.i.	−2.7	−1.8	+0.9

Mean values are given for age, time period and SDS values unless indicated otherwise
n.i. = no information given. *median. **percent patient years spent in each treatment category

Although not a single case of irreversible diabetes mellitus has been observed in children with CKD treated with rh GH through 1998 (427, 428), initial reports of an increased incidence of type-2 diabetes mellitus in non-renal children undergoing GH therapy mandate continued surveillance (429).

An aggravation of secondary *hyperparathyroidism* has been reported anecdotally (430, 431). This effect does not appear to be due to stimulation by GH, but rather result from slight decreases of ionized calcium as a consequence of GH-stimulated bone apposition or an increase in serum phosphate concentration secondary to improved appetite. Furthermore, pre-existing renal osteodystrophy may be unmasked by an increased growth rate and may become radiographically apparent (432).

Concern has also been raised that rhGH may cause glomerular hyperfiltration and accelerated *renal failure progression*. However, long-term observations over up to 8 qears showed no evidence of an accelerated GFR loss in CKD patients prior to or after transplantation other renal disorders and in renal allograft recipients (259, 401, 403, 409).

Since growth hormone is an immunomodulatory hormone (433), allograft rejection might be triggered by rhGH administration in post-transplant patients. Several controlled trials have ruled out a major impact of rhGH on the overall risk of rejection, with the possible exception of high-risk patients who experienced more than one acute rejection episode prior rhGH therapy (413, 415, 434, 435).

Extensive surveys in thousands of patients failed to disclose any significant relationship between rhGH treatment and the occurrence of malignancies (436–438). A review of adverse events in 583 patients with CKD and ESRD treated with GH revealed one solid tumor and one B-cell lymphoma among patients undergoing PD and one lymphoma in a transplant recipient (439). However, reports on renal cell carcinoma in two children 9 and 11 years post-transplant who had received rhGH, premalignant

tubuloepithelial changes in a pediatric renal allograft (414), and a borderline increase in the risk of post-transplant lymphoproliferative disorder associated with pre-transplant rhGH use among 41 cases reported to the NAPRTCS database (440) raise the suspicion that tumor risk may be selectively increased by rhGH in children receiving maintenance posttransplant immunosuppression.

GH induces an IGF-1 mediated increase in distal tubular sodium reabsorption and upregulates the renin-angiotensin system (441, 442). As a consequence, a transient, usually mild retention of sodium and water occurs during the first few days of treatment.

Benign intracranial hypertension has been reported as a rare adverse effect of GH treated patients with various underlying diseases. In CKD, the risk of developing this complication appears to be increased up to 10-fold compared to children receiving rhGH due to other underlying disorders (443, 444). A survey of 1,670 children with CKD treated with rhGH reported intracranial hypertension in 15 subjects (0.9%) within a mean treatment period of 13 weeks (445). Symptoms generally abated upon rhGH discontinuation, but two patients had persistent blindness and in at least 4 patients the symptoms recurred after reinitiation of GH therapy. While this uncontrolled survey suggested a particular risk of CKD patients to develop intracranial hypertension while on rhGH, a prospective, controlled cohort study of patients in the NAPRTCS database noted intracranial hypertension in only 3 out of 1,376 rhGH-treated CKD patients (incidence 1 per 3,000 patient years), 2 of whom were not receiving rhGH at the time when the condition developed (425). Intracranial hypertension was not seen in any of 957 rhGH-treated, but in 7 of 3,983 untreated children on dialysis or post-transplantation. These data suggest that the incidence of the condition may be generally increased in CKD, but whether rhGH further increases the risk is questionable. Nonetheless a baseline fundoscopy should be performed in children initiating rhGH. Since hypertension and fluid overload may be predisposing factors, the state of hydration should be well controlled at start of treatment. Headache, vomiting and other clinical signs of increased intracranial hypertension mandate careful clinical investigation including fundoscopy.

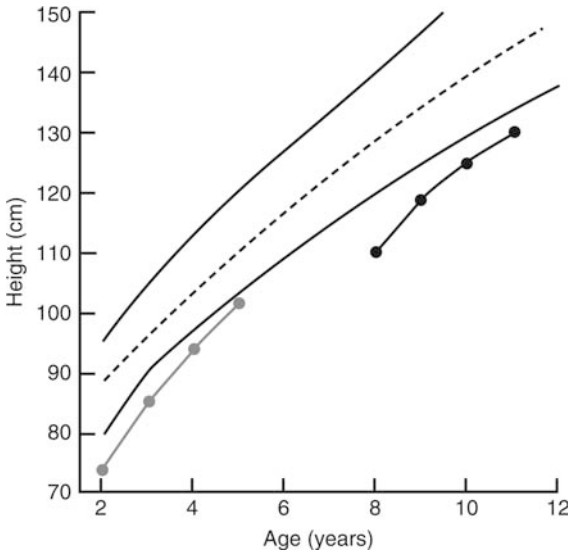
Strategies to Optimize Growth by rhGH Treatment

The response to rhGH is positively influenced by the residual GFR, target height, the initial target height deficit and the duration of GH treatment, and negatively by the age at start of treatment (416). A curvilinear dose-response relationship appears to exist. Although a dosage of 4 IU/m^2/day was more efficient than 2 IU/m^2/day in a double-blind trial (446), no further improvement of the growth response was observed with 8 IU/m^2/day, at least in a pubertal cohort (417). Daily dosing is more efficacious than three applications per week (447). Discontinuation of rhGH treatment will result in loss of height SDS in 75% of children with CKD on conservative treatment (448), whereas catch-down growth seems to be uncommon when rhGH is discontinued due to renal transplantation (420, 422).

Since the absolute growth response to rhGH (in cm height gain per year) is independent of age but the reference range increases with age (401), rhGH treatment should be started as early as possible in the course of renal disease (*Fig. 70-15*). A fixed daily dose of 4 IU/m^2

Figure 70-15

Age-dependent relative efficacy of GH treatment exemplified by individual growth curves predicted for two patients aged 2 and 8 years, started on GH at a basal height SDS of −3.5 and a height velocity of SDS −2.0. The reference lines indicate the 3rd, 50th and 97th percentile of a normal population. Growth is accelerated over baseline height velocity in both patients by 4.5 cm in the first, 1.9 cm in the second, and 1.0 cm in the third treatment year (empirical means observed in patients on conservative treatment followed for 3 years). The young child reaches the 3rd percentile within 3 years, whereas the older child does not. (From (401) with permission.)



(or 0.05 mg/kg) should be used. Treatment should not be stopped if the annual gain of height SDS diminishes after the third treatment year to avoid the risk of catch-down growth. It should also be continued when the patient becomes dialysis-dependent. If rhGH is initiated in a small child already on dialysis, transplantation should not be postponed for the sake of approved rhGH treatment, since the efficacy of rhGH in dialysis patients is limited and short-lasting. Treatment should be stopped at time of renal transplantation to observe the spontaneous evolution of growth. If no or insufficient catch-up growth occurs within 12 months, glucocorticoid withdrawal is the first option. If unsuccessful, rhGH reinstitution should be considered. Treatment decisions should be made without delay to save growth potential. The question of rhGH continuation through puberty is still controversial. The evaluation of treatment efficacy is difficult due to the dynamic changes of growth rate as part of the pubertal growth spurt, which occurs independently of concomitant rhGH treatment. Controlled prospective study results are not available. Hence, the decision to continue or restart rhGH in puberty must be made on an individual basis, taking into account the degree of growth retardation, the residual growth potential and the expected therapeutic compliance of the adolescent.

It remains uncertain whether a low growth rate (e.g., height velocity below the 10th–25th percentile over several years) should also be a treatment criterion in a child with a relative height still in the normal range. Such "preventive" therapy might prove to be more effective in increasing adult height than initiation of treatment when short stature is already established. This treatment strategy appears justified if a growth arrest is noted which is not explained by non-endocrine circumstances. It is also an issue of current consideration whether in patients with imminent or early puberty skeletal maturation should be delayed by pharmacological intervention by GnRH analogues or aromatase inhibitors to prolong the prepubertal growth phase for GH treatment.

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71 Chronic Kidney Disease Mineral and Bone Disorder

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The kidney plays a major role in bone and mineral homeostasis by regulating calcium, phosphorus, parathyroid hormone (PTH), fibroblast growth factor-23 (FGF-23) and calcitriol (1,25 dihydroxyvitamin D_3, $1,25(OH)_2D_3$) metabolism. Disordered regulation of mineral metabolism occurs early in the course of chronic kidney disease (CKD) and results in alterations in bone modeling, remodeling, and growth. These alterations have been a focus of CKD management in children for decades. However, a growing awareness that cardiovascular calcifications accompany CKD, that cardiovascular disease is the leading cause of mortality in both adults and children with kidney disease, and that therapies designed to treat the skeletal consequences of CKD affect the progression of vascular pathology, has led to a reclassification of the mineral, skeletal, and vascular complications associated with progressive kidney disease. Together, these alterations are termed "CKD Mineral and Bone Disorder" ("CKD-MBD") (1).

The CKD-MBD is defined as a systemic disorder of mineral and bone metabolism due to CKD that is manifested by either one or a combination of the following: (1) abnormalities of calcium, phosphorus, PTH, or vitamin D metabolism, (2) abnormalities in bone histology, linear growth, or strength, or (3) vascular or other soft tissue calcification. "Renal osteodystrophy" is the specific term used to describe the bone pathology that occurs as a complication of CKD and is therefore one aspect of the CKD-MBD. Traditionally, such lesions have been defined according to alterations in bone turnover, ranging from high bone turnover (secondary hyperparathyroidism, osteitis fibrosa) to lesions of low bone turnover (adynamic bone disease and osteomalacia). However, alterations in skeletal mineralization and volume are also common in patients in CKD (1) and may contribute to such outcomes as fractures, skeletal deformities, and poor growth which persist despite normalization of bone turnover (2). Bone histomorphometry continues to be the gold standard for the assessment of three essential aspects of bone histology: turnover, mineralization, and volume (1). This chapter summarizes the major aspects of the pathogenesis, clinical manifestations, histologic features, and therapeutic interventions currently used in the management of CKD-MBD. The clinical and histologic features of bone diseases after successful kidney transplantation are also described.

Pathogenesis of CKD-MBD

Abnormalities of Calcium, Phosphorus, PTH, Vitamin D, and FGF-23 Metabolism

Calcium and Phosphorus

1,25 dihydroxyvitamin D_3 is the most active form of vitamin D and regulates calcium balance and serum calcium levels by increasing intestinal calcium absorption. The kidney generates the majority of circulating $1,25(OH)_2D_3$, converting 25(OH) vitamin D to $1,25(OH)_2D_3$ by means of the enzyme 1α-hydroxylase. As renal failure progresses, calcitriol levels and intestinal calcium absorption decline. However, at the same time, rising PTH levels increase 1α-hydroxylase activity and also release calcium and phosphorus from bone, thus maintaining serum calcium levels until late in the course of CKD (*Fig. 71-1a*) (3).

Likewise, serum phosphorus levels are usually maintained in the normal range throughout mild to moderate CKD (*Fig. 71-1a*). Elevated serum PTH and FGF-23 levels increase single nephron phosphate excretion, thus maintaining overall phosphate balance until GFR declines to 25–30% of normal (4, 5). In late (stage 4) CKD, hyperphosphatemia ensues and contributes to secondary hyperparathyroidism (3).

FGF-23

A recently described phosphaturic hormone, FGF-23, was first identified in patients with tumor-induced osteomalacia, autosomal dominant hypophosphatemic rickets, and X-linked hypophosphatemic rickets. In these

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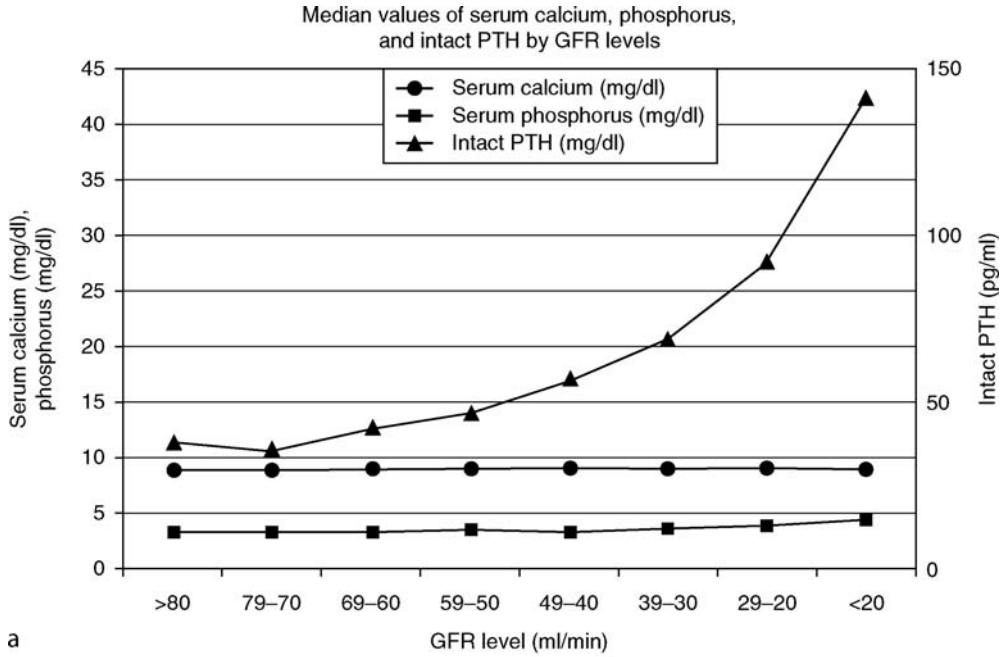
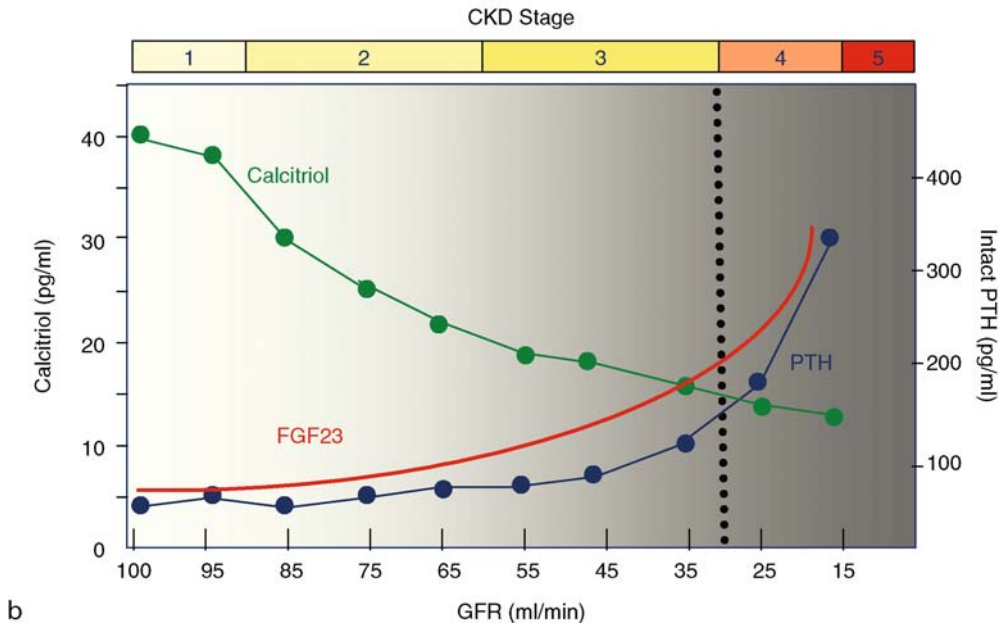
Figure 71-1

Median levels of calcium, phosphorus, PTH, FGF-23 and PTH per stage of CKD. (a) Median serum levels of calcium and phosphorus stay constant until late in the course of CKD; PTH levels rise before any changes are seen in calcium and phosphorus. (Reprint by permission from Levin A et al., *Kidney International* 2007. vol 71 pp31–38). (b) Rising serum FGF-23 and decreasing levels of 1,25 dihydroxyvitamin D occur prior to a rise in serum PTH. (See color plate 46)





conditions, elevated circulating levels of FGF-23 result in renal phosphate wasting and suppression of $1,25(OH)_2D_3$ production. The protein is made in bone (6, 7) and the presence of a cofactor, Klotho, is essential for its action in many tissues (8). FGF-23 levels may be regulated by phosphorus intake (9, 10) and serum values increase as CKD progresses, becoming markedly elevated in individuals with end-stage kidney disease (*Fig. 71-1b*) (11).

In animals with normal renal function, FGF-23 suppresses 1α-hydroxylase activity (12) and, in patients with CKD, $1,25(OH)_2D_3$ levels are inversely related to levels of circulating FGF-23, suggesting that the hormone may play a significant role in mineral metabolism – in declining active vitamin D levels in CKD, specifically (13). FGF-23 levels have been implicated in parathyroid gland regulation; higher levels of FGF-23 have been shown to suppress PTH release (14, 15). FGF-23 has also been shown to act directly on PTH secretion in vitro, through a mechanism independent of its actions on vitamin D metabolism (14, 15).

Vitamin D

In patients with CKD, serum $1,25(OH)_2 D_3$ levels decline early in the course of kidney dysfunction, before any changes in serum calcium or phosphorus concentrations occur and prior to any rise in serum PTH levels (*Fig. 71-1b*) (3, 16). $1,25(OH)_2D_3$ levels decline as 1α-hydroxylase activity decreases and low levels have been implicated as the initial event of the altered mineral and bone metabolism associated with CKD (3, 16). In late stages of CKD, phosphate retention and increased serum phosphorus levels directly suppress 1α-hydroxylase activity (9). However, while these factors contribute to declining 1α-hydroxylase activity, current evidence demonstrates that rising FGF-23 levels may be of even greater importance (13).

Low circulating levels of $1,25(OH)_2D_3$ have consequences for many tissues. Aside from its effect on intestinal calcium absorption, $1,25(OH)_2D_3$ plays a direct role in the suppression of PTH gene transcription. Animal studies also indicate that $1,25(OH)_2D_3$ is essential for normal skeletal physiology – particularly in growing animals – and that this effect may not be mediated by the vitamin D receptor (VDR). Mice who lack the VDR (i.e., mice unable to respond to the actions of $1,25(OH)_2D_3$ through its classical receptor) are phenotypically similar to those lacking the 1α-hydroxylase gene itself (i.e., mice unable to generate $1,25(OH)_2D_3$); both sets of mice are hypocalcemic with markedly elevated serum PTH levels, parathyroid gland hyperplasia, and rickets (17). However, a diet replete in calcium, phosphorus, and lactate is sufficient to normalize the serum calcium, phosphorus, and PTH levels, and to prevent the development of rickets in VDR deficient animals (18). By contrast, this "rescue diet" is unable to completely reverse growth plate abnormalities in 1α-hydroxylase deficient mice, suggesting that $1,25(OH)_2D_3$, acting through a receptor other than the classical VDR, may be essential for proper growth plate development (19). $1,25(OH)_2D_3$ has also been shown to regulate the renin-angiotensin system; 1α-hydroxylase deficient mice demonstrate cardiac hypertrophy and dysfunction which are reversed with angiotensin converting enzyme blockade (20, 21). Thus, $1,25(OH)_2D_3$ may be essential for cardiac health, a finding that may explain observational data suggesting that active vitamin D sterol therapy improves survival in patients treated with maintenance dialysis (22, 23).

Native 25(OH) vitamin D (25(OH)D) deficiency is prevalent in patients with CKD; low levels of this form of the hormone also contribute to altered mineral metabolism. Vitamin D is either made in the skin or ingested from the diet (24, 25). UVB (290–315 nm) photons penetrate the skin and are absorbed by 7-dehydrocholesterol to form previtamin D_3 which then spontaneously converts to vitamin D_3. Vitamin D_3 is extruded from the skin cell into the extracellular space where it binds vitamin D-binding protein (26). While the vitamin D created in the skin is exclusively of the D_3 form, dietary sources of vitamin D as well as food supplements may contain either vitamin D_2 (created through the UV irradiation of ergosterol in yeast) or D_3 (from animal sources – particularly fish). Vitamin D (both D_2 and D_3) undergoes hydroxylation by the liver, forming 25(OH)D (27). Subsequently, 25(OH)D is taken up by the renal tubular cells by a megalin dependent process and undergoes a second hydroxylation, facilitated by renal 1α-hydroxylase, to $1,25(OH)_2D_3$, a more potent stimulator of gut calcium absorption (28, 29). Although conversion of 25(OH)D–$1,25(OH)_2D_3$ is, in the general population, a substrate-independent process, it becomes a substrate dependent process in patients with CKD (30). Furthermore, although the actions of 25(OH)D have been underemphasized in CKD, extrarenal 1α-hydroxylase activity may significantly contribute to $1,25(OH)_2D_3$ production, even in anephric patients (31–33). Thus low levels of the precursor, 25(OH)D may exacerbate $1,25(OH)_2D_3$ deficiency in the context of CKD.

Since levels of 25(OH)D below 32 ng/ml are associated with increased PTH levels, reduced bone mineral density (BMD) (34), and increased rates of hip fractures (35) in the general population, such levels are therefore considered to represent insufficient vitamin D storage. Levels between 15 and 30 ng/ml are defined as vitamin D "insufficiency", levels between 5 and 15 ng/ml are characterized as vitamin D "deficiency" and levels less than 5 ng/ml as "severe deficiency" (36). Interestingly, 25(OH)D levels in the vast majority of the general population meet the definition of D insufficiency and a large percentage – as many as 57% in one series of medical inpatients (37) – have serum levels less than 15 ng/ml,

thus qualifying as vitamin D deficient. A high prevalence of vitamin D deficiency is also present in the pediatric population; 24% of adolescents with normal kidney function in Boston display 25(OH)D levels less than 15 ng/ml (38). The prevalence is higher in individuals with darker skin pigmentation; 52% of Hispanic and black adolescents from the same cohort demonstrating evidence of D deficiency (38).

Several studies have also documented a high prevalence of 25(OH)D deficiency in patients with CKD; this prevalence increases as renal function declines (39, 40) and the vast majority of patients treated with maintenance dialysis have insufficient vitamin D storage (41,42). Patients with CKD are at increased risk of vitamin D deficiency for several reasons: 1) many are chronically ill with little outdoor (sunlight) exposure. 2) CKD dietary restrictions, particularly of dairy products, curtail the intake of vitamin D rich food and lead to decreased dietary calcium intake (43), resulting in greater conversion of 25(OH)D–$1,25(OH)_2D$, thus necessitating higher vitamin D intake and/or production (44); and 3) patients with CKD display decreased skin synthesis of vitamin D_3 in response to sunlight compared with individuals with normal kidney function (45). Decreased skin synthesis of D is particularly prominent in individuals with darker skin due to higher skin melanin content (46). Proteinuric diseases further exacerbate D deficiency in the CKD population, as 25(OH)D, in combination with vitamin D binding protein, is lost in the urine (47, 48).

Apart from its conversion to $1,25(OH)_2D_3$, 25(OH)D may have its own effect on tissues. Indeed, supplementation with ergocalciferol has been shown to decrease serum PTH levels in patients with CKD (49, 50). Recent evidence demonstrates that 1α-hydroxylase is present in the parathyroid glands; thus, 25(OH)D is converted inside the gland to $1,25(OH)_2D_3$, suppressing PTH (51). Furthermore, 25(OH)D administration suppresses PTH synthesis even when parathyroid gland 1α-hydroxylase is inhibited, indicating that 25(OH)D may contribute to PTH suppression, independent of its conversion to $1,25(OH)_2D_3$ (51).

Parathyroid Hormone (PTH)

Due to alterations in calcium, phosphorus, FGF-23, and $1,25(OH)_2D_3$ metabolism, PTH levels increase as CKD progresses (*Fig. 71-1a*). Calcium is the primary stimulus for PTH release, however, changes in serum calcium are not common until late in the course of CKD while serum PTH levels begin to rise before any changes in serum calcium are evident. Altered $1,25(OH)_2D_3$ metabolism is a primary factor in the development of secondary hyperparathyroidism early in the course of CKD (52–54). $1,25(OH)_2D_3$ has been shown to suppress PTH gene transcription, both *in vitro* (bovine parathyroid cell culture) and *in vivo* (intact rats). In conjunction with the vitamin D receptor (VDR), $1,25(OH)_2D_3$ binds negative vitamin D response elements in the parathyroid gland which inhibit pre-proPTH gene transcription (55, 56). In a positive feedback loop, $1,25(OH)_2D_3$ itself increases VDR gene expression in the parathyroid gland, further suppressing PTH gene transcription. $1,25(OH)_2D_3$ also increases the expression of the calcium sensing receptor (CaSR), the expression of which is reduced in hyperplastic parathyroid tissues obtained from patients with renal secondary hyperparathyroidism (57). Vitamin D deficiency in animals is associated with decreased expression of CaSR mRNA in parathyroid tissue, while $1,25(OH)_2D_3$ therapy increases CaSR mRNA levels in a dose dependent manner (58). Thus, in CKD, reduced circulating $1,25(OH)_2D_3$ contributes to secondary hyperparathyroidism and parathyroid gland hyperplasia in a number of ways: through decreased intestinal calcium absorption, decreased binding to the VDR, reduced CaSR expression, and decreased VDR expression.

Phosphorus retention and hyperphosphatemia have also been recognized for many years as important factors in the pathogenesis of secondary hyperparathyroidism. The development of secondary hyperparathyroidism is prevented in experimental animals with chronic kidney disease when dietary phosphorus intake is lowered in proportion to the glomerular filtration rate (GFR) (59). Dietary phosphate restriction can also reduce previously elevated serum PTH levels in patients with moderate renal failure (9, 60). Phosphorus retention and hyperphosphatemia indirectly promote the secretion of PTH in several ways. Hyperphosphatemia lowers blood ionized calcium levels as free calcium ions complex with excess inorganic phosphate. The ensuing hypocalcemia stimulates PTH release. Phosphorus also impairs renal 1α-hydroxylase activity, which diminishes the conversion of 25(OH)D to $1,25(OH)_2D_3$ (9). Finally, phosphorus can directly enhance PTH synthesis by the parathyroid cell by interrupting the normal calcium sensing receptor (CaSR) signal cascade. High serum phosphorous levels decrease cytosolic phospholipase A2 (normally increased by CaSR activation), leading to a decrease in arachidonic acid production with a subsequent increase in PTH secretion (61). Hypophosphatemia also decreases PTH mRNA transcript stability *in vitro* (62), suggesting that phosphorous itself affects serum PTH levels,

probably by increasing the stability of the PTH mRNA transcript.

Alterations in parathyroid gland CaSR expression also occur in secondary hyperparathyroidism and may, in turn, contribute to parathyroid gland hyperplasia. The CaSR is a seven transmembrane G protein-coupled receptor with a large extracellular N-terminus, which binds acidic amino acids and divalent cations (63). Low extracellular calcium levels result in decreased calcium binding to the receptor, a conformational relaxation of the receptor and a resultant increase in PTH secretion (64), while activation of the receptor by high levels of serum calcium decrease PTH secretion (65, 66). The expression of the CaSR is reduced by 30–70% as judged by immunohistochemical methods in hyperplastic parathyroid tissue obtained from human subjects with renal failure (57, 67). Decreased expression and activity of CaSR has been linked to decreased responsiveness in PTH secretion due to altered calcium levels (68). This decreased expression of the CaSR results in an insensitivity to serum calcium levels with subsequent uncontrolled secretion of PTH. Increased stimulation of the CaSR by calcimimetics has been shown to decrease PTH cell proliferation, implicating the CaSR as a regulator of cell proliferation, as well as PTH secretion (69).

The link between the CaSR and vitamin D in cell cycling in the parathyroid gland is incompletely understood. However, there is some evidence that vitamin D may work to decrease parathyroid hyperplasia by activating the CaSR. CaSR gene transcription has been shown to be regulated by vitamin D through two distinct vitamin D response elements in the gene's promoter region (70), suggesting that alterations in vitamin D metabolism in renal failure could account for changes in calcium sensing by the parathyroid glands and that vitamin D may act upstream of the CaSR in preventing parathyroid cell hyperplasia (17).

The temporal relationship between the duration and/or the severity of renal failure and the decrease in parathyroid CaSR expression has yet to be determined. In vivo studies of parathyroid gland function in patients with end-stage renal disease indicate that calcium sensing by the parathyroid glands is altered in advanced, but not in mild to moderate, secondary hyperparathyroidism (71,72). However early *in vivo* findings have yet to be confirmed by *in vitro* assessment of either calcium-regulated PTH release or CaSR expression. It remains uncertain, therefore, whether alterations in CaSR expression fully account for disturbances in PTH secretion in mild to moderate chronic renal failure.

Recent evidence suggests that FGF-23 may also regulate PTH secretion. *In vitro* experiments indicate that increased FGF-23 levels suppress PTH release. This effect appears to be independent of the action of FGF-23 on vitamin D metabolism (14, 15).

Pathogenesis of Renal Bone Disease

Abnormalities in Bone Turnover, Mineralization, Volume, Linear Growth, or Strength

Evaluation of skeletal histology by bone histomorphometry provides both a method for understanding the pathophysiology of renal bone disease and a guide to its proper management. The routine assessment of bone histology is not performed in the clinical setting; however, current recommendations from the National Kidney Foundation (KDOQI Guidelines) suggest that a bone biopsy should be considered in all patients with CKD who have fractures with minimal trauma (pathological fractures), suspected aluminum bone disease, or persistent hypercalcemia despite serum PTH levels between 400–600 pg/ml (1, 73). After double tetracycline labeling, bone tissue is obtained from the iliac crest on an outpatient basis with minimal morbidity (74, 75). As recently recommended by the *Kidney Disease Improving Global Outcomes* (KDIGO) workgroup, three areas of bone histology are examined: bone turnover, mineralization and volume, all of which may be altered in patients with chronic kidney disease (1).

Bone Turnover

Traditionally, renal osteodystrophy has been classified primarily by alterations in bone turnover. The primary lesion of renal osteodystrophy in children is one of high bone turnover, also termed "secondary hyperparathyroidism". Since PTH activates the PTH/PTHrP receptor on osteocytes and osteoblasts, increasing cellular activity of both osteoblasts and osteoclasts (76, 77), excessive levels of circulating PTH result in increased bone turnover (78). Serum PTH levels are inversely correlated with GFR, and the majority of patients with GFR less than 50 ml/min have increased serum PTH levels and high turnover bone disease (79–81). These findings are nearly universal in untreated children at the initiation of dialysis (2). Secondary hyperparathyroid bone disease is marked by

an increased numbers of osteoblasts and osteoclasts. Excess osteoclastic activity leads to increased resorption of mineral and matrix along both the trabecular surface and within the haversian canals of cortical bone. Osteitis fibrosa cystica, the advanced lesion of secondary hyperparathyroidism, is also associated with peritrabecular fibrosis (*Figs. 71-2a, b*) (82).

A state of low-turnover bone disease (adynamic renal osteodystrophy) also occurs in children treated with maintenance dialysis, although it has not been demonstrated in children with earlier stages of CKD (80). Adynamic renal osteodystrophy is associated with disorders such as age-related or postmenopausal osteoporosis (in adults), steroid-induced osteoporosis, hypoparathyroidism (idiopathic or surgically induced), and diabetes mellitus. Reversible causes of adynamic renal osteodystrophy include aluminum toxicity, subtotal parathyroidectomy, prolonged treatment with calcium-containing phosphate-binding medications, use of high dialysate calcium concentration, and aggressive vitamin D therapy (83). The prevalence of adynamic lesions of bone is less than 20% in children and adolescents with end-stage renal disease who are treated with daily doses of calcitriol, although adynamic bone is present in as many as 33% of pediatric patients treated with high dose intermittent calcitriol therapy and calcium-based binders for the control of secondary hyperparathyroidism (84). Due to a direct suppressive effect of bone agents on bone turnover, adynamic bone often develops in these patients despite markedly elevated serum PTH levels (84).

On bone histomorphometric analysis, adynamic bone is characterized by normal osteoid volume, an absence of fibrosis, and a reduced bone formation rate, as indicated by a reduced or absent double tetracycline label (*Figs. 71-3a, b*) (84, 85). A paucity of osteoblasts and osteoclasts are present (78). Adynamic bone is associated with low PTH levels, low alkaline phosphatase levels, high serum calcium levels, and a propensity for increased vascular calcification (86, 87). In addition to the increased risk for fractures that is observed in adults with adyamic bone, dialyzed children with low bone turnover display an increased severity of growth retardation (88, 89).

The presence of CKD markedly attenuates the effect of PTH on bone (90, 91). Indeed, serum levels of PTH that are three–five times the normal range are associated with normal bone turnover in patients treated with maintenance dialysis, while similar PTH values in patients with mild to moderate kidney disease are associated with high turnover osteodystrophy (80, 92, 93). Although the precise mechanisms are poorly understood, uremia has been associated with this "skeletal resistance" to the actions of PTH. Uremic animals and humans display decreased PTH/PTH-related protein receptor mRNA expression in bone and growth plate (94, 95). Hyperphosphatemia and alterations in vitamin D metabolism, among other factors, have been implicated in these changes and calcitriol administration has been shown to partially restore the calcemic response to PTH in both experimental animals and in patients with moderate CKD (96).

Mineralization

Although renal osteodystrophy has traditionally been defined by lesions in bone turnover, alterations in skeletal mineralization are also prevalent in children with CKD (93). Increases in unmineralized bone (osteoid) in conjunction with delayed rates of mineral deposition are

Figure 71-2

Bone histology, Osteitis Fibrosa. (a) Under light microscopy, an increase in cellular activity, osteoid accumulation, erosion, and fibrosis are visible. (b) An increase in double tetracycline labeling signifies an increase in bone formation. (See color plate 47)

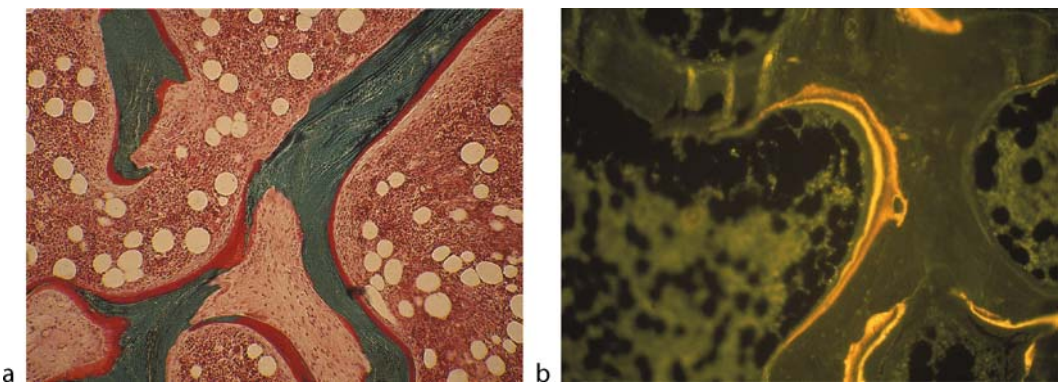
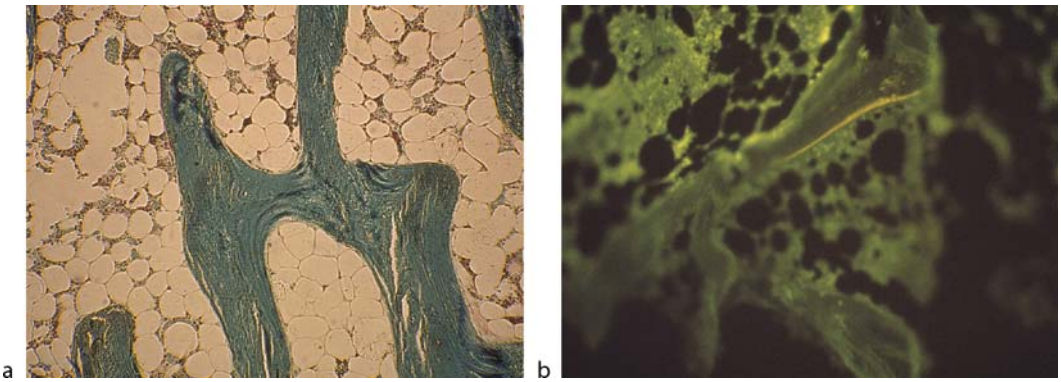


Figure 71-3

Bone histology, Adynamic Bone. (a) Under light microscopy, decreased cellular activity with minimal osteoid accumulation. (b) Very little double tetracycline labeling signifies a decrease in bone formation.



common (82, 93). Defective mineralization that is associated with high turnover bone disease is termed "mixed lesion"; when associated with low to normal bone turnover, it is referred to as "osteomalacia" (1). In children with CKD, osteomalacia may result from inadequate dietary intake of calcium, phosphorus, or vitamin D, particularly during periods of rapid growth, when the skeletal demand for these nutrients is high. During previous decades, aluminum toxicity from the use of aluminum based phosphate binders resulted in low bone turnover and poor skeletal mineralization in many patients.

The skeletal histology of the "mixed lesion of renal osteodystrophy" has features of both osteitis fibrosa and osteomalacia (97). Patients with this lesion often display high serum PTH and alkaline phosphatase levels. Mixed lesions are seen with high-turnover bone disease in patients who are developing aluminum toxicity or in patients with low-turnover aluminum-related bone disease during Desferoxamine (DFO) therapy (98). In these cases, mixed lesion represents a transitional stage between high-turnover and low-turnover bone disease.

Although the mechanisms of skeletal mineralization are incompletely understood, other factors, including 25(OH)D deficiency and altered FGF-23 metabolism, have been implicated in the pathogenesis of osteoid accumulation in the CKD population. In the general population, nutritional 25(OH)D deficiency results in osteomalacia and a similar phenotype may occur in children with CKD. FGF-23 may also play a role; both overexpression (99–101) and ablation of FGF-23 (102, 103) in mice result are associated with abnormal mineralization of osteoid, although by different mechanisms. The phosphaturic effect of increased FGF-23 may cause rickets and osteomalacia through an insufficiency of mineral substrate. The mechanisms leading to impaired mineralization in FGF-23-null animals, which have severe hyperphosphatemia and normal or elevated serum calcium levels, remain uncertain; however, osteomalacia in these animals suggests that FGF-23 may play a direct role in skeletal mineral deposition. While the ramifications of defective mineralization remain to be established, increased fracture rates, bone deformities, and growth retardation are prevalent in patients with CKD despite adequate control of bone turnover. These complications may be due, in part, to alterations in bone mineralization.

Bone Volume

Since PTH is an anabolic steroid at the level of trabecular bone, high levels of serum PTH are typically associated with increases in bone volume, trabecular volume, and trabecular width (75, 93, 104, 105). Thus, children with CKD typically have normal or high bone volume as assessed by bone histomorphometry. Those treated with corticosteroids, however, may display loss of bone volume, termed "osteoporosis". The impact of osteoporosis in childhood may not always be immediately apparent; however, sub-optimal peak bone mass accretion in adolescence is associated with an increased risk of osteoporosis, hip fractures, and mortality in adulthood (106).

Clinical Manifestations

Renal osteodystrophy in children presents with nonspecific signs and symptoms and often goes unnoticed.

Clinically, patients may refrain from physical activity, making early diagnosis difficult, and radiographic changes may not reflect the severity of bone disease. Careful and detailed physical examination must be performed in the evaluation of these patients, since renal osteodystrophy contributes to long-term morbidity (including short stature, deformities, and chronic disability) in the majority of adults who developed renal failure as children (2).

Growth

Growth retardation is the hallmark of CKD in children. Protein and calorie malnutrition, metabolic acidosis, end-organ growth hormone resistance, and renal bone disease are the factors most commonly implicated in growth failure (107). Despite correction of acidosis and anemia, normalization of serum calcium and phosphorus levels, and vitamin D sterol therapy replacement, the majority of children with CKD continue to grow poorly. Growth failure worsens as renal function declines; the average height of children with even mild CKD (GFR 50–70 ml/min/1.73 m^2) is 1 standard deviation (SDS) below the average for healthy children. Moderate CKD (GFR 25–49 ml/min/1.73 m^2) is associated with a height SDS of -1.5, and, at the time of initiation of dialysis, the mean height SDS is -1.8. Boys, younger patients, and those with prior renal transplants are at greatest risk for growth failure (108).

Acidosis has been linked to delayed linear growth in patients with renal tubular acidosis and normal renal function, and correction of metabolic acidosis often leads to acceleration in growth velocity (109). Acidotic rats have been found to have decreased growth hormone (GH) secretion, serum insulin-like growth factor 1 (IGF-1), and hepatic IGF-1 mRNA expression. Moreover, metabolic acidosis has been shown to inhibit the effects of GH in rats with normal and decreased renal function (110–112). Growth plate mRNA levels of GH receptor, IGF-1 receptor, and IGF-1 expression are downregulated, while IGF-binding proteins are upregulated in the face of acidosis (113). In adults treated with maintenance dialysis, correction of acidosis has been shown to decrease the progression of secondary hyperparathyroidism and improve skeletal mineralization (114).

Calcitriol deficiency has also been thought to contribute to growth retardation and bone disease in children with CKD. Secondary hyperparathyroidism remains prevalent in children with advanced renal disease, and osteitis fibrosa continues to be the most common skeletal lesion of renal osteodystrophy in those undergoing regular dialysis despite regular treatment with daily doses of oral calcitriol (75, 115). Secondary hyperparathyroidism contributes to growth retardation, although optimal target values for PTH in children in all stages of CKD remain controversial. In children with moderate CKD, some data indicate that normal growth velocity is achieved when PTH levels are maintained within the normal range (116) while others have demonstrated a linear correlation between growth and PTH levels in the same patient population – those with the highest PTH values maintaining the highest rates of growth (117). Treatment of secondary hyperparathyroidism with large, intermittent doses of calcitriol and calcium-based phosphate binders has been shown to significantly reduce bone formation and suppress osteoblastic activity in both adults and children (96, 118). During such therapy, adynamic bone may develop and linear bone growth decrease, despite serum PTH levels in the KDOQI recommended range (73, 89). By contrast, normal rates of bone formation are achieved and adynamic bone avoided when serum PTH levels are maintained between 300–500 pg/ml during intermittent vitamin D sterol therapy (105).

The mechanisms responsible by which calcitriol inhibits epiphyseal growth plate cartilage remain poorly understood; however, it is well-known that calcitriol exerts dose-dependent inhibitory effects on cell proliferation of chondrocytes and osteoblasts *in vitro*. In addition, vitamin D sterols increase expressions of a number of IGF binding proteins (IGFBPs), IGFBP-2, -3, -4, -5, which sequester IGF-1 and may exert IGF-1-independent antiproliferative effects through their own receptors (119–123).

GH resistance also contributes to impaired linear growth in renal failure. In CKD, poor growth develops despite normal or increased serum GH levels (124, 125). While the underlying molecular mechanisms for this GH resistance are incompletely understood, uremia has been associated with diminished hepatic GH receptor and IGF-1 mRNA expression, defects in post-receptor GH-mediated signal transduction (126, 127), reductions in serum GH-binding protein levels (128), along with increased synthesis and reduced clearance of IGF binding proteins (128–130). Improved growth velocity during recombinant human GH (rhGH) therapy has been ascribed to increased bioavailability of IGF-1 to target tissues. Children who are treated with maintenance dialysis respond less well to rhGH therapy than children with less severe CKD, but the mechanisms for the differences in response to GH therapy remain to be determined.

Bone Pain

In the early course of renal osteodystrophy, bone pain is nonspecific and difficult to distinguish from common

aches and pains, and a great variability in clinical presentation occurs among patients. Pain often localizes to the lower back and to weight-bearing joints, including hips, knees, and ankles. Symptoms worsen with pressure and changes in posture. Limping requires prompt and thorough evaluation in a previously ambulatory child with secondary hyperparathyroidism due to the increased prevalence of fracture and slipped epiphysis in this population (66).

Slipped Epiphyses

Slipped epiphyses are one of the most severe and physically incapacitating manifestations of bone disease in children with CKD. In preschool children, the upper and lower femoral epiphyses are often affected, while the upper femur and the radial/ulnar epiphyses are involved in older children. The distal radius and metacarpal and metatarsal heads may also be affected (66). In one report, slipped epiphyses were found in as many as 10 of 33 children with newly diagnosed CKD and in 1 of 82 of those who are undergoing dialysis therapy (66). The clinical presentation may include limping, waddling gait, limitation of the range of motion, and inability to ambulate. Severe osteitis fibrosa with marked endosteal fibrosis is present on bone biopsy. A dense fibrous tissue develops between the growth plate cartilage and the adjacent metaphysis, where the plane of slippage may occur (131). The diagnosis is usually established by roentgenograms. Total joint replacement is often required when the proximal femur is involved.

Skeletal Deformities

Bone deformities are also common in uremic children due to altered skeletal remodeling and, due to their increased growth velocity, are most evident in children younger than 10 years. The pattern of bone deformities varies with the child's age. Patients younger than 4 years have skeletal abnormalities similar to those due to vitamin D-deficient rickets, including rachitic rosary, metaphyseal widening (leading to wrist and ankle enlargement), craniotabes, and frontal bossing. Slipped epiphyses, genu valgum (*Fig. 71-4*), femoral and wrist deformities (*Fig. 71-5*) are most common in preadolescent children with long-standing CKD (2, 131). Avascular necrosis of the femoral head and pathologic fractures of the extremities and chest wall due to osteoporosis and bone deformities may occur with minimal trauma. In addition, vertebral crush fractures contribute to significant morbidity in this population. Patients may also present with ulnar deviation, pes varus, pseudo-clubbing, and dental abnormalities. The initial management of skeletal deformities requires the normalization of serum calcium, phosphorus, and PTH levels. Surgical correction is often also

Figure 71-4
X-rays of genu valgum.

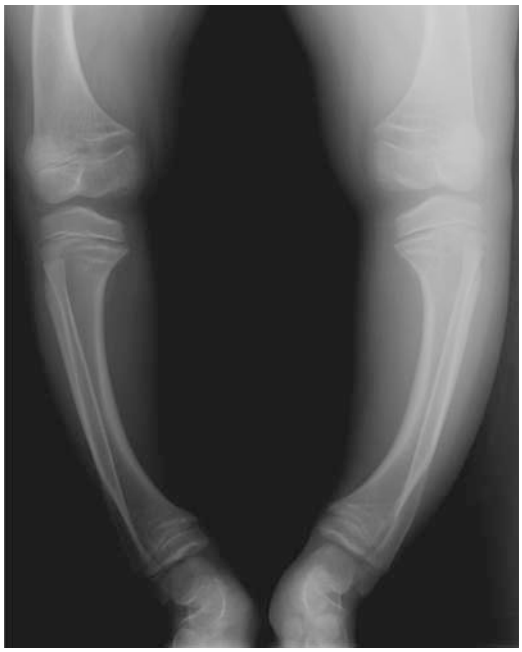
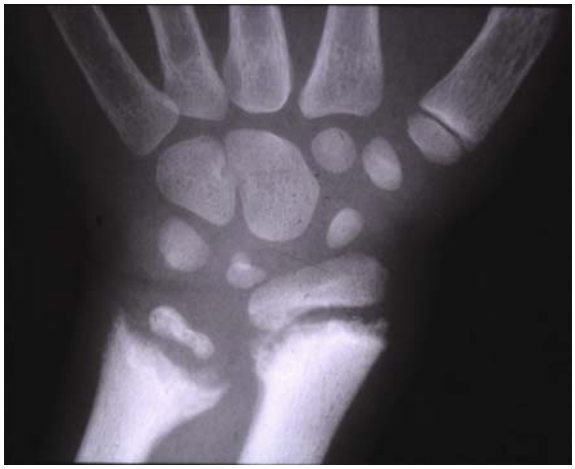


Figure 71-5
X-rays of skeletal erosions in the hand.



necessary but should be performed only after correction of biochemical abnormalities (73).

Muscle Weakness

Myopathy in children with CKD may be progressive and the diagnosis is clinical; no specific tests are available. Electromyographic studies are non-specific and serum levels of creatine phosphokinase, aldolase, and transaminases are usually within normal limits. Initially, patients may complain of nonspecific aches and pains; subsequently they report limited ability to perform activities of daily living. These symptoms may go undiagnosed by the clinician, as patients may self-restrict physical activity, limiting their quality of life and social development. Similar to the proximal muscle weakness seen in patients with nutritional vitamin D deficiency, children with CKD may develop a characteristic waddling gait (132, 133). The mechanism behind this proximal myopathy is not well understood, although secondary hyperparathyroidism, phosphate depletion, aluminum bone disease, and disorders of vitamin D metabolism may contribute (134). Progressive and debilitating myopathy that develops in association with severe bone pain is a common manifestation of aluminum-related bone disease. No specific treatment is available, although an improvement in muscle strength in both proximal and distal muscles has been shown after treatment with vitamin D (135). Subtotal parathyroidectomy also results in clinical improvement.

Extraskeletal Calcification

Visceral, tumoral or periarticular, and vascular calcifications may develop in patient with CKD. Autopsy data from 120 children who had undergone dialysis or renal transplantation identified soft tissue calcifications in 60%, and the most commonly affected sites were blood vessels, lung, kidney, myocardium, coronary arteries, central nervous system, and gastric mucosa (136). Calcification was associated with multiple factors such as treatment with vitamin D, peak calcium-phosphorus product, age at onset of CKD, and male gender. Deposition of calcium and phosphorus in the conjunctiva leads to inflammation (termed the "red-eye syndrome") in 10% of dialyzed patients. Calcifications in the cornea are demonstrated by slit-lamp examination and may result in band keratopathy. Pulmonary calcifications may progress despite optimal dialysis therapy and may lead to restrictive lung disease. Parathyroidectomy and renal transplantation are often the best therapeutic options (137). Calcific periarthritis is characterized by episodes of acute inflammation in and around small joints, with periarticular warmth and erythema. Radiographic findings demonstrate small effusions within the joint and calcifications in adjacent periarticular tissues.

The mortality rate in adults and children with CKD is markedly higher than that of the general population, and cardiovascular disease is the leading cause of death in both children and adults treated with maintenance dialysis (138, 139). The etiology of cardiovascular disease in CKD is multifactorial and includes traditional risk factors, such as hyperlipidemia, hypertension, and inflammation, as well as alterations in mineral metabolism specific to CKD. Furthermore, a link exists between alterations in mineral metabolism, bone disease, and cardiovascular disease (1). Cardiovascular changes associated with increased mortality have their origins in CKD prior to dialysis and these alterations begin in childhood (*Figs. 71-6* and *71-7*) (136, 138, 140). In contrast to the calcifications of atherosclerotic plaques that develop with age in the vascular intima of individuals with normal kidney function, vascular calcification in the uremic milieu develops primarily in the tunica media. This form of calcification does not contribute to plaque instability or rupture, but is associated with decreased distensibility of blood vessels, causing a rigid "lead pipe" pathology which is associated with increased risk of congestive heart failure (1). Electron beam computed tomography (EBCT) is used in the assessment of vascular calcifications in the adult population, and measurements in young adults who were treated with maintenance dialysis as children demonstrated that a significant proportion of this population has evidence of vascular calcification (138, 139). Carotid ultrasound measurement of intimal-medial thickness (IMT) has been validated for the assessment of cardiovascular pathology in children – increased thickness being associated with worsening disease (140, 141). Recent concurrent EBCT and IMT evaluation of pediatric patients treated with maintenance dialysis demonstrated a positive correlation between serum PTH levels and calcification score by EBCT as well as between PTH levels, increased IMT and decreased vascular distensibility, suggesting that both EBCT and IMT are useful in the evaluation of cardiac disease in young patients with CKD (142).

The pathophysiology of cardiovascular changes remains to be fully elucidated but factors such as altered mineral metabolism, hypertension, hyperlipidemia, oxidative stress, and uremic toxins play a role. Hypercalcemia, hyperphosphatemia, elevated levels of the calcium x phosphorus product, and high doses of vitamin D sterols

Figure 71-6

Coronary-Artery Calcification Scores in 39 Children and Young Adults with End-Stage Renal Disease Who Were Treated by Dialysis, According to Age. Coronary-artery calcification was assessed by electron-beam computed tomography. The scale on the y axis is logarithmic. (Reprinted with permission. Goodman W et al., *NEJM* 2000. vol 106(1) pp 100–105).

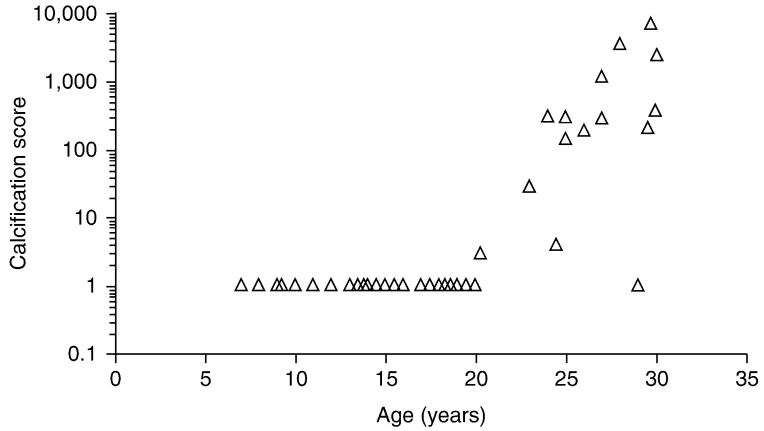
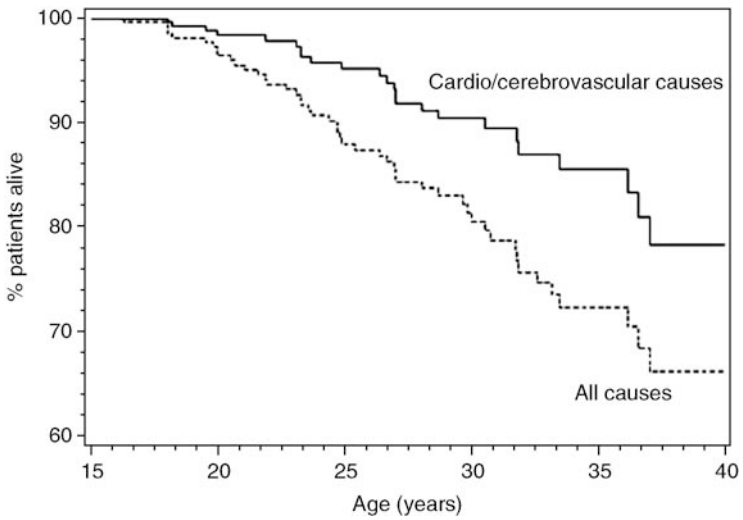


Figure 71-7

Kaplan-Meier actuarial survival curve of 283 patients with childhood-onset CRF. Broken line, survival rate considering all causes of death; solid line, survival rate considering cardiovascular and cerebrovascular causes of death only. (Reprinted by permission. Oh et al., Circulation 2002: 106(1):100–105).



(136, 138) have all been implicated. However, 40% of adult patients with stage 3 CKD, without these risk factors, show evidence of calcification (143), suggesting that factors in the uremic milieu itself, apart from high levels of calcium and phosphorus, contribute to cardiovascular disease. Indeed, vascular tissues in the uremic milieu express osteoblast differentiation factors (144). Osteoblasts and vascular smooth muscle cells have a common mesenchymal origin; Core binding factor-1 (Cbfa1) is thought to trigger mesenchymal cell to osteoblast transformation. Mice that are deficient in Cbfa1 fail to mineralize bone (145), and arteries obtained from patients undergoing renal transplantation show increased levels of this protein (144). Upregulation of the sodium-dependent phosphate transporter PIT-1 likely also contributes to increased calcification (146), and upregulation of

pro-mineralization factors such as osteopontin, bone sialoprotein, osteonectin, alkaline phosphatase, type I collagen, and bone morphogenic protein-2 (BMP-2) is potentiated by the uremic milieu (147–150). By contrast, expression of calcification inhibitors, such as fetuin A, matrix gla protein, and klotho is suppressed (151–154). Levels of circulating FGF-23 may also contribute, as values are inversely correlated with peripheral vascular calcification in adult dialysis patients (155). Klotho, the cofactor necessary for the actions of FGF-23, has also been implicated in vascular calcification. Animals lacking the Klotho gene display elevated levels of calcium, phosphorus, and vitamin D, along with vascular calcification and premature aging. CKD is associated with low circulating levels of Klotho (154), which has been implicated in regulating sodium/phosphate cotransport in the aorta (156).

As the pathophysiology of cardiovascular disease in CKD is multifactorial, treatment strategies are also multifaceted and vary according to stage of CKD. Therapies that are effective in early CKD may not be effective in later stages – lipid lower agents decrease mortality in adults with CKD (157) and in those with stable renal allografts (158) but have not been shown to benefit patients treated with dialysis(159). By contrast, normalization of mineral metabolism, by avoiding hypercalcemia and hyperphosphatemia, limiting calcium intake, and avoiding adynamic bone is effective at slowing the progression of cardiovascular calcification in patients treated with maintenance dialysis (73, 138, 160, 161). Thus, at different stages of CKD, the relative importance of individual risk factors and the value of different biomarkers may vary.

Diagnostic Evaluations

Biochemical Determinations

Calcium

Serum calcium levels are typically maintained within the normal range until late (stages 4 and 5) CKD, when serum calcium levels drop. Hypocalcemia often resolves during treatment with calcium-containing phosphate binders, vitamin D, and with initiation of dialysis. Typical dialysis solutions containing 2.5 mEq/L calcium concentrations are generally adequate to maintain serum calcium within acceptable limits.

Recurrent or persistent hypercalcemia in patients with CKD is uncommon yet may occur with severe secondary hyperparathyroidism, aluminum-related bone disease, adynamic bone, prolonged use of high dose vitamin D sterols, large amounts of calcium-containing phosphate-binding agents, and prolonged immobilization (85, 162, 163). Malignancy and extrarenal production of calcitriol in disorders such as sarcoidosis or tuberculosis result in hypercalcemia, but these conditions are uncommon in the pediatric age group. Patients with adynamic bone lesion without evidence of aluminum toxicity are prone to develop hypercalcemic episodes, which are related to the skeleton's inability to incorporate an acute calcium load (164).

Phosphorus

Serum phosphorus levels are often maintained within normal limits in early CKD. However, when the GFR falls below 30 ml/min/1.73 m^2 (stage 4 CKD), hyperphosphatemia ensues (4). In order to prevent the development and progression of secondary hyperparathyroidism and vascular calcification, measures should be initiated to maintain serum phosphorus levels within age-appropriate levels and calcium phosphorus product lower than 55 mg^2/dl. Normal values for phosphorus are higher in infants and decline to adult values by late adolescence. During the first 3 months of life, values range from 4.8 to 7.4 mg/dl (mean: 6.2 mg/dl), levels decrease to 4.5–5.8 mg/dl (mean: 5.0 mg/dl) at age 1–2 years, and 3.5–5.5 mg/dl (mean: 4.4 mg/dl) during childhood (*Fig. 71-8*) (138).

Alkaline Phosphatase

Serum total alkaline phosphatase is a biochemical marker of osteoblastic activity. Values generally correspond to the histologic severity of osteitis fibrosa, and serial measurements may be helpful in the follow-up of children with renal osteodystrophy. During intermittent calcitriol therapy, serum alkaline phosphatase levels have been found to be good predictors of adynamic renal osteodystrophy (89). Indeed, serum alkaline phosphatase levels often decrease below baseline in patients with bone biopsy-proven secondary hyperparathyroidism, and such changes correspond to marked reductions in bone formation documented by bone biopsy. In addition, changes in Z-scores for height during intermittent calcitriol therapy correlate with serum alkaline phosphatase levels, although a similar relationship is not observed during daily calcitriol therapy (89). Bone-specific alkaline phosphatase activity may be useful in predicting the histologic lesion of renal osteodystrophy, but whether these values are superior

Figure 71-8

Appropriate serum phosphorus and calcium levels by age (reprinted with permission from the KDOQI guidelines).

Representative normal values for serum phosphorus, Total calcium, Blood Ionized calcium, and Alkaline phosphatase concentrations

Age (yrs.)	Serum Phosphorus (mg/dL)	Serum Total Calcium (mg/dL)	Blood Ionized Calcium (mM)	Alkaline Phosphatase (IU)
0–0.25	4.8–7.4	8.8–11.3	1.22–1.40	
1.5	4.5–6.5	9.4–10.8	1.22–1.32	100–350
6–12	3.6–5.8	9.4–10.3	1.15–1.32	60–450
13.20	2.3–4.5	8.8–10.2	1.12–1.30	40–180

to total alkaline phosphatase levels remains to be demonstrated.

Parathyroid Hormone

Accurate measurements of the concentration of PTH in serum or plasma are essential for the proper assessment of renal osteodystrophy (92, 93, 165–167). Indeed, determinations of PTH levels are used as surrogates for bone turnover, although bone histology, remains the most reliable method by which to establish the diagnosis of renal osteodystrophy (75, 97, 168). Serum PTH levels are usually normal in early CKD and rise in patients with moderate and severe CKD (9, 60). Increasing skeletal resistance to the actions of PTH necessitate that serum PTH levels be maintained at higher ranges in more severe renal failure (*Figs. 71-9* and *71-10*).

Because PTH and its fragments are cleared by the kidneys, active and inactive fragments of the molecule accumulate as renal failure progresses. In the 1980s, PTH assays were developed that detected PTH epitopes in the midregion and carboxy-terminal ends of the molecule. These assays therefore detected both full-length (active) PTH as well as many of the inactive fragments retained in renal failure (166), thus yielding spuriously high assessments of the amount of active PTH in circulation. Andress et al. later demonstrated that an assay detecting the amino terminal region of the molecule better avoided inactive fragments and was a better predictor osteitis fibrosa than the inactive carboxy terminal/midregion PTH fragments (169). Subsequently, the first clinically useful two-site immunometric PTH assay (PTH-IMA) was developed using antibodies direct both against amino- and carboxyl-terminal epitopes. These assays were predicted to measure full length PTH (also called "PTH(1–84)") exclusively (170).

During the past 25 years, this first-generation PTH-IMA (1st PTH-IMA) has proven to be a reasonable predictor of the different subtypes of renal osteodystrophy. It has also performed well in assessing the therapeutic response to active vitamin D sterols in patients with renal failure (92, 93, 165). Subsequent observations, however, have highlighted important shortcomings of this and other PTH-IMAs (171, 172). In particular, a series of studies by D'Amour and colleagues (171–173) demonstrated that 1st PTH-IMAs detect not only the intact hormone, but also additional PTH fragments truncated at the amino-terminus (173). 1st PTH-IMAs thus overestimate the true concentration of PTH(1–84) in serum or plasma both in patients with ESRD and in those with normal renal function. By contrast, more recently developed second-generation PTH-IMAs (2nd PTH-IMAs) using detection antibodies raised against the first four amino-terminal amino acids of human PTH recognizes only PTH(1–84) (and possibly PTH fragments that are truncated at the carboxyl-terminus) but not amino-terminally truncated fragments, such as PTH(7–84) (64, 174). Consistent with this assessment, human PTH(1–34) but not human PTH(2–34) or other amino-terminally truncated fragments of human PTH(1–34) cross-reacted with the detection antibody (64, 174).

In addition to interfering with current PTH assays, current *in vitro* and *in vivo* experimental data also indicate that one or more amino-terminally truncated PTH fragments (PTH(7–84) and other fragments) may antagonize the calcemic actions of PTH(1–84) and diminish bone cell activity. These actions may be mediated through a receptor distinct from the type I PTH/PTHrP receptor (174, 175). These fragments may modulate bone metabolism and may contribute to the skeletal PTH resistance that occurs in patients with renal failure (174, 175). It is therefore possible that an independent assessment of PTH (1–84) and amino-truncated PTH fragments may have better value for diagnosis of the different subtypes of renal osteodystrophy than each parameter alone. Indeed, it has been suggested that estimates of the ratio between PTH(1–84) and amino-truncated PTH(1–84) fragments

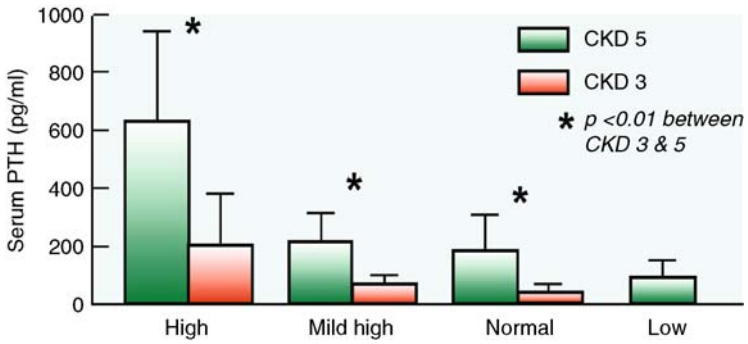
Figure 71-9

Target PTH levels according to stage of CKD (Reprinted with permission from the KDOQI guidelines).

Stage of CKD	GFR (ml/min/1.73m^2)	1st PTH-IMA Target Range (pg/ml)
3	30–59	35–70
4	15–29	70–110
5	Less than 15	200–300

Figure 71-10

PTH levels corresponding to high, normal and low turnover bone histology in two different stages of CKD.



more accurately predicts bone remodeling and turnover in patients with ESRD (176). However, others have failed to confirm such findings (104, 177).

Although 2nd PTH-IMAs are more specific for the concentrations of the full-length, active molecule, current data demonstrate that measurements of PTH using either 1st or 2nd PTH-IMAs are highly correlated and provide similar accuracy for predicting bone turnover in pediatric patients undergoing maintenance peritoneal dialysis (*Fig. 71-11*) (104, 177). Therefore, current recommendations for indications of therapy with active vitamin D sterols should be based on the relationship between indices of bone formation and PTH levels determined by 1st generation immunometric assays.

Aluminum

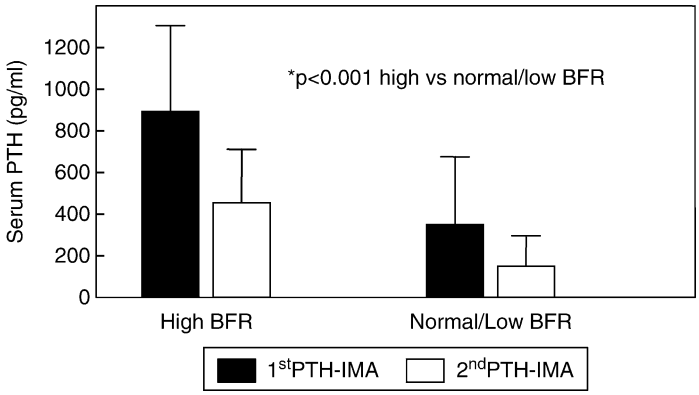
Aluminum toxicity occurs in dialysis patients or CKD patients with GFR less than 30 ml/min/1.73 m^2 because aluminum that is absorbed from the gut, from the dialysate, or from parenteral infusions is inadequately excreted by the diseased kidney. Accumulation occurs in various tissues, including bone, liver, brain, and parathyroid glands, and can produce toxicity such as dialysis encephalopathy, osteomalacia, and microcytic anemia. The gold standard for the diagnosis of aluminum bone disease is a bone biopsy demonstrating increased aluminum staining of the bone surface (greater than 15–25%) with histologic evidence of adynamic bone or osteomalacia. The presence of aluminum deposits in the bone and liver do not correlate with plasma levels (178); however, plasma aluminum levels are useful in monitoring patients who are undergoing chronic dialysis therapy and receiving aluminum-containing phosphate-binding agents for prolonged periods. DFO should be administered to symptomatic patients with aluminum levels between 60 and 200 μg/L or a positive DFO test. The DFO infusion test is performed by infusing 5 mg/kg of DFO during the last hour of the dialysis session. Serum aluminum is measured before DFO infusion and 2 days later, before the next dialysis session. In order to prevent DFO-induced neurotoxicity, DFO should not be administered if serum aluminum concentrations are greater than 200 μg/L (104, 169).

Radiography

Radiographic features of secondary hyperparathyroidism resemble those of nutritional rickets. Subperiosteal resorption may occur at the distal ends of the clavicles, ischial and pubic surfaces, sacroiliac joints, junction of

Figure 71-11

PTH levels by different assays according to bone histology (high vs normal/low bone formation rate-BFR) in patients treated with maintenance dialysis.



the metaphysis and diaphysis of long bone, and in the phalanges (179, 180). A diffuse ground-glass appearance, generalized mottling, focal radiolucencies, and sclerotic areas may be evident in the skull. Metaphyseal changes, called growth zone lesions or rickets-like lesions, are best demonstrated in hand radiographs. Although subperiosteal erosions are a hallmark of secondary hyperparathyroidism, these lesions can also be seen in patients with aluminum-related bone disease, which may represent unhealed lesions from a previous state of secondary hyperparathyroidism (181). To enhance the sensitivity of hand radiographs, the use of fine-grain films and magnification by hand lens has been recommended (180).

The radiographic features of osteomalacia are less evident, particularly in older children and adolescents. In young children, widening of the epiphyseal growth plate and other deformities of the growth plate cartilage are evident, whereas pseudofractures and looser zones, which appear as straight, wide radiolucent bands within the cortex, may be the only findings in older children and adults. The radiographic density of bone on conventional skeletal radiographs is reduced in many patients with renal osteodystrophy, but osteosclerotic changes or localized increases in bone density are a prominent finding in children with chronic renal failure.

Bone Scintigraphy

Bone scans using the technetium-99-labeled diphosphonate are helpful in estimating the severity of bone disease and differentiating between high-turnover lesions of osteitis fibrosa and low-turnover lesions of osteomalacia (182). Uptake is usually diffuse and symmetric in patients with severe secondary hyperparathyroidism, whereas patients with osteomalacia exhibit a less intense uptake (182). Calcifications may also be demonstrated in various organs, including the lungs and heart, using the bone scan.

Bone Biopsy

Iliac crest bone biopsy provides the most valuable diagnostic information of the skeletal lesions of renal osteodystrophy. The procedure is safe and well tolerated in children and is done in an outpatient setting (75). Bone biopsy provides information about the histologic appearance and dynamics of bone formation and mineralization. Although not routinely performed in the clinical setting, a bone biopsy should be considered in all patients with CKD who have fractures with minimal trauma (pathological fractures), suspected aluminum bone disease, or persistent hypercalcemia despite serum PTH levels between 400–600 pg/ml (73).

For bone labeling, a 2 day course of tetracycline is administered at 15 mg/kg/day (divided in twice or thrice daily doses). Fourteen days later, the 2 day course is repeated. For children younger than 8 years, tetracycline dosage is usually kept below 10 mg/kg/day to avoid toxicity. Histochemical staining procedures demonstrate the deposition of abnormal components within bone such as iron, aluminum, and oxalate (75, 134).

Treatment of CKD-MBD

In order to minimize complications on the growing skeleton and to prevent extraskeletal calcifications, particular

attention must be made to the alterations of bone and mineral metabolism in children with CKD.

Dietary Manipulation

Evaluation of dietary intake and growth must be performed at regular intervals to maximize the growth potential of children with CKD. Nutritional requirements are based on the recommended dietary allowances for energy and protein. Nutritional supplements, which may be given orally or through a nasogastric or gastrostomy tube, are necessary when recommended dietary allowances for energy and protein are not achieved by food intake alone or when there is impaired linear growth and/or weight gain, particularly during the first few years of life. The dietary reference intake (DRI) for phosphorus for the different age groups is based on the report from the Food and Nutrition Board (183). The average phosphorus intake of children in the United States is higher than the DRI and is approximately 1,500–2,000 mg/day, of which 60–70% is absorbed. Phosphorus is present in nearly all foods and with highest concentration in meat and dairy products. Dietary phosphorus intake is frequently restricted to 600–1,200 mg/day when serum phosphorus levels exceed age appropriate levels (73). Similar restrictions are recommended for patients with serum PTH levels that are higher than the target range according to the stage of CKD. In dialysis patients, phosphorus removal by peritoneal dialysis (240–440 mg/day) or hemodialysis (600 mg during a 4 h session) is inadequate to maintain target phosphorus levels (184); therefore, dietary phosphorus restriction is usually required. More frequent hemodialysis, however, such as nocturnal hemodialysis, which is performed 6–7 nights per week for 8–10 h during sleep at home, has been shown to remove twice as much phosphate per week than conventional hemodialysis (185). As a result, patients undergoing nocturnal hemodialysis do not require phosphate binders, are able to ingest higher dietary phosphate and protein intake, and may even require the addition of phosphorus to the dialysate (185–187).

Dietary phosphorus restriction is recommended in the majority of patients with more advanced CKD; however, protein requirements for growth and the unpalatable taste of low phosphorous diets make long-term compliance with such restrictions difficult. Accordingly, the use of phosphate-binding agents is integral to the treatment of these children. Since calcitriol enhances intestinal phosphorus absorption, vitamin D sterol therapy may complicate the management of hyperphosphatemia and higher dosages of phosphate binding agents are often required (188).

Serum phosphorus levels should be measured at least every 3 months after starting the phosphorus-restricted diet to prevent hypophosphatemia, particularly in those who are also given large doses of phosphate-binding agents. Infants are particularly at risk for hypophosphatemia due to the use of low phosphorus-containing formulas, aggressive use of phosphate binders, increased peritoneal dialysis phosphate removal (due to a higher peritoneal surface area to body area ratio than in older children) (189). Patients with severe and persistent hypophosphatemia have been reported to develop bone disease such as osteomalacia and rickets, proximal myopathy, rhabdomyolysis, and congestive heart failure (189, 190).

In order to attain peak bone mass and reduce risks of osteoporosis and fractures later in life, the DRI of calcium intake and regular exercise are strongly recommended throughout childhood and adolescence, even in patients with CKD. The main source of calcium is milk and dairy products, although various foods such orange juice and cereals are fortified with calcium. Calcium supplementation is recommended when the DRI is not met by dietary intake. To limit the development of cardiovascular calcifications, however, calcium intake from both diet and calcium containing phosphate binders should be limited to no more 2,500 mg/day in children with CKD stages 3 through 5 (73).

Phosphate-Binding Agents

Phosphate-binding agents are recommended in patients with persistent hyperphosphatemia despite dietary phosphorus restriction. These agents form poorly soluble complexes with phosphorus in the intestinal tract, thereby decreasing phosphorus absorption. Calcium-containing phosphate binders are widely used as the initial agent for the management of hyperphosphatemia. They also provide an additional source of calcium. Several calcium-containing salts, including calcium carbonate, calcium acetate, and calcium citrate, are commercially available. Since citrate increases intestinal absorption of aluminum, calcium citrate should not be administered to patients with CKD who are also receiving aluminum-containing phosphate binders (191).

Calcium carbonate is the most widely prescribed calcium salt, and its effectiveness in reducing serum phosphorus levels has been reported in adult and pediatric patients (85, 192). Calcium carbonate therapy has also been shown to decrease serum intact PTH levels in adult

patients with secondary hyperparathyroidism (193–195). The dose of calcium-based phosphate binders varies widely among children and should be adjusted to maintain normal calcium levels and age-appropriate serum phosphorus levels. Hypercalcemia is a major complication associated with the long-term use of high doses of calcium-based phosphate-binding agents, particularly in patients treated with vitamin D or those with adynamic bone lesions (196, 197). Hypercalcemia usually resolves with lowering doses of vitamin D and calcium-based binders. Furthermore, bedtime administration of vitamin D may decrease intestinal calcium absorption and limit hypercalcemia.

The use of calcium-containing binders has been linked to the development of vascular calcifications in adult and pediatric patients treated with maintenance dialysis (138) as well as in adults with stage 4 CKD (198). Furthermore, there is substantial evidence that abnormalities in mineral metabolism, hypercalcemia, hyperphosphatemia, and elevated calcium-phosphorus ion product are associated with the development of soft tissue and vascular calcifications (136, 138, 140). Although it is difficult to differentiate between the effects of calcium intake and vitamin D sterols on the development of hypercalcemia, intake of calcium-containing phosphate binders may play a role in the development of accelerated cardiovascular disease that occurs in patients treated with dialysis (136, 138).

Alternative phosphate binders have therefore been developed to limit the risks of hypercalcemia and vascular calcification associated with the use of calcium salts. When used with active vitamin D sterols, sevelamer hydrochloride (Renagel^{R}), a calcium- and aluminum-free hydrogel of cross-linked poly(allylamine-hydrochloride), effectively lowers serum phosphorus, PTH, and bone formation rate without inducing hypercalcemia in pediatric patients with ESRD (105, 199–203). Furthermore, treatment with sevelamer hydrochloride, when compared to calcium-containing binders, halts the progression of vascular calcification and reduces mortality in adult patients with CKD stages 4 and 5 (160, 161). Serum total cholesterol and low-density lipoprotein cholesterol levels also decrease, whereas high-density lipoprotein increase, during sevelamer treatment (202). These effects may offer additional benefits in reducing cardiovascular complications in patients with ESRD. Sevelamer carbonate (Renvela^{R}) has recently been introduced and is also an effective phosphate-binder. The carbonate in its composition may decrease the incidence of mild acidosis that sometimes accompanies the use of sevelamer hydrochloride (204).

Additional phosphate binders include magnesium, iron, and lanthanum compounds. Magnesium carbonate reduces serum phosphorus levels, but predisposes dialysis patients to developing hypermagnesemia and diarrhea. Iron compounds, such as stabilized polynuclear iron hydroxide and ferric polymaltose complex, are also effective in short-term studies in adults with CKD (205). Lanthanum carbonate, another new agent, also is an effective phosphate binder that does not induce changes in serum calcium levels (206); its role on the process of vascular calcification has not been defined. Lanthanum is a heavy metal that accumulates in liver and bone in rats with renal failure (207). In dialysis patients, lanthanum has been shown to persist in bone as long as 2 years after discontinuation of the drug (208). Due to the accumulation of lanthanum in tissues, this medication is not recommended for routine use in children.

Aluminum-containing gels are also effective phosphate binding agents. Due to their toxicities (encephalopathy and bone disease), the lowest possible dose should be used for a limited period (4–6 weeks), and plasma aluminum levels should be followed closely. Since citrate enhances aluminum absorption by altering tight junctions in the intestinal epithelium, simultaneous use of citrate (found in such medications as calcium citrate, Alka-Seltzer, Shohl's solution, and sodium citrate (Bicitra/Polycitra), as well as in citrus fruits) with aluminum should be avoided (191). Current guidelines for maximum safe dosages of aluminum hydroxide are: 30 mg/kg/day for children and 2,000–3,000 mg/day for adults (209, 210). Despite the recommended dosages, aluminum accumulation still occurs, as seen by a rise in plasma aluminum level after deforoxamine (DFO) infusion and by histologic evidence of aluminum deposits in the bone (211).

Vitamin D Therapy

Despite control of serum phosphorus levels, secondary hyperparathyroidism is present in the majority of pediatric patients treated with maintenance dialysis; vitamin D sterols are therefore used to control serum PTH levels. Vitamin D therapy inhibits PTH release by two mechanisms: directly, by inhibiting pre-pro-PTH gene transcription, and indirectly, by increasing intestinal calcium absorption and increasing serum calcium levels. In patients with CKD stages 2–4 whose PTH levels are greater than the recommended target range, 25(OH) vitamin D levels should be measured and, if less than 30 ng/ml, should repleted with cholecalciferol or ergocalciferol (73).

Active vitamin D sterol therapy is indicated when PTH levels are above the target range despite repletion of 25 (OH)vitamin D stores (73). It is important to ensure that serum phosphorus levels are within the normal range for age before starting vitamin D sterol therapy. Calcitriol or alfacalcidol are started at a daily dose of 0.25–0.5 μg, and the dose is gradually titrated in 0.25–0.5 μg increments to achieve 1st PTH-IMA levels within the normal range for stage of CKD. In patients with CKD stage 5 or those requiring regular dialysis, vitamin D therapy is recommended when 1st PTH-IMA levels exceed 300 pg/ml and serum phosphorus concentrations are at age-appropriate levels (73). Similar to patients with moderate CKD, daily calcitriol or alfacalcidol therapy may be initiated at a dose of 0.25–0.5 μg, with gradual dosage increases of 0.25–0.5 μg in order to achieve 1st PTH-IMA levels between 200 and 300 pg/ml. Paricalcitol and doxercalciferol therapy is typically initiated at 2.5–5 μg and titrated upwards in 2.5 μg increments. Serum levels of calcium, phosphorus, and 1st PTH-IMA should be monitored at regular intervals after start of therapy (73). In all dialyzed children, PTH levels below 200 pg/ml, have been associated with an increased risk for adynamic bone and subsequent growth retardation (88).

Intermittent (thrice weekly) doses of vitamin D sterols may be considered when serum PTH levels are greater than 500 or 600 pg/ml. Intravenous vitamin D sterols given in a thrice weekly dosing regimen are also effective at reducing serum PTH levels in children treated with maintenance dialysis; starting doses depend on serum PTH levels and, for calcitriol, are typically between 0.5 and 1.5 mcg/kg. Paricalcitol is also effective at starting doses of 0.04–0.08 μg/kg (212, 213). Vitamin D should be titrated to maintain serum calcium levels less than 10.2 mg/dl, serum phosphorus levels within the age-appropriate range, and PTH levels between 300–400 pg/ml, since PTH levels in this range have been associated with normal rates of bone formation during intermittent vitamin D sterol therapy (105).

Hypercalcemia and hyperphosphatemia are undesired consequences of active vitamin D therapy. Hypercalcemia is a particular problem when active D sterols are administered in conjunction with calcium-based phosphate binders and have been associated with the progression of vascular calcification in the CKD population (160, 161), calling into question the safety of this form of therapy. Thus, newer active vitamin D sterols, with lower calcemic activity, have been developed. In the United States, 19-nor-1,25-$(OH)_2D_2$ (paricalcitol) and $1\alpha(OH)D_2$ (doxercalciferol) have been introduced, while 22-oxa-1,25 $(OH)_2D_3$ (maxacalcitol) is used in Japan. These newer sterols offer the potential for reduced episodes of hypercalcemia (214, 215), though their superiority over calcitriol has not been consistently demonstrated in controlled trials in humans (105). When active vitamin D sterols are used with sevelamer, the skeletal and biochemical features of secondary hyperparathyroidism are markedly improved without inducing changes in serum calcium levels. Thus, the use of calcium-free phosphate binders may enhance the margin of safety during therapy with active vitamin D sterols.

Despite its calcemic properties, current evidence suggests that active vitamin D sterol therapy may in fact confer a survival benefit to patients treated with maintenance dialysis. Cross sectional data from several large dialysis databases suggest that the administration of active vitamin D sterols confers a survival benefit over no therapy with active vitamin D regardless of serum calcium, phosphorus and PTH levels (22, 216, 217). Furthermore, it appears that there may be differences in survival benefits of some newer active vitamin D sterols (paricalcitol and doxercalciferol) over calcitriol in patients treated with maintenance dialysis (23, 217). Although prospective trials remain to be performed and the mechanism involved remain to be elucidated, these results are intriguing. The systemic effects of vitamin D on the immune and cardiovascular systems may contribute to these survival benefits.

A role for vitamin D has long been implicated in organ systems other than the bone/mineral axis; indeed, a growing body of evidence suggests health benefits for both active vitamin D sterols and native vitamin D in the general population. In addition to the parathyroid gland and bone, arterial smooth muscle cells, pancreatic islet cells, macrophages, T and B cells, hepatocytes, lung alveolar cells, dermal keratinocytes, and renal parenchymal cells have been shown to respond to vitamin D (218). Many of the effects on these organ systems appear to be due to the immune-regulatory role of vitamin D. Evidence from as far back as the nineteenth Century has implicated 25(OH)D in immune regulation. Sanitoriums located at high elevations, and thus with increased UV light to facilitate dermal vitamin D production, were central to the treatment of infectious diseases, including tuberculosis (26). Recent investigations suggest that 25(OH)D has a direct effect on macrophage function that cannot be reproduced by active $1,25(OH)_2D$ administration. Indeed, macrophages have their own 1α hydroxylase and require sufficient ambient levels of 25 (OH)D substrate in order to internal $1,25(OH)_2D$ (219). Striking evidence of macrophage 1α hydroxylase activity is found in granulomatous conditions such as tuberculosis,

sarcoidosis, and inflammatory bowel disease, in which $1,25(OH)_2D$ levels may be markedly elevated (220). Serum from patients who are vitamin D deficient induce a lower bactericidal response than serum from vitamin D replete individuals, suggesting decreased macrophage function under conditions of vitamin D deficiency (219).

Not only may the immune system require sufficient vitamin D in order to fight foreign antigens but suboptimal vitamin D levels may lead to dysregulation of the immune system and autoimmunity. Indeed, type I diabetes, an autoimmune disorder, has been associated with vitamin D deficiency (221) and a reduced incidence in type I diabetes associated with vitamin D repletion in pregnant women (222). Moreover, immune-surveillance of tumor activity may be a vitamin D dependent process. Calcitriol has demonstrated regulatory active of over 200 genes and is thus thought to be responsible for maintaining cellular health and providing antitumor activity (26, 223) in many cell types, including skin, prostate, breast, colon, lung, brain, and placenta that express 1α hydroxylase suggesting that therapy with 25(OH)D itself may be required to generate sufficient internal calcitriol levels for proper immune surveillance and control of cell proliferation.

Aside from immune regulation, active vitamin D therapy has been associated with protective effects on both the kidney and the heart. Administration of active vitamin D sterols results in less proteinuria, decreased fibrosis, and a decrease in podocyte hypertrophy in sub-totally nephrectomized rats (224). Moreover, paricalcitol treatment of CKD patients decreases proteinuria (225). Studies in rats have also demonstrated a beneficial effect of active vitamin D sterols on cardiac hypertrophy (20). Furthermore, the constituative left ventricular hypertrophy found in VDR knockout animals are successfully treated with captopril (226). These effects may be mediated by suppression of the renin-angiotensin system (RAS); indeed *in vitro* studies have demonstrated that calcitriol, paricalcitol, and doxercalciferol all suppress the RAS to a similar degree (227).

The systemic benefits of 25(OH)D in patients with CKD are less well established, but current intriguing observations suggest a role for the native vitamin in improving survival in patients treated with maintenance dialysis (216). Recent studies have demonstrated that supplementation with ergocalciferol in patients with all stages of CKD increased both 25(OH)D levels and $1,25(OH)_2D$ levels and suppress PTH levels (41, 49, 228). Furthermore, dialysis patients require lower doses of epogen and active vitamin D sterols during ergocalciferol supplementation (41). However, the long term effect of this form of therapy in patients with CKD remains to be carefully evaluated as well as the effects of combined therapy with active vitamin D sterol administration, on target organs remain to be more completely defined.

Calcimimetic Agents

Cinacalcet, an allosteric activator of the calcium sensing receptor, is now available for the treatment of hyperparathyroidism. This small organic molecule reduces serum PTH levels and has also been shown to decrease the calcium-phosphorous ion product in adult patients treated with maintenance dialysis (69, 229). Experiments in uremic rats have demonstrated that calcimimetics are able to halt the progression of parathyroid cell hyperplasia (69); the antiproliferative effect of this agent shows promise for use of the molecule as a "medical parathyroidectomy". Studies in animals also suggest that the use of calcimimetic agents may play a role in reversing the process of vascular calcification (230); however such effects need to be further evaluated in humans. Due to the presence of the calcium sensing receptor on the growth plate, these agents are not approved and should be used with caution in growing children.

Growth Hormone Therapy

Recombinant growth hormone (rhGH) should be considered in children with growth failure that does not respond to optimization of nutrition, correction of acidosis, and control of renal osteodystrophy. Serum phosphorus and PTH levels should be well controlled prior to the initiation of rhGH in children with CKD. Serum phosphorus levels should be less than 1.5 times the upper limit for age and 1st PTH-IMA levels less than 1.5 times the upper target values for the CKD stage prior to rhGH therapy (73). Growth hormone therapy will increase serum PTH levels during the initial months of therapy; therefore, serum PTH levels should be monitored monthly. GH therapy should be temporarily discontinued if PTH levels exceed three times the upper target value for the CKD stage (73).

Parathyroidectomy

Parathyroid gland hyperplasia, which is characterized by serum PTH elevations and osteitis fibrosa on

bone biopsy, is a result of poorly controlled secondary hyperparathyroidism. Calcitriol resistance may develop in the presence of an enlarged nodular hyperplastic parathyroid gland because of the decrease in the number of VDRs and the presence of monoclonal proliferation in nodular areas (231, 232). When vitamin D therapy fails to correct the signs of secondary hyperparathyroidism, parathyroidectomy must be considered. Urgent indications for parathyroidectomy include persistent or recurrent hypercalcemia (particularly when associated with intractable pruritus not responding to intensive dialysis), progressive extraskeletal calcifications, bone pain, multiple and recurrent fractures, and the appearance of calciphylaxis (136). However, prior to parathyroidectomy, care must be taken to ensure that these symptoms are attributable to secondary hyperparathyroidism and high turnover renal osteodystrophy, since all may also be associated with adynamic bone disease (233). Before the 1980s, the development of hypercalcemia in patients receiving little or no vitamin D suggested the presence of aluminum-related bone disease. Currently, hypercalcemia is more commonly due to the development of adynamic bone disease from the administration of high doses of vitamin D and calcium containing compounds (75, 88, 89).

Hypocalcemia may develop after parathyroidectomy in patients with severe secondary hyperparathyroidism. This condition, called "hungry bone syndrome", is caused by a high rate of skeletal calcium uptake, which may continue for some time after serum PTH levels are lowered by parathyroidectomy. Serum calcium decreases 24–36 h after surgery; daily doses of calcitriol at 0.5–1.0 g/day for 2–6 days before surgery can be administered to prevent hypocalcemic episodes. Other forms of active vitamin D sterols are also effective and may be used. Calcium gluconate infusion should be started immediately after parathyroidectomy to provide 100–150 mg of elemental calcium per hour during the 1st 4–6 h. The infusion rate should be adjusted thereafter according to serum calcium measurements. Daily doses of calcitriol or other active vitamin D sterols should be continued in the post-operative period and may be increased to several micrograms per day in order to maintain serum calcium levels. Intravenous administration of these compounds is generally preferred in the immediate post-operative period due to its higher bioavailability better tolerability. Hypophosphatemia may also develop post-operatively, but supplemental phosphorus may aggravate hypocalcemia and should be avoided unless serum levels of phosphorus are below 2.0 mg/dl.

Bone Disease After Successful Kidney Transplantation

Successful kidney transplantation corrects many of the metabolic abnormalities associated with the development of renal osteodystrophy. Despite a well-functioning graft, however, osteopenia, growth failure, spontaneous fractures, and avascular necrosis remain prevalent in adult and pediatric kidney recipients (234–239). Significant bone loss has been shown to occur as early as 3–6 months after kidney transplantation (238, 239). Several factors are implicated in the development of bone disease after transplantation, including persistent alterations in mineral metabolism, prolonged immobilization, and the use of immunosuppressive agents required to maintain graft function.

In both adult and pediatric kidney recipients, bone histologic changes associated with secondary hyperparathyroidism have been shown to resolve within 6 months after kidney transplantation (239). However, some patients have persistently elevated rates of bone turnover, while others develop adynamic lesions, despite moderately elevated serum PTH levels (240). Bone biopsy data from pediatric kidney recipients indicate that 67% of patients with stable graft function have features of normal bone formation, while 10% have adynamic bone lesion, and 23% have bone lesions characteristic of secondary hyperparathyroidism (241). Bone resorption is typically increased (242), leading to a net loss of bone mass over time. Serum PTH levels are unable to discriminate between adynamic, normal, and increased bone turnover in the pediatric transplant population (241). The use of maintenance corticosteroids have been implicated in these alterations; steroids decrease intestinal calcium absorption, enhance urinary calcium excretion, inhibit osteoblastic activity, decrease bone formation, and increase osteoclastic activity and bone resorption (243–246). Likewise, cyclosporine has been reported to increase both bone formation and bone resorption and reduce cancellous bone volume in the rat (247, 248). By contrast, azathioprine has been shown to have minimal impact on skeletal remodeling (249). The role of other immunosuppressive agents, such as mycophenolate mofetil, as potential modifiers of bone formation and bone resorption has not been evaluated.

While bone turnover may return to normal, defective skeletal mineralization is present in the majority of pediatric transplant recipients (241). Osteoid volume is increased while mineral apposition rate is markedly

reduced (241). Although the factors responsible for the persistent increase in osteoid formation remain to be fully explained, corticosteroid use may contribute, as may persistent imbalances in PTH, vitamin D, and FGF-23 metabolism (250).

After successful kidney transplantation with standard immunosuppressive regimens (daily corticosteroids, calcineurin inhibitor, and anti-metabolite), growth may be accelerated by an improvement in kidney function, but catch-up growth may not be observed even in children who undergo transplantation very early in life (108). Moreover, height deceleration occurs in approximately 75% of patients who undergo transplantation before the age of 15 years (251). The etiology of persistent growth retardation is not completely understood, but immunosuppressive agents, persistent secondary hyperparathyroidism, altered vitamin D and FGF-23 metabolism, and the persistence of defective skeletal mineralization may all contribute. Children receiving steroid-free immunosuppressive regimens, those treated with alternate day steroids and those with better height SDS at the time of transplant attain the greatest final adult height (108, 251–253). Recombinant human growth hormone has been used in children with significant height deficit after kidney transplantation. A substantial increase in linear growth occurs within the 1st year of rhGH therapy, but the magnitude of growth response may decline thereafter (254).

Cardiovascular disease continues to be prevalent after renal transplantation. In the post-transplant period, the presence of hypertension is strongly linked to increased IMT and poor vessel distensibility in children (255). EBCT data also indicate that vascular calcifications, present in young adults on dialysis, do not regress post-transplantation and may contribute to the burden of cardiovascular disease in this population (256).

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72 Peritoneal Dialysis

Enrico Verrina

Introduction

Peritoneal dialysis (PD) represents a well-established dialysis modality for pediatric patients with end-stage renal disease (ESRD). Chronic peritoneal dialysis (CPD) currently is the dialysis treatment modality most commonly prescribed for children and adolescents throughout much of the world, and the preference for CPD over hemodialysis is most pronounced among infants and young children 0–5 years of age (1–3). PD has evolved substantially during the past three decades (4).

Since the subject of PD is extremely wide, and textbooks have been published on its use in adult (5) as well as in pediatric patients (6, 7), an effort has been made in this chapter to summarize accurately current knowledge specific to this field. However, readers are encouraged to consult references cited throughout the chapter for more details on specific topics. In addition, issues such as anemia, growth, and osteodystrophy, which are pertinent to all pediatric patients, are addressed by other chapters of this book.

Brief History of the Development of Pediatric Peritoneal Dialysis

Experience with PD in children has been reported for the first time in 1948 (8), when the worldwide reported clinical experience with PD was less than 100 patients; the technique consisted of a *continuous peritoneal lavage* with large volumes of warmed dialysate. At that time, widespread use of PD was still hindered by its practical requirements: keeping sterile water in 40-L drums and preparing and sterilizing dialysate. During the 1950s, the development of disposable nylon catheters and commercially prepared dialysis solutions made PD a practical short-term treatment for ARF, known as *acute intermittent PD (IPD)*. Successful adaptation of the technique for use in infants and children with ARF was first reported in 1961 (9). However, early acute IPD techniques required reinsertion of the catheter for each treatment, making prolonged use in small patients impractical and hazardous because of frequent infections. This all changed in the 1960s, when Henry Tenckhoff developed a "permanent," indwelling peritoneal catheter (10). The "Tenckhoff catheter," combined with a close loop reverse osmosis automated delivery system ("cycler") for purifying dialysate, that freed patients from their dependence on the huge drums of water and could be used at home (11), made *chronic IPD* an accessible form of renal replacement therapy (RRT) for pediatric ESRD patients. A new era in the history of PD started with the introduction of a "novel portable/wearable equilibrium dialysis technique," called *continuous ambulatory PD* (CAPD) (12). The glass bottle were replaced by plastic bags containing 2 L of dialysate which were folded and fixed on the abdomen until the next exchange; the dwell time was extended to 4–8 h and four exchanges of the dialysate volume per day usually proved to be adequate (13). In children CAPD was first applied in Toronto, Canada in 1978 (14), and pediatric nephrologists were quick to recognize the potential advantages that CAPD offered to their young patients. Importantly, CAPD made possible the routine treatment of very young infants with ESRD, thereby extending the option of RRT to an entire patient population previously considered too young to be suitable for chronic treatment.

At the beginning of the 1980s, there was a rediscovery of IPD techniques, which were now performed with the help of new automatic machines to assist the delivery and drainage of dialysate. *Automated PD* (APD) is a broad term that is used to refer to all forms of PD employing a cycler. The various forms of APD include *intermittent PD* (IPD), *continuous cycling PD* (CCPD), *nightly intermittent PD* (NIPD) and *tidal PD* (TPD). The evolution of these modalities has been closely linked with the development of new ad hoc cyclers, incorporating microchips and computer technology, and with recent advances in prescription and monitoring of PD treatment. The benefits of the APD technique results from the fact that it can deliver different dialysis regimens, thus meeting various dialysis needs and increasing treatment efficacy; moreover, it provides children and parents with more daytime freedom (15, 16).

CAPD has the undoubted advantage of ease of use and limited cost of equipment, while there can be limitations to the use of APD in many developing countries owing

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to cost constraints and technical problems (reliability of electricity supply, for instance). However, a recent report from Mexico showed how the organizational effort to provide the desired PD treatment to a large pediatric patient population can be successfully performed (17).

The Peritoneal Dialysis System

PD System Components

The PD system has three major components: peritoneal microcirculation, peritoneal membrane (PM), and dialysis fluid (18).

Peritoneal microcirculation: Peritoneal capillary blood flow has been reported to vary between 50 and 100 ml/min; however, the effective amount of this flow that is involved in peritoneal exchanges is unknown, and is subject of controversy. The peritoneum has an active lymphatic system, which is comprised of specialized structures located on the undersurface of the diaphragm (the lacunae).

Peritoneal membrane: PM lines the inner surface of the abdominal and pelvic walls (parietal peritoneum), covers the intraperitoneal organs, forms the visceral mesentery and omentum, and connects loops of bowel (visceral peritoneum) (19). PM represents the barrier that solutes and water have to cross, and is composed of:

1. Capillary wall; peritoneal capillaries are mainly of the continuous type, with less than 2% of fenestrated capillaries (20); endothelial cells are linked to each other by tight junctions and surrounded by a basement membrane; healthy endothelium plays a central role in the control of vascular permeability (21).
2. Interstitium, which is made of extracellular matrix, containing a limited number of cells (fibroblasts, mononuclear cells) and some lymphatic vessels; hyaluronan, a major component of the extracellular matrix, was reported to be an important determinant of the resistance to fluid and solute transport (22).
3. A layer of mesothelial cells, which have a system of tight and gap junctions, microvillous projections at the free surface and several organelles in their cytoplasm; mesothelial cells were reported to participate in glucose transport and regulation of water and solute fluxes through tight junction modulation, but their actual role as rate-limiting barrier is still debated (23, 24).

Dialysis fluid compartment: This compartment includes the composition of the solution and the modalities of delivery. Dialysis fluid is infused in the peritoneal cavity in an amount that is scaled on patient's body size and clinical conditions; standard dialysis solutions contain an osmotic agent to produce the osmotic gradient required to obtain ultrafiltration (UF), a buffer to correct patient's metabolic acidosis, calcium, magnesium and electrolytes.

Driving Forces of Solute and Water Exchange

The driving forces of solute and water exchange across the peritoneal membrane, between the dialysis solution and the capillary blood and surrounding tissues (24) are represented by diffusive transport, ultrafiltration and convective mass transfer.

Diffusive transport: Diffusion consists in a solute exchange between two solutions (blood and dialysis fluid) separated by a semipermeable membrane (peritoneal membrane). Main factors affecting the rate of solute diffusion are represented by:

1. Concentration gradient between blood and dialysate; since blood flow through the peritoneal membrane is relatively stable, concentration gradient is best maintained by changing the dialysate in the abdomen as often as is feasible.
2. Molecular weight (MW); since diffusion is a size-selective process, small molecules (urea, creatinine) diffuse more rapidly than larger molecules (vitamin B_{12}, "middle molecules").
3. Effective surface area and permeability of peritoneal membrane. Indeed, it is the functional and not the anatomic peritoneal surface area that is important in peritoneal exchange. The vascular surface area available for dialytic exchange can be determined using the so-called three-pore permeability model (25). According to this model, the peritoneum is characterized as a heteroporous three-pore membrane with few ($\sim$ 1–2%) water-exclusive ultra-small pores (aquaporins) (radius 2–4 Å), a small percentage ($\sim$ 5%) of large pores (radius 200–300 Å) and a majority ($\sim$ 90–95%) of small pores (radius 40–60 Å). Small hydrophilic solute transport occurs primarily by diffusion across the small pores (although convection also plays a certain role), while the movement of proteins and other macromolecules occurs across the large pores and is driven by hydrostatic forces. Fluid transport can occur across all the three pathways and is determined by crystalloid and colloid osmotic pressures. Total membrane pore area that is engaged in exchanges can be influenced by fill volume, patient posture, and PD fluid composition (26, 27). The impact of dialysate volume is felt to rest on the principle of

geometry of diffusion (28), which simply states that the larger the dialysate volume, the longer the transperitoneal concentration gradient will persist to drive diffusion. The permeability of the tissue between the capillary lumen and the peritoneal space can be altered by disease: it increases during acute peritonitis, while can be progressively impaired by peritoneal fibrosis. The concentration gradient and hence diffusive transport are also impacted by the presence of residual peritoneal volume from previous exchanges. Small solutes in the residual fluid will likely have equilibrated with serum; this will lead to a time "zero" solute concentration that is much greater than zero, despite the fact that the instilled dialysate concentration of a solute was zero. This will impact fluid flux and solute transport. Residual volume can be substantial and of clinical relevance in children (29).

Ultrafiltration (UF): UF consists in the bulk movement of water along with permeable solutes across the peritoneal membrane; in PD the driving force for UF is primarily represented by the osmotic pressure of dialysis fluid that is exerted by glucose or other osmotic agents; the effects of hydrostatic pressure gradient are usually of minor importance in PD unless exceedingly high levels of intraperitoneal pressure (IPP) are reached (30). Other factors that can affect UF are membrane surface area and hydraulic permeability. The flux of water (J_F) across the membrane can be expressed by the following equation (31):

$$J_F = K_f\left([P_c + s_f] - [p_c + P_f]\right)$$

where: K_f = peritoneal membrane permeability coefficient; P_c = hydraulic pressure in the capillary; s_f = osmotic pressure of the peritoneal fluid; p_c = oncotic pressure in the capillary; P_f = hydraulic pressure of the fluid under flux.

Net UF results from the balance between osmotic UF and peritoneal fluid absorption, which can play a role in some patients in whom net UF is reduced, as well as in the absorption of a significant amount of macromolecules. Lymphatic absorption was estimated to account for 20% of fluid absorption (32), which is believed to move primarily into interstices in the peritoneal cavity and to be driven by intraperitoneal hydraulic pressure (33). The limited data on lymphatic absorption in children are conflicting (32, 34).

Fluid absorption rate can be determined when a PD exchange is modeled using the three-pore model. In one pediatric study, the absorption rate increased with body size in absolute terms but decreased when normalized to body size. The decrease was slight when scaled to body surface area (BSA), but marked when scaled to body weight (BW) (35).

Convective mass transfer: As water moves from capillaries to peritoneal cavity on a pressure gradient, the dissolved molecules are dragged along (solvent drag). The convective transport of a solute depends on the amount of UF and on membrane permeability, that can be expressed by the sieving coefficient and calculated by dividing the concentration of solute in ultrafiltrate by its concentration in plasma water (in the absence of a concentration gradient). During PD exchanges, the contribution of convection to solute removal is limited for small molecules, but significant for large molecules, such as proteins.

Relationship Between Peritoneal Surface Area and Dialysate Fill Volume

Whereas peritoneal membrane surface area per unit (Kg) of BW is twice as large in infants than in adults, the relationship between BSA (m^2) and peritoneal membrane surface area is constant and age-independent (36). Scaling IPV by BSA maintains the ratio of dwell volume to peritoneal surface area constant across patient age groups, thus avoiding the false perception of peritoneal hyperpermeability in infants and small children while performing peritoneal function tests (36), and has become a standard in pediatric PD prescription (37, 38). BSA can be calculated from weight and height by use of the Gehan and George formula (39):

$$\text{BSA}\ (m^2) = 0.0235 \times (\text{height},\ \text{cm})^{0.42246} \times (\text{weight},\ \text{kg})^{0.51456}.$$

Both IPV and patient posture are factors that can dynamically affect the recruitment of effective peritoneal membrane area available for dialytic exchange, which corresponds to the unrestricted pore area over diffusion distance ($A_0/\Delta X$) as determined using the three-pore model (25, 26). The peritoneal vascular surface area is maximized as IPV is raised from 800 to 1,400 ml/m^2 BSA (26). An excessive IPV may cause patients discomfort, as well as a series of complications, such as pain, dyspnea, hydrothorax, hernia, and loss of UF due to an increased lymphatic drainage. Measurement of the hydrostatic intraperitoneal pressure (IPP) can help to determine fill volume tolerance of the individual patient (30). Maximum tolerable IPV was defined as the volume causing an IPP of 18 cm H_2O in the supine position; at and above this limit, abdominal pain and/or a decrease in respiratory

vital capacity may occur (40, 41). While IPP is influenced by factors such as gender and ponderosity, and gradually adapts to a given IPV, the level of IPP in general is a reproducible patient-characteristic parameter (42).

Methods to Assess Peritoneal Membrane Transport Characteristics and Function

Peritoneal solute and fluid transport may vary considerably from patient to patient and in the same patient during different phases of CPD treatment, as a consequence of the recurrence and/or severity of peritonitis episodes, or of the exposure of PM to CPD solutions and materials. Therefore, PM transport characteristics should be assessed at the beginning of CPD and then monitored every 6–12 months, and in case of recurrent or particularly severe peritonitis episodes, or of any other clinical event that may have altered transport capacity, thus requiring a re-evaluation of patient's PD prescription (38, 43).

The application of PM function tests to the pediatric patient population has long been hampered by a lack of standardization of dialysis mechanics during the test (44). Scaling the exchange volume by BSA maintains the relationship between dialysate volume and PM surface area across populations, and makes comparison of peritoneal transport properties between patients of different body sizes possible (36, 45). An exchange volume of 1,100 ml/m^2 BSA approximates the standard 2,000 ml/1.73 m^2 volume applied to adult patients.

Mass transfer area coefficient (MTAC): This parameter is an expression of the diffusive permeability of the peritoneal membrane, and describes the maximal clearance theoretically achievable at a constantly maximal gradient for diffusion (i.e., when dialysate solute concentration is zero), and is independent of dialysate osmotic agent concentration. Calculation of MTAC requires rigorously performed PD exchanges and solutions of complex equations. In pediatric patients, Warady et al., by using a standardized exchange volume of 1,100 ml/m^2 BSA, found non-linear decrements of the MTAC with age for creatinine, glucose and potassium, indicative of a greater transport capacity in children younger than 3 years, as a consequence of higher peritoneal permeability, or larger effective surface area of the peritoneal membrane (29). In a study based on the three-pore model, MTAC values for children, when scaled for BSA, were similar to those of adults (46).

Peritoneal equilibration test (PET): PET is the most commonly employed means of characterizing PM transport capacity in adult as well as in pediatric patients (29, 47–49). This test measures the rate at which solutes, usually creatinine (Cr), urea and glucose, come to equilibration between the blood and the dialysate. In order to reach a satisfactory level of reproducibility of results, standardized PET procedure in children should include the use of a 2.5% dextrose PD solution with a dwell volume of 1,100 ml/m^2 BSA (29). A 4.25% dextrose solution can be used to obtain a more accurate assessment of UF and of sodium sieving (50). Dialysate to plasma (D/P) ratio of Cr, and dialysate glucose concentration to initial dialysate glucose concentration at time 0 (D/D_0) are calculated at 2 and 4 h of the test; a serum sample is obtained at time 2 h. Dialysate Cr concentration must be corrected for the interference of the high glucose levels in the dialysate by the formula:

$$\text{Corrected Cr (mg/dl)} = \text{measured Cr (mg/dl)} - \text{correction factor} \times \text{dialysate glucose (mg/dl)}$$

The correction factor should be determined in the laboratory of each dialysis center, by dividing measured Cr of a fresh PD solution by glucose concentration. In addition, measured plasma solute concentrations must be divided by 0.9 to account for their presence in plasma water only and not in whole plasma.

Urea and Cr D/P ratios, and glucose D/D_0 ratio can be compared to the reference kinetic data developed from the results of a large pediatric study in which the same standard pediatric PET was adopted (29), in order to characterize patients as having a high, high average, low average or low PM solute transport capacity (*Figs. 72-1* and *72-2*). High transport for Cr (and/or urea) implies its fast removal from blood, while high transport for glucose denotes its fast elimination from dialysate, thus dissipating the osmotic gradient required for UF. High transporter status may be associated with poor treatment outcome, and has been identified as a significant risk factor for inadequate weight control, poor statural growth (51), and low-turnover bone disease (52).

Recently, Warady and Jennings reported that the PET results obtained at 2 and 4 h, based on either creatinine or glucose transport in 20 PD pediatric patients, provided identical characterization of peritoneal membrane transport capacity for the same solute (53). Therefore, the Authors proposed the use in children of a simplified, 2-h PET procedure, the so-called short PET, as already described in adults by Twardowski in the original PET publication (47). However, further study with a larger patient cohort is required to confirm the accuracy of the short PET in the characterization of membrane transport capacity in this setting (54).

Figure 72-1

Peritoneal equilibration test results for creatinine. Colored areas represent high, high-average, low-average, and low transport rates. The four categories transport are bordered by the maximal, mean + 1 standard deviation (SD), mean, mean −1 SD, and minimal values for the population. D/P, dialysate:plasma ratio (from (29) with permission).

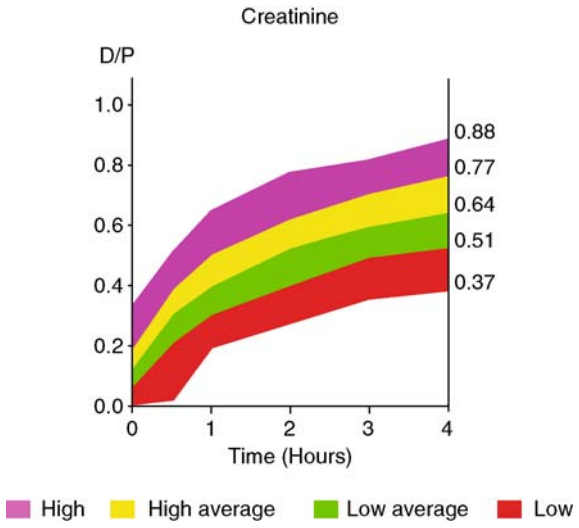
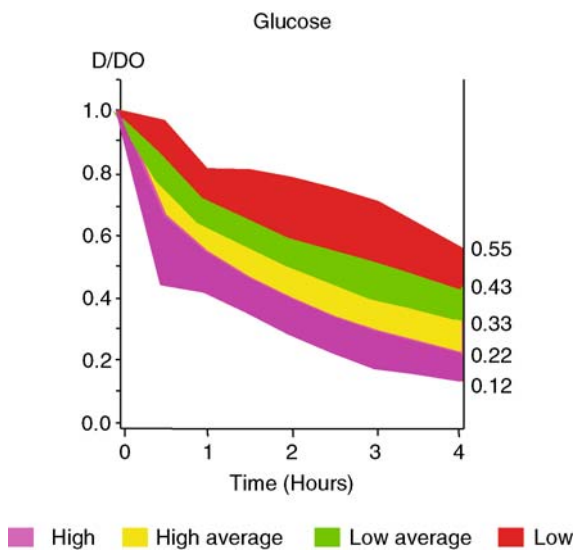


Figure 72-2

Peritoneal equilibration test results for glucose. Colored areas represent high, high-average, low-average, and low transport rates. The four categories transport are bordered by the maximal, mean + 1 standard deviation (SD), mean, mean −1 SD, and minimal values for the population. D/D_0, dialysate glucose: initial dialysate glucose concentration ratio (from (29) with permission).



Standard permeability analysis (SPA): In this test, poly-disperse dextran-70 is added to the PD solution employed to perform a PET in order to obtain the simultaneous measurement of transcapillary ultrafiltration (UF), markers clearance rate, and intraperitoneal volume (IPV). SPA conducted with a test IPV of 1,200 ml/m^2 and a 1.36 or 3.86% glucose PD solution gave comparable results in adult and pediatric patients (46, 55).

Personal dialysis capacity (PDC) test: In this test, which is based on the three-pore model of solute and fluid transport across the peritoneum, the following three parameters are calculated: (1) the effective peritoneal surface area, or unrestricted pore area over diffusion distance ($A_0/\Delta X$), corresponding to the diffusion capacity for solutes; (2) absorption, i.e., the final rate of fluid reabsorption from the abdominal cavity, and (3) the large pore volume flow, that represents the rate of protein-rich fluid passing through the large pores from blood to dialysate (25). PDC protocol includes five exchanges to be performed in 24 h using different dwell times and two glucose solutions for patients on CAPD; a simplified protocol for patients on APD is also available (35). PDC test has been successfully employed in children to model individual peritoneal membrane function (35). In one pediatric study, D/P or D/D_0 ratios derived from PET analysis were used to estimate $A_0/\Delta X$ by using a specific computer program (26).

Peritoneal Dialysis Equipment

Peritoneal Catheters for CPD

A reliable peritoneal catheter is the cornerstone of successful CPD. Careful attention should be paid to:

- A planned approach to PD initiation
- The choice of catheter type
- Technical details of placement procedure, that should be performed by a competent and experienced surgeon or nephrologist (56)
- Optimal postoperative care of the catheter and exit-site by a dedicated nurse team also involved in the training of patient's caretakers (57).

There are multiple types of PD catheters presently available, with a variety of configurations. In general, most long-term PD catheters are constructed of soft material, such as silicon rubber or polyurethrane. The catheters can be thought of as having two separate regions: the intraperitoneal portion and the extraperitoneal portion. The intraperitoneal portion contains holes or slots to

allow passage of peritoneal fluid. The optimal position of the catheter tip is the most dependent portion of the pelvis, a few degrees lateral to the midline. The shape of the intraperitoneal portion is straight or curled, the latter configuration often associated with less patient pain with dialysate inflow and a decreased predisposition to occlusion and omental wrapping of the catheter. The most common catheters have been the straight and curled Tenckhoff catheters. The extraperitoneal portion of these catheters has one or two Dacron cuffs to prevent fluid leaks and bacterial migration and to fix the catheter's position. The first or single cuff is positioned between the posterior and anterior fascia of the rectus sheath; the second cuff should be located within the subcutaneous tunnel, 1.5–2.0 cm from the catheter exit site. The shape of the extraperitoneal portion of the catheter may be straight or have a preformed angle (e.g., swan neck or pail handle) to help create a downward-directed exit site and facilitate drainage (58).

In children, a midline incision through the skin, with a paramedian fascial incision is more effective than a strictly midline approach (56). Infants and young children with vesicostomies, ureterostomies, colonostomies, or other sources of contamination require placement of the catheter exit site as far from the stoma as possible to prevent contamination and infection. Placement of the exit site on the chest wall has successfully limited the number of infections in such high-risk situations in two small series of pediatric patients (59, 60).

Laparoscopic catheter placement has been successfully employed in children (61, 62). This procedure allows careful inspection of the abdominal cavity, and the performance of adhesiolysis and inguinal hernia repair, in addition to guiding catheter placement; it can also be employed to rescue blocked catheters.

The use of intravenous antibiotic prophylaxis at the time of catheter placement has been shown to significantly reduce the risk for peritonitis within the first 4 weeks, but not the risk for exit site and tunnel infection (63, 64). The routine use of mupirocin as part of exit site care has been shown to reduce *Staphylococcus aureus* related exit site infection and peritonitis (58); however, concern regarding the emergence of mupirocin resistant organisms has arisen (65). Since mupirocin is not effective against *Pseudomonas*, which is frequently involved as causative organism in peritonitis and exit site infection among PD children (66, 67), topical application of gentamicin cream to exit site daily has been recently recommended in adult patients (68).

The primary reason for catheter revision is catheter malfunction (69), often caused by omental wrapping of the catheter. Although there are no prospective studies to support routine omentectomy, the majority of pediatric surgeons recommend that a partial omentectomy be performed in conjunction with the initial catherer placement (56, 70). Catheter occlusion by fibrin may also occur but can usually be successfully relieved by the instillation of fibrinolytic agents into the catheter (71, 72).

Catherer tip migration in the peritoneal cavity may occur as a result of too much torque placed on the catheter at the time of surgical placement, since the "memory" of the synthetic materials used to manufacture the catheter makes the catheter itself to assume again its original curvature. Occasionally, tip migration may be due to constipation or excessive bladder distension.

Dialysate leakage more often occurs in the immediate post-operative period, is usually external in nature, and is evident at the catheter exit or incision site. Early leakage may be related to poor catheter implantation technique, and can occur when initial exchange volumes are too large, when the catheter is frequently manipulated (as in too aggressive exit site care), or when traction is placed to it. Therefore, catheter implantation should be timed to allow 2–6 weeks for healing prior to dialysis initiation; if dialysis is required early, small volume exchange in the supine or recumbent position can be performed with frequent check of leakages. During this time, catheter immobilization (without the use of surgical sutures) and weekly exit site dressing changes conducted by trained dialysis personnel using aseptic technique have been recommended (57, 58). Late leakage (>30 days from catheter implantation) usually occurs into the abdominal wall, and can cause genital edema; exploration of the incision site or evaluation for an anatomical defect, resulting in the need for catheter revision, are often required.

Data from the 2007 NAPRTCS report have shown that the time to the initial episode of peritonitis is longer and peritonitis annualized rate is lower in association with catheters characterized by two cuffs, a swan neck tunnel and a downward oriented exit site (69). In the experience of the International Pediatric Peritonitis Registry (IPPR), the use of Tenckhoff catheters with a straight ending was associated with an increased rate of post-peritonitis technique failure (66).

Connection Technology

Over the years, a number of dialysate transfer sets and associated devices have been developed with the aim of reducing the risk of bacterial contamination during either

the catheter-to-transfer set or the transfer set-to-dialysate bag connections, and has contributed to simplify PD connecting maneuvers, thus shortening patient and partner training (73).

Catheter-to-transfer set connector: To prevent cracking or accidental disconnection of old plastic plug-in connectors, industry developed a Luer-lock catheter adapter made of titanium, which was chosen for its light weight and resistance to electrolyte-containing PD solutions, or of more durable plastics.

Transfer set-to-container connection: The original spike-and-port connecting system has been replaced in many systems with a Luer-lock system, resulting in easier insertion and a low chance of accidental dislodgement.

Transfer set: Starting from 1980, the standard straight Oreopoulos System was replaced by the Y set to free the patient from the need to remain attached to the empty bag between exchanges, and to allow a flush-before-fill phase after the connection, thus significantly reducing the incidence of peritonitis episodes due to touch contamination. A further evolution of the Y set was represented by the double bag system, where the Y set is attached to the PD solution bag and to an empty bag, which are connected to an adapter tubing during the exchange and discarded after each use. With this system, the patient has only to wear a small adapter tubing, that is closed with a disinfectant containing cap.

Peritoneal Dialysis Solutions

The composition of standard, commercially available PD solutions has been designed to achieve a satisfactory removal of fluid and waste products, and to maintain acid-base and calcium balance, and electrolyte homeostasis. Clinical concerns about the harmful effects of prolonged exposure of the peritoneal membrane to standard PD solutions with high glucose and lactate concentration, low pH, high osmolarity, and high level of glucose degradation products (GDPs) has led to the development of more biocompatible, second generation PD solutions. In fact, the results of a series of experimental studies support the hypothesis that peritoneal membrane hypervascularization and fibrosis observed during long-term PD are correlated to acute and chronic toxicity of conventional PD fluids. In addition, progressive decline of residual renal function, that is considered a major determinant of PD treatment outcome, can be exacerbated by the metabolic and cardiovascular burden related to glucose load, GDPs accumulation, and oxidative stress (74, 75).

Osmotic Agents

Glucose: Glucose is still the most widely employed osmotic agent in the clinical setting. It removes fluid from the extracellular volume by a mechanism known as crystalloid osmosis, which is exerted through the system of ultrasmall pores (the aquaporins), and can be effectively enhanced by increasing glucose concentration in the PD fluid. Rapid glucose absorption rate from the peritoneal fluid dissipates the osmotic gradient and makes it unsuitable to obtain adequate UF during long dwells and in patients with high peritoneal transport. Glucose absorption may worsen the anorexia, hyperglycemia, dyslipidemia, and insulin resistance as well as the increased oxidative stress that are often associated with the uremic syndrome. Moreover, long-term exposure to the elevated glucose concentration of PD fluids contributes to structural changes of the peritoneal membrane, such as submesothelial thickening and fibrosis and vascular proliferation, which are associated to functional impairment, represented mainly by UF failure. The main mechanisms by which glucose-based PD fluids induce these deleterious effects on peritoneum are represented by (76–79):

- Hyperosmolar stress
- Highly reactive glucose degradation products (GDPs), that impair mesothelial cell functions and modulate cytokine generation
- Glycation of structural proteins and formation of Amadori adducts and advanced glycosylation endproducts (AGE)
- Effects on peritoneal cell metabolism via the polyol pathway, protein kinase activation and gene induction.

Reduced GDPs formation has been obtained by the separation of glucose from the other constituents in a double chamber bag system, which allows glucose sterilization at a lower pH than is possible in single chamber bags (78, 80). In pediatric patients, significant reduction of plasma AGE levels has been reported by administration of low-GDP PD solutions (81).

In summary, glucose is effective in UF induction along short dwells, but the lowest glucose concentration of PD solution should be used while still being compatible with patient's clinical needs (82).

Icodextrin: Icodextrin consists of a family of glucose polymers with an average MW of 16,200 Da and exerts its colloid osmotic effect through the small pore system. A 7.5% icodextrin solution is able to obtain a sustained UF during a prolonged dwell, and when compared to a 3.86% glucose solution, it yields similar volumes after

8 and 14 h (83). Studies in pediatric patients showed the same UF profile than in adults and an increase in solute removal, with rare and mild side effects mainly manifesting by a mild to moderate skin rash, which usually resolves without any sequelae after icodextrin discontinuation (84–86). However, a positive correlation between age and net UF was reported in a small group of patients, with negative UF in infants characterized as high transporters on PET (87). Rusthoven et al. (88) found that with a 3.86% glucose solution the increase in IPP was positively correlated with transcapillary UF and inversely correlated with patient BSA, while by using an icodextrin solution, IPP hardly increased and no correlation was found with fluid kinetics or patient BSA. Since colloid osmotic effect exerted by icodextrin does not induce sodium sieving, sodium removal is usually higher than that obtained with glucose-based solution (89). In pediatric patients icodextrin absorption, which occurs via convective pathways, such as peritoneal lymphatics, was reported to be 45% over 14 h (86). Icodextrin is metabolized by amylase to maltose and a number of oligosaccharides, whose serum levels usually reach a steady-state within 2 weeks from the start of treatment, and go back to zero 1–2 weeks after its discontinuation (84). Sterile peritonitis was reported in some patients treated with icodextrin, and was caused by peptidoglycan contamination of dialysate by thermophilic, acidophilic bacteria (90). In vitro and ex vivo studies have shown that icodextrin solution is more biocompatible with the peritoneal membrane than glucose-based solutions, possibly due to its iso-osmolar property, lack of glucose, and lower GDP content (91). However, icodextrin may restrain the normal process of mesothelial cell repopulation and induce connective tissue formation (92). In adult patients, the use of icodextrin PD solutions to increase fluid removal was associated with less functional deterioration of the PM as the use of solutions with high glucose concentrations could be avoided (93).

Icodextrin is currently licensed for use in not more than one dwell per day out of concern for the potential side effects of its low molecular weight metabolites.

In practice, icodextrin solution is indicated for:

- Long night-time dwell in CAPD
- Long daytime dwell in CCPD
- Patients with type I UF failure
- Patients with transient UF failure associated with peritonitis.

Amino acids (AA): In children on CAPD the use of AA solution in a long dwell gave controversial results on patient's nutritional status and was associated with increases of blood urea nitrogen and worsening of acidosis (94). On the other hand, combined intraperitoneal infusion of AA and glucose during a nocturnal APD session in children was reported to promote utilization of AA for protein synthesis and to improve anthropometric parameters (95, 96). A 1.1% AA solution is as osmotically efficient as a 1.36% glucose solution; in adult patients an increase in UF and solute removal was observed, since AA tend to induce peritoneal vasodilatation and hence recruitment of microvascular surface area to a greater extent than glucose (97).

Buffer

Lactate: Lactate has long been the standard buffer traditionally applied in PD solutions. Lactate is absorbed from dialysis fluid along its concentration gradient, and is metabolized to bicarbonate in the liver. Use of acidic (pH 5.5–6.5) lactate-buffered PD solutions is associated with a series of clinical, metabolic and biocompatibility drawbacks (98): bicarbonate back diffuses in dialysate; some patients experience pain during inflow; lactate may induce local release of growth factors that stimulate fibrogenic processes and neoangiogenesis, thus contributing to peritoneal fibrosis and to the impairment of peritoneal membrane transport function.

Bicarbonate: Bicarbonate, the physiological buffer of the body, has been made available in PD solutions by the use of multi-compartment bag systems, which allow bicarbonate and calcium to be separated during sterilization and storage. Neutral pH (7.0–7.6) PD solutions contain 34 mmol/l of bicarbonate, or 25 mmol/l of bicarbonate plus 15 mmol/l of lactate. Both in adult and pediatric patients, the results of experimental and clinical studies showed that the use of these bicarbonate buffered PD solutions is associated with better biocompatibility, more effective correction of acidosis, and lower incidence of infusion pain than that of conventional lactate buffered solutions (99–101). Schmitt et al. (102) found that peritoneal mass transfer kinetics were similar with bicarbonate and lactate for water and most solutes, except for slightly lower phosphate and creatinine transport rates at 1-h dwell time with bicarbonate solution. These more physiological PD fluids have been shown to prevent hyperperfusion, and to reduce the loss of proteins into dialysate; their use was associated with lower intraperitoneal pressure reflecting enhanced fill volume tolerance, but also with a reduction of the unrestricted area over diffusion distance and of the vascular exchange area (101). APD solution with a neutral pH combined with a reduced

lactate concentration, partially replaced by bicarbonate, significantly increased UF in a group of adult PD patients, conceivably by causing less peritoneal vasodilation than conventional acidic lactate-based PD solutions (103), while no difference in net UF has been reported in children (100–102).

Sodium

Since most of the commercially available PD solutions have sodium concentration (132–134 mmol/l) that is slightly lower than plasma sodium concentration, diffusion of sodium is usually less important than its convective transport, which can be accomplished by transcapillary UF, colloid osmosis induced backfiltration and absorption into the lymphatic system. Sodium sieving associated with the transcellular water transport through ultrasmall pores can contribute to a reduction in the dialysate concentration of sodium during the initial phase of the dwell. By this mechanism, more water than sodium may be removed during the shorts dwell of APD. In patients on APD, PD solutions with lower sodium concentration than in CAPD patients may be employed, especially when a large amount of high glucose solutions is prescribed. In infants, higher sodium concentration PD solutions (137–138 mmol/l), or oral sodium supplementation, may be required in case of increased sodium loss in the dialysate and in the residual urine volume associated with congenital uropathies.

Calcium

Since calcium transfer across the PM is driven by both diffusion and convection, its peritoneal flux depends on serum ultrafiltrable calcium concentration, PD solution concentration, dwell duration, and UF rate. PD solutions contain: (1) 1.75 mmol/l of calcium; in these solution ionized calcium level is higher than that normally present in blood; therefore, diffusion of calcium from dialysate to blood would lead to a positive calcium balance, (2) 1.25 mmol/l; these solutions are frequently employed with the goal of reducing the risk of hypercalcemia, especially in children receiving calcium carbonate or calcium acetate as phosphate binders, and treated with vitamin D analogues (82). Hypercalcemia and/or a high calcium $\times$ phosphate product should be avoided for the potential risk of inducing vascular and soft tissue calcification. Use of non-calcium containing phosphate binders is indicated in these cases.

Peritoneal Dialysis for End-Stage Renal Disease

Indications for End-Stage Renal Disease Therapy

The indication for initiating dialysis therapy in children with ESRD depends on a combination of biochemical, clinical, and psychosocial assessments that should be individualized for each child. When possible, dialysis should be initiated early enough to prevent the development of malnutrition and/or any significant uremic symptomatology. Although there is no definite level of blood urea nitrogen or serum creatinine concentration that mandates dialysis initiation, this should be considered when the residual glomerular filtration rate (GFR) (104) has declined to a value between 9 and 14 ml/min/1.73 m^2 BSA, and should be recommended when GFR is 8 ml/min/1.73 m^2 or less (49). Decreased school performance and restricted daily activities are also important factors in children (105).

Selection of Peritoneal Dialysis or Hemodialysis for Children with End-Stage Renal Disease

Because CPD proceeds over prolonged periods (i.e., 24 h/day for CAPD and CCPD; 8–12 h for NIPD), body fluid composition and volume change slowly, resulting in a near steady state. Thus, the disequilibrium syndrome does not occur in children receiving CPD, which can be safely performed in children who have cardiovascular instability. Almost all children receiving CPD may be encouraged to eat an appropriate diet, relatively high in protein, and with reasonable allowances for fluid and sodium. The relative simplicity and safety of CPD, also in its automated form, allow performance at home, thereby returning the child with ESRD to regular school attendance and other normal childhood activities; this is particularly advantageous for children living far from a pediatric dialysis center. CPD avoids the many difficulties associated with the maintenance of a vascular access, especially in very small patients, and eliminates the need for regular dialysis needle punctures.

There have been no randomized comparative studies of CPD and HD outcome in children. Pediatric dialysis programs that once focused almost entirely on CPD have recognized that the availability of a chronic hemodialysis designed for pediatric patients is of equal importance to the provision of optimum care for children with

ESRD (106). While the preference for CPD is most pronounced among younger patients, older children are almost equally distributed between CPD and hemodialysis (2), and are more likely to receive maintenance hemodialysis than CPD if treated in nonpediatric units (107).

Patients and families must be given the opportunity to actively participate in the selection of the chronic dialysis modality that is best suited to their individual needs and lifestyle (49, 105). Action points of patient/family selection for home CPD treatment should include:

- Early patient/family referral to dialysis staff.
- Evaluation of patient's clinical needs and patient and family life-style.
- Structured, unbiased information on dialysis modalities.
- Evaluation of physical and psychological ability of the caregiver(s) to perform dialysis tasks.
- Assessment of patient's home environment.

Table 72-1

Absolute and relative contraindications to the use of chronic peritoneal dialysis (CPD) in pediatric patients

Absolute contraindications
• Omphalocele
• Gastroschisis
• Bladder extrophy
• Diaphragmatic hernia
• Obliterated peritoneal cavity
• Peritoneal membrane failure
Relative contraindications
• Inadequate living situation for home dialysis
• Lack of appropriate caregiver
• Impending/recent major abdominal surgery
• Imminent living-related donor transplantation (within 6 months of dialysis initiation)

Patient Selection

CPD can be attempted in any child whose peritoneal cavity is intact and will admit a sufficient volume of dialysate. Experience has shown that CPD can be used successfully in children even with the following conditions: polycystic kidney disease (usually after unilateral or bilateral nephrectomy), vesicostomy, cutaneous ureterostomy, colostomy, prune belly syndrome, bilateral Wilms' tumor, recent abdominal surgery (if no draining wounds are present), ventriculoperitoneal shunt, spina bifida, and concurrent immunosuppressive therapy. However, absolute and relative contraindications to the use of CPD in children still exist, and are listed in *Table 72-1* (49).

CPD is the clear treatment of choice for infants (2, 108–111). It is now widely recognized that CPD can be an effective maintenance RRT in babies who develop ESRD as early as the first few days or weeks of life (112, 113). The short cycles, high dialysate flow rates, and high intraperitoneal volumes that can be delivered during APD make this modality appropriate to manage the high fluid intake of the infant diet. The development of cycling machines with smaller tubing dead space and recirculation of dialysate, that allow drainage at low flow rate without alarming, has further facilitated the PD procedure in infants. In a recent report (114), peritonitis rate in PD patients under 2 years of age was comparable to that in older children. Despite these favorable results, mortality rate among infants on CPD is as much as four times that of children beyond infancy, with most deaths occurring in the first year of life (111, 115). In infants, a survival rate of 85% at 1 year, 74% at 2 years and 68% at 3 years was reported, as compared with 95, 90, and 86% at the same time intervals in children starting dialysis after infancy (108, 116). The presence of non-renal disease, particularly pulmonary disease/hypoplasia, and oliguria or anuria, is a major risk factor for patients receiving CPD during the first 2 years of life (117, 118). Similarly, neurodevelopmental outcome is better nowadays than in the past (119), but it may be conditioned by extrarenal disorders such as neonatal hypoxia or certain syndromes and chromosomal abnormalities (111). Poor nutritional status and growth are frequently observed in infant phase, and should be managed intensively by supplemental feeding and growth hormone therapy (110, 120, 121). The hospitalization rate for infants on CPD may be higher than that for older children (122), and is mainly due to inpatient treatment of peritonitis, catheter revision, hernia repairs and re-evaluation of therapy (123).

Preparation of the Patient and Family

A treating facility that provides CPD to children should be able to provide the necessary multidisciplinary services required by the child and family. The team consists of CPD nurse specialists, nephrologists, urologists, general surgeons, renal dietitians, renal social workers, child psychologists, child psychiatrists, child development specialists, child life therapists, speech pathologists, school teachers, and chaplains, all of whom are pediatric specialists.

Table 72-2

Patient and family preparation for home chronic peritoneal dialysis (CPD)

Patient and family preparation for home CPD should:
→ Start well before dialysis initiation
→ Involve a specialized, multidisciplinary team
→ Make use of appropriate written information and other teaching aids
→ Encourage contacts with similar-aged children on home dialysis
→ Include a home visit to ensure safe delivery of home dialysis, and a liaison with the nursery/school/college, and the family doctor
→ Include a nutritional assessment and the evaluation of any clinical conditions that could be susceptible of surgical correction before, or at the moment of peritoneal catheter placement

Preparation of the patient and family for CPD and their education to perform home dialysis should be accomplished through a structured comprehensive program, involving the whole multidisciplinary team of the dialysis facility (*Table 72-2*). Training for home PD procedures should involve two family members and may be completed in the home environment (105, 106). Once PD treatment has started, regular telephone contact and support for the family should be planned, in order to avoid exhaustion and burnout. Moreover, acquired knowledge and skills of performing home PD should be assessed at regular intervals according to a formal home update program (105).

According to the recommendations of the NKF K/DOQI Guidelines for peritoneal dialysis adequacy in pediatric patients (49), each home CPD training unit should establish quality improvement programs with the goal of monitoring clinical outcomes and implementing programs that result in improvements in patient care. Since any pediatric center takes care of a relatively small number of patients, single-center clinical outcomes should be compared with the results reported by large pediatric databases.

Children require a great investment of time and resources from the CPD team, and the effort that is involved is often several orders of magnitude greater than that required to care for the typical adult CPD patient (106).

Peritoneal Dialysis Prescription

Optimal PD prescription for each individual child should be tailored on his/her age, body size, residual renal function (RRF), nutritional intake, and peritoneal membrane transport capacity. In addition, PD schedule should be compatible to the psychological and social needs of the patient and family. The importance of evaluating PM transport status by means of validated functional tests and selecting PD solution according to its biocompatibility and potential UF capacity has been already underlined. Two other technical parameters that should be primarily considered in the prescriptive process are represented by exchange fill volume and dwell time (38, 49, 124).

Fill volume prescription: As previously described, scaling IPV by patient BSA has become a standard in pediatric PD. Both IPV and patient posture dynamically affect the recruitment of effective peritoneal membrane area available for dialytic exchange, which corresponds to the unrestricted pore area over diffusion distance ($A_0/\Delta X$) as determined using the three-pore model (25, 26). Maximization of peritoneal vascular surface area has been obtained by raising IPV from 800 to 1,400 ml/m^2 BSA (26). On the other hand, excessive IPV may cause patients discomfort, abdominal pain, dyspnea, hydrothorax, hernia, gastroesophageal reflux, and loss of UF due to increased lymphatic drainage. Hydrostatic IPP is a reproducible, patient-characteristic parameter, and its measurement helps to evaluate fill volume tolerance in the individual patient (30). Fill volume leading to an IPP of 18 cm H_2O in the supine position is considered the maximum tolerable IPV, above which abdominal pain and a decrease in respiratory vital capacity may occur. An IPV of 1,400 ml/m^2 BSA seems to be optimal to ensure optimal recruitment of vascular pore area in children; however, this should be considered as a maximal limit, the safety of which has not been validated in children. In clinical practice, in order to obtain as high recruitment of vascular pore area as possible, fill volume can be increased in steps, remaining under the limit of 1,400 ml/m^2 BSA for a night exchange while the patient is lying down, and monitoring patient's clinical tolerance and IPP (37).

Dwell time prescription: Dwell duration should always be adjusted on individual patient's transport status and on required small and middle-sized molecule removal and UF. (38, 49, 124). Short dwells are more efficient for small solute clearance and UF, which can be further enhanced by increasing dialysate glucose concentration. High transport patients would benefit from short exchanges, but dissipation of osmotic gradient due to fast glucose absorption should be taken into account. Removal of solute of relatively higher molecular weight, such as creatinine and phosphate, is favored by prolonged exchanges, but these can be associated with impaired UF or even with dialysate reabsorption while using glucose solutions. Icodextrin solution is more appropriate for long dwells (82, 85).

A potentially useful way to individualize dwell duration in pediatric patients on automated PD (APD) according to peritoneal transport capacity is the calculation of the so-called APEX time. While performing a PET, APEX time corresponds to the point at which D/P urea and D/D_0 glucose equilibration curves cross, and should represent the optimal length of APD cycles (125). Similarly, phosphate clearance, which is usually insufficient to obtain a satisfactory control of hyperphosphatemia, thus requiring strict dietary restriction and phosphate binder administration, can be improved by optimizing exchange duration through the calculation of the so-called phosphate purification dwell time (PPT) (125).

PD Treatment Modalities

CPD can be performed manually (CAPD), or by means of a cycler (APD). APD regimens can be continuous, with dialysis solution present in the peritoneal cavity evenly throughout 24 h, or intermittent, with empty abdomen for part of the day, usually during daytime. Continuous regimens allow complete equilibration of small solute as well as a certain removal of middle molecules. However, the presence of a large volume of dialysate in the abdomen during the day can be associated with patient discomfort, the occurrence of abdominal hernias (especially in infants and young children), and problems of body image (especially in adolescents). Moreover, continuous absorption of glucose from the dialysate compromises appetite and aggravates uremic dyslipidemia.

CAPD: This continuous PD modality has the advantage of ease of use and limited cost of the equipment. The guidelines of the European Committee on adequacy of the pediatric PD prescription (38) suggest to employ an initial fill volume of 600–800 ml/m^2 during the day and 800–1,000 ml/m^2 overnight; then, dwell volume can be gradually increased according to patient tolerance and IPP measurements. An icodextrin-based solution can be used for the prolonged night-time dwell. In order to further increase the delivered dialysis dose, there is no other means than increasing the number of exchanges. CAPD is usually effective in patients with RRF, but its decline should be closely monitored. Patients with a low-average or high-average peritoneal transport status at the PET can be maintained on CAPD with close monitoring of dialysis adequacy indexes.

APD: During the past 15 years, APD has progressively expanded as the PD modality of choice for children and has largely replaced CAPD, at least in those countries where it is not limited by cost constraints (2, 17, 69). The preference for APD has mostly been a lifestyle choice, since the night-time APD course enables children to attend school full-time and reduces the impact of dialysis treatment on the way of life of the patients and their families (16). The wide range of APD schedules help in tailoring dialysis prescription to patient's age, body size, clinical conditions, growth-related metabolic needs, residual renal function and PM transport status. In addition, performing the night-time exchanges in the lying position allows the use of large fill volumes, thus increasing the recruitment of functional peritoneal surface area (26).

The selection of an individualized APD prescription can be facilitated by kinetic modeling. Mathematical modeling software programs, that have a specific individual peritoneal function test as data entry, have been developed to calculate kinetic parameters, to simulate the results of APD regimens, and to rapidly find the best personalized dialysis schedule (126). Two of these software programs have been validated in children (35, 127, 128), and their accuracy in predicting solute removal is good. The accuracy of these programs in UF prediction was less good, owing to the inability of kinetic modeling to account for changes in residual dialysate volumes, the marked day-to-day variability of UF, the large variability of daily fluid intake, and the confounding effects of residual diuresis in non-anuric patients (129, 130). Therefore, computer-assisted kinetic models represent useful tools to help selecting the optimal dose of dialysis for a given patient, but the evaluation of the actually delivered dialysis dose by direct measurement of solute clearances and UF rate is always needed.

Nightly intermittent PD (NIPD): NIPD consists of a number of short nocturnal cycles, without a daytime dialysate dwell, and is primarily indicated in patients characterized by a high-transport peritoneal membrane, which allows rapid solute equilibration. The main advantages of a dry abdomen during the day include normal

IPP, and the reduction of glucose absorption, of AA and protein loss, and of membrane exposure to glucose. On the other side, the absence of a daytime dwell is a limitation for solute clearance (especially for middle-size molecules), and makes NIPD not suitable for patients with low and low-average peritoneal transport. Even in NIPD a small daytime fill volume is often prescribed in order to allow the flush of the catheter and of the lines at the start of the night PD session, thus reducing the risk of peritoneal contamination. NIPD is frequently adopted as the first APD regimen in patients with a significant RRF. As clearance and UF requirements increase, mainly as a consequence of the decline of residual renal function, NIPD efficiency can be improved by: enhancing dwell volume according to patient's tolerance (37); extending total treatment time; increasing the number of exchanges, up to a point beyond which clearance solute and water removal may decrease as the non-dialytic time, corresponding to the fill and drain phases, becomes more important than the benefit of further increasing total dialysate volume.

Continuous cycling PD (CCPD): In this continuous form of PD, a fresh exchange of dialysis solution, ranging in volume from 50 to 100% of the fill volume applied at night, is left in the abdomen at the end of the night APD session. Daytime exchange dialysate can be drained at bedtime when the cycler is reconnected, so that patient involvement is reduced to one session for preparation of the equipment and connection to cycler, and one short disconnection in the morning. Prolonged daytime dwell significantly contributes to dialytic clearances since removal of middle-sized uremic toxins, which is poorly influenced by short cycles of APD, is increased (131). Daytime solute clearances are also influenced by convective transport associated with net UF, which in turn depends on the type of osmotic agent, fill volume related IPP, and membrane transport status. Colloid osmotic effect exerted by icodextrin solution is able to obtain net UF during a long daytime exchange in the majority of patients (82, 84, 86, 87).

A continuous PD regimen is particularly indicated in patients with high-average peritoneal transport, and should be considered when the contribution of RRF to solute removal and UF has become negligible. If a further increase of solute clearance and UF is desired, more than one diurnal exchange can be performed, optimizing the length of each dwell according to patient's peritoneal transport and the type of employed osmotic agent *(continuous optimal peritoneal dialysis, COPD)* (38, 131). With this schedule, an exchange is usually performed at mid-day or after school, using the cycler in a disconnectable manner.

Tidal PD (TPD): In this modality, an initial infusion of PD solution into the peritoneal cavity is followed by only partial dialysate drainage, thus leaving always an intra-abdominal reserve volume. Tidal drain volume is replaced with fresh dialysis fluid to restore the initial IPV with each cycle, while the entire dialysate volume is drained at the end of the PD session (sometimes also once in the middle of the session). The expected amount of ultrafiltrate during each cycle must be estimated and added to the drain volume to prevent overfilling of the peritoneal cavity. With tidal PD, enhanced clearances are expected as a result of the continuous contact between dialysate and peritoneal membrane, which maintains a sustained diffusion of solutes. The efficiency of the dialysis modality can be further increased by reducing inflow and outflow dead times, especially if high dialysate flow rates are employed. Tidal PD is also adopted to avoid repeated cycler alarms of low flow rate in case of catheter malfunction, and to reduce pain occurring during the drainage phase. Major determinants of TPD efficiency are total volume of delivered PD fluid and individual peritoneal transport status. Patients with high transport status can reach adequate solute clearances with intermittent nightly TPD, while high average transport patients would better benefit from a continuous regimen of TPD with one or more daytime dwells. Studies on pediatric patients showed that TPD efficiency was equal or higher than that of standard APD, even requiring larger total dialysate volumes (132, 133). Optimization of TPD efficiency can be attained by adapting tidal volume to the drainage profile of each patient, thus reducing the fill and drain dead times (134). In fact, peritoneal catheter drainage profile is not linear, since a high flow rate is maintained until a critical intraperitoneal volume is reached. After this point, also called breakpoint, the flow rate drops and during the final, slow-flow portion of the drainage phase peritoneal cavity is almost empty and solute clearance greatly reduced (135). Since the critical intraperitoneal volume is an individual characteristic, tailoring tidal volume to the drainage profile of each patient reduces idle time and improves the overall efficiency of the system.

Peritoneal Dialysis Adequacy

The correlation between the delivered dialysis dose and the adequacy of dialysis treatment was first analyzed in hemodialysis patients by studies mainly based on urea kinetics evaluation. The resultant concept of "adequate" dialysis was originally created to define a minimum hemodialysis dose, below which a clinically unacceptable

rate of negative outcome might occur (patient hospitalization, morbidity, mortality). During the 1990s, the influence of small solute clearance on the outcome of patients on PD was a major focus of interest. Observational studies in adult CAPD patients suggested that better patient survival and lower morbidity was associated with higher clearances of small molecules, as urea and creatinine (136, 137). As a consequence, small solute clearance was considered the key criterion of PD adequacy in clinical practice guidelines such as those of the Kidney Disease Outcomes Quality Initiative (K/DOQI) (138). Subsequently, a re-analysis of the data from the original Canusa study, as well as the results of prospective randomized interventional trials (139–141) were unable to demonstrate any clear survival advantage with increases in peritoneal small solute clearances and showed that RRF is a much stronger predictor of patient survival than peritoneal clearance. Possible, speculative explanations for failure of increased PD dose to improve outcomes are: higher IPP associated with larger exchange volume; failure to increase clearance of middle molecules; increased exposure to glucose-based dialysate (142). Moreover, some recommendations proved difficult to be fully applicable in clinical practice, especially among pediatric patients.

Indeed, in children, even more than in adult patients, adequacy of PD treatment cannot be solely defined by targets of solute and fluid removal. Clinical assessment of treatment should take into consideration a series of clinical, metabolic and psycho-social aspects, such as:

- Hydration status
- Nutritional status
- Dietary intake of energy, proteins, salts and trace elements
- Electrolyte and acid-base balance
- Calcium phosphate homeostasis
- Control of anemia
- Blood pressure control
- Growth and mental development
- Level of psycho-social rehabilitation

In clinical practice, delivered dialysis dose can be adjusted and monitored following the guidelines by an ad hoc European committee on adequacy of the pediatric PD prescription (38) and the 2006 update of the NKF-K/DOQI clinical practice recommendations for pediatric peritoneal dialysis adequacy (49).

The following issues will be specifically, even briefly, addressed: small solute clearance; clearance of middle-sized molecules; fluid balance and UF; clinical correlates of PD adequacy.

Small Solute Clearance

In the absence of definitive outcome data in pediatrics to indicate that any measure of dialysis adequacy is predictive of well-being, morbidity, or mortality, 2006 K/DOQI guidelines (49) stated that by clinical judgment the "delivered" small solute clearance in children should meet or exceed adult standards.

A minimal "delivered" dose of small solute clearance should be at a Kt/V_{urea} of at least 1.8 per week. Data from pediatric and adult studies found serum albumin level to be a predictor of patient survival, and a Kt/V_{urea} of 1.8 or greater in adult PD patients has been associated with better serum albumin values (49, 143).

The above reported target should be intended as total (peritoneal plus renal) clearance, or peritoneal clearance alone in patients without RRF (defined as urine Kt/V_{urea} <0.1 per week). For practical reasons peritoneal and renal clearance can be added to determine total clearance, even if they have a different impact on patient's outcome. The term "delivered" refers to the actual dose the patient is receiving based on direct measurement, not to an estimated value obtained by using a kinetic modeling program. Solute clearance should be measured within the first month after starting PD, and at least once every 6 months thereafter, when the patient is clinically stable, and at least 1 month after resolution of a peritonitis episode. More frequent measurements should be conducted when dialysis clearance may have been compromised (e.g., after peritonitis), there is a progressive loss of RRF, or there is clinical evidence of inadequate dialysis. However, if a patient is not doing well and has no other identifiable cause other than kidney failure, a trial of increased dialysis is indicated (49).

Historically, both Kt/V_{urea} and creatinine clearance (CrCl) have been employed to evaluate PD clearance. A discrepancy between urea and creatinine PD adequacy parameters was often reported (144–147), especially in patients on APD and in children (38). Indeed, urea clearance is mostly related to dialysate volume and number of exchanges, while CrCl is predominantly affected by the duration of the dwell time, and by the presence of residual renal function. The 2006 K/DOQI recommendations suggested the determination of dialysis and urine Kt/V_{urea} alone for follow-up, based upon the simplicity of its calculation, and because studies on adult PD patients have not provided evidence of a benefit in terms of patient outcome when expressing clearance in any manner other than Kt/V_{urea} (49, 144, 147).

Kt/V_{urea} is normalized for urea distribution volume (V), which is assumed to equal total body water (TBW).

Therefore, accurate estimation of TBW is a critical component of dialysis dose measurement. Since gold-standard isotope dilution technique to determine TBW are laborious, costly and not widely available, anthropometric prediction equations based on height and weight are commonly used to estimate TBW. Equations derived from healthy children (148) systematically overestimates TBW in pediatric patients receiving PD, and recently a new set of anthropometric TBW prediction equations have been developed in this patient population, and validated by comparison with the determination of TBW by means of heavy water (H_2O^{18} or D_2O) dilution technique (149). These formulae are based on a new anthropometric parameter called "*height times weight*," that correlates linearly with TBW when both values are log-transformed, and are as follows:

$$\text{Boys}: \quad \text{TBW} = 0.10 \times (\text{HtWt})^{0.68} - 0.37 \times \text{weight}$$

$$\text{Girls}: \quad \text{TBW} = 0.14 \times (\text{HtWt})^{0.65} - 0.35 \times \text{weight}.$$

An alternative measure of PD dose, other than Kt/V urea and CrCl, is represented by the solute removal index (SRI), which normalizes removal by the solute content of the body at the beginning of treatment, so that the ratio between net urea removal and pre-dialysis urea body pool is calculated. By SRI different dialytic treatments, or the results of the same treatment in patients differing in body size, can be compared (150).

In conclusion, numerical targets of small solute clearance, as defined by currently available guidelines, should be interpreted cautiously and in the context of patient clinical assessment.

Clearance of Middle-Sized Molecules

Failure to achieve an adequate clearance of the so-called middle-sized molecules (from 300 to 5,000 Da MW) is one of the possible explanations for the lacking effect of increased dialysis dose on patient survival (142). Small solute and middle-sized molecule clearances respond differently to changes in PD prescription; while the former is mainly determined by the frequency and volume of dialysate dwell, the latter depends more on the duration of contact of the peritoneum with dialysate (151, 152). Removal of middle-sized molecules and low-molecular weight proteins, such as β_2-microglobulin and leptin, mainly depends on RRF (153, 154). Moreover, an increase of the restriction coefficient for macromolecules was reported in relation to time on chronic PD, which is associated with an increased size selectivity and a reduced peritoneal permeability for higher molecular weight solutes (46). Hence, particular attention should be paid to middle molecule clearance especially in children on NIPD, and as RRF is declining. In these cases a continuous PD regimen (CCPD or CAPD) should be adopted even if small solute clearance is above target without the longer dwell (49). Increased β_2-microglobulin and leptin clearance have been reported in patients receiving a long dwell with icodextrin solution (155).

Fluid Balance and UF

PD prescription should be continuously adjusted in order to achieve and maintain fluid balance and normal blood pressure. PD has been considered an optimal approach to reach this therapeutic result thanks to its continuous nature, which avoids fluctuations of volume and offers better homeostatic stability than intermittent therapies. Nevertheless, PD population surveys show a high prevalence of hypertension and cardiovascular mortality, and UF proved a significant predictor of survival in anuric adult patients (156, 157). Data from the North American Pediatric Renal Transplant Cooperative Study (NAPRTCS) (158) showed that 57% of nearly 4,000 patients on dialysis had blood pressure values higher than age-, sex-, and height-specific 95th percentile. Left ventricular hypertrophy was documented by echocardiography in 68% of 38 long-term pediatric patients on PD therapy (159). Hypertension and cardiac impairment were most frequently found in the younger and nephrectomized PD patients (160). Even if the cause of hypertension is multifactorial, volume overload is likely to play an important etiologic role in a relevant percentage of patients on PD therapy (49).

Routine evaluation of volume status, daily UF volume and daily residual diuresis volume is therefore essential in the process of attaining adequate PD, and can be performed according to the recommendations for PD adequacy in pediatric patients (38, 49). In the absence of validated, readily applicable indicators of volume status, the assessment of patient "target weight" mainly relies on clinical judgement. In clinical practice, the desirable target weight of a PD patient could be reasonably approximated to that weight at which the patient is edema free and normotensive with minimal need for antihypertensive medications. Since fluctuations in patient weight secondary to growth and to changes in nutritional status may

occur, re-evaluation of target weight at regular intervals is mandatory.

Recommended interventions to maintain patient fluid balance include:

- Dietary counseling on sodium and fluid restriction; this recommendation should take into account renal and/or dialysis-related sodium losses, since sodium depletion may result in hypotension and impaired growth
- Use of loop diuretics in children with RRF
- Evaluation of PM transport characteristics; these affect net fluid removal at a given dwell time by determining the osmotic gradient time curve; a modification of the standard PET utilizing 4.25% dextrose solution can be employed to better evaluate the UF kinetics (129, 130)
- Tailoring of dwell time and PD solution tonicity; these parameters are interrelated and should be considered jointly; for instance, low dialysate dextrose concentration and prolonged dwell time will inevitably lead to inadequate fluid removal in high transport patients (130); an increase of dextrose tonicity is associated with enhanced UF, but the osmotic gradient dissipates over time; therefore, dextrose solutions are indicated for short dwells, while for the night-time dwell in CAPD and the daytime dwell in APD, icodextrin solution may be more appropriate; icodextrin is also effective in maintaining adequate UF rate during peritonitis episodes (161); the observation that icodextrin may behave differently in young children, in whom UF may not be as successful as in older patient, requires further confirmation (87)
- Correction of peritoneal catheter malfunction leading to incomplete dialysate drainage, especially after prolonged dwells on CAPD and CCPD.

In practice, PD prescription strategies to improve UF rate could be summarized as follows:

- During short dwells on the cycler: increase number of cycles and/or overall treatment time and/or glucose concentration (even if any effort should be done to employ the lowest possible dextrose concentration required to achieve the desired UF rate
- During prolonged dwells: utilize icodextrin solution; replace single long exchange with two or more exchanges.

Clinical Correlates of PD Adequacy

Large-scale, prospective outcome studies on pediatric PD patients are lacking owing to the small number of patients per center, and the relatively short period of time on dialysis prior to renal transplantation. Nevertheless, some pediatric studies have effectively addressed the issue of the correlation between PD dose and selected clinical aspects.

Growth is a potentially valuable outcome measure specific to pediatrics. Multivariate analysis of the data of a multicenter study (162) showed a weak positive correlation of height standard deviation score (SDS) with dialytic creatinine clearance, and a negative correlation with peritoneal transport status (i.e., children with high transport on PET had a lower change in height SDS). Accelerated height velocity was reported in 62% of the patients who met or exceeded DOQI target clearances (163). Chadha et al. (164) presented data showing that growth correlates with renal solute clearance, but not with peritoneal clearance. Similar to adult studies, these data may suggest that peritoneal and residual renal small solute clearances are not equivalent.

Nutrition is an issue of particular interest in pediatric PD, since it can significantly affect growth and development of children. Dietary protein intake is inconsistently correlated with delivered Kt/V urea (165–167). However, the relationship between Kt/V urea and the normalized protein equivalent of nitrogen appearance (nPNA) has often been criticized as merely being the result of mathematical coupling (168). Finally, a higher Kt/V was associated with a lower serum albumin level in children, suggesting that enhancing PD dose may reach a point of no further benefit (i.e., a Kt/V value of more than 2.75), owing to an increased loss of albumin in the dialysate (169).

A study in 18 children on PD showed that increasing weekly Kt/V and CrCl was positively correlated with cardiac function and inversely with left ventricular mass (170).

Preservation of Residual Renal Function

Prospective randomized trials of dialysis adequacy and observational studies in adult patients have confirmed that RRF is a much stronger predictor of patient survival than peritoneal clearance (139, 140, 141). In pediatrics, no data from large-scale trials on the correlation between RRF and patient outcome are currently available. However, the already cited single-center observational study on PD pediatric patients by Chadha et al. reported that

growth velocity was higher in a group of children with RRF than in a group of children without RRF even if the same mean total solute clearance was achieved in the two groups (164).

The rate of RRF decline in pediatric patients on PD was reported to be slower than in patients on HD (171, 172). PD prescription should be aimed to preserve RRF as long as possible, by gradually increasing the dialysis dose in steps, accurately targeting UF rate to maintain patient's dry body weight, and using the lowest possible dialysate glucose concentration required to achieve the desired UF volume (49, 172). Prevention of RRF loss also involves avoidance of nephrotoxic insults (medications, radiocontrast agents, urinary tract obstruction and infection); in particular, aminoglycoside antibiotics should be employed in the treatment of PD related peritonitis taking into account their nephrotoxicity, as well as ototoxicity and vestibular toxicity.

Data on adult PD patients support the role of angiotensin-converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARBs) in slowing RRF decline (173, 174), but no experience on the use of these agents is yet available in children with ESRD. ACE inhibitors and/or ARBs can be prescribed to PD patients who require antihypertensive therapy, but close monitoring for the occurrence of hyperkalemia is recommended (175).

In summary, interventions that may contribute to the preservation of RRF should be adopted whenever possible (49). At the same time, RRF should be routinely measured by means of 24-h urine collection and PD prescription should be adjusted to its decline in a timely fashion, in order to anticipate the occurrence of signs and/or symptoms of inadequate treatment.

PD Adequacy Monitoring: Practical Schedule

The assessment of PD delivered dose is fundamentally based on the direct measurement of dialytic and renal clearances, through a 24-h collection of dialysate and urine (49). All dialysate discharged during 24 h should be accurately collected (including daytime exchange, if present), total volume precisely measured, and a sample obtained after mixing effluent thoroughly. The same attention should be paid to perform complete 24-h urine collection, although this may be difficult to do accurately in children, requiring good cooperation by caregivers. Urine collection requires a preservative, such as thymol, to be added to the collection or refrigeration to inhibit the growth of bacteria that can degrade urea; dialysate does not require refrigeration or preservative.

Weekly peritoneal Kt/V urea can be calculated with the following formula (176):

$$(24\ \text{h D/P urea} \times 24\ \text{h dialysate volume} \times 7)/\text{V}$$

where D/P represents the dialysate to plasma concentration ratio.

In patients with RRF, renal Kt/V corresponds to:

$$(\text{ml/min urea clearance} \times 1440\ \text{min/day} \times 7)/(1,000\ \text{ml} \times \text{V}).$$

CrCl calculation is normalized to BSA, that can be calculated from weight and height by use of the Gehan and George formula (39).

The following formula can be employed to calculate dialytic CrCl per week (176):

$$(24\ \text{h D/P Cr} \times 24\ \text{h dialysate volume} \times 7 \times 1.73\ \text{m}^2)/\text{BSA}\ (\text{m}^2)$$

Residual renal clearance is better expressed as the average of CrCl and urea clearance, each of which can be calculated by the standard formula (and normalized to patient's BSA):

$$\text{solute clearance (ml/min)} = (24\ \text{h urine volume in ml} \times \text{urine solute concentration})/(1,440\ \text{min/day} \times \text{serum solute concentration}).$$

PD dose assessment should be coupled with an evaluation of nutritional status, which will be discussed in the following paragraph.

A practical schedule of routine outcome evaluations in pediatric patients on chronic PD can be organized as indicated in *Table 72-3*.

Methods of Monitoring Patient Adherence

Non-adherence is an important obstacle to achieving an adequate dialysis dose, and a significant cause of morbidity, patient hospitalization and technique failure (177, 178). Several methods to assess patient adherence to PD prescription have been proposed, based on comparison of measured versus predicted creatinine excretion (177); home visits to check dialysis solution supply inventories (179); patient self-report confidential questionnaires (180); or the comparison of self-reports of compliance with the rate of predicted versus measured Kt/V urea and CrCl (181). However, none of these methods provides a complete assessment of non-adherence in patients on home PD.

Table 72-3

Timetable of routine clinical and biochemical evaluation in pediatric patients on stable chronic PD (UF = ultrafiltration; CrCl = creatinine clearance)

Parameters to be evaluated	Frequency
• Clinical and physical examination	Every month
• Height	
• Weight	
• Head circumference (in infants)	
• Blood pressure	
• Blood urea and creatinine	
• Serum electrolytes	
• Acid-base status	
• Hemoglobin/hematocrit	
• Serum albumin	
• Daily urine volume and UF	
• Serum ferritin	Every 3 months
• Serum iron	
• Total iron binding capacity	
• Alkaline phosphatase	
• Parathyroid hormone	
• Kt/V urea and CrCl from 24-h dialysate and urine collection	
• Neurodevelopment assessment	Every 3 months
• Ambulatory blood pressure monitoring	Every 12 months
• Echocardiography	
• Hand and wrist X-ray for bone age	

Thanks to the introduction of microchips and computer technology, modern APD cyclers can store the patient's prescription, medical history and treatment events on an electronic device. This system provides information on the home dialysis PD treatment and an objective means to monitor patient adherence. Comparison of the prescribed versus the actually delivered therapy shows any change the patient and/or caregiver may have done in the prescribed dialysis schedule on his/her initiative. More frequent changes include skipping treatment cycles; shortening overall treatment time; manually changing treatment parameters; bypassing therapy phases or cycles; or, reducing fill volume by performing manual drains.

The preliminary results reported on the use of telemedicine in a pediatric PD program (178), show that this type of tele-communication allows to identify and successfully address clinical and psychosocial problems, and increase patient and family satisfaction with home PD treatment. Whether the so-called "teledialysis" is able to significantly reduce the need for patient hospitalization or the incidence of technique failure in a population of children on home APD has still to be evaluated in large-scale studies.

Nutritional Management of Children Receiving Continuous Peritoneal Dialysis

The achievement of normal growth is uncommon among children treated with CPD, despite access to an essentially unlimited diet. Compared with normal healthy children, pediatric patients receiving CPD have significantly lower energy intake, as well as diminished height, weight, triceps skinfold thickness, and mid-arm muscle circumference (182–184). Hypoalbuminemia, hypertriglyceridemia and hypercholesterolemia are commonly seen (185).

Children on CPD commonly suffer from protein and calorie malnutrition with loss of muscle mass and protein stores, and this condition is associated with increased morbidity and mortality (184). The term malnutrition would infer that dietary replacement would be curative, which is not always the case in ESRD patients, which usually have a loss of lean body mass combined with normal or even increased fat mass, high resting energy expenditure and inadequate response to nutrient supplementation (183, 184, 186, 187).

Assessment of nutritional status: In pediatric patients on PD, an accurate assessment of the nutritional status is essential to provide the most appropriate diet as well as the most effective dialysis prescription. However, although many indices have been proposed for this assessment, no single or easy measure of inadequate nutritional status does exist, and no gold standard has been yet defined (183, 184). Anthropometric measures that are routinely obtained in clinical practice are height (Ht), weight Wt), and head circumference (in younger children), and are plotted on percentile charts to evaluate weight changes and height velocity. Another way to express the relative weight and height is the body mass index (BMI, Ht/Wt^2), that should be calculated according to height age (183). Extreme values of BMI have been associated with increased morbidity and mortality, but it may not reflect body composition precisely, since is not able to distinguish between fat mass and fat-free mass (FMM) (183, 188). Direct measurements of tricipital skinfold thickness and arm circumference are used to calculate mid arm muscle circumference (MAMC), arm muscle area (AMA) and arm fat area (AFA) by means of appro-

priate formulae (189). These parameters can be compared with the corresponding age- and gender-specific reference values and then expressed as standard deviation score (SDS). In pediatric patients, anthropometric variables standardization by chronological age is questionable, and correction for height age has been suggested (190). Patient's nutrient intake should be estimated by means of retrospective dietary recall or, even better, by prospective food record. The latter is usually performed over 3 days and requires the cooperation of parents, who should receive detailed instructions by a pediatric renal dietician. Frequency of dietary reviews should be every month in PD children under 2 years of age, and every 3–4 months in older patients. Their accuracy and the role of the pediatric renal dietician, who should work with the patient and family, are crucial to the successful management of nutrition in pediatric patients on chronic dialysis (183, 184, 191).

In steady state patients, protein intake can also be estimated by calculating the protein catabolic rate (PCR), which is also called protein nitrogen appearance (PNA), by means of the modified Borah equations, as suggested by the KDOQI guidelines (191), or the formula proposed by Mendley and Majkowski (192).

Among biochemical parameters, serum albumin is the most frequently used nutritional index, and has been identified as a surrogate marker for nutritional status as well as for morbidity and mortality in ESRF patients. Low serum albumin is associated with an increased risk of death in ESRF children, and is more common in patients on PD, who suffer from protein malnutrition more frequently than their peers treated with hemodialysis (143, 193). The rate of protein and AA loss with dialysate varies with peritoneal transport status, and is inversely correlated with body weight and BSA, so that it may impair nutritional status and even normal growth especially in infants (183, 194, 195).

Metabolic status of patients on chronic dialysis can be evaluated by calculating the nitrogen balance, which represents the difference between nitrogen intake and nitrogen losses. Since direct measurement of nitrogen losses in urine, stools and dialysate is quite complicated, a mathematical model for its estimation in pediatric patients treated with PD has been developed (196).

Body composition of children on PD has been evaluated by means of a series of methods (DEXA; isotope dilution techniques; total body nitrogen; densitometry), all of which are difficult to use on a regular basis in clinical practice. Bioelectrical impedance analysis (BIA), that is non-invasive and quite easy to perform at the bedside, has been widely employed to assess patient's dry weight. The interpretation of the results obtained by using the parameters that are measured by BIA (resistance and reactance) and the indices directly derived from them (phase angle; distance; BIA vector) to estimate body composition is still debated. Reference values of the whole-body BIA vector have been determined in healthy children by a multicenter, cross-sectional study (197), but more data on pediatric patients on dialysis are required. Specific equations to predict free fat mass (FFM) and TBW from BIA data have been provided (198); however, their use in the clinical setting should take into account a certain degree of unpredictable variability of some parameters. In a group of children on APD, Edefonti et al. (199) found that the BIA indices (reactance, phase angle and distance) were able to detect alterations in body composition earlier than anthropometry. The comparison of the values measured by BIA with previous data relating to the same patient, taking into account patient's height increase and fluid status, allows to identifying changes in body composition with a satisfactory level of accuracy.

Since none of the above mentioned methods can stand alone to define the nutritional status of children on PD, the use of a combination of them is reasonably advisable in the everyday practice. Edefonti et al. (200) recently proposed the so-called ABN score, that is based on nine anthropometry and BIA parameters: height, weight, BMI, MAMC, AMA, AFA, reactance, phase angle, and distance. These indices are expressed as SDS and elaborated by means of a dedicated software program to calculate an ABN score that may vary from 3 to 15. Distribution percentiles were calculated in healthy children, and an ABN score of 10.33 was established as the limit of normality (third percentile). A multicenter study on PD pediatric patients (200) showed that 48.8% of them had an ABN score lower than 10.33 as well as significantly lower levels of serum albumin, hemoglobin and creatinine. Therefore, the ABN score seems a valuable tool for monitoring nutritional status in children on chronic PD, even if further studies on a larger patient population are required to validate it.

The NKF-K/DOQI clinical practice guidelines for nutrition in children with chronic renal failure (191) recommend to including in the minimal nutritional assessment of dialysis patients an estimate of nutrient intake by dietary interview, diary or PNA calculation, serum albumin and a complete anthropometric and growth evaluation.

Nutritional requirements of children on PD: Current guidelines from the NKF-KDOQI program suggest the need to provide an energy intake of at least 100% of the recommended daily allowance (RDA) for children of the same gender and chronologic age (191). The cal-

ories derived from the absorption of glucose contained in the PD solutions should be taken into account while calculating daily total energy intake, since it can increase calorie intake by as much as 7–12 kcal/kg/day (201, 202). Dietary protein intake in PD pediatric patients should provide 100% of RDA plus an allowance for the loss of proteins and AA in the peritoneal effluent. Protein losses of 100–300 mg/kg/day have been reported in children on PD (195). Without an energy source nitrogen will not be effectively incorporated into protein (201, 202). In a study on 31 children on dialysis, growth velocity was negatively correlated with daily protein intake, while it positively correlated with total energy intake (203).

Dietary energy and protein prescription should be adjusted on the individual child's response by routine assessment of nutritional status. Moreover, energy and protein intake should be increased in the case of enhanced metabolic needs associated with acute illness or conditions of stress (184).

Poor appetite as well as early satiety, nausea or vomiting are quite common in children with ESRD and may contribute to an inadequate spontaneous oral intake, thus requiring nutritional interventions. The K/DOQI guidelines recommend that supplemental nutritional support be considered when a child does not have a normal height velocity or is failing to consume the RDA for energy and/or protein (191). Oral nutrient supplementation is obtained by using modular carbohydrate, fat and protein components which can be added to infant formulas, or provided in liquid or bar form to the older children. In patients who fail to meet their nutritional requirements by the oral route alone, enteral nasogastric or gastrostomy tube feeding should be considered. Nasogastric tubes are easy to insert, but must be periodically replaced and may cause vomiting. Gastrostomy tubes or buttons are usually well tolerated, but are also associated with emesis as well as with an increased risk of exit-site infection, leakage and peritonitis in patients on PD (120, 121, 204). Feeding dysfunction were reported in infants with severe chronic renal failure after long-term use of nasogastric tube (205), but oral feeding is successfully resumed in the majority of children after renal transplantation (206, 207).

Intraperitoneal AA supplementation has given controversial results in children on CAPD (94, 208, 209). APD with a combined intraperitoneal infusion of AA and glucose has been proposed as a means of providing an extra amount of readily available nitrogen and calories thanks to an adequate non protein calorie/nitrogen ratio. The use of this APD regimen in eight children for a period of 6 months (95) was associated with an improvement of anthropometric measures (MAMC, AMA, AFA), and of the weight for height percentile. No modification of nitrogen waste products was observed, while nitrogen balance significantly increased from 47 $\pm$ 33 mg/kg/day at baseline to 126 $\pm$ 74 mg/kg/day at 6 months. These data support the hypothesis that AA, when infused simultaneously with glucose, can be more efficiently utilized for protein synthesis, rather than catabolized for energy production. The main advantages of administering supplemental AA by the peritoneal route are the good compliance, without modification of the normal dialysis procedure, and that the supplementary nitrogen by AA carries no additional phosphorus. However, further studies are needed to confirm the potential of intraperitoneal AA infusion to improve the nutritional status of children on chronic PD.

The potential need for sodium, potassium, and phosphorus supplementation requires special attention. Young infants may have significant sodium loss in dialysate, as well as from their native kidneys as a result of underlying obstructive uropathy. Hypophosphatemia is especially common in association with the use of low phosphorus infant formulas (210), even if in any other condition there is the need to restrict phosphate dietary intake and to administer phosphate binders. Iron supplementation is usually needed, together with advice to increase dietary iron (183). Low dietary intake of zinc and copper is also reported (211), and their monitoring and, if necessary, supplementation is recommended by the K/DOQI guidelines (191).

Little prospective evidence is available to support supplementing water-soluble vitamins above the RDA or the dietary intakes for age (191, 212, 213). In adult PD patients, blood concentrations of such water soluble vitamins as vitamin C, vitamin B_6 and folic acid have been reported to be low, as a consequence of inadequate intake, peritoneal loss and increased needs. According to K/DOQI guidelines (191), supplements of these vitamins should be considered if dietary intake alone does not meet the RDA, if blood vitamin levels are below normal values, or if there is clinical evidence of deficiency (212, 213). If folic acid is given to lower plasma homocystein levels, which is considered an independent risk factor for cardiovascular disease, administered doses largely overcome daily requirement and dialysate loss of folate (214). Supplements of the fat-soluble vitamin A should be avoided, because elevated levels of vitamin A have been reported in children and can lead to hypercalcemia, anemia and hyperlipidemia (215).

Peritonitis

Despite a series of improvements in connection technology and procedure, peritonitis remains the single most common complications that occurrs in CPD children, and the most important cause of morbidity, patient hospitalization and technique failure (2, 216). Peritonitis annualized rate varies in different part of the world, from 0.68 in North America (69) to 0.58 in Italy (217), and to the exceptional rate of 0.31 found in Japanese children (218). The same registry data show an inverse relationship between patient's age and peritonitis rate; in North America the youngest patients (0–1 years) have an annualized rate of 0.86, while the adolescents have a rate of 0.61 (69).

Reductions in observed peritonitis rate have been reported in association with use of a two-cuff catheter with a swan neck tunnel and a downward oriented exit site; intravenous antibiotic prophylaxis at the time of catheter placement; the flush-before fill technique; and a well structured, prolonged PD training (58, 63, 64, 69, 219–221). The questions whether local prophylaxis, by antibiotic placement in the nares and/or at the exit-site, should be provided to all patients or only to those patients/parents who are determined to be carriers by screening, and whether the preferred agent should be mupirocin or gentamicin requires further studies (58, 68, 222, 223). Prompt diagnosis of exit-site/tunnel infections, based on objective criteria, and effective antibiotic therapy, chosen according to the susceptibilities of the cultured organism, are also important elements of peritonitis prevention measures (219). Finally, time to first peritonitis episode was reported to be shorter in CAPD versus APD children (69).

Recent data from the International Pediatric Peritonitis Registry (IPPR), an initiative designed to prospectively evaluate the clinical application of the pediatric treatment guidelines (66, 67, 219, 224), showed that 44% of 501 reported peritonitis episodes were caused by Gram-positive and 24% by Gram-negative bacteria, while 2% were fungal peritonitis and in 30% of the episodes the culture remained negative. Staphylococci were the most frequently isolated organisms, with *S. epidermidis*/other coagulase negative staphylococcal organisms accounting for 24% and *S. aureus* for 22%. In comparison with previous pediatric peritonitis surveys (108, 225), there have been an overall shift from Gram-positive to Gram-negative causative organisms, that largely reflects a decreased absolute frequency of Gram-positive peritonitis, secondary to improved connection technology and decreased touch contamination, as well as to the implementation of *S. aureus* prophylaxis programs (58, 219). However, the proportion of Gram-negative organisms varied widely between regions (66). The likelihood of acquiring Gram-negative peritonitis was inversely related to patient age and its clinical presentation is more severe (224). *Pseudomonas* was the most frequent organism simultaneously isolated from the peritoneal cavity and exit-site, and 70% of peritonitis episodes caused by this organism developed in association with patient's previous exposure to antibiotics (67). The high rate of culture negative peritonitis, that should not account for more than 20% of peritonitis episodes (219, 226), could be the result of incubation of insufficient effluents volumes, long transport times, or extreme ambient temperature; therefore, the diagnostic work-up of peritonitis should be performed according to a standardized protocol (219).

In the year 2000, a set of 15 pediatric-specific peritonitis treatment guidelines that incorporated the specific risk factors and unique clinical aspects of children were developed by an international committee of physician and nurses under the auspices of the International Society of Peritoneal Dialysis (219). These treatment recommendations were largely opinion based as a result of the limited evidence on the topic that exists in the pediatric nephrology and infectious disease literature. They were also influenced by the concern that had arisen regarding the potential development of vancomycin-resistant organisms and of ototoxicity, vestibular toxicity, and nephrotoxicity of aminoglycosides, with the possible loss of residual renal function. Empiric intraperitoneal antibiotic therapy consisted of a first-generation cephalosporin combined with ceftazidime for children without risk factors for severe infections, while for children with certain risk factors, a combined administration of a glycopeptide and ceftazidime was recommended. Recommendation for treatment of Gram-positive, Gram-negative, and fungal peritonitis, and indications for catheter removal and replacement were also given.

Based on the first available data collected by the IPPR on the results of the application of the guidelines into clinical care (66, 67, 224), the following issues should be considered while developing the upcoming set of new ISPD peritonitis treatment guidelines for children:

- Local variability of causative organisms and, even more, of antibiotic sensitivities; the choice of antibiotic for empiric therapy should take into account patient- and center-specific history of organisms and their sensitivities;
- Only 80% of Gram-negative organisms were sensitive to ceftazidime as compared with 88% aminoglycoside sensitivity; therefore, empiric antibiotic therapy rec-

ommendation should include the initial use of aminoglycosides with subsequent, prompt modification of antibiotic management based on culture and susceptibility results (monitoring of blood aminoglycoside concentration should be considered for therapy exceeding the first 72 h);
- Identification of initial treatment response as an independent predictor of final outcome strengthened the importance of adequate empiric therapy;
- The observed increased risk of *Pseudomonas* peritonitis associated with topical mupirocin, and frequent exit-site care raises doubts about the current concept of topical prophylaxis;
- Special consideration should be given to the management of culture negative peritonitis.

Relapsing peritonitis is defined as the recurrence of peritonitis with the same organism as in the preceding episode, according to antibiotic susceptibilities within four weeks of completion of antibiotic treatment (216, 219). The most common organisms involved are slime-forming coagulase-negative staphylococci, which can survive antibiotic treatment in fibrinous adhesions and biofilm matrix on the catheter surface, *S. aureus*, and *Pseudomonas aeruginosa*, which may cause subclinical microabscesses in the tunnel region or in intra-abdominal adhesions. Catheter decontamination by local instillation of fibrinolytic agents and high-dose antibiotics can be attempted in case of relapsing peritonitis with coagulase-negative staphylococci. Early catheter removal should be considered as part of relapsing peritonitis management when the origin of the re-infection can be localized to the catheter tunnel and in case of *Pseudomonas* infections.

Finally, sclerosing encapsulating peritonitis is a rare but extremely serious clinical entity characterized by the presence of continuous, intermittent, or recurrent bowel obstruction associated with gross thickening of the peritoneum (227). Although primarily diagnosed in adult patients, it may also occur in children, typically those who have been on CPD for more than 5 years (228, 229). The presence of peritoneal calcifications on abdominal computed tomography scan in association with ultrafiltration failure is highly suggestive of the diagnosis, and may be an indication to discontinue CPD.

Peritoneal Catheter Exit Site Infection

Peritoneal catheter exit site infection (ESI) represents an important risk factor for peritonitis, and frequently requires catheter removal and replacement, especially after its recurrence (219, 230).

Since the subjective evaluation of an exit site status may differ widely, the diagnosis of an ESI should be based on objective criteria by using an objective scoring system of such infectious symptoms as swelling, crust, redness, pain on pressure, and secretion (219, 225). It should be remembered that a positive culture is not required for the diagnosis of ESI, and a positive culture in a non-inflamed exit site indicates colonization, not infection. Accordingly, *Staphylococcus epidermidis* is frequently cultured, but is rarely causative of ESI. On the contrary, *Staphylococcus aureus* is the most frequent involved organism, followed by *Pseudomonas*, enterococci, *E. coli*, and *Klebsiella*. ESI due to *Pseudomonas* species has become a frequent predisposing factor for peritonitis, especially in patients with previous antibiotic exposure (67).

Antibiotic treatment of ESI should be based on the susceptibilities of the cultured organism, and close monitoring of clinical response should be applied, especially for ESI that are treated with an empiric therapy in the absence of a positive culture or Gram stain results (57, 219).

Nonocclusive sterile dressing should be changed daily (or twice daily) as long as discharge from the sinus tract is evident. Exuberant granulomatous tissue can be removed by cauterization with silver nitrate.

Duration of antibiotic treatment should be of 2–4 weeks and for at least 7 days following complete clinical resolution of the ESI (219). After this period of treatment, refractory exit site and/or subcutaneous tunnel infection, and/or the development of a peritonitis episode secondary to the same organism are indications to peritoneal catheter removal. Catheter replacement during the same surgical procedure is possible unless infection is severe with purulent discharge, and/or a tunnel infection is present.

Mechanical Complications of Peritoneal Dialysis

Pain: After the catheter insertion wound has healed, PD should be a painless procedure. Apart from peritonitis, the presence of pain on inflow can be due to dialysate that is not at body temperature, local reaction to the low pH of commercial dialysate, local irritation from rapid dialysate inflow when straight catheters are used, omental or adhesion trapping of the catheter tip, and excessive distension of the abdomen. Rectal or suprapubic pain is usually due to migration or faulty placement of the catheter tip too deep in the pelvis between the bladder and the rectum.

Peritoneal pain may be referred to the shoulder, and in this case it may be due to air under the diaphragm. Outflow pain can be seen early in the course of dialysis when the peritoneal cavity is drained too completely (catheter irritation in the dry abdomen). Abdominal pain may also be due to processes not directly related to PD procedure, such as peptic ulcer disease, pancreatitis, or diffuse peritoneal calcification.

Investigation of painful dialysis should include abdominal radiographs that provide a three-dimensional picture of catheter location. Constipation is a frequent contributing factor to both poor catheter drainage and painful dialysis. Prescription of bicarbonate or bicarbonate/lactate-buffered PD solutions results in the resolution of pain that is secondary to the low dialysate pH (99). Tidal PD may be successful in reducing infusion pain, or pain related to peritoneal calcifications (231).

Hemoperitoneum: The incidence of hemoperitoneum in children is likely lower than in adult PD patients, but is difficult to ascertain accurately (231). Italian registry data reported an incidence of 1.3% in 363 children of less than 15 years of age at dialysis initiation (232).

Hemoperitoneum can be observed in female PD patients in the reproductive age group. It can be mild and can occur either 2–3 days before the onset of menses, or as a mid-cycle bleeding. A more severe bleeding can be associated with the rupture of ovarian corpus luteum cysts.

Causes of hemoperitoneum that are not associated with the female reproductive apparatus are several and include (231): post.catheter insertion; coagulopathy or anticoagulant therapy; intra-abdominal malignancies; physical activity; pancreatitis; cholecystitis; gastric ulcer; rupture of liver or renal cysts; retroperitoenal bleeding; cytomegalovirus infection; slerosing peritonitis; post-radiation, following laparoscopic cholecystectomy, and post-colonoscopy. In children, trauma to the abdomen or the catheter is a relatively common cause of bleeding.

Abdominal hernias: The incidence of hernias is higher in pediatric than in adult PD patients, and is inversely proportional to age (114, 233, 234). Hernias can be incisional, umbilical, or inguinal. Incisional hernias occur more commonly when the catheter is placed through the midline instead of the paramedian approach through the rectus muscle. Inguinal hernias are more common in boys, become clinically evident at a younger age than in girls, and are frequently bilateral. A patent processus vaginalis can be found in up to 80–90% of newborns, and its incidence progressively falls with increasing patient age. With instillation of PD fluid in the abdomen, a patent processus vaginalis may progress to frank hernia quite early in the course of PD treatment (233). Laparoscopic inspection of the abdominal cavity at the time of catheter insertion allows to detect the presence of patent processus vaginalis and/or of inguinal hernias, and to conduct prophylactic herniorraphys, if needed (61, 62).

Since IPP for a given intraperitoneal fill volume presents marked interindividual variations, the optimum dwell volume to maximize dialysis clearances while reducing the risk of hernia cannot be easily defined, and should be personalized according to each individual patient characteristics (41, 235). Hernias left untreated tend to increase in size as PD treatment continues, and almost all hernias must eventually be repaired surgically. Before and for 1–2 weeks after repair, dwell volume should be reduced by up to 50%, and patient should receive APD (without or with small daytime dwell) if possible.

Hydrothorax: The incidence of hydrothorax is reported to be 2.8–3% in children on PD (236–238). Pleural effusion is usually unilateral (more commonly on the right), and can develop within hours to days of initiating PD, or may be delayed up to several months. It may be responsible for a gradual onset of inadequate UF, or it can dramatically present with dyspnea in the absence of volume overload. In many patients, fluid effusion occurs through a small, often congenital, diaphragmatic defect, while in others no communication can be demonstrated, and the etiology is postulated to be via sub-diaphragmatic lymphatics (231).

Management of hydrothorax is to stop PD temporarily, or to reduce dwell volumes, changing the patient to an NIPD regimen. Since recurrent hydrothorax may result in PD discontinuation and transfer to hemodialysis, surgical repair of diaphragmatic defects has been successfully performed in pediatric patients (237). Suggested treatments also include pleurodesis with tetracycline (239), talcum powder, or autologous blood.

Treatment Outcome

Long-Term Preservation of Peritoneal Membrane Function

The stability of the peritoneum as a dialyzing membrane is of particular interest because of the potential for the long-term need for CPD, especially for children going back to dialysis after renal transplant failure. In pediatrics, limited data are available on the long-term viability of the PM in terms of UF capacity and solute transport. Infection would seem to be a likely contributor to decreased long-term membrane function, but data are conflicting.

In a retrospective study in children, peritonitis caused by *Pseudomonas aeruginosa* or alpha streptococcal organisms was an independent predictor of membrane failure, defined as the severe loss of UF capacity (240). When two groups of children were assessed by means of solute D/P ratios derived during the performance of a PET conducted without standardized study mechanics, a decrease of the D/P creatinine ratio over time was observed in those with a history of peritonitis, while the D/D_0 glucose ratio was unchanged over time in both groups (241). The peritonitis history of the cohort of children studied to develop the pediatric PET curves was reanalyzed and revealed higher MTAC for glucose and creatinine in those who had previous episodes of peritonitis (29, 242). Similarly, in a pediatric prospective evaluation of the PM as assessed by PET testing over a mean of 20 months between studies, there were no changes in MTAC for glucose or creatinine over time. However, in the children with a history of peritonitis, the MTAC values increased (243). The role of the duration of CPD treatment was studied in a group of children who did not experienced peritonitis; no remarkable change was found for D/P creatinine or D/D_0 glucose during the first 24 months of CPD but thereafter, D/P creatinine increased gradually and D/D_0 decreased gradually (244). Finally, in one of the few studies evaluating peritoneal biopsy material from children, a reduced density of mesothelial microvilli presumably related to the duration of CPD treatment and the presence of peritonitis was documented, as was fibrosis of the PM. However, the latter did not correlate with the frequency of peritonitis or the duration of CPD therapy (245).

Standard PD solutions with low pH and containing high concentrations of lactate and glucose have been demonstrated to negatively affect the peritoneal membrane integrity, mesothelial cell viability, residential peritoneal cells and also to inhibit phagocytic functions. An increasing body of experimental evidence supports the idea that the peritoneal hypervascularization and fibrosis observed in long-term PD are causally related to the acute and chronic toxicity of conventional PD solutions (74–76, 78–80, 82, 98). The potential of dialysis regimens based on more biocompatible low-GDP, pH-neutral PD solutions to slow down peritoneal damage associated with long-term CPD treatment should be evaluated in extended clinical trials.

Health-Related Quality of Life of Children on Peritoneal Dialysis

Health-related quality of life (QOL) refers to the measure of a patient's functioning well-being, and general health perception in each of three domains: physical, psychological, and social (246). In turn, a patient's QOL is an important indicator of the effectiveness of the medical therapy he or she receives (247). Children on chronic dialysis have specific challenges such as physical changes related to illness and to dialytic access, chronic dependence on medical equipment to sustain life, maintaining a restricted dietary and fluid regimen, time away from school and peers, and the knowledge that they will live their whole lives with the recurrent cycle of dialysis and transplantation (248, 249). The ability to attend school every day may be one of the most beneficial features of CPD for children. Data from the North American Pediatric Renal Trials and Collaborative Studies (NAPRTCS) 2007 Report showed that among school-age patients maintained on CPD, 78% were attending school full-time and 9% part-time, compared to 53 and 28% of children on hemodialysis (69). Very few restrictions to physical activities are necessary. This philosophy also facilitates the ability of these children to participate in summer camping experiences that are commonly coordinated by dialysis team personal (250). Nevertheless, data suggesting a decline in the QOL over time for adult patients on CPD mandate the performance of formal investigations of QOL in pediatric patients to better evaluate this critical aspect of patient care (247–249). So far, the results of the studies describing health-related QOL in children with ESRD are often conflicting, reporting either significantly lower QOL scores than in healthy controls (251, 252), or scores similar to that of the general population despite lower physical functioning (253). No difference was noted between hemodialysis and PD patients (251). A potential limitation of those studies was that the instruments used were not ESRD specific and cannot yield detailed information for the specific factors that impact on QOL in children with ESRD. Recently, an ESRD-specific health-related QOL instrument has been proposed (254); initial data support the feasibility, reliability, and validity of the PedsQL 3.0 ESRD Module, but additional validation testing is still in course to further establish the psychometric properties of this instrument.

Patient and Technique Survival

Technique failure: Data on termination of CPD therapy are available from national registries' reports. A NAPRTCS study in 2003 (255) showed that 194 (20%) of the 997 incident PD patients transitioned to hemodialysis over the 6-year study period. Excessive infections largely represented the main reason for CPD failure (43%), followed by inadequate UF or solute clearance, patient/family choice, and dialysis access failure. Similar data have been reported by the Italian Registry in a study on a smaller group of patients (n = 295) that had started their first course of CPD from 1989 to 2000; among 44 (15%) patients who were switched to hemodialysis, peritonitis and/or catheter exit-site infection accounted for up to 66% of technique failures (2). In both reports, patients showed a slow and steady increase in CPD termination over time as they transitioned to hemodialysis. In the Italian study (2), actuarial technique survival of CPD patients resulted significantly higher than that of 160 patients on hemodialysis; PM transport failure was reported in 6 out of 44 cases of CPD abandonment (13.6%), and was registered after a mean of 46 months of CPD (range 7–102 months). A higher percentage of transfer to hemodialysis due to UF failure and/or underdialysis (27% of total transitions) was reported by the Japanese Registry (256). This figure might be explained by the fact that in Japan, where cadaveric transplantation is rare, the percentage of children maintained on CPD for longer than 5 years was 24.8% compared with 5.9% in North America (227) and 7.1% in Italy (2). In a single center study, Gulati et al. found the likehood of technique failure to be increased in PD patients with hypoalbuminemia 1 month after dialysis initiation (257).

Patient mortality: Dialysis in children carries a significantly higher mortality rate than that for the age-matched healthy population, and also than that for children who have received a renal transplant (258–260). An overall mortality rate between 5.8 (2) and 8.5% (108) was noted for the pediatric CPD population. An assessment related to age of NAPRTCS data (4) revealed a mortality rate of 14.9% for patients younger than 5 years at dialysis initiation, which was significantly greater then the mortality rate of 5.7 and 4.5% for patients in the 6–12 and older than 13 years age groups. The mortality rate of patients 0–1 years and 2–5 years was 18.9 and 8.3% with 1- and 3-year post-initiation mortality rates of 15.3 and 32.5% for the youngest patients, and 5.8 and 13.7% for the 2- to 5-year age group. Data from the Italian pediatric dialysis registry showed that actuarial patient survival was significantly lower in the group of children of less than 5 years at the start of CPD than in the group of 5- to 15-year-old patients (2). These reports, as well as the data collected from children in Japan (261), confirm the high-risk status of the infant and young child with ESRD. At the same time, the outcome of dialysis initiated in the neonatal period (<1 month of age) is comparable to that of dialysis in children <2 years of age (113).

The results of several studies confirmed the importance of non-renal co-morbidities, such as multi-system involvement from inherited disorders, prematurity, pulmonary hypoplasia, severe development delay, congenital cardiac disease, inherited metabolic disorders, malignancy, or chronic renal disease following overwhelming infection with multi-organ damage, as significant risk factors for increased mortality in infants and young children as well as in older CPD pediatric patients (2, 115, 117, 118).

Differences in the rate of mortality with the type of dialysis were reported by McDonald et al. (259), that found an overall mortality rate of 4.8 (95% confidence interval, 4.2–5.6) per 100 patient-years among children receiving hemodialysis, and of 5.9 (95% confidence interval, 4.9–7.2) per 100 patient-years among those receiving PD. On the other hand, the survival rates of two groups of age-matched patients (5–15 years of age at the start of dialysis) treated in Italy either with CPD (n = 193) or with hemodialysis (n = 160) were not significantly different (2). Similarly, Wong et al. (143) did not find any difference in adjusted relative risk of death for 1,723 pediatric patients (<18 years at dialysis initiation) treated with HD versus PD.

Peritoneal membrane function was reported to be an independent predictor of patient survival, with patients with high transporter status and, therefore, decreased UF capacity demonstrating worse outcomes (260, 262, 263).

The primary reported causes of death for pediatric PD patients of all ages are cardiovascular disease and infection. Cardiovascular disease is increasingly recognized as a major cause of morbidity, and a life-limiting problem in young patients with chronic kidney disease, giving a 1,000-times higher risk of cardiovascular death than in healthy age-adjusted population (264, 265). Indeed, long-term dialysis may be associated with an increasing incidence of cardiovascular diseases, as a result of prolonged exposure to cardiovascular risk factors, such as hypertension, dyslipidemia, the presence of a pro-inflammatory state and endothelial dysfunction, and vascular calcification due to calcium phosphorus metabolism disturbances (266). Pediatric PD registries reported cardiovascular disease as the cause of patient's death in 20.9% (108), 43% (260), and 58,8% (2) of cases.

Infection is the second leading cause of death for PD pediatric patients, accounting for 17.6% (2) to 27.9% (108) of cases, and is the primary cause of death for patients 0–5 years of age (108). Warady et al. (224) reported 5 (0.9%) lethal outcomes in the follow-up of 548 episodes of peritonitis that were recorded in 392 patients by the International Pediatric Peritonitis Registry; three patients died from uncontrolled hypervolemia and one from venous access complications when switched to hemodialysis, while in one case the cause of death remained unclear. Broad categories for cause of death are currently reported by dialysis registries and multicenter studies, while a better characterization of these diagnoses would be necessary in order to attempt preventive measures.

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73 Hemodialysis

Lesley Rees

Chronic dialysis, including both hemodialysis and peritoneal dialysis, is now technically feasible in children of all ages, including infants (1). In countries with active pediatric transplant programs, however, it is not used as the first choice of chronic renal replacement therapy, as most pediatric nephrologists would aim for pre-emptive transplants for their patients. There are exceptions to this, including the neonate, in whom a period of dialysis may be necessary until adequate size for transplant is reached, the child needing urgent treatment because of presentation in end-stage kidney disease, and children needing native nephrectomies, or other corrective surgery pre-transplant. This means that at any time, around 20% of the pediatric end-stage kidney disease population is being dialyzed (2, 3). Only a minority of children are able to escape a period of dialysis altogether, either while waiting for their first transplant or because of subsequent graft failure (4).

The choice of dialysis modality varies from country to country. In Europe, peritoneal dialysis is the commonest modality choice, with a ratio of 2:1 peritoneal dialysis to hemodialysis (2, 5), whereas in the US this ratio is reversed (3). Of course, many children will need to switch modality, usually from peritoneal to hemodialysis because of peritoneal membrane failure. Total numbers of children on hemodialysis throughout the world are small, for example being only 70 in the UK (6) and around 900 in the US at any time (3). Despite these international differences in choice of dialysis modality, there are some general rules, including the avoidance of chronic hemodialysis in the infant due to difficulties with vascular access, and the use of hemodialysis when there is technique failure, intrabdominal pathology or social difficulties that preclude peritoneal dialysis. Because of this bias towards the use of hemodialysis in preference to peritoneal dialysis in the more complicated patient, it might be expected that outcome would be inferior in hemodialysis patients, but there is no evidence to suggest a difference either in morbidity (7) or transplant outcome (8). There is good evidence, however, that the longer the duration of dialysis, the greater the risk of cardiovascular disease and premature death, regardless of the dialysis modality (9).

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There is no prescribed GFR at which children should start dialysis. The optimum time varies between individual patients and requires assessment not only of renal function, fluid status and biochemical abnormalities but also of wellbeing, both physical and psychosocial. Some children can be managed extremely successfully with diet and medications, and continue to remain well with good growth for prolonged periods with very poor renal function. This is particularly true for young children with structural renal abnormalities, in whom renal function can remain stable for many years, and who often continue to produce large volumes of urine. However, uremic symptoms frequently begin when the GFR falls below 15 ml/min/1.73m^2. In the US, registry data (USRDS) shows that the mean GFR of 4,808 children who were initiated on dialysis was 8.2 + /−4.1 ml/min/1.73m^2; 49.6%, had an estimated GFR greater than 10 ml/min/1.73m^2 and 7.3% an estimated GFR less than 5 ml/min/1.73m^2 (10).

What is clear is that the care of children on hemodialysis requires input from a large multidisciplinary team including pediatric nephrologists, renal nurses and dieticians, transplant surgeons, urologists, interventional radiologists, anesthetists, pharmacists, play specialists, schools teachers, psychologists and social workers, all of whom need the special skills necessary to treat such children. Availability of hospital school teachers and liaison with local schools is particularly important for children on hemodialysis, who spend so much of their time at the hospital. Many children with CKD have associated comorbidities, which can include congenital abnormalities in other organ systems. This amount of expertise can only be provided by bringing patients together in specialist pediatric nephrology centers generally sited in major cities. This inevitably means that some families have to travel long distances to obtain the best possible care for their child, placing particular stresses on the family with a child on hemodialysis.

Principles of Hemodialysis

In the normal kidney, water is removed from the blood by ultrafiltration (UF) and solutes by convection. Solutes of

molecular weight below 40,000 daltons are able to pass freely across the glomerular basement membrane allowing the passage of low molecular weight molecules and retention of larger molecules such as plasma proteins. The purpose of hemodialysis is to mimic the role of the kidney, removing waste products and prescribed quantities of solutes and fluids that have accumulated between dialysis sessions. The semi-permeable membrane in the dialyzer allows the passage of water and small molecular weight molecules and inhibits the movement of larger molecules. Solute transfer (clearance) occurs by diffusion and convection and water is removed by ultrafiltration.

Diffusion

Diffusion is the movement of a solute down a concentration gradient. The rate of diffusion of a solute is inversely proportional to its molecular weight and directly proportional to the temperature of the solution, in this case the dialysate. It is also affected by the electrical charge of the solute. The hemodialysis membrane itself will also affect rate of diffusion; its permeability is a reflection of its thickness and the number of pores in it and their density, and diffusion across it will also be affected by its surface area. The transmembrane concentration gradient is maintained and maximized by high flow rates of blood and dialysate in opposite directions (countercurrent). As well as being the main mechanism for the removal of solutes, diffusion is also responsible for the replenishment of bicarbonate. As they are buffered in plasma, hydrogen ions are in low concentration so are not readily removed by dialysis. Fluid can stagnate on either side of the dialysis membrane, and this is referred to as 'unstirred' fluid, which can decrease diffusion. This can be minimized by maintaining high flow rates, and by dialyzer design. Clearly the dialysate only comes into contact with the intravascular compartment, so, although rapid equilibrium can take place with circulating solutes, predominantly intracellular solutes such as phosphate take time to move into the blood and clearance is less effective. Furthermore, protein bound solutes will not be removed.

Ultrafiltration (UF)

Ultrafiltration is the process whereby water is moved across the membrane by convective flow down an osmotic gradient or a pressure gradient, which is created by generating a transmembrane pressure (TMP) within the dialysate compartment by the dialysis effluent pump. Large molecular weight molecules are removed better by convection than diffusion. The net rate of ultrafiltration is affected by the surface area, structure and thickness of the dialyzer, blood flow rate and the transmembrane hydrostatic pressure and osmotic pressure.

Convection

Convection (solute drag) is the passive movement of solute 'dragged' by water moving down an osmotic or pressure gradient. It is independent of the concentration gradient but dependent on the ultraflitration rate and the sieving properties (coefficient) of the dialyzer, i.e., if the molecular weight of the molecule is such that it is not held back and sieving does not occur, it is swept across the membrane by the ultrafiltrated water.

Mass Transfer

Removal of toxins depends on their distribution in the body compartments: those in the intravascular space will be rapidly removed, but those that are predominantly intracellular will need time to move into the intravascular space as concentration gradients change. Molecular size will also affect removal: *Table 73-1* shows the definitions of uremic retention solutes by molecular weight, and their method of removal during dialysis. Uremic

Table 73-1

Definitions of uremic retention solutes by molecular weight (MW), and their method of removal during dialysis

Solute	Molecular weight	Example (MW)	Method of removal
Small solutes	<500	Urea (60), creatinine (113)	Diffusion
Middle molecules	300–5000	Vitamin B12 (1355)	Diffusion/convection
Low Molecular Weight proteins	5000–50000	β2-microglobulin (11800)	Diffusion/convection
Large proteins	>50000	Albumin (60000)	Convection

retention solutes such as urea and creatinine are rapidly removed. Large numbers of biochemically active, potentially toxic 'middle molecules' have been identified. Many are peptides that affect many organs and may contribute to the symptoms and morbidity of uremia, such as poor appetite, anemia, inflammation and cardiovascular disease. Well over 100 have been identified. The best known is ß 2-microglobulin, which causes dialysis related amyloid. Recently interest has focused on the dinucleotide polyphosphates, structural variants of angiotensin II, interleukin-18, p-cresylsulfate and the guanidines. Large pore dialyzers and hemodiafiltration may improve their removal by increasing convection. Some protein bound middle molecules may also be toxic, one example being leptin, and are particularly difficult to remove by any dialysis technique (11).

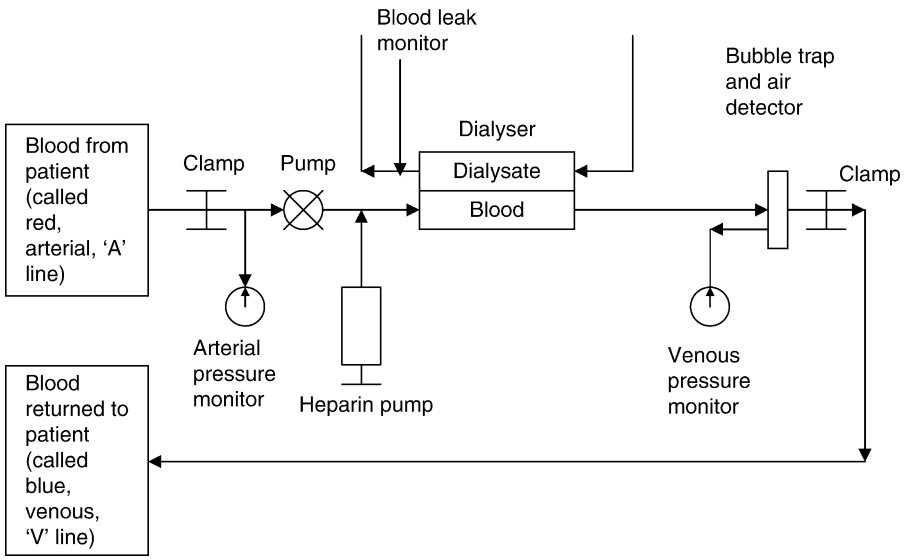
The HD Machine, the HD Circuit, Dialysate, Water and Dialyzers

The Hemodialysis Machine and Blood and Dialysate Circuits (Fig 73-1)

There are two circuits, the blood circuit and the dialysate circuit, that run in opposite directions, separated by the semi-permeable membrane of the dialyzer. The blood circuit includes lines through which blood is pumped from the patient to the dialyzer, and then back to the patient. Conventionally, the lines taking blood from the patient are called arterial, 'red' lines, and those returning blood to the patient are the venous, 'blue' lines. Pressures in the blood lines are monitored in the 'arterial' segment between the blood pump and the dialyzer, and in the 'venous' segment at the venous bubble trap and air detector, so that vascular access problems are indicated by arterial or venous pressure alarms. Low arterial pressure alarms indicate that there is an insufficient blood flow reaching the blood pump. This is commonly referred to as 'sucking' and is usually a result of poor access position in the vessel. Arterial pressure between 150 and 200 mmHg limits trauma to the vascular endothelium. A low venous pressure alarm also indicates poor blood flow. A high venous pressure alarm indicates that there is an occlusion to the flow of returning blood. It is due either to poor access position, fistula stenosis or the presence of clot formation. If an alarm is activated, the blood pump is stopped and the lines are clamped. Precise occlusion of tubing by the pump is vital in order to prevent backflow, foaming and hemolysis. There is a separate system for delivery of anticoagulant to the blood circuit before the dialyzer. Sample ports in either side of the circuit allow blood samples to be obtained whilst the child is on the machine (Fig 73-1).

The dialysate circuit is separated from the blood circuit by the semi-permeable membrane of the dialyzer.

Figure 73-1
The blood and dialysate circuits.



Dialysate is run in the opposite direction to the blood to maintain the concentration gradient between the blood and the dialysate. There is a blood leak detector (to detect rupture of the dialyzer fibers with leakage of blood into the dialysate) just distal to the dialyzer. The machine is able to further purify the water supply using reverse osmosis, and to prepare the dialysate by mixing the water with prescribed quantities of electrolytes and bicarbonate.

There are certain specific features that are necessary in a pediatric hemodialysis machine: it must be capable of low blood flow speeds, and be able to use lines of varying blood volumes and to measure and remove very small amounts of fluid so that it can be used even in infants. A volumetric fluid removal system allows accurate fluid removal. It does this by measuring the inflow and outflow volumes from the dialyzer so that ultrafiltrate volume is measured directly, allowing the automatic calculation and application of a pressure across the dialysis membrane, the transmembrane pressure (TMP) and prescribed fluid removal throughout the dialysis session. New machines have systems for continuous on-line monitoring of the hematocrit and therefore blood volume, oxygen saturation, blood pressure and pulse and for Kt/V urea.

Dialysate

Dialysate is prepared during the dialysis session. Sodium, potassium, magnesium, calcium, chloride, dextrose and bicarbonate are added to the purified water by the machine and their concentrations can be varied within certain prescribed limits. They are mixed and proportionated by the dialysis machine. Standard settings are 138–140 mmol/l for sodium, 2 mmol/l for potassium, 1.25–1.75 mmol/l for calcium, 0.5–1 mmol/l for magnesium and glucose 1g/l. The dialysis machine monitors the electrical conductivity of the dialysis solution to ensure the correct proportion of water to concentrate is occurring before it is delivered to the hemodialyzer. Bicarbonate is used as a buffer. The bicarbonate preparation has a separate acidic component to prevent precipitation of calcium and magnesium carbonate and is added by a second proportionating pump. Inevitably with time there will be deposition of calcium and magnesium salts, so the dialysate system needs daily decalcification. The dialysate is warmed before delivery to the dialyzer. Temperature selection is between 34.5 and 37.5 °C. Lower temperatures are associated with less hypotensive events, but diffusion is increased at higher temperatures. Typical dialysate flow is 500 mls/min (range 300–800 ml/min).

Water

Although water from the mains supply is satisfactory for human consumption, it is not suitable for dialysis. At each session the patient's blood is directly exposed to 120 liters of water and all its potentially hazardous contaminants, including particles, dissolved substances such as ions, trace elements, organic substances, nitrogen compounds, microorganisms and their toxins. Therefore filters of progressively smaller sizes to remove particulate matter, activated carbon for chlorine and chloramines and water softeners to remove calcium and magnesium must be in the water circuit before the water reaches the dialyzer. Finally, a reverse osmosis unit purifies softened filtered water before it is of a quality suitable for dialysate production by removing residual particulates, dissolved inorganic and organic substances, microorganisms and toxins.

There are detailed standards for the design of the water delivery circuit and its management, and for all aspects of water purity (12, 13). Water quality is defined as 'pure' or 'ultrapure' (*Table 73-2*). 'Pure' water is adequate for conventional dialysis, but ultrapure water is preferable, and is essential for high flux dialyzers, when back filtration can occur, and in high flow hemofiltration, when large volumes of replacement fluids are used (see below). Even very low levels of endotoxin in the water can cause cytokine mediated inflammation which, in turn, may contribute to the increased risk for cardiovascular disease seen in patients on dialysis (14). Water quality standards need to be maintained and regularly checked to ensure bacterial contamination and mineral content are within acceptable limits. Microbiological contamination of the delivered water should comply with published recommendations and acceptable levels of all other recognized contaminants have also been defined (12, 13).

Table 73-2

European Pharmacopoeia definitions for the upper limit of water quality (12)

	Bacterial growth (cfu/mL)	Endotoxin (EU/mL)	Cytokine induction
Mains water	200	5	+
Regular water	100	0.25	+
Ultra-pure	0.01	0.03	–
Sterile	10^{-6}	0.03	–

The Hemodialyzer

The hemodialyzer is composed of two compartments, one for blood and one for dialysate, which are separated by the semi-permeable membrane. A capillary (hollow fiber) configuration achieves the maximal membrane surface area over which blood and dialysate make contact in a relatively low fill volume with low compliance.

The membrane can be composed of modified cellulose or a synthetic material. Unmodified cellulose membranes are the least biocompatible, and may cause activation of complement and leucocytes and a severe allergic reaction within minutes of starting dialysis. Sterilizing solutions (e.g., ethylene oxide) may also cause allergic reactions.

Each type of dialyzer has an ultrafiltration coefficient (KUf), which describes its ability to remove water. For example, a KUf of 2.0 means that 2 ml/hr of UF will occur for each mmHg of TMP at a blood flow rate of 200 ml/min. KUf depends on the surface area of the dialyzer as well as its membrane characteristics. Dialyzers with KUfs of less than 10 are referred to a low-flux and those with a rate of 15–60 ml/hr/mmHg are called high flux. Synthetic membranes tend to be high-flux.

Solute transport properties of dialysate membranes are expressed as the mass transfer-area coefficient (KoA). Dialyzers of usual efficiency (for removal of small solutes) have a KoA of 300–500; high-efficiency dialyzers may have a KoA of more than 700. Clearance values, generally achieved at blood flow of 200 ml/min, for creatinine, urea, vitamin B_{12} and phosphate are given for all dialyzers in the manufacturer's specification sheet. Clearance of a solute is inversely proportional to the molecular size; most dialyzers allow the passage of solutes of up to 5–10,000 Daltons.

Dialyzers may be sterilized with irradiation, heat or ethylene oxide. The latter is particularly likely to cause reactions. Priming the circuit with 1–2 L of saline to expel air and prepare the capillaries for use will also help flush out remaining ethylene oxide and other soluble compounds in the circuit, which may be toxic or cause allergic reactions at the commencement of dialysis.

Factors Affecting the Dialysis Prescription

Types of Dialysis

Conventional hemodialysis uses a low flux (small pore size) membrane and solute removal is primarily by diffusion. High efficiency hemodialysis refers to a more rapid removal of urea, K^+ and other small solutes. This is achieved by using a low flux membrane with a high efficiency (KoA) for removal of small solutes. It is also achieved by using a larger surface area membrane and a high blood flow. Because conventional dialysis is principally diffusive based, even when using high flux dialyzers it is limited in clearing middle-sized molecules (MW 200–20,000), which are better removed by convection.

High flux hemodialysis utilizes high flux (large pore size) membranes. It is more efficient in removing solutes that are substantially larger than urea (middle and large molecules such as vitamin B_{12} and ß-2 microglobulin respectively), but may not be more efficient than conventional hemodialysis in removing small solutes. Better clearance of ß-2-microglobulin may reduce the risk of amyloidosis. A more effective form of dialysis is to superimpose convection upon standard diffusive blood purification using hemodiafiltration. It is possible to use a high flux hemofilter to ultrafilter up to 30% of the blood volume passing through it. The desired volume of replacement fluid is then infused into the blood circuit. Ultrapure dialysate is necessary. Care must be taken that excess fluid removal does not occur, so a volumetrically controlled machine is essential. High flux dialyzers should be considered in larger children who are likely to be on hemodialysis for more than a few years and those showing evidence of amyloidosis. For other children the balance of evidence favors low flux synthetic and modified cellulose over unmodified cellulose membranes (15).

Online hemodiafiltration machines are available which generate ultrapure dialysis fluid. High flux membranes (Kuf $\geq$ 50ml/h/mmHg, solute permeability KoA urea >600, ß-2 microglobulin >60 ml/min) are necessary. The surface area of the membrane needs to be large and blood flows high, which restricts use in small children as the UF volume is limited by the blood flow rate. In this technique, up to 20 L of 'extra' fluid, over and above the patients' interdialytic fluid gain is removed through the dialyzer and an equal volume of physiological 'replacement' fluid is returned to the blood either before (predilutional) or after (post-dilutional) the dialyzer. If the replacement fluid is given before the dialyzer the UF can be even as high as the blood flow rate. If it is given after the dialyzer the volume that can be removed is less, because of hemoconcentration and the risk of clotting in the dialyzer.

Size of the Extracorporeal Circuit

The lines and the hemodialyzer are selected on the basis that the child can tolerate 8% −10% of their total blood

Table 73-3

Examples of the volumes of lines available for dialysis according to the size of the patient

Patient size	Venous (mls)	Arterial (mls)	Total (mls)
Mini-neonatal (<6 kg)	21	8	29
Neonatal (6–12 kg)	22	18	40
Pediatric	42	30	72
Adult	70	62	132

volume (TBV, 80 ml/kg estimated dry weight) in the extracorporeal circuit. For example, a child weighing 10kg has a TBV of 800mls (10 × 80 ml), therefore the extracorporeal circuit can be 64–80 mls. The total volume of the lines and hemodialyzer therefore must not exceed 64–80 mls. There are lines that are made in a variety of sizes by different companies. Some examples are shown in *Table 73-3*.

Lines are primed with saline. However, in the very young, even the smallest circuit may exceed the safe extracorporeal volume. In this situation, the circuit must be primed with blood. The blood is not washed back into the child at the completion of dialysis to prevent hemoconcentration. Obviously this is not ideal because the repeated prescription of blood increases the risks of infection and HLA sensitization, with its consequent difficulties for transplantation. This is one of the reasons for opting for peritoneal dialysis in infants.

The dialyzer is selected on the basis of its surface area and the priming volume. Roughly, the surface area should be equal to but not exceed that of the child's. At present, hemodialyzer surface areas range from 0.25 m^2 up to 1.7 m^2 and above. The greater the surface area, the greater the clearance of water and solutes.

Frequency of Sessions

It is conventional to dialyze for 4 hours 3 times a week. Middle molecules diffuse slowly into dialysis fluid, so treatment times that are less than this have a proportionately greater deleterious effect on their clearance than other molecules. This may have implications for the long-term health of dialysis patients. There is increasing evidence that, even in children, more intensified dialysis is beneficial. This can vary from long intermittent hemodialysis ranging from 6 to 8 hours 3–7 times per week, or short frequent hemodialysis for 2–3 hours 5–7 times per week, and can be carried out in center or at home. Intensified dialysis has been reported from several pediatric centers, but, for resource reasons, is usually reserved for those on conventional dialysis who would benefit from longer hours because of chronic fluid overload or hyperphosphatemia, because of poor growth, or for infants, whose predominantly liquid diet requires removal of relatively large fluid volumes. However, some children and families are not prepared to commit to the increased time spent on the dialysis machine. Home hemodialysis is possible for children who have adequate housing and a family member who is prepared to take on the responsibility. Reports demonstrate better phosphate and BP control, with many patients being able to come off all their medications, improved appetite and growth, and, despite increased time spent dialyzing, improved quality of life (16–20).

Blood Pump Speeds

The speed at which the blood is pumped out of the child and around the circuit is calculated as the equivalent of their extracorporeal volume total i.e., up to body wt (kg) × 8 ml/min. Thus, the 10kg child, with an extracorporeal circuit of 64–80 mls can have blood speeds of up to 80 ml/minute. The blood pump flow rate is a very important determinant of solute clearance, allowing maximum diffusion and convection.

Estimation of Dry Weight and Fluid Removal

The aim is to end the hemodialysis session with the child at their target weight, which is the weight below which the child will become symptomatically hypotensive. However, estimation of dry weight can be difficult and needs to be reassessed at least monthly, and more often in very small children, in particular infants, to allow for growth. It can only be determined by careful but persistent fluid removal to achieve a normal BP after dialysis. The child who is always hypertensive is likely to be above their dry weight; antihypertensives can usually be discarded when this is achieved. However, attainment of dry weight with conventional three times a week dialysis can be difficult in the child who has high interdialytic weight gains requiring large UF volumes. Much better results have been obtained with daily dialysis, with most children no longer needing antihypertensives at all (16–18).

The amount of fluid to be removed is calculated by the weight gain since the previous session (assuming dry

weight had been achieved then), the volume of saline required for the 'washback', and any drinks to be consumed during the session. The hemodialysis machine will adjust the TMP accordingly, depending on the time (in hours), and the venous pressure (which is affected by the blood speed, and peripheral resistance), to give an hourly UF rate.

The greater the TMP that is set, the greater the amount of fluid that will be removed from the child, and the more likely it is that the child will feel unwell. High UF rates whilst diffusion is occurring are not well tolerated. To counteract this, isolated UF can be performed, in which the flow of dialysate is halted, therefore dialysis and hence diffusion ceases so that the osmolality of the intravascular space is maintained. This allows more fluid to be removed more quickly from the child and is useful when there are large volumes requiring ultrafiltration. As dialysis does not occur during isolated UF, the length of time on the dialysis machine will increase.

The amount each child will tolerate losing per hour varies but 10 ml/kg/hr is a safe starting point. Up to 600 ml/hr can be removed in children weighing > 40 kg who are consistently volume overloaded. No more than 5% of body weight should be removed in one session, or 0.2 ml/kg/min.

Fluid loss (UF) can only be achieved if the fluid is in the vascular space. As the vascular space empties, refilling must occur from the other compartments, to allow ultrafiltration to continue. The child will show signs of hypovolemia if ultrafiltration (from hemodialysis or isolated UF) continues unchecked. If hypovolemia occurs, the UF rate should be decreased and the child given a drink or bolus of saline to correct hypotension, if necessary. Many patients collapse having had no prior warning of feeling unwell, therefore close monitoring of blood pressure and other observations (including peripheral temperatures) is important during isolated UF. However, chronic hemodialysis children are often able to recognize the early warning signs and can prevent such episodes.

Another way to prevent intravascular volume depletion due to slow refilling from the extravascular space is to vary the concentration of the dialysate sodium throughout the course of the session. The machine can be programmed to deliver a sodium concentration higher than that of the plasma at the beginning of the session so that sodium diffuses into the plasma and balances the change in osmolality caused by diffusive urea removal. The sodium concentration in the dialysate is then progressively reduced. This is important as leaving the patient with a high plasma sodium will stimulate thirst between sessions. Although this technique, which is has been called sodium ramping, profiling or modeling, helps intradialytic hypotension, the danger is that there is inadequate sodium removal, hence contributing to chronic fluid overload and hypertension.

Newer machines are able to monitor circulating blood volume by determining changes in hematocrit. It has been shown that a decrease in blood volume of >8% in the first 90 minutes or >4% thereafter is likely to lead to hypovolemia (21).

The Electrolyte Concentrations in the Dialysate and Blood Biochemistry

Sodium – the dialysate sodium must be within 10mmols of the child's plasma sodium to avoid disequilibrium. The sodium dialysate concentrate level can be altered on the machine within preset parameters. If it is set below that of plasma, then more sodium and therefore water will be removed, although this may cause intradialytic hypotension. Sodium modeling can improve these symptoms but may results in more salt and water retention (see above).

Potassium – the standard dialysate potassium is 1–2 mmol/l. Adjustment may be needed for children with low plasma potassium levels or in those requiring a long dialysis session, when a dialysate potassium of 3–3.5 mmol/l can be used; if the child has a very high potassium, a zero-potassium dialysate can be used for a short period of time, before reverting to the standard potassium dialysate. There is a danger of severe hypokalemia if a zero-potassium is used for too long. The use of serum potassium monitoring equipment (ionometer) facilitates the management of hyperkalemia.

Bicarbonate – the dialysate level can be adjusted on the machine, within preset limits. The level is usually around 35 mmol/l.

Calcium – the standard dialysate level is 1.75 mmol/l. This does, however, result in an influx of calcium into the patient and a rise in serum calcium post dialysis. Dialysates containing calcium concentrations from 1.25 mmol/l (which is equivalent to the blood in the normal child) are available, and can be used in situations of hypercalcemia in order to remove calcium from the patient.

Phosphate – clearly there is no need for phosphate in dialysate. After an initial fall in plasma levels during the first 1–2 hours, movement from the intracellular compartment is slow, so very little is removed thereafter and levels are back to 80% of pre-treatment values by 12 hours post dialysis so that dietary phosphate restriction and phosphate binders are almost always necessary. Long

dialysis sessions or frequent short sessions result in the best phosphate clearance.

Urea – care needs to be taken if serum urea levels are over 40 mmol/l because a rapid reduction in serum levels can result in disequilibrium syndrome (see complications). Mannitol can be infused (1G/kg) during hemodialysis, through the bubble trap to counteract this. The best way to avoid disequilibrium is to keep the dialysis session short, at less than 2 hours.

Creatinine – will fall rapidly during the session as there is none in the dialysate, but it will rebound and rise rapidly following the end of dialysis.

Administration of Blood Products

Albumin – a low serum albumin will result in edema and difficulty in removing excess fluid. If the child is oligoanuric 20% albumin should only be given when on dialysis, as the resultant fluid shifts can cause pulmonary edema. It must be given in small boluses through the arterial infusion port at the beginning of the session, to allow time for movement of fluid from the extravascular into the intravascular compartment.

Blood – blood should only be required to prime the lines if the volume of the dialyzer and lines exceeds the safe extracorporeal circuit volume i.e., in infants. This blood prime is not washed back into the child at the end of the session. If blood is required for the treatment of anemia, the calculation for number of mls of blood required = weight (kg) × 3 × number of grams that the Hb is to be raised. The blood is infused in small boluses at the beginning of dialysis, through the arterial infusion port, so that potassium will be dialyzed out.

Resulting fluid shifts with blood or albumin may lead to the need for ultrafiltration towards the end of the session.

Anticoagulation

Heparin is the standard anti-coagulant used during hemodialysis. It can be infused slowly and continuously throughout the session to prevent the blood clotting in the circuit. It is given at a rate of 5–50 units/kg/hour through the arterial side of the circuit. Some units use low molecular weight heparin, given as a bolus of 1mg/kg at the beginning of the session.

The circuit needs to be constantly monitored for the formation of clots. If suspected, a bolus of 50–100 ml of saline flushed through the circuit with the arterial lines clamped may reveal clot formation. Clots may form when there is slow blood flow because of access problems, a raised hematocrit, a long period of ultrafiltration as the hematocrit is raised as fluid is removed, and inadequate heparinization. If a circuit clots off completely, the blood in the lines is lost. This will not have a detrimental effect on the child, providing the extracorporeal rules have been observed. However, UF will need to be increased to remove the extra saline. The heparin dose may be increased and/or a bolus of heparin given. The venous side of the blood circuit can be changed during the session to prevent total clotting. If clotting problems persist, long-term aspirin and/or dipyridamole or warfarin or treatment may need to be considered.

The heparin infusion needs to be stopped 30 minutes prior to the end of dialysis if a fistula is being used, to prevent bleeding after the needles have been removed. The heparin dose will need to be adjusted in the patient with abnormal clotting or low platelets. Heparin free dialysis can be used, for example if dialysis is taking place just before surgery. In this situation the circuit can be primed with heparinised saline (3000–5000 u/l) as this will bind to the dialyzer. The dialyzer must then be flushed before connecting to the patient. The dialyzer must be checked regularly for signs of clotting. High blood flows will help prevent clotting. Heparin may induce thrombocytopenia in some patients, which resolves on stopping it.

Complications of HD

Intradialytic Hypotension

The commonest complication of hemodialysis during the treatment is hypotension. Hypotension occurs because of the movement of fluid from the extracellular to the intracellular space due to a decrease in serum osmolality, impaired sympathetic activity, vasodilation in response to warm dialysate, and splanchnic pooling of blood while eating during dialysis. Hypotension may also be due to excessive UF requirements because of a high interdialytic salt and water intake or to the use of anti-hypertensive agents, although it is usual to omit the dose of antihypertensive medication on the morning before dialysis.

The treatment is the prescription of normal saline 5 ml/kg and cessation of UF. It is important to reassess the dry weight in case this has been underestimated, and also the daily salt intake and fluid allowance, which may be too high so that too much fluid needs to be removed. Another cause is the use of a dialysate sodium lower then plasma, as this leads to hyponatremia in blood returning

to the patient and, therefore, the movement of water into cells from the intravascular compartment. Sodium modeling (use of decreasing dialysate sodium concentration during a session, see above) to optimize vascular refilling; UF separate from dialysis; and on-line blood volume monitoring may help patients with recurrent hypotension. Symptoms such as nausea, vomiting, itching, pains and cramps are also common, frequently occurring during hypotensive episodes.

Disequilibrium

A less common complication is disequilibrium, which is due to the plasma urea falling more rapidly than brain cell urea with the resultant movement of water into brain cells by osmosis. It is particularly likely to occur if the plasma urea is high at the start of dialysis, especially when the patient is initiating a course of hemodialysis. Disequilibrium can present with headache, nausea, dizziness and progress to disorientation, seizures and coma. Symptoms resolve spontaneously but if severe can be treated with intravenous mannitol using 1 gm per 10% of body weight. Disequilibrium should be avoided by assuring that the fall of urea during treatment is not too rapid or too great. In some units, mannitol is infused during the beginning of the treatment or evenly throughout it to prevent a rapid change in serum osmolality.

Hemolysis

Hemolysis may occur due to overheating, contamination or hypotonicity of dialysate, kinking of the lines or a malfunctioning pump. Dialysis should be stopped and the potassium should be checked immediately. Hemolysis may continue for some hours. It presents with pains and nausea and a dark appearance to the venous blood.

Air Embolism

Air may enter the circuit and this is particularly likely to happen before the blood pump as there the blood is under negative pressure. Air embolism, however, is rare, as air detectors will clamp the return lines. One ml/kg may be fatal. Air embolism presents with fitting or coma in the upright patient, and chest symptoms if recumbent. Treatment is to clamp the lines, stop the pump, put the patient head down in the left lateral position, give 100% oxygen (to enhance nitrogen diffusion out of air bubbles) and resuscitation as necessary. Air may need to be aspirated from the ventricle.

Anaphylaxis

An anaphylactic reaction to the dialyzer can occur at any time, but is more common after first use ('First use syndrome'). It occurs soon after the start of dialysis and disappears with dialyzer reuse and predialysis rinsing. It causes hypotension (or sometimes hypertension), angioedema, pulmonary symptoms, chest and abdominal pain, vomiting, fever, urticaria, and pruritus and results from activation of plasma complement or kinin systems by the dialysis membrane, or the release of noxious materials which may have contaminated the dialyzer during manufacture or the sterilization process (e.g., with ethylene oxide). Dialysis must be stopped and blood should not be returned to the patient. Normal saline for hypotension, epinephrine subcutaneously or intramuscularly (1:1000 concentration) and/or hydrocortisone may be necessary. Symptoms may be milder, for example presenting with just urticaria, which can be treated with an antihistamine or hydrocortisone. Severe reactions necessitate a change of dialyzer.

Amyloidosis

Dialysis-related amyloidosis is unusual in childhood but can occur in those who have been dialyzed for a long time; symptoms are typically first reported 7–10 years after commencing hemodialysis, although tissue accumulation of dialysis-related amyloid can be demonstrated much earlier. It is a disabling, progressive condition caused by the polymerization within tendons, synovium, and other tissues of beta-2-microglobulin, a large (molecular weight (MW) 11,600) molecule, which is released into the circulation as a result of normal cell turnover but is not excreted in renal failure and is not removed by cellulose membranes. Exposure to bioincompatible membranes may increase beta-2-microglobulin generation.

Vascular Access

Good vascular access is crucial to the success of dialysis, and is more important than any other factor. The best form of access is an arterio-venous (a-v) fistula, otherwise

a line that is tunnelled subcutaneously is used, or, rarely, shunts or grafts (22). The life of a fistula is superior to a tunnelled line; over two thirds are still functioning after 4 years, whereas reports of the survival of tunnelled lines vary from 30–85% at one year (23). Vascular access problems are indicated by the arterial and venous pressure alarms. Low arterial pressure alarms indicate that there is an insufficient blood flow reaching the blood pump. This is commonly referred to as 'sucking' and is usually a result of poor access position in the vessel. A low venous pressure alarm also indicates poor blood flow. A high venous pressure alarm indicates that there is an occlusion to the flow of returning blood. It is due either to poor access position, fistula stenosis or the presence of clot formation. Tissue Plasminogen Activator, TPA, can be safely used to dissolve suspected clots in the lumen of central lines (24, 25). A solution of 1mg/ml is instilled in the dead space of the catheter and left for at least one hour, preferably overnight or between dialysis sessions. A randomized, controlled trial has shown that TPA is significantly more effective than heparin in preventing clot formation in central hemodialysis lines (26). TPA should be completely aspirated before the line is next used for dialysis. This is important, not only because of its anticoagulant properties, but also because of its phosphate content, so that a spuriously high phosphate level can be obtained if it is not thoroughly removed before blood sampling (27).

Access Recirculation

Adequacy of dialysis may be compromised if the dialyzed blood that returns from the venous line to the circulation is directly taken back into the arterial line to the blood circuit to be redialyzed. This is called recirculation. It can occur if the distance between the site of blood withdrawal and return is small, if there is abnormal blood flow through a fistula, if the needles are wrongly placed or during single needle/lumen dialysis. Recirculation can be measured and expressed as a percentage, which should be less than 10%. If the result is higher than this, then the fistula needs to be assessed with venography for the presence of a venous stenosis.

The procedure for assessment of recirculation is simple. Arterial (A) and venous (V) samples are taken from the access lines 30 minutes after the start of dialysis (without UF). The pump speed is then halved and then switched off. The arterial line is clamped above the port and a sample taken from it (S). Alternatively, this sample can be obtained from the other arm or other site of the patient. The dialysis is restarted, and the urea in the arterial (A), venous (V) and systemic (S) circulations is measured.

$$\text{recirculation } (\%) = \frac{S - A}{S - V} \times 100$$

Arterio-Venous (a-v) Fistule

Children on short-term dialysis (e.g., awaiting a living-related transplant) may elect to be dialyzed via a tunnelled line. An arterio-venous (a-v) fistula is the preferred method of vascular access in children on chronic hemodialysis because of the decreased risk of infection (in comparison to a line); line infection is the most important cause of vessel stenosis (28). Preservation of vessels is particularly important in children, who have a lifetime of renal replacement therapy ahead of them. An a-v fistula can be used in children who are able to co-operate with needling; education and play therapy may enable this even in small children and those with needle phobia. A fistula is created by surgically anastomosing an artery to a vein, so that the higher pressure within the vein causes it to expand to allow large enough needles to be inserted to enable the high blood flows required for dialysis. It may be created at the wrist (radio cephalic or radio basilic), the elbow (brachio-cephalic) or by basilic vein transposition to create a brachio-basilic fistula (29). It is preferable to start distally at the wrist to preserve more proximal vessels for future use. Clearly the larger the vessels the greater the chance of success, but some groups are able to operate on even very young children using microsurgery (30).

The success of a fistula depends on adequate run off distal to the anastomosis. Any distal vessel stenosis will result in high venous pressures and oedema of the arm. For this reason, the child who has had previous central lines will need upper limb venography to establish patency of the arm and central vessels (31). A thrombosed subclavian vein will preclude a fistula being created in that arm as venous return may be obstructed, although balloon dilatation or stenting of the stenosis may be possible. Ultrasound examination of the proximal vessels can be misleading and should not be relied upon because collaterals may be mistaken for patent upper limb vessels, and the veins cannot be easily seen under the clavicle. It is preferable to select the non-dominant arm for the fistula if at all possible.

A fistula should be created at least 6 weeks before it is needed as it takes some time to mature. Prior to surgery, the child must be well hydrated, or left at slightly above dry weight if already on dialysis and antihypertensives

adjusted to decrease the chances of clot formation due to low circulating blood volume or BP. Post operatively the fistula needs to be checked regularly for the presence of a thrill, which, if lost, needs to be assessed urgently as clots can be removed by catheter, surgery or locally instilled TPA but only if this is undertaken as soon as possible (32). Clot removal after 48 hours is rarely successful in restoring flow. It is usual to increase the child's target weight for the next week or so after the surgery. Prophylactic antiplatelet doses of aspirin (1–5 mg/kg/day) may reduce the risk of clotting.

When the fistula is 'mature' for use, needles should be placed proximal to the fistula. It may be necessary to start with a single needle, but it is usual to use two needles, with the arterial needle distal to the venous one, which should be as far away as possible. The arterial needle can point in either direction, but the venous needle should be towards the heart. The needle sites should be changed as repeated needling in the same place will cause weakness of the vessel wall and aneurysm formation. The use of anesthetic creams have reduced the physical and psychological trauma associated with fistula use in children.

Fistula Stenosis and Other Complications

Arterial and venous pressure alarms suggest the presence of stenosis, which can occur in the arterial or venous side or in the fistula itself. Sites of stenosis occur where there is turbulent blood flow, which causes intimal hyperplasia of the vessel wall. The commonest place for stenosis is within a few centimeters of the fistula, just distal to the anastomosis. Low blood flow and arterial pressure with 'sucking' suggests stenosis at the inflow to the fistula. High venous pressure suggests stenosis distal to the fistula. Slow blood flow through the fistula predisposes to thrombosis.

In the first instance, much can be determined by clinical examination of the fistula. A palpable thrill is present if the blood flow through the fistula is >450 ml/min. In the case of a stenosis, the thrill is distal to the pressure drop. With a basilic vein fistula stenosis, the thrill will be further up the arm towards the heart. Another common site of stenosis is where the cephalic vein goes through the clavipectoral fascia. The vein may then be pulsatile all the way up the arm.

Doppler ultrasound studies can be used to examine flow, and can be followed by tests of recirculation which if >10–15% suggests venous stenosis. Arteriography is necessary to examine the arterial flow into the fistula and a fistulogram, also using Xray contrast, is used to demonstrate the blood flow into and out of the fistula. It is usual practice to anticipate the formation of stenosis by screening with Doppler US every 6 months. The risk of clotting has been found to increase when flows are <650 ml/min, but the finding of a downward trend in blood flow is important. If a stenosis is >50% of vessel diameter it needs angioplasty, stenting or surgical repair (33). A stenosis at the clavipectoral junction is much harder to dilate than a basilic vein stenosis.

Other complications include ischemia of the hand or 'steal syndrome', and pseudoaneurysm, due to communication of the fistula with an enclosed area of surrounding tissue. The latter may lead to prolonged bleeding and needs to be repaired. Infection of the fistula can also occur. It is also possible for the fistula to become too large with unsightly forearm veins and flows that are too high, predisposing to heart failure.

Grafts and Shunts

These are less commonly used in children, and shunts are really a last resort. An artificial conduit can be inserted between an artery and vein subcutaneously (graft) where it can be needled, or may be brought out externally (shunt) where the loop can be disconnected to attach to dialysis lines. Grafts and shunts are, like a-v fistule, also liable to stenosis (particularly at the anastomosis site) and to clotting. Stenosis can be suspected by the presence of a thrill, which would not normally be present. Although 1, 3 and 5 year survival are similar to a-v fistule, at 90%, 50–60% and 40% respectively, the need for surgical intervention is higher in grafts (34). They also have an increased risk over fistule of infection, which can be difficult to eradicate. Shunts carry the further risk of disconnection and blood loss.

Tunnelled Lines

Tunnelled lines are used in children who are too young for an a-v fistula, or in children who are not expected to be on dialysis long; e.g., when a parent is being prepared as a donor. They can be inserted so that the tip is in the right atrium using US guidance (35). The success rate is superior to non-tunnelled lines but inferior to an a-v fistula (34). The internal jugular veins are the first choice. The presence of a line, particularly if it becomes infected, can lead to vessel stenosis, so the subclavian vein should not be used if possible as a subclavian vein stenosis would preclude the use of that arm for future fistula formation.

Table 73-4

Catheter size and siting according to the weight of the child

Size of child	Catheter size	Siting
Neonate	5Fr (single lumen)	Femoral vein
3–6 kg	7Fr	Internal or external jugular or femoral vein (preferably not subclavian)
6–15 kg	8Fr	
>15 kg	9Fr	
>30 kg	≥ 10Fr	

The bigger the gauge of the access the faster the blood flow that can be obtained. The majority of vascular access is either 7–8 FG or 11FG in differing lengths (12 or 18 cm or 12 to 19 cm for smaller or bigger gauges respectively) and is chosen according to the size of the child and their vessels. Some examples are shown in *Table 73-4*.

Most lines are dual lumen, allowing a continuous flow of blood around the circuit. However, hemodialysis can also be achieved using single lumen access. In very small children single lumen access may be more appropriate as the lumen of the catheter will be larger and therefore the flow that can be obtained through it is relatively greater, as flow is proportional to the fourth power of the radius. In order to obtain two directional blood flows with a single lumen line, the dialysis circuit has to be modified. This can be achieved by the double-pump method, using two blood pumps which pump alternately, or by using a single pump which pumps intermittently, using gravity to let blood flow back in to the child. Disadvantages of the single lumen catheter are that an expansion chamber is necessary in the circuit to allow for the pressure changes and this increases the volume of the blood circuit; also the superior blood flow rates are compromised by a greater degree of recirculation.

Infection in Vascular Catheters

Infection in vascular catheters may be at the exit site, in the subcutaneous tunnel or in the catheter. The development of biofilm within the catheter makes bacterial eradication particularly difficult. Line sepsis may present as a rigor soon after starting dialysis, fever with raised CRP or septicemic collapse. The factors increasing the risk of catheter infection include: exit site and/or tunnel infection or contamination of the hub, failure of aseptic technique, frequent need to access the catheter during dialysis and long duration of use, use of non-tunnelled rather than tunnelled lines, immunosuppression, hypoalbuminemia, diabetes and nasal and cutaneous colonization with Staphylococcus aureus. Post dialysis heparin or alteplase into the line decreases infection risk by decreasing clot formation, which predisposes to infection (26).

Treatment of Catheter Related Infection (*Fig 73-2*) (36)

Figure 73-2 demonstrates the steps in the management of exit site, tunnel and catheter infections. Exit site infection presents with erythema and tenderness within 2 cm of the exit site, and discharge from the exit site itself. Great care should be taken to immobilize the catheter as far as possible as movement within the exit site encourages infection. There are different types of dressings but there is no evidence to suggest superiority of any particular type. It is important to check for carriage of Staph aureus so the exit should be swabbed every month, along with 3 monthly nasal swabs. Staph aureus carriage can be treated with topical exit site and nasal mupirocin for 5 days every month. If there are clinical signs of infection then swabs should be taken, daily cleaning instituted and oral antibiotics commenced. If there is no response after 4 weeks, removal of the catheter with replacement after 24–48 hours is warranted.

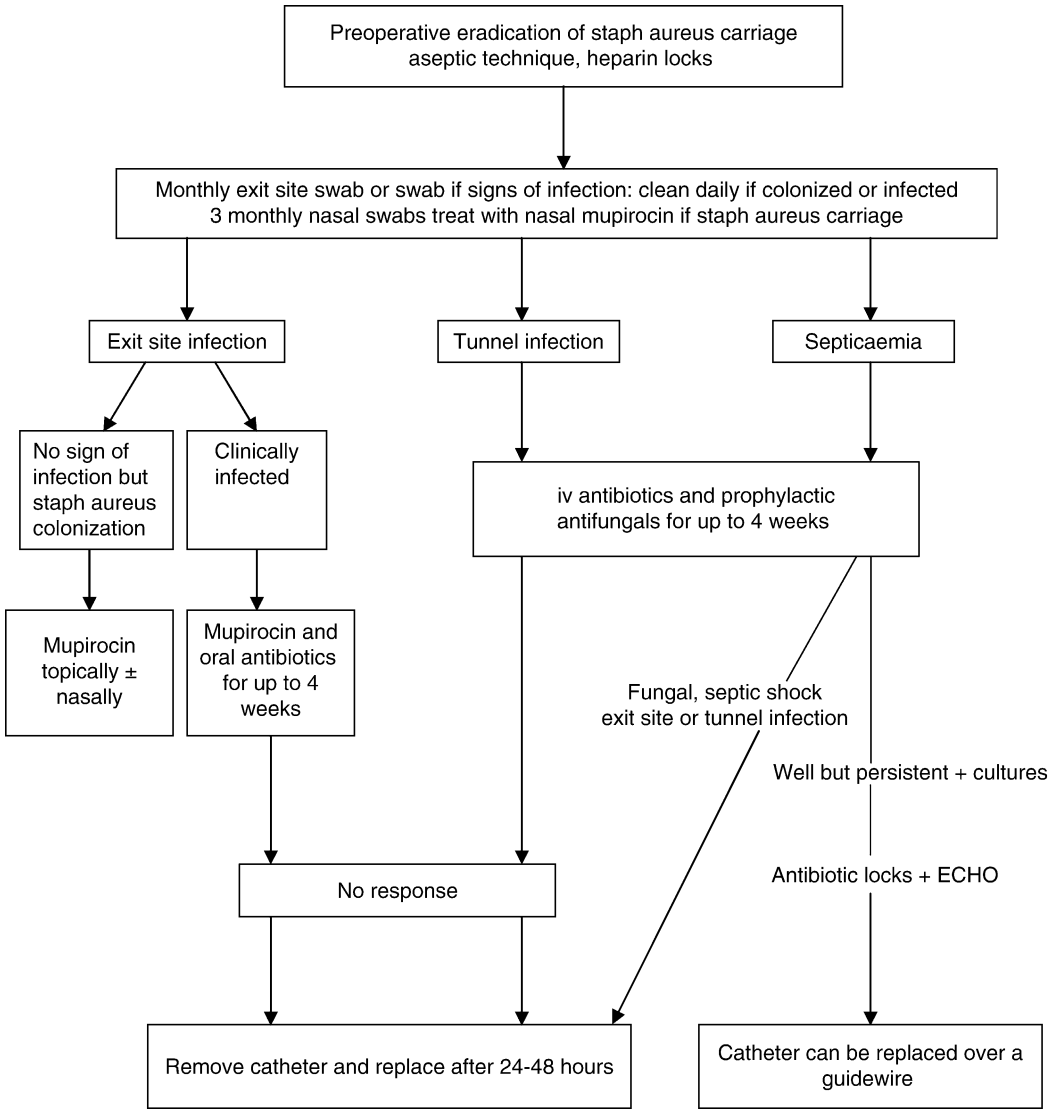
Exit site infection can spread down the subcutaneous tunnel of the catheter, when in addition there will be tenderness, erythema and induration along the subcutaneous tract >2cm from the exit site. A tunnel infection is much more difficult to eradicate, and may track into the blood stream, resulting in septicemia. Intravenous antibiotic therapy is warranted, along with antifungal prophylaxis, and if there is no response after 4 weeks, the catheter should be removed and replaced after 24–48 hours.

The catheter itself may become colonized, so that there is repeated growth of the same organism on blood drawn through the line, but without signs of sepsis. Antibiotic locks may be helpful in eradication of line colonization (37). Antibiotic locks use an antibiotic to which the organism is sensitive and are instilled into each lumen after dialysis. The antibiotic is then removed at the start of the next dialysis session. With ongoing positive growths but no systemic symptoms, it has been shown to be safe and effective to replace the line over a guidewire, as long as antibiotics have been started and there is no tunnel or exit site infection (38). This is preferable as it means that the access point in the vessel is not lost.

The presence of septicemia, bacteremia or fungemia with at least one positive culture of the same organism from the catheter and a peripheral vein, clinical signs of

Figure 73-2

Prevention and treatment of hemodialysis catheter related infection.



infection (fever, hypotension), no other cause of infection and a raised C-reactive protein indicate catheter infection. Antibiotics need to be started immediately, but in the presence of septic shock, the catheter should be removed.

Septicemia treatment is usually started with Vancomycin in units with a significant incidence of MRSA. If there is severe systemic illness, two antibiotics are used. An example could be to initially use both intravenous vancomycin and ciprofloxacin until the gram stain/culture are available, when the treatment can be modified accordingly. However, antibiotic policy will depend on local resistance patterns, which are changing, with the emergence of more resistant organisms being seen (39). It is wise to use antifungal prophylaxis when broad-spectrum antibiotics are used. Antibiotic therapy (intravenous) would usually be continued for 2 weeks, but may need to be longer if there is fungal infection. If there is persistent fever, endocarditis is a possibility so an echocardiogram should be performed.

If there is septicemia the catheter should be removed, particularly if the infection is occurring in a non-tunnelled line or one that is no longer being used or if there is septic shock. If the septicemia is caused by a fungus or if the same organism as is infecting the exit

site or tunnel, if there is persistence of fever after 48 hours of therapy or ongoing positive blood cultures, then the chances of clearing the infection are low and again this is an indication to remove the line.

Dialysis Adequacy

The concept of dialysis adequacy was introduced in order to define the dialysis prescription (blood flow rate, dialyzer clearance, treatment time) required to minimize patient morbidity and mortality. Obviously such correlations can only be made in adults, as large numbers of patients with significant complications are required for statistical analysis. However, attempts have been made to correlate adequacy with features such as hospitalization rates in children (40).

The clearance of urea has been selected as the basis for all the calculations of dialysis adequacy, although of course urea represents small molecule clearance and not clearance of other larger molecules that move more slowly across the dialysis membrane. Dialysis adequacy is defined as the minimum amount of urea clearance and nutritional intake that prevents adverse outcomes. The figures that have been calculated for dialysis adequacy represent a minimum acceptable level; determination and calculation of what is optimal, i.e., the dose above which no further improvement in outcome occurs, is less easy to achieve. Some hemodialysis machines provide on-line calculation of urea clearance.

Urea is evenly distributed throughout the body fluid compartments. During dialysis, urea is removed from the extracellular space. However, movement from the intracellular space into the intravascular compartment occurs more slowly so that there is a difference in levels between the two compartments that persists for an hour or so after dialysis. If this difference is not taken into account in calculations, then the amount of urea removed is overestimated and the dialysis dose appears greater than it is. Thus calculations of adequacy may be either 'single pool' or 'double pool'. As well as urea clearance, nutritional status is also assessed as part of the adequacy measurement. The interdialytic accumulation of urea reflects protein catabolism so that, in a steady state, the protein catabolic rate is equivalent to the amount of protein ingested and can be taken, therefore, as an indication of nutritional status. When calculations of dialysis adequacy use both urea clearance and patient nutritional status (i.e., urea generation rate), this is called urea kinetic modeling (UKM).

The amount of urea that is removed during a hemodialysis session is affected by the urea clearance coefficient of the dialyzer, the pre and post treatment blood urea, the treatment time, the total body water, the UF, residual renal function and the interdialytic urea generation rate. Therefore these factors are fed into the UKM equation to obtain the measure of dialysis adequacy. UKM requires samples over two sessions, and is less commonly used because of its complexity

A more common assessment of dialysis adequacy is Kt/V, where K is the urea clearance of dialyzer, t the treatment time and V is the volume of distribution (0.6 × body weight in kg). It can be predicted from the pre and post dialysis urea, weight loss and duration of dialysis. For the single pool Kt/V the blood urea can be obtained straight after the end of the dialysis session. Although urea rises rapidly post dialysis, it is impractical to wait to take the post dialysis blood urea sample until after urea rebound is complete, which takes approximately one hour; mathematical calculations can be used to allow for this (double pool Kt/V). Methods of standardization of post-dialysis sampling that aim to reduce variability in the timing of blood sampling are called the slow-flow and stop-flow methods. The stop dialysate flow, when dialysate flow is stopped but the blood pump is kept running for five minutes, is the most commonly used, but gives higher results. There are several different formula available for the calculation. Most would use the Daugirdas II formula:

$$\mathrm{Kt/V} = -\ln(\mathrm{C_1/C_0} - 0.008^{*}\mathrm{t}) + (4 - 3.5^{*}\mathrm{C_1/C_0})^{*}\mathrm{UF/W}$$

Where C_0 and C_1 = pre and post dialysis blood urea (mg/dl) respectively, t = time (hrs), UF = ultrafiltration volume (Kg), W = post dialysis weight

The simplest way to assess dialysis adequacy is the urea reduction ratio (URR), which is calculated as follows:

$$\frac{\text{Pre dialysis urea} - \text{post dialysis urea}}{\text{pre dialysis urea}} \times 100\%$$

URR underestimates the dose of dialysis as it does not take into account convective losses. Recirculation, on the other hand, will give a falsely high Kt/V as the urea result will be underestimated. Residual renal function is an extremely important contributor to urea clearance and should be included in all calculations of dialysis adequacy. However, 24 hour urine collections may be extremely difficult to obtain in children on dialysis with poor urine output.

Measures of adequacy in children have not been defined, but consensus standards propose that they should be equal to or better than adult recommendations

of > 1.2 for Kt/V and > 65% for URR (23). One study in children demonstrated an improvement in the risk for hospitalization up to a Kt/V of that level, but above 1.4, no further improvement occurred (40). However, the improvement in all aspects of patient well-being with daily or frequent short hemodialysis suggests that a higher Kt/V is likely to be better (16–20).

The normalized protein catabolic rate (nPCR) is a reflection of protein intake, and is more sensitive and specific than albumin as a marker of nutritional status because many processes unrelated to nutrition can affect albumin concentrations. nPCR may be useful for monthly nutrition status in adolescent patients receiving maintenance HD: adolescents with nPCR less than 1 g/kg/d may be at increased risk for subsequent weight loss (41).

Of equal, or perhaps even more importance in the assessment of dialysis adequacy is the well being of the patient, which can be objectively assessed in children by height and weight gain. Control of BP, anemia, acidosis and bone disease are also part of the overall assessment of dialysis adequacy.

Blood-Borne Viruses

Hemodialysis unit patients and staff are at risk from blood-borne viruses, particularly hepatitis B, hepatitis C and HIV. Transmission may result from percutaneous exposure to blood or other fluids, via droplets or through contaminated equipment. Universal precautions should be followed as for all patients, and the entire dialysis circuit should be decontaminated after each use by heat or chemical disinfection. External surfaces should be wiped over between patients using a chlorine based disinfectant.

All staff and patients should be immunized and/or show immunity to hepatitis B. It is preferable to administer the vaccine before dialysis is necessary for the best chance of a good response. The vaccine can be given at any age at intervals of 0, 1 and 6 months. An accelerated course can be used so that the third dose is given 2 months after the first dose (i.e., doses at 0, 1 and 3 months and a booster dose at 12 months). The anterolateral thigh (intramuscular) is the preferred site in infants and young children. The deltoid muscle is the preferred site in older children. It should not be injected into the buttock as vaccine efficacy is reduced.

If the antibody level is <10 iu/L 2–3 months after the last vaccine, the course of vaccine is repeated and hepatitis B surface antigen (HBsAg) is measured 3 monthly until there is a satisfactory antibody response (>10 iu/L 2–3 months after last vaccine). If antibody levels are >10 iu/L, then it can be assumed that immunity is sufficient and antibody levels can be measured annually, with booster doses as necessary. The usual dose of hepatitis B vaccine is doubled for patients on dialysis. If a patient is exposed to hepatitis B or has been to an endemic area, such as the Middle and Far East, and has antibody titers < 100 iu/L in the last year, then hepatitis B immunoglobulin and vaccine should be given by intramuscular injection, and the patient should be screened weekly for HbsAg for 3 months. Patients who are or who might become HbsAg positive should be dialyzed in a separate room with their own machine.

Although screening for hepatitis C or HIV is not universally recommended at present, many units do so. Hepatitis C can be spread nosocomially, so a separate room is recommended for the patient who is hepatitis C positive, but a dedicated machine is not necessary. HIV is less infectious but the same criteria apply. Testing for hepatitis C antibody every 3 monthly and for HIV annually is a reasonable compromise (42).

Nutrition in Hemodialysis

Nutrition is fully covered in chapters 12 and 68, but there are some nutritional issues that are specific to hemodialysis. Malnutrition is common in children on hemodialysis, and is associated with increased mortality (43). Both UK (44) and US (45) guidelines advise an increase in the recommended protein intake for age in children on hemodialysis, varying from around 20% in very small children to up to 50% in older ones. This increase is not as large as that recommended for children on peritoneal dialysis. It is to allow for losses of amino acids into the dialysate, which depend on their plasma concentrations and molecular weights.

It may be difficult to achieve an adequate intake of protein without dietary supplementation, which can be administered either orally or enterally. Studies of the effect of dietary supplementation on nutritional status and growth have given variable results (46), so because of this, some centers have explored the use of intradialytic parenteral nutrition (IDPN). There are only four studies of IDPN during hemodialysis in children, and these involve very small numbers so it is difficult to draw conclusions about its effectiveness (44, 46).

There may be specific deficiencies occurring in patients on hemodialysis, one of which is carnitine. Carnitine has a MW of 162 daltons and is water-soluble and unbound. It may therefore be cleared by dialysis, and deficiency in dialysis patients has been reported to be

common. The biologically active L-carnitine plays an important role in fatty acid metabolism and energy production in cardiac and skeletal muscles, and carnitine deficiency has been associated with poor response to erythropoietin, intradialytic hypotension, cardiomyopathy and muscle weakness. However, studies of the benefits of carnitine supplementation have been inconsistent (47).

CKD-MBD

Abnormal mineral metabolism and altered bone structure and composition is almost universal in children on dialysis (48) and bone related problems which adversely affect quality of life, including bone and joint pain and fractures, are the most common complaint in young adult survivors of pediatric renal failure programs (9). It is not only morbidity but also mortality that are affected by abnormal mineral metabolism: the association with extra skeletal, and in particular vascular calcification, is strongly suggestive of a causative link, although the pathological mechanisms are yet to be unraveled. In order to reflect the complex issues surrounding these areas it has been suggested that CKD-mineral and bone disorder (CKD-MBD) should be used as an encompassing definition (49).

Current management of CKD-MBD hinges on the concept of an optimum range for plasma parathyroid hormone (PTH) levels, which is a range that maintains normal bone turnover without increasing the risk for ectopic calcification. The risks of extra skeletal calcification are thought to be increased with both low and high bone turnover because both types result in high plasma calcium and phosphate: low bone turnover because of the inability of bone to buffer changes in plasma calcium and phosphate, and high turnover because high PTH levels mobilize calcium and phosphate from bone, increase tubular reabsorption of calcium, and promote gut absorption of calcium and phosphate by hydroxylation of 25, OHD3. PTH itself is thought to be an independent risk factor for myocardial fibrosis, arteriolar thickening, and hypertension (50). There are detailed guidelines from the US (KDOQI) (51) and Europe (52) on PTH management, and all aspects of calcium, phosphate and vitamin D control in order to achieve these aims. European recommendations for children on dialysis are that the plasma PTH should be kept at 2 to 3 times the upper limit of normal and KDOQI sets higher levels of 3 to 5 times the upper limit of normal. However, there is limited evidence for these recommendations (50), and review of the evidence that is available suggests that high PTH levels are associated with abnormalities of vascular structure and function and vascular calcification, and indeed these vascular abnormalities are less common in children on dialysis with PTH levels $< 2\times$ the upper limit of normal (53). What is known is that hyperphosphatemia and a high calcium $\times$ phosphate product are toxic to the vasculature and should be prevented. It is also known that both too much and too little intake of calcium is bad, and that high PTH levels, and plasma levels of vitamin D that are either too low or too high, are associated with increased cardiovascular morbidity (54). This is discussed further in the chapter on renal bone disease.

Drug Prescribing in Patients on Hemodialysis

Great care needs to be taken when prescribing for children with renal failure. Drug handling in hemodialysis is made more complicated by reduced bioavailability due to abnormal gastrointestinal motility, nausea, vomiting and anorexia. The drug volume of distribution may be altered by the presence of volume overload (edema and ascites) and reduced if there is volume depletion or muscle wasting. Protein binding is altered by acidosis, malnutrition and inflammation. This can result in high levels of the free drug despite normal blood levels (e.g., phenytoin).

Many drugs will be cleared by hemodialysis. This depends on the molecular weight of the drug and the degree of protein binding. Drugs with a large volume of distribution are generally lipid soluble and not confined to the circulation; they are not well cleared by hemodialysis. It is logical to administer drugs known to be cleared by hemodialysis immediately at the end of the dialysis session.

There is a tendency within units to administer drugs intravenously during the hemodialysis process. This is particularly helpful with iron therapy, which is often poorly tolerated orally. However, there is no evidence that other drugs such as erythropoietin or activated vitamin D are more effective when administered intravenously, although this route does overcome concordance issues.

Hemodialysis for Acute Renal Failure

Hemodialysis is less commonly used as treatment for acute kidney injury in the intensive care unit as it is better managed by continuous veno-venous hemofiltration-dialysis, which does not cause major fluid shifts and

disturbances to BP and cardiac output. Situations in which hemodialysis may be useful are when there is a need to rapidly remove large volumes of fluid, particularly if there is pulmonary edema, or an urgent need for the fastest possible clearance of toxic metabolites, such as with inborn errors of metabolism, drug poisoning or tumor lysis syndrome.

Emergency hemodialysis in ARF is usually started with a temporary percutaneous dual lumen non-tunnelled catheter. The principles of management are not different from chronic hemodialysis. A short session may be the best starting point, followed by daily sessions. Dry weight assessment may be particularly difficult in the child presenting acutely, and awareness of potential reactions to the procedure are necessary as these are unpredictable in the new patient. A more complete description of treatments for acute kidney injury is given in Chapter 67.

Long-Term Outcome

Despite all the improvements that have taken place over the years, such as more biocompatible and high flux dialysis membranes and UF controlled machines, and better understanding of the management of nutrition, anemia and CKD-MBD, mortality in adults is showing no signs of improvement. Amyloidosis, malnutrition, LVH and accelerated atherosclerosis remain common. Although mortality in children on dialysis remains low, it is still around 30 times greater than that of the age-matched normal population (55), and in young adults on dialysis is equivalent to that of an 85 year old (56). Most of this excess mortality is due to CVD. Age at the start of dialysis also affects outcome: dialysis before 1 year of age increases the mortality risk by 2.7, and between 1 and 5 years by 1.8. However, more importantly co-morbidity, which affects a significant proportion of patients, increases mortality risk 7.5 times (4, 57). Lifespan is reduced by 40–60 years in children on dialysis, with about 50% of deaths due to CVD (58). The challenge to pediatric nephrologists is to improve the long-term outcome for these children. Much can be gained by careful attention to fluid overload and LVH, to metabolic abnormalities, particularly CKD-MBD and prevention of anemia, but further research is needed into the benefits of more frequent dialysis, types of dialysis and the prevention of CVD. Most importantly of all, bypassing dialysis altogether by pre-emptive renal transplantation is currently the best option for our patients.

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74 Transplantation Immunobiology

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Introduction

Allograft rejection is an inflammatory reaction that occurs rapidly after transplantation and is associated with a characteristic cellular and humoral attack on the graft. However, allograft rejection can also occur more chronically, as a result of an insidious immunological process involving delayed type hypersensitivity mechanisms. Both the early acute, and the later chronic rejection processes are mediated by the recipient's immunological response to donor antigen, which is initiated and coordinated by T cells. All forms of rejection also require the activation of other cell types including B cells and macrophages as well as the induced expression of molecules that enable the trafficking of destructive effector cells into an allograft. New discoveries in the field of immunology have provided many insights into mechanisms that function in the alloimmune response and in the development of rejection. Several of these discoveries have been translated into the clinic, and have resulted in new therapeutics that have the potential to promote long term graft survival. In this chapter, we will review the cellular and molecular basis for the alloimmune response, and we will discuss mechanisms and concepts that have resulted in new targeted therapeutic strategies.

Allorecognition

When a tissue or organ, such as skin, is transplanted from one animal into another, there are two possible outcomes. One possible outcome is that the skin will engraft and will function over time as normal skin. The other outcome is that the skin will not engraft and will be rejected. The immunological basis for these outcomes and the observation that allogeneic tissues are "rejected" has served as a basis for our understanding of concepts about immunity itself, and how our immune system is capable of recognizing foreign tissues as self or non-self. Approximately 50 years ago, a major breakthrough led to the identification of Histocompatibility Antigens (1). Cells or organs transplanted between identical strains of mice or between identical human twins were not rejected. However, cells or organs transplanted between different strains of mice or between unrelated humans are almost always rejected. By inbreeding of mice, it was further discovered that several gene products could determine the rejection of skin between parents and offspring; and the molecular basis for these observations led to the identification of specific "rejection genes" encoded by the major histocompatibility complex (MHC) (1, 2). It is now understood that interactions between a recipient T cell and individual MHC molecule(s) is the basis for allorecognition. In addition, it has been determined that peptides derived from allogeneic tissues or proteins, which are expressed in association with the MHC molecule, mediate T cell activation responses. Furthermore, it is known that peptide interactions with a T cell receptor complex (TCR) results in a signaling cascade that is key to the elicitation of an effective T cell activation response. However, the TCR signal alone is not sufficient to elicit the full activation of T cells. Full activation requires interactions with additional molecules called costimulatory molecules (commonly called signal 2). Once activated, T cells initiate and coordinate a variety of pro-inflammatory events called "effector mechanisms," all of which result in inflammation within an allograft. These include the activation of monocyte/macrophages, cytotoxic $CD8^+$ T cells and alloantibody-producing B cells. Activated T cells also co-ordinate delayed-type hypersensitivity (DTH) responses in part through the activation of vascular endothelial cells, and the induction of a variety of chemoattractants, that serve to facilitate local inflammation (3–7). We will describe these concepts in more detail below.

Major Histocompatibility Complex

The major histocompatibility complex (MHC) is a cluster of genes located on chromosome 6 in humans, which encode the family of human leukocyte antigens (HLA). MHC molecules are divided into two main subgroups: class I (HLA-A, -B, -C) and class II (HLA-DR, -DP, -DQ) molecules. Proteolyzed peptide fragments of proteins derived from allogeneic cells (called allopeptides) bind within the groove of MHC molecules on the surface of

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antigen presenting cells (APCs). Once expressed, interaction with the T cell receptor complex of proteins expressed on the surface of recipient T cells results in the antigen-dependent signal. $CD8^+$ T cells recognize peptide/MHC class I complexes, which are constitutively expressed on the surface of virtually all nucleated cells. MHC class I molecules consist of a heavy chain non-covalently associated with a β_2-microglobulin (β_2 m) light chain (8). β_2 m is encoded by a non-polymorphic gene separate from the MHC complex. The peptides that associate with MHC class I molecules are eight to eleven amino acids in length and are derived from the cell cytosol. Some studies have suggested that longer allopeptides can also bind within the groove of the MHC class I molecule and mediate the activation of $CD8^+$ T cells (9).

In contrast, $CD4^+$ T cells selectively recognize allopeptides in the context of MHC class II molecules. MHC class II molecules are constitutively expressed by a relatively small number of cell types including B lymphocytes, monocyte/macrophages, and dendritic cells, but they can be induced in expression on many cell types including vascular endothelial cells. MHC class II molecules are divided into three subgroups: -DR, -DP, and -DQ which are structurally and functionally distinct and are composed of non-covalently associated α and β chains. The length of the peptide bound within the grove of MHC class II molecules is typically twelve to nineteen amino acids in length and is longer than those that associate with MHC class I molecules. Further, while MHC class I peptides are endogenous, MHC class II peptides are classically derived from exogenously supplied alloantigens. Once expressed, MHC class II molecules present peptide antigen to $CD4^+$ T lymphocytes, and elicit signals through the T cell receptor/CD3 complex. These signals ultimately result in proliferation and cytokine production that serve to initiate and coordinate the effector immune response.

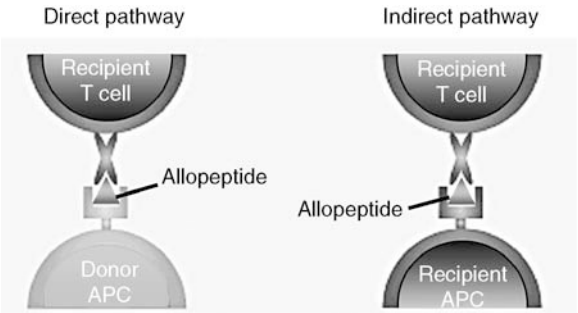
Minor histocompatibility antigens are non-MHC proteins that are also capable of eliciting an immune response and allograft rejection (1). Any non-MHC gene that encodes epitopes capable of binding to both MHC class I and class II molecules and inducing $CD8^+$ and $CD4^+$ T cell response(s) respectively, can be considered a minor histocompatibility gene (10). In principle, polymorphisms among common proteins expressed in the donor can be processed and presented by self-MHC. When presented by self-MHC molecule(s) on self-APCs, peptides derived from these polymorphic proteins can mediate an effective immune response and can promote alloimmunity. Furthermore, in the context of alloimmunity, some minor histocompatibility antigens have been found to be more immunodominant than others (11). For instance, a well characterized minor MHC antigen is the male-specific Y chromosome antigen, also known as the H-Y antigen. When this antigen is processed by MHC molecules in a female, it can induce an anti-Y response against a male donor graft (12–14). $CD8^+$ T cells (15, 16) and $CD4^+$ T cells (17) specific for minor antigens have been isolated from humans and rodents, and have have been shown to play an important role in the rejection of solid organ transplants. Responses to minor antigens have also been shown to be associated with the development of chronic rejection, graft-versus-host disease after bone marrow transplantation and corneal allograft rejection (13, 14, 18, 19).

Pathways of Allorecognition

The recognition of allopeptide by recipient T cells is central to the initiation of the rejection process. Several studies have determined that there are distinct pathways of allorecognition, called the "direct," the "indirect" and the "semi-direct" allorecognition pathways (*Fig. 74-1*). Activation of T cells through the "direct" pathway is unique to the transplantation immunological response. It involves the recognition of intact allo-MHC molecules on the surface of donor cells, including donor-derived APCs and endothelial cells. In contrast, activation of T cells via the "indirect" pathway occurs in an analogous

Figure 74-1

Direct and indirect pathways of allorecognition. In the direct pathway, recipient T cells recognize donor allopeptide presented by donor MHC molecules on donor cells. In the indirect pathway, recipient T cells recognize donor allopeptide presented by self-MHC molecules on self-APCs. The direct pathway is unique to transplantation, where as the indirect pathways is analogous to delayed-type hypersensitivity mechanisms, where foreign proteins are processed by self-APCs.



manner as self-restricted recognition of nominal antigens in delayed type hypersensitivity. In this latter indirect allorecognition pathway, processed allopeptides are presented to T cells by self-MHC molecules on the surface of self-APCs (*Fig. 74-1*). The mechanisms and interactions between these pathways and the relative contribution of either pathway to acute versus chronic rejection is an area of intense research. However, as we will discuss below, it is likely that direct pathway activation is functional in the development of acute rejection, and indirect allorecognition is functional in the development of chronic rejection (20, 21). Finally, T cells can also be activated by a third pathway called the "semidirect" pathway, in which intact allo-MHC molecules can be transferred to recipient APCs where they function to stimulate a recipient T cell activation response (22).

Direct Pathway of Allorecognition and Allograft Rejection

T cells characteristically respond vigorously in vitro in the mixed lymphocyte culture resulting in marked proliferation and overproduction of cytokines. Furthermore, the intensity of the recipient MHC disparate alloresponse to donor cells is thought to reflect the propensity of a recipient to mediate acute rejection (23, 24). The cellular basis for this intense allo-MHC response is associated with allorecognition via the direct pathway. All humans have a high precursor frequency of circulating T cells that are primed to respond through the direct pathway of allorecognition. The direct pathway precursor frequency of T cells is estimated to be between 1–10% of all circulating cells in peripheral blood (25). Circulating T cells survive a process of selection in the thymus that ensures that their T cell antigen receptor has a low but significant affinity for self-peptide/self-MHC molecules. At the same time, selection ensures that circulating T cells maintain a high affinity for foreign peptides in association with *self*-MHC molecules (26, 27). The relatively weak interaction between self-MHC and the TCR is required for the survival of naive T cells during development (28) and in the periphery (29).

Several models have evolved to explain the structural basis for allorecognition and they suggest that direct allorecognition does not conform to classical rules of self-MHC restriction. In the direct pathway, recipient T cells respond to allo-MHC molecules on donor antigen presenting cells. One proposed model suggests that direct pathway alloactivation of T cells can be an allopeptide-independent process (30), while another model suggests that direct alloactivation is peptide-dependent (31). For instance, when donor MHC is structurally different from recipient MHC, alloreactive T cells may directly recognize polymorphic residues with in the allo-MHC molecule itself, independent of allopeptide (32–36). Alternatively, effective allorecognition may require typical presentation of peptide to the TCR, especially in circumstances where donor MHC is structurally similar to recipient MHC (37–39). This latter model suggests that the greater the similarily between allogeneic (donor) MHC molecule(s) and self-MHC molecule(s), the greater the likelihood that the direct T cell allogeneic response will be dependent on allopeptide (40). It is important to note that the crystalline structure of MHC molecules and their analysis of function favor a role for peptide binding in the effective alloactivation of T cells (41–43). Therefore, peptide-dependent activation of recipient T cells through the direct pathway may occur via individual MHC molecule (s) that are similar in structure to self-MHC, while simultaneously, activation of T cells may occur by dissimilar MHC molecule(s) though peptide-independent mechanisms. Collectively, these mechanisms and pathways will result in a profound polyclonal T cell activation response, involving many T cell subsets. It is thus not surprising that we observe such a marked proliferative and activation response in the mixed lymphocyte reaction.

Acute allograft rejection is likely coordinated by T cells activated through the direct pathway (44–46). This possibility is based on the observation that there is a high precursor frequency of T cells in the circulation that are primed to respond through this pathway. Another reason may relate to the observation that the transplanted graft contains a significant number of donor-derived passenger APCs or passenger leukocytes that have a high density of allo-MHC molecules on their cell surface(s). These donor derived APCs also provide the necessary costimulatory signals for the full activation of recipient T cells (see below). In contrast, at later times post transplantation (>1 year), allo-activation via the direct pathway is minimal. This relative hyporesponsiveness, compared to pretransplantation responses, is most notable in the mixed lymphocyte reaction where there is a decreased proliferation/cytokine production of recipient T cells to donor cells. This is called "donor specific hyporesponsiveness" (21). Nevertheless, as will be outlined below, T cells are still activated by donor-specific allogeneic proteins at these later times, only the response is mediated through the indirect pathway of allorecognition (20, 21, 47–49).

Direct allorecognition requires the migration of allo-MHC bearing, and graft-derived cells into secondary lymphoid tissue (50, 51). Early studies showed that the

removal of donor-derived passenger APCs from the graft prior to transplantation resulted in prolonged graft survival (51–54). In contrast, the injection of donor dendritic cells into the recipient could induce acute rejection (55, 56). Dendritic cells are the most potent professional APC (57) and they have been implicated to be the "passenger leukocyte" (58) that functions in the initiation of the direct pathway of allorecognition, and thus in acute rejection. Immature dendritic cells are abundant within peripheral tissues where they are ideally positioned to capture antigens. Upon receiving inflammatory or "danger" signals, APCs undergo a maturation process, reverse transmigrate out of the graft into the afferent lymphatics, and eventually migrate to the paracortex of lymph nodes where T cells primarily reside (59–67). Some "danger" or inflammatory signals that result in APC maturation and migration are unique to transplantation and include characteristic ischemia-reperfusion injury. Ischemia-reperfusion induces the generation of reactive oxygen species (ROS) (68), which can result in the activation of chaperoning proteins, some of which are ligands for toll-like receptors (TLRs) (69–71). Following the stimulation of TLRs, immature TLR-expressing dendritic cells are activated, and respond by migrating from the graft into secondary lymphoid tissues (72–75). Oxidative injury also facilitates signaling through TLR-associated adaptor molecules such as MyD88 and TRIF, which have been shown to induce donor-derived dendritic cell migration into lymph nodes as well as the development of acute rejection (76–78). Knockout of intragraft TLR(s) reduces the degree of ischemia-reperfusion injury. In addition, ischemia-reperfusion injury as well as ROS may be directly toxic to intragraft cells and can result in cellular apoptosis and/or necrosis (79). These processes serve as additional stimuli for dendritic cell maturation and migration into lymph nodes (80, 81). Therefore, ischemia-reperfusion and the generation of ROS and its associated activation of inflammatory events within the allograft (82, 83) can be a factor in the early and rapid activation of the immune system and/or the subsequent activation of T cells throughout the direct pathway.

It is important to note that direct pathway alloactivation of cytotoxic $CD8^+$ T cells may serve as specific initiators of the acute rejection process (3, 84). If donor APCs are removed from allografts (and thus, $CD4^+$ T cell activation is negligible), $CD8^+$ T cells have been shown to mediate acute rejection by direct interactions with the graft (84). Under these circumstances, $CD8^+$ T cells recognize donor MHC class 1 expressed on graft vascular endothelial cells and mediate endothelial cell destruction leading to allograft rejection (3, 84, 85).

Over time however, donor APCs are depleted from the graft and thus activation of T cells via the direct pathway becomes less dominant, and persistent T cell activation is predominantly mediated by recipient dendritic cells, and/or self-MHC restricted alloactivation (20, 21, 49, 55, 86, 87). Furthermore, even in the absence of direct pathway alloactivation, T cells have the potential to mediate acute rejection, presumably as a result of alloactivation through the indirect pathway (88). In general, however, activation through the indirect pathway is thought to be of pathophysiological importance in the development of chronic rejection (89).

Indirect Pathway of Allorecognition

The indirect pathway of allorecognition describes a mechanism whereby T cells recognize processed alloantigens, presented as allopeptides on self APCs (*Fig. 74-1*). This self-restricted mechanism for the activation of T cells is not unique to the transplantation setting, and is the major physiological mechanism for the processing of foreign antigens and the presentation of foreign peptide antigens to T cells. This mechanism of T cell activation is characteristic, for instance, in delayed type hypersensitivity following exposure to bacterial antigens. However, the finding that the indirect pathway allorecognition is functional for T cell activation in the alloimmune response is most important, as this pathway is now proposed to be associated with the development of chronic rejection (90). The alloactivation of T cells via the indirect pathway is a result of processing of donor alloantigens by recipient APCs, and the subsequent presentation of allopeptide to recipient $CD4^+$ T cells in lymphoid organs (91). This process occurs through three mutually exclusive mechanisms. First, donor cell membranes and/or alloantigens may be shed from the graft into the circulation and phagocytosed by recipient dendritic cells that reside within secondary lymphoid tissue. Second, donor cells that migrate to secondary lymphoid tissues may be phagocytosed by recipient dendritic cells, and third, recipient APCs that migrate into the graft may phagocytose intragraft alloantigens (perhaps from apoptotic or necrotic intragraft cells) and then reverse transmigrate out of the graft into the secondary lymphoid tissue, where they serve to activate recipient T cells in a self-restricted manner. Any intragraft protein antigen of donor origin that differs from that expressed in the recipient is potentially a significant alloantigen capable of inducing an indirect anti-graft T cell response. Therefore, the indirect pathway of alloactivation has every potential to be a potent

mechanism to induce anti-graft T cell responses. Furthermore, it is likely that dominant alloantigen(s) driving the indirect response will change from time to time, such that the associated indirect T cell response will also change over time, a process called epitope shifting (92). Thus, the indirect alloresponse can result in ongoing, insidious and chronic T cell alloactivation.

In contrast to the direct pathway discussed above, the precursor frequency of T cells activated via the indirect pathway is very low (less than 1/million T cells), and the magnitude of the T cell response following indirect alloactivation is small (93). However, epitope shifting is a means whereby the indirect pathway elicits polyclonal and persistent activation responses (92, 94, 95).

Persistent alloactivation of T cells though the indirect pathway has been found to be associated with the development of chronic rejection following renal transplantation (95, 96). Furthermore, when donor specific hyporesponsiveness occurs through the direct pathways, T cells activated through the indirect pathway can be dominant, and can be associated with rejection, for instance following human cardiac transplantation (94). Neverthless, although T cells activated via the indirect pathway have the potential to elicit acute rejection, it is generally believed that activation through the indirect pathway results in the development of chronic rejection. Therefore, one might conclude that in the future the inhibition of T cell activation through the indirect pathway will be a means to prevent of chronic allograft rejection.

It is important to note that activation through the indirect pathway (95) and not the direct pathway (97) is also dependent on the degree of immunoregulation. As will be discussed below, self-restricted T cell activation can result in an expansion of low numbers of immunoregulatory T cells which serve to inhibit effector T cell activation responses. These immunoregulatory $CD4^+$ T cells, which are typically identified by high levels of expression of CD25 and FOXP3, inhibit indirect T cell activation. Therefore, indirect alloactivation (and thus chronic rejection) can only occur in those patients with limited numbers of CD25 and FOXP3 immunoregulatory cells (94, 95). Indeed, it has been reported that patients without indirect T cell responses have high numbers of immunregulatory cells in sufficient numbers to suppress the response. Also, patients with active indirect T cell responses and chronic rejection were found to have coincident low numbers of immunoregulatory T cells (95). Thus, one might conclude that one limiting factor for the expansion of T cells with indirect specificity (and thus chronic rejection) is the presence of immunoregulatory cells.

Other studies have suggested that costimulation is necessary for the activation of indirectly activated graft-specific T cells, and it has been demonstrated that some accessory costimulatory molecules inhibit the expansion of immunoregulatory T cells (98). These observations imply that costimulatory blockade might hinder the expansion of T cells activated through the indirect pathway (99).

There may be interplay among T cells activated through the direct and indirect pathways. For instance, directly primed $CD8^+$ T cells can target the graft and elicit an anti-graft destructive response. This response serves to stimulate recipient monocytes to process allontigens, which subsequently results in, and stimulates, an indirect response (3, 12, 84). Also, it is possible that both $CD4^+$ and $CD8^+$ indirect responses occur in the context of transplantation. Dendritic cells have the unique ability to process and produce not only peptide/MHC class II complexes from exogenous antigens, but also peptide/MHC class I complexes (100–103). It is therefore conceivable that both kinds of peptide/MHC complexes derived from donor antigens could be presented to recipient $CD4^+$ and $CD8^+$ T cells. Both indirectly primed $CD4^+$ and $CD8^+$ T cells can elicit rejection, and in some circumstances each specific response may be dominant to mediate ongoing rejection (12, 92, 95).

Semi-direct Pathway of Allorecognition

Intact allo-MHC molecules can be transferred to recipient dendritic cells and are capable of stimulating allo-T cell responses (22). The acquisition of MHC and/or other molecules can occur by direct cell-to-cell contact and/or via the stripping or "nibbling" of molecules from one cell membrane to another (104, 105). For instance, this can occur when recipient dendritic cells transmigrate across allo-MHC expressing graft endothelium (22, 105). Exosomes (nanovesicles < 100 nm) are intriguing in this process, as they are generated by reverse budding of the plasma membrane, and are capable of being secreted into the surrounding mileu and/or transferred between cells. Transfer of allo-MHC via exosomes has been shown to occur both in vitro and in vivo (106–108), but exosome transfer alone may not be sufficient to mediate the activation of naïve T cells (107). The transfer of allo-MHC molecules and processing by self-APCs is a notable mechanism for the stimulation of an indirect response. Thus, the semi-direct pathway may also be a mechanism that links the direct pathway to the indirect pathway of allorecognition (109).

Co-Stimulation/Co-Inhibition of T Cell Activation

The engagement of the T cell receptor with the MHC/allopeptide complex on APCs alone is not sufficient to elicit an effective T cell activation response (110, 111). Full T cell activation requires additional signals, commonly called "costimulatory signals," which are provided to T cells by one or more accessory molecules typically expressed on professional antigen presenting cells (111–115). When T cells receive an alloantigen-dependent signal as well as a costimulatory signal, several intracellular pathways are activated that synergize to result in proliferative, cell survival and migratory responses. Furthermore, the combined signals result in an overproduction of cytokines including proinflammatory IL-2 and IFNγ, and a marked proliferative response result in clonal expansion of the T cell. Inhibition of costimulation, while coincident antigen-presentation is permitted has been shown in cell culture as well as in several models of inflammatory diseases to result in T cell deletion, unresponsiveness (anergy), immune suppression or regulation and/or immune deviation. Together, the effect of costimulatory blockade is to dampen the immune reaction.

In addition, it is increasingly becoming appreciated that co-inhibitory signals are simultaneously transmitted to the T cell through other accessory molecules. Thus, the outcome and the resultant T cell activation response is dependent on the combined effects of both "positive" as well as "negative" accessory signaling. Furthermore, overexpression of co-inhibitory or "negative" signals suppresses activation and are thought to function to maintain a balance that regulates the effective immune response. This balance is somewhat complex as the temporal expression of costimulatory and co-inhibitory signals change over time. In general, costimulation occurs early and co-inhibition occurs late and serves as a physiological means to dampen the outcome of any antigen-dependent interaction. Dysfunction in this balance whereby immunoregulation fails to occur efficiently has been found to result in chronic inflammatory disease states as well as some forms of autoimmunity (116, 117). Alternatively, overexpression of co-inhibitory molecules have been found to augment immunoregulation and are thought be a major mechanism underlying allogeneic fetal tolerance (118). Pharmacological manipulations to block co-stimulation or to augment co-inhibitory signals can be used to change the outcome of alloimmune T cell activation. These types of manipulations have generated significant interest within the transplant community with the recent introduction of costimulatory blockers as therapeutics. We will next review several co-stimulatory and co-inhibitory signaling pathways that have been well characterized in the literature (summarized in *Table 74-1*).

CD28/CTLA-4/B7-1/B7-2 Pathway

Co-stimulation mediated by CD28 is one of the most important and intensely studied of all costimulatory signals. CD28 is a glycoprotein receptor that is constitutively expressed on resting T cells, but its expression is increased following activation. Signaling through T cell CD28 has been demonstrated to be critical in the primary activation and clonal expansion of naïve T cells. In the absence of TCR engagement, ligation of CD28 has no known immunological effect. However, CD28-inducible signals lower the threshold for TCR-inducible signaling so that together

Table 74-1
Costimulatory signaling pathways

Costimulatory receptor	Expression	Ligand	Function
CD28	Naive and activated T cells	CD80/CD86	Required for naïve T cell activation, IL-2 production and IL-2R expression
CD154 (CD40L)	Activated T cells	CD40	Promotes T-B cell interactions to induce B cell activation, antibody production and isotype switching Activation of APC
ICOS	Activated T cells	ICOS-L	Promotes effector function
CD134 (OX40)	Activated T cells	OX40L	Promotes effector function; negative regulator of FOXP3 regulatory cells
CTLA4	Activated T cells	CD80/CD86	Downregulates T cell activation responses
PD1	Activated T cells	PD-L1	Negatively regulates T cell activation and effector function

the signals synergize and promote several T cell activation responses. The biological basis for costimulation was in part defined by the CD28 pathway, whereby CD28 ligation was found to lower the threshold requirement for antigen-dependent and TCR-mediated signaling as well as IL-2 production (110, 119). Indeed, the intracellular signaling cascade mediated by both TCR/CD28-mediated events has profound synergistic effects upon T cell clonal expansion (120), cytokine production, mRNA stability (121–123) and cell cycle progression (124, 125). CD28 signaling also promotes T cell survival through enhanced expression of the anti-apoptotic protein, for instance, Bcl-XL (126). The enhanced proliferation and survival of T cells following CD28-mediated activation also influences immune deviation, including T helper type 1 and T helper type 2 cell differentiation, and thus, the effector immune response (discussed below) (127, 128). In addition, co-ligation of CD28 has biological responses on cytotoxic T lymphocyte (CTL) responses (129) and on $CD4^+$ T cell-dependent B cell effects that facilitate germinal center formation and antibody production (130, 131). Finally, CD28 signaling influences the expression of other costimulatory molecules, including CD154 (CD40L), ICOS, and OX40 (132, 133) (*Table 74-1*), thereby providing for amplification of proinflammatory loops, all of which serve to sustain the response. Together, these biological effects of CD28 are profound and have major implications for the development of transplant rejection as well as the production of alloantibody following transplantation.

The major ligands for CD28, called B7-1 (or CD80) and B7-2 (or CD86), are glycoprotein members of the immunoglobulin family. They are expressed on professional antigen-presenting cells (APC's), including B cells, monocytes, and dendritic cells. B7 molecules are induced in expression on APCs, following activation and/or following differentiation, and each molecule has a distinct patterns of expression. In general, B7-2 is expressed at early times following activation of an APC, whereas B7-1 appears at later times. The higher the level of expression of B7-1 and B7-2 on APCs, the more potent they become in their ability to activate T cells. For instance, immature dendritic cells have low levels of these costimulatory molecules and this correlates with their limited ability to promote alloactivation. In contrast, both B7-1 and B7-2 increase in expression in the course of dendritic cell maturation. Mature dendritic cells have very high levels of expression, which correlates with their characteristic potent APC function (134).

The effect of B7 family molecules on T cell activation is complex, as these molecules also bind an important inhibitory receptor called cytotoxic T lymphocyte-associated antigen 4 (CTLA4), a homologue of CD28. CTLA4 is not expressed on resting T cells but is upregulated after activation and is induced in expression following activation of CD28 signaling. CTLA4 binds to B7-1 and B7-2 with greater affinity than CD28, and thus, once expressed it is likely to function (135). In general, as CTLA-4 expression increases on a T cell, interactions between CD28 and B7 decrease, and thus it functions indirectly to suppress CD28-mediated costimulation. Signaling through CTLA4 also directly antagonizes costimulation via CD28 by inhibiting IL-2 production, IL-2R expression and cell cycle progression (136–138). CTLA4 also promotes expression of idoleamine 2,3 dioxygenase (IDO) in dendritic cells, which stimulates the degradation of tryptophan, thus limiting T cell proliferation (139). Studies using mice deficient in CTLA-4 have confirmed a major immunoregulatory function for this molecule (140). In CTLA-4 knockout mice, T cell proliferation is unrestrained and massive lymphoproliferation from unchecked costimulation occurs and results in autoimmune disease and early death (140, 141). Regulatory T cells have been shown to contain large intracellular stores of CTLA-4 which is thought to influence their suppressive properties (142). These data support the notion that CTLA-4 plays a critical role in the maintenance of self-tolerance, and that agents that interfere with CTLA-4 signaling may augment T cell activation and prevent tolerance induction (143). In contrast, agents that inhibit CD28-mediated activation will limit the T cell activation response to antigens, including alloantigens. Collectively, these observations imply that augmenting CTLA-4 signals while inhibiting CD28-induced responses augment regulatory immune function(s).

As a final note, we wish to point out that physiologically it is most important to keep an immune response "in check," and to suppress constant ongoing activation within the body. CTLA4 may be a prototype molecule in this regard to maintain immune regulation. Without these "checks" it would be difficult to maintain physiological immune regulation over time. It is thus of great interest that there can be an interplay between co-stimulatory and co-inhibitory receptor-ligand interactions. As discussed above, B7 family molecules, which provide for activation via interactions with CD28, also bind CTLA4 to provide for negative costimulatory signals. CTLA-4-mediated inhibition of T cell activation is enhanced at late times allowing for regulation to occur. Recently, it was found that human B7-1 can additionally interact with Programmed Death-1 ligand-1 (PD-L1), another negative co-inhibitory molecule (144). B7-1 binds to PD-L1 with

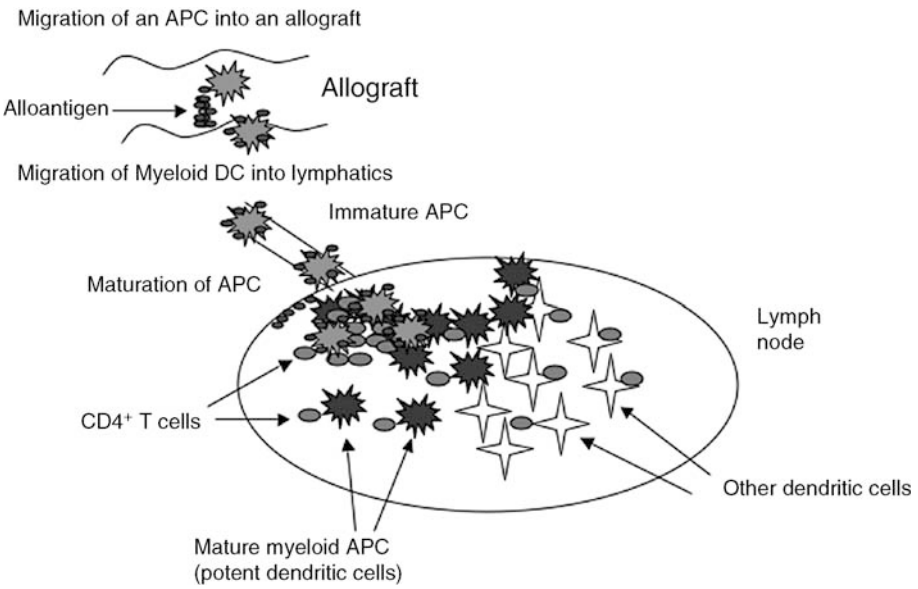
a greater affinity than it binds to CD28, but with less affinity than it binds to CTLA-4 or the interaction between PD-L1 and its co-inhibitory ligand PD-1 (145, 146). Thus, it is important always to consider costimulation and coinhibition as complementary interactions that serve to maintain an immune regulatory balance (147).

CD154/CD40 Pathway

CD40, and its ligand CD154 (also called CD40L, gp39, TRAP, T-BAM) are also important and well characterized costimulatory signaling molecules (148). CD40 is a 50-kDa integral membrane glycoprotein member of the TNF receptor family that has a wide distribution of expression on different cell types including T cells, B cells, monocyte/macrophages, dendritic cells, vascular endothelial cells (EC) and epithelial cells (149–151). The expression of CD40 is highly regulated by cytokines, and the intracellular signaling pathways resulting from the ligation of CD40 have been well characterized (148). CD40 ligand, or CD154 (also called CD40L, gp39, TRAP-1, TBAM) is 39 kDa type 2 membrane glycoprotein that is predominantly expressed on activated T cells and platelets (148, 152, 153). It is also expressed on other cell types within the immune system including B cells, dendritic cells, natural killer cells and endothelial cells. Interactions between CD154 and CD40 promote T cell and B cell activation, as well as the maturation of myeloid-derived APCs into mature dendritic cells (*Fig. 74-2*) (62, 154–156). CD154 is expressed on T cells after TCR signaling and is enhanced following CD28 dependent costimulation. CD154/CD40 interactions were initially shown to be important for T helper-dependent B cell responses, promoting B cell proliferation and isotype switching as well as germinal cell formation (149, 157–160). CD40 signals have also been shown to enhance T cell responses (161, 162). The potent physiological function of CD154/CD40 interactions in immunity were clearly defined when it was found that humans with genetic mutations of CD154,

Figure 74-2

Maturation of myeloid-derived antigen presenting cells. Recipient monocytes are recruited into allografts following transplantation, where they may differentiate into resident macrophages or into dendritic cells. Exposure to, and phagocytosis of, allogeneic necrotic tissue serves to initiate the maturation process. Immature dendritic cells reverse transmigrate out of the allograft into the lymphatics. Ongoing stimulation of these cells by cytokines, growth factors and cell surface molecules further stimulates their differentiation into mature dendritic cells. When they enter lymph nodes, they encounter naïve T cells, and they present allopeptide and stimulate the indirect allogeneic response. Although not illustrated in this figure, donor APCs present within an allograft immediately after transplantation, can reverse transmigrate into the lymphatics using identical mechanisms; when they enter the lymph node, they encounter recipient T cells and stimulate them via the direct pathway of allorecognition.



rendering the protein incapable of binding to CD40, are immundeficient. This human immunodeficiency disease is called the Hyper IgM syndrome (HIM) (163–167), because the dominant effect of this mutation/deficiency was found to be high serum levels of IgM and reduced levels of IgG, IgA, and IgE. The immmunological defect was subsequently identified to be related to an inability of B cells to switch from IgM production to IgG production. Furthermore, patients with CD154 deficiency and the HIM syndrome were also found to have defects in T cell function, and fail to mount appropriate cell-mediated and delayed type hypersensitivity responses (163–167). Thus, the in vivo dysregulation of CD154/CD40 interactions result in an immune deficiency with abnormalities in both cellular and humoral responses (151, 168, 169). Additional studies using CD154- as well as CD40-deficient mice confirmed a major role for CD40 in immunity as well as in several immune inflammatory diseases (170–172). Collectively, these observations have defined CD154 and CD40 as critical molecules in immune responses; and suggest that limiting these interactions alone (in humans) will have biological properties that regulate antibody production as well as effector responses all of which are associated with the rejection process.

Another important aspect of CD154/CD40-dependent costimulation relates to its interplay with the CD28/B7 pathway. Ligation of CD40 on APCs has been found to result in the expression of B7 family molecules. Furthermore, the functional costimulatory effect of CD40 signaling has been found to be associated in part with B7 expression, and with B7-CD28-induced signaling, discussed above. Also, the absence of CD40-induced signaling results in reduced CD28-dependent costimulation, suggesting that these molecules interact for costimulatory responses. In addition, there is an interplay between CD40 ligation and other proinflammatory molecules including, for instance, the overexpression of cytokines, such as IL-12. As discussed above, CD40 signaling has also been found to promote the antigen presenting function of APCs, and to promote dendritic cell maturation and survival (154–156). Other signals such as those mediated by Toll-like receptors (TLRs) have been found to synergize with CD40 to facilitate the maturation of dendritic cells, as well as their function as APCs (173–178). Blocking CD40-induced activation of dendritic cells can thus have pluripotent effects on immunity, resulting in the inhibition of T cell and B cell activation, inhibition of cytotoxic T lymphocyte development (162), reduced autoimmunity (179–184) and the augmentation of peripheral tolerance (185–188). Inhibition of CD40/CD154 signaling has also been shown to be associated with the expansion of immunoregulatory T cells, as well as populations of anergic $CD4^+$ T cells with potent immunosuppressive functions (189).

The functions of CD40 indicate that it is an important costimulatory molecule and that it functions in both humoral and cellular immunity either directly or indirectly via its ability to regulate the several accessory molecules and inflammatory mediators. Therefore it is not surprising that inhibition of CD154 alone has been shown to inhibit both acute and chronic rejection in animal models (190–196); and further, it is not surprising that the biological effect of CD154 blockade is most profound in combination with CD28/B7 blockade (190, 192). Unfortunately, initial studies using CD154 antagonists following human transplantation were unsuccessful as they were found to have significant side-effects (197, 198). However, newer CD40 antagonists are currently under development, and it is possible that they may be useful therapeutically in the future.

ICOS/ICOSL Pathway

As its name suggests, the costimulatory molecule ICOS (inducible T cell costimulator) is expressed selectively on activated and memory subsets of T cells but is not expressed on unactivated/naïve T cells (199–205). ICOS has structural and functional similarities to CD28, but it does not bind to B7 family molecules. Rather, it interacts with its own ligand, ICOS-L, also called B7H, GL50, B7RP-1, and B7-H2 (202, 206–208). The inducible expression of ICOS shortly after T cell activation suggests that it may be particularly important in providing ongoing costimulatory signals to activated T cells. Furthermore, during the course of an immune response, ICOS levels have been found to remain high on subsets of T cells (132, 209, 210).

ICOS-L is typically expressed in peripheral tissues, especially on endothelial cells where it is induced by inflammatory mediators (202, 206, 207). In this manner, ICOS-L may be functional in association with inflammation, and in association with several diseases including allograft rejection (206). The expression of ICOS-L in peripheral tissues is thought to be important for local reactivation responses, as ICOS-mediated costimulation may occur at the local site (206).

Although ICOS-L does not bind to CD28 or CTLA-4, there are similarities in the functions of ICOS and CD28. There also appears to be an interplay between the expression of ICOS and costimulation by CD28, especially since ICOS itself is induced in expression following CD28-dependent costimulation (205). Thus, some of

the functions previously ascribed to CD28, may in part be due to ICOS. For instance, while naïve T cells require CD28 signaling for the initiation of proliferation and cytokine production, it has been suggested that optimal activation of recently-activated T cells is less dependent of CD28, but is dependent on ICOS-ICOS-L interactions. Thus, different than that observed for CD28, ICOS selectively functions at later times in the immune response, and has little effect(s) on the initial stages of T cell activation. In activated T cells, ICOS-mediated signaling appears to induce cytokine production, especially T helper type 2 cytokines (209, 210). ICOS costimulation has also been found to be important for humoral immunity (211, 212). Furthermore, blockade of ICOS can inhibit IL-10 production as well as the development of regulatory T cells (213). Thus, there are several properties of ICOS that make it distinct from CD28.

In the experimental transplant setting, ICOS deficiency or blockade of ICOS can prolong cardiac allograft survival (214) especially when administered later in the course of the immune response (215). Although ICOS blockade does not effect memory T cell expansion, it has been shown to reduce the recruitment of T cells into allografts (216) and inhibits the effector function of memory T cells (217). Inhibition of ICOS-ICOS-L interactions in combination with blockade of CD154/CD40 has been found to prevent the development of chronic allograft vasculopathy (214). In summary, whereas CD28/B7 interactions are functional at early times post transplantation in the initiation of the alloimmune response, ICOS–ICOS-L interactions function to sustain alloimmune inflammation and memory responses.

OX40/OX40L Pathway

Similar to ICOS-ICOS-L, the OX40 (CD134)/OX40L (CD134L) costimulatory pathway does not function in the initiation of alloimmunity, but rather functions in the effector arm of the response. OX40 is expressed on activated T cells after several rounds of division, as well as on cells with potent effector function (218, 219). OX40L, a member of the TNF superfamily, is expressed on activated B cells, dendritic cells and on endothelial cells (220–222). OX40 has been found to possess potent costimulatory properties to promote T cell proliferation and effector cell generation (223–225). Costimulation through OX40 has a profound effect on the generation of memory T cells, and some studies suggest that it is of critical importance in the expansion of populations of memory T cells (226). Stimulation of OX40 can also promote the development of effector function from previously anergic (autoreactive) T cells (227). OX40 costimulates allogenic $CD4^+$ and $CD8^+$ T cell responses and can function in skin allograft survival (219). Stimulation of OX40 on memory T cells promotes their reactivation (228), and enables them to develop effector function and an anti-graft response (229).

Of importance, OX40 is also expressed on regulatory populations of $CD4^+$ T cells expressing FOXP3 (98, 230). Costimulation via OX40 has been shown to inhibit the generation of FOXP3-expressing cells from activated effectors and inhibits their suppressor function (98, 231). Thus, OX40 can act directly on T effector cells to support their expansion and survival or it can function indirectly to enhance the immune response by inhibiting T regulatory cells as well as immunoregulatory mechanisms.

PD-1/PD-L1/PD-L2 Pathway

PD-1 (programmed death 1) accessory molecule and its ligands PD-L1 (B7-H1) and PD-L2 (B7-DC) are members of the CD28/B7 family and have been shown to transmit inhibitory signals to T cells and play a role in peripheral tolerance induction (205). PD-1 is induced on activated T cells and B cells as well as on activated APCs. Its ligand, PD-L1 is expressed on several cell types but is up-regulated in expression on activated T cell and B cells. It is also induced in expression on other cells including, dendritic cells and endothelial cells, where its expression is thought to be important in the regulation of immune responses at peripheral sites (232). In contrast, the expression of PD-L2 is restricted to antigen presenting cells and is induced upon activation by inflammatory mediators (233–235).

Antigen-dependent activation of T cells and co-ligation of PD-1 results in decreased T cell proliferation, cytokine production (i.e., IL-2) and failure to progress through cell cycle (233–235). In addition, PD-1 ligation inhibits B cell activation responses (236). Murine knockouts of PD-1 have been found to have accelerated autoimmunity similar to that observed with CTLA-4 deficiency but to a lesser degree (237). In rodent studies, lack of PD-1 leads to increased numbers of B cells and a lupus-like syndrome with uncontrolled T cell and B cell activation, the development of arthritis and glomerulonephritis (238) and an autoimmune myocarditis (239). Collectively, these observations indicate that PD-1 is critical for the maintenance of peripheral tolerance.

In transplantation models, blockade of this pathway has been found to result in accelerated graft rejection and graft-versus-host disease (240–243). In circumstances

where costimulatory signal blockade has been used to inhibit rejection, augmenting PD-1 signaling can prolong survival, prevent the development of allograft vasculopathy, as well as modulate T cell and B cell dependent alloimmunity and augment peripheral tolerance (244–246). In other studies, blockade of the PD-1/PD-L1 pathway resulted in accelerated rejection of fully MHC-mismatched cardiac allografts (240), and knockout of the pathway within donor allografts was found to result in accelerated allograft loss and chronic rejection (247). In general, it is thought that the novelty of this co-inhibitory pathway relates to the possibility that manipulating its overexpression at peripheral sites will serve to augment peripheral tolerance and could be beneficial in to inhibit inflammation within allografts.

T Cell Activation Responses/Immune Deviation

Upon activation, $CD4^+$ T helper cells produce multiple cytokines and utilize cytokine-responses to elicit distinct effector functions and an inflammatory response (7, 248, 249). $CD4^+$ T helper type 1 cells (also called Th1 cells) predominantly produce IL-2 and IFNγ, whereas T helper type 2 $CD4^+$ cells (also called Th2 cells) produce IL-4, IL-5, IL-10, IL-13, but do not produce IL-2 and IFNγ. In general, T cell activation responses can be considered to be Th1-like or Th2-like depending on the cytokines produced. Furthermore, the nature of the response, Th1- or Th2-, can change over time in the course of a cell-mediated immune inflammatory reaction. The profile of cytokine production (Th1 and/or Th2), and thus the immune response is of importance in the outcome of any inflammatory reaction including allograft rejection. Th1-responses are associated with classical delayed-type hypersensitivity and cell-mediated immune inflammation. In contrast, Th2-like responses are generally thought to be associated with immune regulation and perhaps tolerance induction following transplantation (249–252). Th2 responses are also important in immunoglobulin switching and in eosinophilic inflammation. Thus, it is proposed that when an immune response deviates from a proinflammatory Th1-type response towards a Th2-type response, in general, it will be associated with immune regulation and the suppression of acute inflammatory responses, including allograft rejection.

$CD4^+$ T helper type 1 responses are typically elicited according to the strength of the antigen-induced signal and positive costimulation (253). In addition, the phenotype of the antigen presenting cell has a major effect on the T helper response (254, 255). Mature dendritic cells express high levels of MHC and high levels of positive costimulatory molecules, and thus are most potent to induce Th1 responses and pro-inflammatory cytokines (154, 255, 256). In contrast, immature dendritic cells or semi-professional antigen presenting cells (such as endothelial cells) with low levels of MHC, and/or low levels of costimulatory molecules are less potent to induce Th1 responses, and facilitate higher levels of Th2 responses (187, 255, 257). Furthermore, professional APCs such as dendritic cells that produce high levels of IL-12 tend to stimulate Th1 responses, whereas subsets of dendritic cells that fail to produce IL-12 and perhaps produce other immunregulatory cytokines can facilitate Th2 responses (254, 256). Although, further research is needed in this area, it is important to note that an APC can facilitate proinflammatory Th1-, or immunoregulatory Th2-type immune responses. Furthermore, the concept that it is possible to pharmacologically manipulate a dendritic cell such that it selectively stimulates a Th1- or a Th2- type immune response has significant therapeutic implications (255, 258, 259).

As discussed above, some costimulatory pathways such as, for instance, CD28/B7 and OX40/OX40L can facilitate the deviation of an immune response towards a Th1 profile (224, 225, 260). Nevertheless, interactions with costimulatory molecules for immune deviation are complex. For instance, while CD28 signals drive Th1 responses, OX40 costimulation promotes Th1 and Th2 responses. Furthermore, B7, which augments Th1 responses via CD28-dependent costimulation also binds to CTLA4 and PD-L1, which may serve to suppress IL-2 production and deviate the response towards Th2. Also, it is important to note that the state of activation of a T cell at the time of costimulation is also a determinant of whether costimulation can result in T helper type 1 cytokine production (261). Costimulation via pathways such as ICOS/ICOSL and OX40/OX40L (214, 262) may be critical to sustain Th1-effector responses, and their absence may, by default, result in Th2 cytokine expression.

The activation of $CD4^+$ T cells and the resultant Th1- or Th2 –type immune response has a profound effect on the outcome of many acute and chronic inflammatory diseases including allograft rejection (263). Acute allograft rejection is predominantly associated with Th1-type immune responses. However, acute rejection can occur in the absence of the classical Th1 cytokines IL-2 (264, 265) and IFNγ (266). Although the dominant Th1 cytokine, IL-2, classically drives T cell proliferation and expansion, it also promotes activation induced cell death (AICD). Thus, in its absence AICD is less pronounced, and memory cell

survival is higher, such that immune regulation may be more difficult to achieve (265–268). Another interpretation is that immunoregulation is dependent on alloreactive T cell clonal size and thus, when IL-2 deficiency hinders apoptosis/AICD of effectors, it will change the ratio of effector memory cells to regulatory cells resulting in an associated deficiency in immune regulation (263, 267, 268). In contrast, Th2 responses have been found to inhibit cell-mediated immune reactions and delayed type hypersensitivity and promote immune regulation. Several studies in transplant models have demonstrated that immune deviation to Th2-type responses is associated with long term graft survival following treatment with costimulatory blockade (269, 270). Therefore, it is proposed that Th2 cells may have a functional role as immunoregulatory cells in association with the development of tolerance.

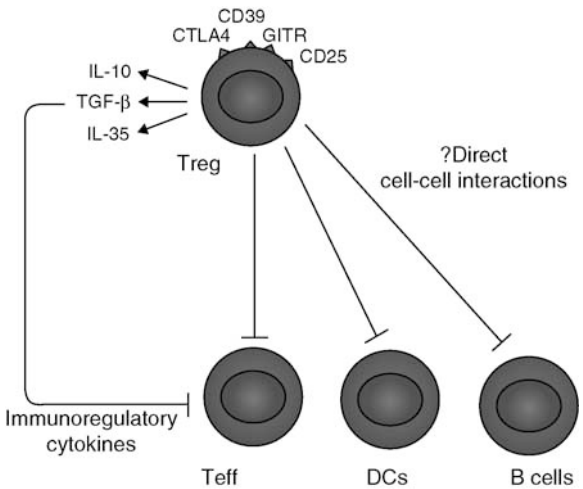
As discussed above, at baseline there is a high precursor frequency of alloreactive T cells primed to respond via the direct pathway, which typically results in a Th1 proinflammatory cytokine profile (251, 271). In contrast, at baseline, there is a relatively low precursor frequency of alloreactive T cells primed to respond via the indirect pathway of allorecognition. In order to sustain indirect pathway alloactivation over time, new naive T cells may be activated towards a Th1 and/or a Th2 response at different times post transplantation. Clonal size, the degree of costimulation and immune regulation may all be factors which determine the dominant T helper response, that in turn is important for long term graft survival. The principle that therapeutics might be used to deviate an immune response over time towards a Th2 profile has been thought to be clinically relevant to promote long term graft survival (263, 269). Nevertheless, it is important to appreciate that most of these studies relate to associations, and thus, it is still controversial whether a Th2 immune deviation alone is sufficient to mediate long term survival and transplant tolerance (127, 272, 273).

Immunoregulation/Regulatory T Cells

It has been well documented for many years that there are naturally occurring circulating T cells that promote immune regulation, prevent autoimmunity and can inhibit acute graft rejection. Furthermore, these cells can be transferred with lymphocytes from one animal to another. Initial studies, attempting to identify the nature of these regulatory cells demonstrated that they were $CD8^+$ T cells (274). More recently however, studies defined a subset of $CD4^+$ T cells expressing CD25 (the IL-2 receptor) and FOXP3 as the potent regulatory lymphocyte cell subtype (275–277) (*Fig. 74-3*). It is now known that naturally occurring regulatory T cells expressing CD25 and FOXP3 represent approximatly 5% of all circulating peripheral $CD4^+$ T cells; and isolation of these cells have confirmed that they exhibit potent regulatory activity against several types of T cell responses in vitro. $CD4^+$ $CD25^+$ $FOXP3^+$ T cells prevent autoimmunity in mice, and their depletion can induce the de novo onset of several immune inflammatory diseases (142, 276, 278, 279). FOXP3 was confirmed as a critical molecule for immune regulation in vivo, when it was identified that mutations in FOXP3 result in an autoimmune disease in humans called the IPEX syndrome (Immunodysregulation, polyendocrinopathy, enteropathy, X-linked) (280). Mutations in the FOXP3 gene in the IPEX syndrome result in defective

Figure 74-3

Immunoregulation by $CD4^+$ $CD25^+$ T cells. $CD4^+$ $CD25^+$ T cells expressing FOXP3 have been found to possess immunoregulatory function (Tregs). It is now established that these cells can regulate the activity and/or the function of effector T cells (Teff), dendritic cells (DC) as well as B cells. The mechanism(s) underlying their immunoregulatory function is an ongoing area of research. Several studies indicate that they may function via direct cell-cell contact, and others suggest that they function via the production of immunoregulatory cytokines. It is possible that different subsets of Tregs mediate immunoregulation via different mechanisms. Treg subsets can be identified by the expression of select cell surface molecules (CTLA4, GITR and CD39).



development of $CD4^+$ $CD25^+$ regulatory T cells and their deficiency leads to severe autoimmune phenomena including autoimmune enteropathy, dermatitis, thyroiditis, and type 1 diabetes, frequently resulting in death within the first 2 years of life (281). In rodent models, the transfer of FOXP3 into $CD4^+$ T cells in vivo, induces regulatory behavior and enables T cells to protect against autoimmune disease in a dominant fashion (282). During development, $CD25^+$ $FOXP3^+$ cells are selected in the thymus and a high proportion of the cells co-express other regulatory molecules including GITR and CTLA-4 (283, 284). FOXP3, is a transcription factor that induces both CTLA-4 expression as well as regulatory activity (282, 285, 286). Thus, FOXP3 controls the generation of regulatory T cells as well as their function. $FOXP3^+$ expressing regulatory T cells can be induced from non-regulatory cells by immunoregulatory cytokines such as TGFβ (287). In addition, following antigen-dependent activation, a subset peripheral $CD4^+$ T cells can be induced to express regulatory markers and subsequently develop functional regulatory activity (286, 288, 289). Indeed, it is established that TGFβ induces the differentiation of naïve $CD4^+$ $CD25^-FOXP3^-$ T cells into $FOXP3^+$ regulatory T cells, typically under conditions of low costimulation and CTLA-4-mediated negative costimulation/signaling. In vivo it has also been found that TGFβ signaling and B7 costimulation are required for expansion and peripheral conversion of $CD4^+$ T cells into regulatory subsets.

$CD25^+$ $FOXP3^+$ regulatory T cells appear to utilize a number of mechanisms to limit immune activation and to induce tolerance. These include the production of cytokines including TGFβ, IL-10 and IL-35 (290). They also have been found to expand following the stimulation of cell surface molecules including CTLA-4, ICOS, PD-1 and CD39 (291–293). Their regulatory function is exerted through T cells, B cells and dendritic cells. Much of their function is thought to involve direct cell-cell contact, although the expression of TGFβ and IL-10 may also be central to their regulatory function (*Fig. 74-3*).

Finally, it is important to note that although $FOXP3^+$ expression appears to dominate as a marker and factor enabling regulatory function, other regulatory cells appear to exist some of which are called Tr1, Tr3, Th3, Qa-1 restricted $CD8^+$ regulatory T cells. It is likely that new therapies in transplantation will focus on the selective expansion of $CD4^+$ $CD25^+$ $FOXP3^+$ T cells, as they are most potent in immune regulation. For instance, if it is possible to sustain their expansion in vivo in the long term with novel drugs or immunosuppressants, then it is hoped that this will translate into the clinic and promote long term graft survival.

Allograft Rejection

Effector Mechanisms

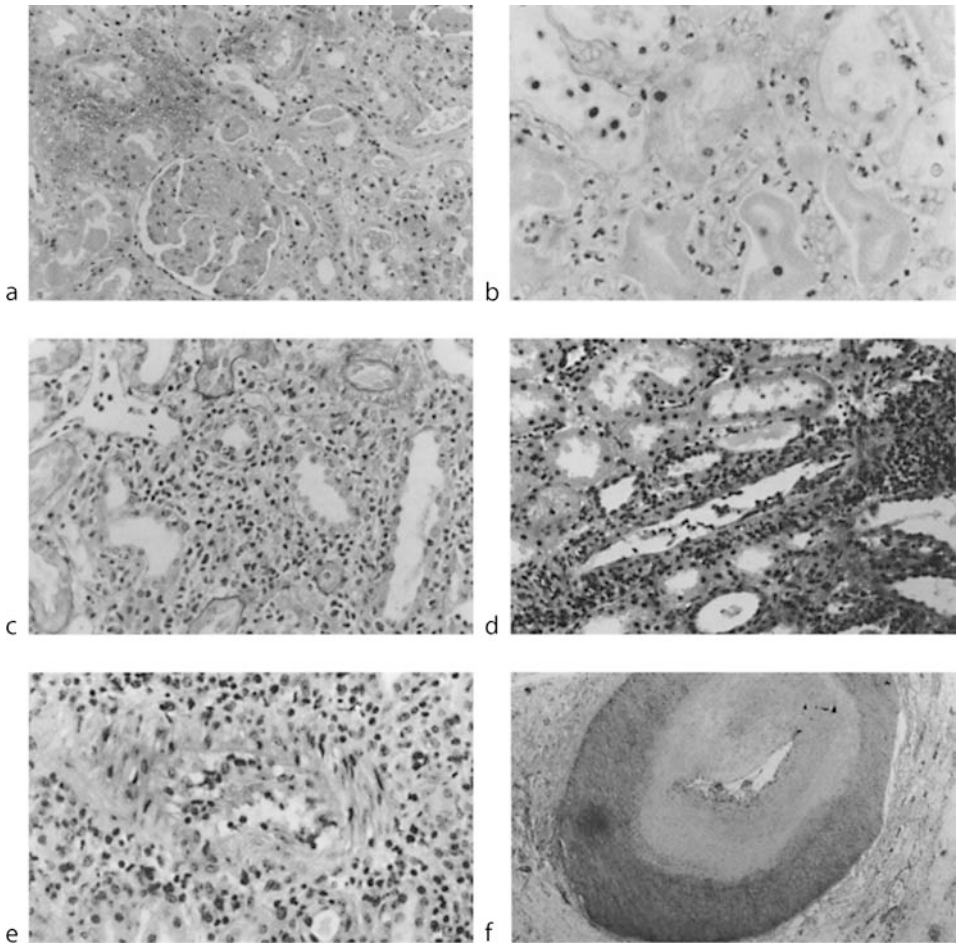
By definition, the development of allograft rejection involves a marked inflammatory reaction, characterized by the recruitment of leukocytes and an intense cellular and humoral attack on the graft (7) (*Fig. 74-4*). As discussed above, the rejection process is mediated by the recipient's immunological response to donor antigen, initiated and coordinated by $CD4^+$ T cells but also involves other cell types including $CD8^+$ T cells, B cells and macrophages (3, 115, 251, 271). The rejection response additionally involves other complex issues including the expansion of effector cells (294, 295), clonal size (267, 268, 296), the mode of allorecognition ("direct" or "indirect" pathways) (49, 271, 297, 298), the degree of immunoregulation (252, 299, 300), and adaptive responses that can occur within the graft (301–303). Nevertheless, activated effector cells and/or memory T cells must be recruited into an allograft in order to mediate graft destruction (3, 4, 84, 304–308). Indeed, several factors within allografts have the capacity to facilitate the rejection process; and the graft itself has the capacity to be dominant to determine the phenotype of rejection (acute or chronic), even in states where immunological tolerance is achieved (304).

$CD4^+$ T cells elicit and coordinate these responses using effector mechanisms (*Fig. 74-5*). They also provide "help" that coordinates the activation of cytotoxic $CD8^+$ T cells as well as alloantibody-producing B cells and mediate the expression of proinflammatory molecules by intragraft cells, such as endothelial cells. Infiltrating mononuclear cells produce a variety of cytokines and chemoattractants which are delivered into the graft. Macrophages also produce fibrogenic growth factors, such as TGF-β, that along with matrix proteins contribute to progressive scarring of the vessels and interstitium. Recently, it has been found that alloantibody development and targeting of graft vessels is of major importance in the destruction of allografts. The binding of alloantibody to cytokine-activated endothelial cells is associated with complement (C4d) deposition, which may result in local vasculitis and endothelial cell death (309). Studies indicate that the production of alloantibody following transplantation is an immunologic risk factor for the development of chronic rejection.

Activated graft vascular endothelial cells function in rejection by facilitating the recruitment as well as the activation of recipient leukocytes, including $CD4^+$ and $CD8^+$ T cells and monocyte/macrophages (3, 12, 84, 306, 308).

Figure 74-4

Pathology of transplant rejection. (a) Hyperacute rejection with edema of stroma, focal hemorrhage, thrombus formation, polymorphonuclear cell infiltration, and mesangialysis, (b) hyperacute rejection 1 h later, with tubular and epithelial cell necrosis and increased polymorphonuclear cell infiltration, (c) acute cellular rejection with inflammatory cell infiltration, (d) acute cellular rejection with ''endothelitis,'' inflammatory cells permeating the vascular intima, (e) acute cellular rejection with vessel wall infiltration, (f) Chronic rejection with dense intimal fibrosis. (Courtesy of Robert Colvin, M.D., Massachusetts General Hospital, Boston, Massachusetts and of Alan Krensky M.D. and Carol Clayberger, Ph.D., Stanford University Medical, Stanford, California).

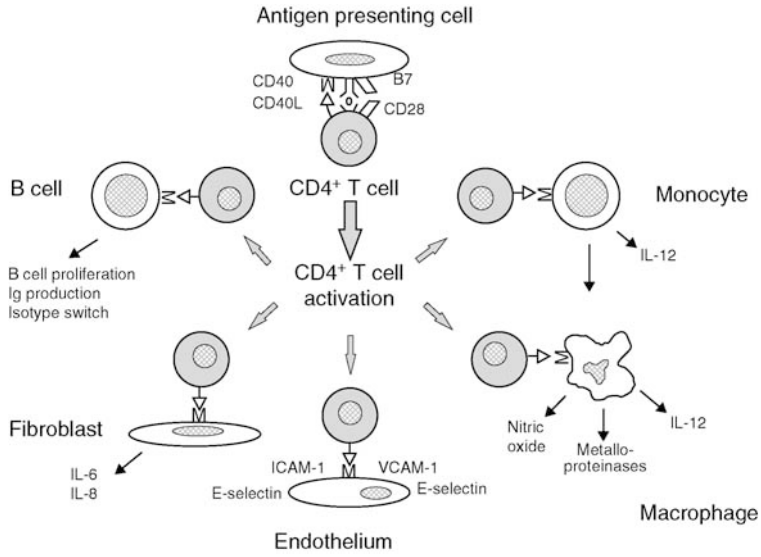


An extensive body of literature has established that endothelial cell activation is a characteristic feature of the rejection process. Ischemia/reperfusion injury, early inflammatory infiltrates and rejection result in endothelial cell expression of several adhesion molecules including E-selectin, ICAM-1 and VCAM-1, as well as chemokines, including the T cell chemoattractants IP-10 and RANTES and the monocyte chemoattractant MCP-1, all of which serve to augment leukocyte recruitment and activation (*Fig. 74-6*). In the course of rejection, it has been reported that endothelial cell activation responses are dynamic, some molecules such as E-selectin are induced at early times and precede rejection. Others such as VCAM-1, IP-10 and Mig are expressed in association with rejection itself (306, 310–312) (*Fig. 74-7*). In addition, activated graft endothelial cells have been found to persistently express HLA-DR, and its expression correlates with the development of rejection (306, 313) (*Fig. 74-8*).

Chemokine-chemokine receptor interactions function in the recruitment of leukocyte subsets, but also

Figure 74-5

Effector CD4$^+$ T cell responses. CD4$^+$ T cell recognition of alloantigen is the primary event that initiates alloimmunity. However, activated T cells mediate graft rejection through a variety of effector mechanisms. These include their ability to provide "help" which promotes the activation of alloantibody-producing B cells as well as other cell types including monocytes and macrophages and vascular endothelial cells. Together, all of these effector response serve to elicit a delayed-type hypersensitivity reaction and rejection.



have direct effects in immunity by facilitating APC function and T-cell activation responses (314–318). Chemokines and chemokine receptors are expressed in allografts; and blockade of one or many of these interactions inhibits the rejection process. Furthermore, chemokines have effects on angiogenesis and vascular repair, which may be of major importance in early destructive events as well as in the development of chronic rejection. For instance, the T cell chemoattractant chemokine IP-10 serves to facilitate recruitment and allograft injury (319), but its anti-angiogenic effects may also serve to sustain injury via the inhibition of vascular repair (320).

Nevertheless, these effector mechanisms account for only some of the well-characterized pathologic and clinical patterns of rejection (321, 322). The rejection process is more complex involving additional growth factors, such as vascular endothelial growth factor (VEGF), angiogenesis, the deposition of extracellular matrix, endothelial to mesenchymal transition and fibrogenesis, all of which are associated with vascular and interstitial disease (*Fig. 74-4*) (5, 90, 323–327). Collectively, these immunologic and nonimmunologic factors contribute to the destruction of allografts, which has been simply called "rejection."

Types of Rejection

Allograft rejection has been traditionally defined according to the timing of the anti-graft inflammatory response as: (1) hyperacute rejection that occurs within minutes to hours following transplantation, (2) acute rejection that occurs over a period of days at any time after transplantation, and (3), chronic rejection that typically occurs insidiously over several years following transplantation.

Hyperacute Rejection

Hyperacute rejection is characterized by rapid thrombotic occlusion of the graft vasculature that begins within minutes after recipient blood vessels are anastomosed to donor graft vessels (328). Hyperacute rejection is mediated by pre-existing antibodies that bind to graft vascular endothelial cells and activate complement. Pre-existing antibodies to blood antigens (ABO) most commonly cause hyperacute rejection, but it can also occur due to prior sensitization to HLA antigens and/or other polymorphic antigens expressed on the allograft endothelium. Following the binding of antibody to vascular

Figure 74-6

Leukocyte recruitment into allografts. Endothelial cells of donor origin play a critical role in the recruitment as well as in the activation of recipient T cells and monocytes/macrophages in vascularized organ allografts. Following transplantation and ischemia-reperfusion injury, endothelial cells express adhesion molecules and chemoattractant chemokines that function to augment leukocyte recruitment. The adhesion molecules E-selectin intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1 are well established to be associated with rejection. Chemokines are produced locally by endothelial cells within the allograft, but also are produced by infiltrating monocytes. When infiltrating monocytes secrete chemokines, they feed back to further amplify the inflammatory reaction. Finally, infiltrating T cells produces many cytokines, which can feedback to activate endothelial cells and promote adhesion molecule expression. Cytokines, such as IFNγ can induce the expression of HLA-DR on endothelial cells (see *Fig. 74-8*). Endothelial cell expression of HLA molecules can facilitate local T cell activation.

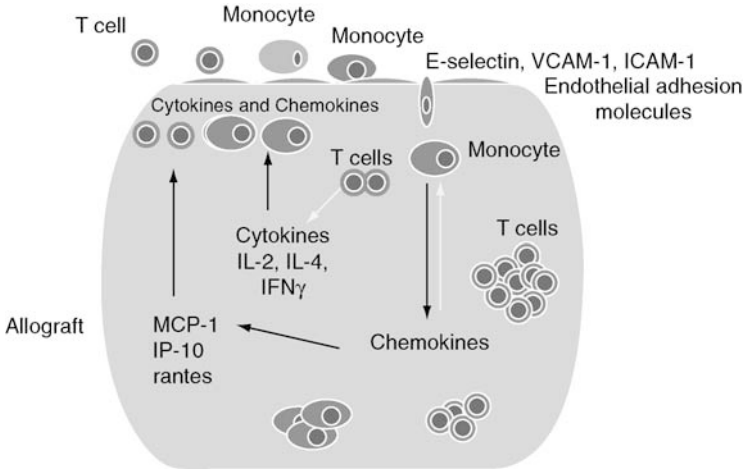
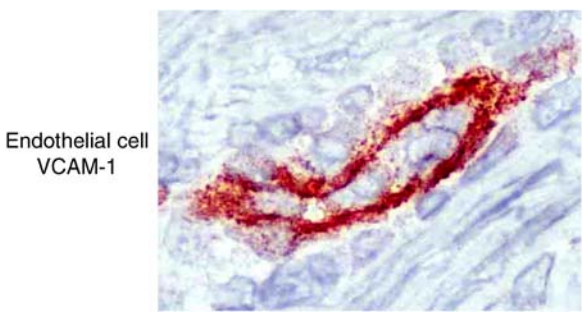


Figure 74-7

VCAM-1 expression. Illustrated is the immunohistochemical localization of the adhesion molecule VCAM-1 within a rejecting human cardiac allograft. Note that VCAM-1 is expressed on endothelial cells of this microvessel (rose brown color staining) and co-localizes with leukocytic infiltrates within the vessel. It is possible that VCAM-1 expression on these endothelial cells may function to promote local mononuclear cell infiltration. (See color plate 48)



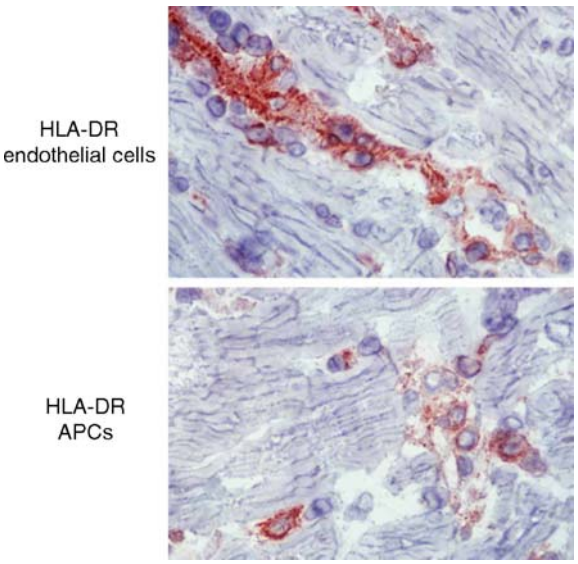
endothelial cells, they are stimulated to secrete high molecular weight forms of von Willebrand factor that mediate platelet adhesion and aggregation. Complement activation also leads to endothelial cell injury, and the exposure of subendothelial basement membrane proteins activates platelets, promotes mononuclear and polymorphonuclear cell recruitment and ultimately mediates destruction of the graft. These processes contribute to thrombosis and vascular occlusion, and the organ suffers irreversible ischemic damage. Although the cause of sensitization (pregnancy, transfusion, previous transplants) is not always apparent, routine crossmatching has virtually eliminated this fulminant type of rejection.

Acute Rejection

Acute rejection is characterized by necrosis of parenchymal cells and is usually accompanied by lymphocyte and macrophage infiltrates (329, 330). Several different

Figure 74-8

HLA-DR expression. Immunohistochemical localization of HLA-DR within a rejecting human cardiac allograft. Note that HLA-DR is expressed on vascular endothelial cells (*upper panel*) as well as on mononuclear cells (APCs) within the graft (*lower panel*). Also, it is noteworthy, that recipient infiltrates appear to encounter donor HLA-DR (and probably allopeptide) in the course of their recruitment into an allograft. (See color plate 49)



mechanisms may be involved in the development of acute cellular rejection including $CD4^+$ T helper cell activation responses (discussed above), cytotoxic T cell-mediated targeting of the graft, activated endothelial cells, chemokine production, monocyte/macrophage-mediated events as well as natural killer (NK) cell-mediated lysis of graft cells. Several lines of evidence suggest that recognition of alloantigen by $CD4^+$ T cells is critical for the initiation and coordination of all of these events. Furthermore, effector responses mediated by $CD4^+$ T cells, discussed above, are characteristic of acute rejection. Inhibition of $CD4^+$ T cell activation using immunosuppressive agents (discussed in Chapter X), and the targeting of T cells with lytic antibodies have been found to markedly inhibit the development of acute rejection.

Importantly, the lysis of graft cells by alloreactive $CD8^+$ cytotoxic T lymphocytes is a critical mechanism in the development of acute cellular rejection. Most vascular and parenchymal cells express MHC class I molecules and $CD8^+$ T cells can directly target the graft to elicit destruction (3, 84). In addition, endothelial cells, as well as other cells within allografts express MHC class II molecules (*Fig. 74-8*), which can be recognized by circulating $CD4^+$ T cells. This antigen-presenting function of endothelial cells may result in reactivation responses within the graft, further amplifying the inflammatory and rejection process (4, 306, 308, 331). There is also considerable evidence that B cells participate in the acute rejection process and produce alloantibody, which also targets the graft vascular endothelium. The binding of alloantibody results in direct lysis of cells, or can result in complement deposition (including C4d), which can be identified in allograft biopsies (332). The identification of C4d deposition is a poor prognostic biomarker.

The production of alloantibody can result in a form of rejection called acute humoral rejection that is characterized by necrosis of cells within graft vessels (309). It is often mediated by IgG alloantibodies directed against endothelial cell alloantigens, especially MHC class I and II molecules. Following the binding of alloantibody to endothelial cells and complement deposition, lymphocytes are recruited into the site. Together, these events result in profound proinflammatory amplication loops leading to activation responses and cytokine production that serve to further accelerate cell death. Thus, the histologic pattern of acute humoral rejection is one of vasculitis and is different than the thrombotic occlusion seen in hyperacute rejection or focal/multifocal inflammation and interstitial disease found in association with acute cellular rejection.

Chronic Rejection

Chronic allograft rejection is a process that occurs in all solid organ transplants, including kidney, heart, lung, pancreas and to a lesser extent liver allografts, and is the most common cause of late allograft failure. It is a poorly understood process and is mechanistically and functionally associated with both alloantigen-dependent and alloantigen-independent processes (90, 115, 251, 333). Histologically, chronic rejection is associated with variable degrees of interstitial mononuclear cellular infiltrates, the deposition of extracellular matrix and the development of fibrosis. It is characteristically associated with the progressive narrowing of vessels following heart and kidney transplantation (commonly called chronic allograft vasculopathy), bronchioles following lung transplantation (commonly called bronchiolitis obliterans) or bile ducts following liver transplantation (chronic cholangitis) (90, 334, 335). While alloantigen has generally been thought to be critical for the initiation of chronic rejection,

improvements in the treatment of acute rejection have failed to impact the development of this process (336, 337). Thus, it is currently proposed that chronic rejection either results from a fundamentally distinct alloantigen-dependent process (e.g., indirect allorecognition, discussed above) and/or is a multi-factorial process.

Despite extensive research, the precise mechanisms involved in the development of chronic rejection remain unclear. It proposed that chronic rejection results from persistent insults to the graft, from the initial ischemia-reperfusion injury and early acute rejection (even low levels of acute rejection, called "silent" acute rejection), the chronic use of nephrotoxic medications (e.g., calcineurin inhibitors) and the chronic insidious inflammatory response associated with delayed type hypersensitivity mechanisms. It has been proposed that persistent ongoing injury to allografts and chronic inflammation will inevitably result in vascular obliteration and parenchymal interstitial fibrosis (*Fig. 74-4*). Consistent with this possibility, it has been observed that there is an association between the incidence of acute rejection and the development of chronic rejection (336); and further, there are correlations between the degree of injury at any time post transplantation and the development of chronic rejection (338).

Pretransplant sensitization and the development of alloantibody post transplantation is also predictive of a high risk for the development of chronic rejection (309). In addition, alloantigen-independent processes including organ injury secondary to brain death in the donor, viral infections, hypertension, hyperlipidemia, cellular senescence and toxicity of immunosuppressive medications are associated with the progression of chronic rejection (339). All of these factors alone may not be sufficient to mediate chronic rejection but each may serve as a component of "chronic persistent injury" to sustain graft destruction (338).

Histopathologically, chronic rejection is also distinct from acute rejection. Extracellular matrix proteins are destroyed by inflammatory proteases, and debris is phagocytosed by macrophages and granulocytes. Fibroblasts undergo morphological changes and produce scar formation by elaborating collagen, fibronectin, and proteoglycans that are organized into new extracellular matrix (326, 327). These highly regulated events probably evolve to maintain the structural integrity of damaged organs, but in this circumstance result in progressive graft dysfunction and eventually graft loss. Cytokines produced by macrophages and lymphocytes, including platelet derived growth factor (PDGF), basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF) induce smooth muscle proliferation and angiogenic responses and facilitate the narrowing blood vessels (90, 323, 340). Cytokines such as TGFβ, which is expressed in allografts undergoing chronic rejection, mediate endothelial to mesenchymal transition and fibrosis (326, 341). Thus, the injury itself, the response to injury as well as the repair process all contribute to the loss of graft function.

Transplantation Tolerance

Following transplantation, clinical tolerance implies that immune unresponsiveness to an allograft is achieved such that it is not rejected by the recipient in the absence of immunosuppression. Tolerance also implies that the recipient will mount a normal immune response to other antigens, including infectious pathogens, and that alloresponses to a third-party is maintained. Our understanding of the immunology of tolerance is based on a long history of basic research and clinical observations.

Historical Observations

The understanding of how an individual discriminates between self and non-self laid the foundation for transplantation immunology. Owen first identified immune responsiveness and "tolerance" as we know it today, when he noted that freemartin cattle, who share a common placenta, become hematologic chimeras and do not recognize each other's blood cells as foreign (342). This observation led to the concept of "neonatal tolerance" and suggested that tolerance is an active process. Burnet and Medawar hypothesized that there was a critical period in neonatal development when the fetal immune system learns to discriminate self from nonself (343, 344). In seminal experiments, Medawar demonstrated that neonatal tolerance could be developed actively in mice. He injected fully allogeneic donor cells into a mouse fetus, and found that the mice subsequently developed tolerance to these donor cells after birth, in as much as they accepted donor strain skin grafts. Furthermore, these same mice rejected skin allografts from a third party mouse strain. This response, which is unique to transplantation, was termed immunological tolerance, and has been studied ever since as a major mechanism by which the immune system learns to recognize self. Brent and Medawar also demonstrated that there is a window of opportunity for the development of neonatal tolerance and that this window varies from animal to animal. Furthermore,

it was found that tolerance was not tissue-specific in as much as injections of leukocytes or other cell types were capable of conferring tolerance to later skin grafts. Altogether, these observations demonstrated that the immune response could be manipulated to lose reactivity to a transplanted organ; and laid the foundation for modern transplantation immunobiology as we know it today.

Many factors promoting immune evasion and neonatal tolerance have been explored in recent years and several theories have been proposed to explain the process. These include clonal deletion, apoptosis, immune regulation, immune deviation and anergy. We will next consider how some of these specific mechanisms are involved in tolerance induction.

Clonal Deletion

This model suggests that all of the T cells capable of recognizing a particular antigen are deleted from the T-cell repertoire. For example, mice expressing a certain HLA class II molecule (e.g., I-E) delete all T cells expressing a particular T-cell receptor variable region (V β 17) (345). This type of tolerance is induced while thymocytes are maturing in the thymus. When whole tissue (e.g., glomeruli, pancreatic islets, or spleen cells) or cells such as dendritic cells, or antigens (e.g., alloantigens or allopeptides) are injected directly into the thymus, they have been found to result in transplant tolerance. These thymic or "central" mechanisms are thought to induce clonal deletion of recipient T cells capable of recognizing the injected cells (346, 347).

Apoptosis and Immune Regulation

Although T-cell deletion during ontogeny leads to immune tolerance, T cells that have exited the thymus into the peripheri can also be deleted. This process is called "peripheral tolerance." In the peripheri, mature T cells can undergo apoptosis by two distinct pathways: activation-induced cell death (AICD) and passive cell death. A1CD occurs in repetitively stimulated T cells and acts to limit the extent of the immune response. AICD is largely mediated through Fas and other members of the TNF receptor superfamily and appears to require IL-2. Once activated, T cells upregulate the expression of Fas and Fas ligand, and when cell-cell contact occurs, the interaction of Fas and Fas ligand results in apoptosis (348). The intracellular events leading to apoptosis/death are mediated though cell signaling pathways (called death pathways) that result in the activation of caspase enzymes which result in DNA breakdown (267). Passive cell death occurs when activated T cells are deprived of growth factors. This pathway results in cell death due to the inhibition of cell survival signaling and the downregulation of anti-apoptotic factors such as Bcl-2 leading to mitochondrial damage and cell death. Both AICD and passive cell death appear to be required for tolerance induction following transplantation (296). In addition, the phagocytosis of apoptotic cells by macrophages often leads to the production of IL-10 and TGF-β (349), which contribute to the development of peripheral tolerance (350).

It is also well established that that there are subsets of cells in the immune system, called regulatory cells, that have the potential to suppress active immune responses (discussed above). This observation was initially discovered as a result of studies in which it was noted that T cells from a tolerant animal can be transferred to another animal to subsequently render the animal tolerant (300). This observation, called "infectious tolerance" led Gershon and Kondo to hypothesize that there were cells (which they thought were $CD8^+$ suppressor T cells) actively involved in the dampening of the immune response (274). Moreover, it now appears that long-term maintenance of peripheral tolerance depends on self-perpetuating immunoregulatory mechanisms that actively inhibit alloreactive T cells. $CD4^+$ regulatory T cells, first identified in 1990 (275, 351), are now recognized to be the critical effectors of peripheral tolerance and the control of autoimmunity (142, 279, 352, 353). As discussed above, these $CD4^+$ T regulatory cells are defined by the expression of CD25 and FOXP3, and they express a range of other regulatory molecules including CTLA4, GITR as well as other receptors such as CD39. FOXP3 is a transcription factor that induces regulatory activity and protects against autoimmune disease. The mechanisms by which peripheral $CD4^+$ regulatory T cells exert their effects remain controversial, but in part involve the inhibition of IL-2 production by the responding T cells (354, 355). Suppression of T-cell proliferation by regulatory T cells may also involve the secretion of anti-inflammatory cytokines such as IL-10 (356) and TGF-β and IL-35 (357), and/or may require direct cell-cell contact (*Fig. 74-3*).

Finally, another mechanism leading to immune regulation and peripheral tolerance is immune deviation. Strategies that result in the deviation of an immune response from a Th1- to a Th2-type also skews the inflammat with immune suppression. Although not necessarily

causal, Th2 immune responses in general are associated with a lesser immune regulation.

Anergy

Anergy is another important peripheral mechanism by which renders mature T cells to be tolerant. The interaction between the T-cell receptor and specific antigen in the context of self-MHC activates the T cell only if a costimulatory signal is also provided (see above and (358, 359)). If a T cell does not receive the costimulatory signal, it will not be activated, proliferate, or differentiate into an effector cell. These cells become anergic; that is, for a prolonged period of time, they will not respond to the appropriate antigenic stimulus even in the presence of an effective costimulatory signal. Clinically, anergy appears to be inducible with soluble antigens, through either donor-specific transfusions (360) or soluble HLA molecules that may be released, for example, following liver transplantation (361).

Clinical Tolerance and Chimerism

Clinical tolerance has been observed rarely in patients following liver and kidney transplants. One study suggested that tolerance can be achieved in 5–10% of patients receiving bone marrow transplantation followed by organ transplantation from the same donor, particularly the liver (362). Tolerance following renal transplantation is extremely rare and while observed, frequently appears to be associated with an insidious form of chronic rejection. Also, it has been associated with an immunosuppressed state in patients with concurrent malignancy (363). Several research groups including Starzl (364, 365), Wood (299, 366), Sachs (367) and Sykes (368) have suggested that donor specific transfusions can serve to facilitate a form of immunological tolerance. These groups pioneered the concept that administration of donor alloantigen in the peri-transplantation period can govern the extent to which post transplant hyporesponsiveness (or tolerance) is achieved. Along with others, these groups have clearly demonstrated that one of the main mechanisms by which a donor specific transfusion can induce allogeneic hyporesponsiveness involves the establishment of mixed chimerism.

Mixed chimerism is defined as a state in which donor and host populations of hematopoetic cells co-exist in the recipient (368). Starzl and Sykes have proposed that the presence of donor cells in a transplant recipient may augment immunological tolerance and is a favorable outcome following transplantation (364, 368). Thus, chimerism may be initiated by "passenger leukocytes" which are inevitably transplanted along with a solid organ allograft. These passenger donor cells, which include stem cells and antigen presenting cells (APCs), migrate from the transplanted organ into the recipient (364, 365, 369).

The mechanism by which mixed chimerism might result in allogeneic hyporesponsiveness involves central (or deletional) tolerance (368, 370). This occurs when donor-derived APCs take up residence within the recipient thymus and mediate deletion of recipient donor-reactive T cells. The degree of chimerism post transplantation could be dependent upon the number of passenger donor leukocytes that are transferred into the recipient from the graft; and this can be different among kidney, heart and liver allograft recipients (364, 369). Liver allografts provide significant numbers of donor cells suggesting that chimerism could be most likely in these recipients. However, even following liver transplantation, chimerism is short-lived and in the absence of persistence should not lead to immunological hyporesponsiveness or tolerance (365, 371, 372). This observation led investigators to initiate studies involving the adoptive transfer of donor cells into the recipient at the time of transplant (or/and following transplantation) to promote and enable the persistence of chimerism. If chimerism is a mechanism underlying tolerance, then these studies could identify if chimerism actually causes immunological hyporesponsiveness as opposed to being simply associated with long-term allograft survival.

One possible clinical approach is to induce hematological mixed chimerism pre-transplantation. Initially this strategy was used in patients with malignancy receiving identical renal transplants (373, 374). In a recent study, this strategy has also been used in the setting of HLA mismatched kidney transplantation (374). Five patients with end-stage renal disease received combined bone marrow and kidney transplants from HLA single-haplotype mismatched living related donors. Transient chimerism developed in four of the five recipients and it was possible to discontinue all immunosuppressive therapy at different times following transplantation. In some of these patients, renal function has remained stable for up to 5 years post transplantation. These trials are the only successful studies that have achieved long term tolerance in patients receiving HLA mismatched kidney transplants. There are however other reports of the development of mixed chimerism and tolerance following HLA-mismatched liver transplantation (375).

Tolerance and Immunosuppressive Agents

Importantly there is also evidence that immunosuppressive medications have major effects on the induction of tolerance. Calcineurin inhibitors reduce regulatory T cell numbers and function (376). Similarly anti-CD25 antibodies cause a temporary depletion of regulatory T cells (377). However, the mTOR inhibitor drug rapamycin, which is efficient to inhibit effector T cell survival, allows for the selective expansion and survival of regulatory T cells. In part this effect relates to the ability of regulatory cells to utilize novel anti-apoptotic mechanisms (378, 379).

Clinical Tolerance and Costimulatory Blockade

In the early 1990s a soluble fusion protein was developed consisting of the extracellular binding domain of CTLA4 fused with the Fc domain of human IgG1, i.e., abatacept (CTLA4Ig) (380). Abatacept binds to both CD80 and CD86 blocking CD28 engagement and signaling (381). The expectation was that graft-specific tolerance would be induced in the clinical transplant setting when CD28-dependent costimulation was inhibited, as was demonstrated in rodents transplant models. However, graft-specific tolerance failed to occur following its use in nonhuman primates (382). Abatacept has been very effective as an immunosuppresant and is currently approved by the U.S. Food and Drug Administration for the treatment of moderate to severe rheumatoid arthritis (383). The failure of abatacept in the transplant setting was thought to be secondary to a fast off-rate of binding to CD86. A second generation agent, LEA29Y or belatacept, was genetically engineered to bind with a higher affinity to CD80 and CD86 compared to abatacept (384). In nonhuman primate renal transplant studies, belatacept is better at preventing acute rejection episodes than abatacept and may offer an alternative to the current maintenance immunosuppressive regimens available (385). Belatacept is currently in phase III human clinical trials to determine if costimulatory blockade can replace calcineurin inhibitors in immunosuppression protocols (383). Preliminary data suggests it may also provide an advantage in reducing the development of chronic rejection.

Targeting other costimulatory pathways such as the CD154/CD40 pathway are also very appealing because of their potent ability to block T cell activation as well as alloantibody production. Initial studies in nonhuman primates demonstrated long-term kidney allograft survival using anti-CD154 (195). However, studies using anti-CD154 (hu5C8) treatment in humans resulted in thromboembolic complications not observed in small animal models (198). This has been attributed to the expression of CD154 on human but not mouse platelets. Nevertheless, newer agents are again being developed that do not have this effect.

Collectively, new costimulatory blockers and tolerance induction studies represent the "tip of the iceberg" in terms of efforts to promote clinical tolerance in humans. Over the next few years, it is likely, that several additional agents will become part of clinical immunosuppressive regimens, and it is hopeful that they will represent a major leap forward to enhance efforts to promote long term graft survival in humans.

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75 Pediatric Kidney Transplantation

William E. Harmon

Introduction

Pediatric kidney transplantation is an extraordinary treatment for End Stage Renal Disease (ESRD) which requires the active and collaborative participation of a team of medical and surgical specialists, including pediatric nephrologists, transplant surgeons, transplant coordinators, dialysis personnel, nutritionists, social workers, psychologists, activity therapists, tutors, pharmacists, infectious disease specialists, and many other consultants. Successful treatment, therefore, requires substantial resources at specialized centers. Nonetheless, since ESRD is relatively rare in children, even the busiest of these centers performs no more than several dozen transplant procedures annually. Thus, the most accurate and reliable information about transplantation requires registries that accumulate information from many centers; and research requires multi-center collaborative study groups. Several voluntary and mandatory registries, therefore, now exist in the field. This chapter is based principally on two of those registries, whose databases are freely available on the World Wide Web: The Organ Procurement and Transplant Network (www.optn.org) and the Scientific Registry of Transplant Recipients (www.ustransplant.org) contain the database of every organ donation and transplant event occurring the in United States since 1986 and appropriate scientific analyses of that data. The North American Pediatric Renal Trials and Collaborative Studies (www.naprtcs.org) has collected information on almost 17,000 children with ESRD treated in 147 North American centers since 1987, including 9,854 index transplant procedures. Based on the size of these databases and the extensive analysis available from them, the information they have produced does provide an appropriate and accurate perspective about the field of pediatric kidney transplantation.

Role of Kidney Transplantation in Treatment of ESRD in Children

Renal transplantation is widely recognized as the treatment of choice for children with end stage renal disease (1–3). The life expectancy benefit of renal transplantation over chronic dialysis for these children may be as much as 25–30 years (4); and a recent report has demonstrated that transplanted children have a survival benefit when compared to those on the waiting list that is even more pronounced than what is found in adults (5). A functioning renal transplant enables children to develop almost normally, grow reasonably well and improve their school performance levels (6–9). Both peritoneal dialysis, delivered as CAPD or CCPD, and hemodialysis lead to a deceleration of growth. Data from the dialysis component of the NAPRTCS registry (10) show that dialyzed childrens' overall height deficit of −1.8 S.D. became more negative reaching a value of −2.16 S.D. at 24 months. Additionally children do not tolerate being "dependent" on the treatment and maintenance dialysis induces loss of self-esteem and emotional maladjustment (11). Cognitive achievement testing may diminish with prolonged time on dialysis (8). In contrast, the mobility and freedom from dietary restrictions afforded by a functioning renal transplant enable children to live nearly normal lives. Although renal transplantation has not lived up to the promise of normal growth for all children, dramatic short-term improvements in height can be seen in many and final adult height is improving after transplantation (7, 12–14). Most importantly, successful transplantation permits the child to attend school and to develop normally; school function testing improves dramatically following transplantation (6, 15). And, importantly, young children now have the best long-term outcomes of all ages of transplant recipients, verifying the utility of transplantation in this age group (16). For all of these reasons, successful renal transplantation remains the primary goal of programs that care for children with ESRD.

There has been a substantial change in long-term renal allograft outcome for children during the past decade (17–19). Previously, young children were thought to have poor short- and long-term graft survival related to several factors, most prominently a proposed heightened immune response, especially in infants (20, 21). Conversely, however, adolescents were subsequently noted to have a higher rate of late acute rejections (22) and infants may have a lower rate of acute rejection than older

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children (23). An important analysis of the UNOS data demonstrated that short-term pediatric renal transplant survival rates became comparable to those in adults about 10 years ago (22). The most recent comprehensive registry reviews have clearly demonstrated a dramatic reversal in outcomes. Improvements in surgical technique (24–28), donor selection (29), immunosuppression practices (30–34), and the enhanced experience of specialized pediatric transplant teams (35), as well as the development of multi-center research consortia have all led to marked improvements in patient and kidney graft survival for infants and young children (17, 18, 36). Indeed, several analyses have identified these very young recipients as now having the best long-term survivals of all age groups (17, 22, 37, 38). In fact, young recipients of adult-sized kidneys who have immediate graft function have been reported as having the longest projected graft half-lives, exceeding even those of adult recipients of 2-haplotype matched living donor transplants (39).

Currently, pediatric recipients under age 11 who receive living donor kidney transplants have 3-year graft survival rates that are as good or better than older age groups (93% for those aged 0–5 years, and 92% for those aged 6–10 years) (38). The results of young recipients of deceased donor kidney transplants are similar to those seen in adults, with recipients aged 1–5 years and those aged 6–10 years having 3-year graft survival rates of 82% (38). Unfortunately, this excellent outcome is not seen in adolescents whose 3-year graft survivals for both living donor (85%) and deceased donor (76%) grafts are worse than all other age groups. As shown in *Fig. 75-1*, 5-year graft survival rates for both living and deceased donor kidney transplants for children less than 11 years of age are better than all other age groups of children and adults (38).

Incidence and Frequency of Pediatric Kidney Transplantation

By the end of 2008, there were about 75,000 individuals registered on the UNOS kidney transplant waiting list. Of these, about 1,800 were children, representing 2.4%. In 2008 16.514 kidney transplants were performed in the United States, of which 773 were children; thus pediatric patients comprise about 4.6% of all transplant recipients in the United States. Pediatric recipients received 293 or 5% of all living donor kidney transplants and 480 or 4.5% of all deceased donor transplants. Although the number of pediatric transplants performed each year has not varied by more then 10%, the donor origin has undergone substantial changes (*Fig. 75-2*).

The Scientific Registry of Transplant Recipients (SRTR) data show that living kidney donation has expanded substantially during the last decade and the number of living donors exceeded the number of deceased donors in the United States for the first time in 2001 (40). Living donation has accounted for 35–42% of all annual kidney transplants in the United States in the past decade. In 1987, only 40% of all transplants performed in children were from living donors; by 1991, the figure had risen to 53%, and until recently living donors accounted for over 60% of all pediatric renal transplants (*Fig. 75-2*) (36, 38). This recent shift of donation to a majority of deceased donors is likely related to a change in the Organ Procurement and Transplant Network (OPTN)

Figure 75-1

Five-year graft survival by age at the time of kidney transplantation. (Adapted from (38)).

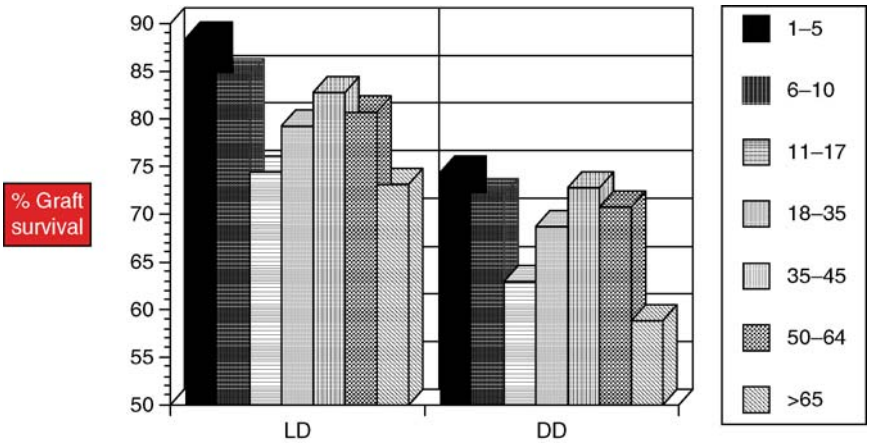
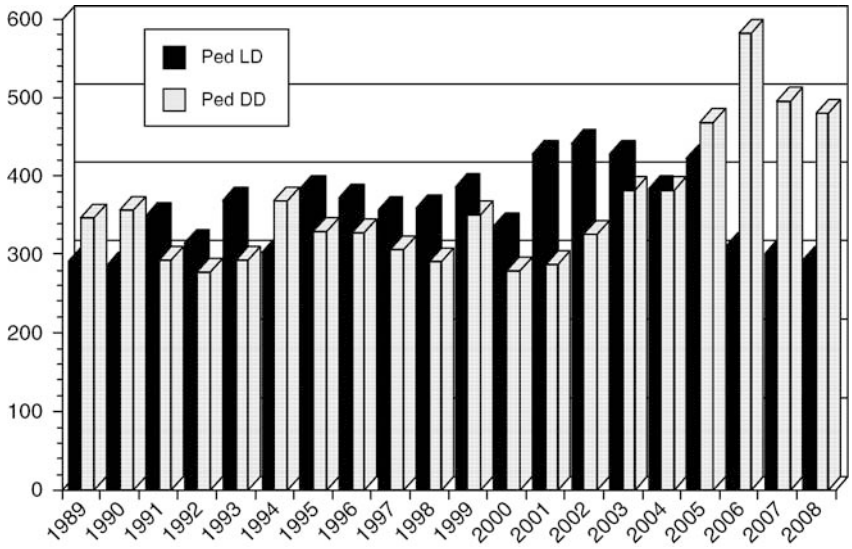


Figure 75-2

Pediatric living (LD) and deceased (DD) donor kidney transplants by year of transplantation.



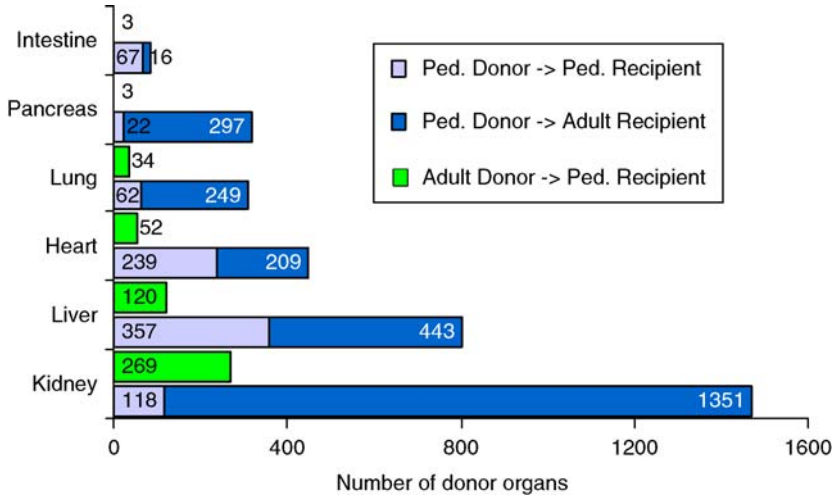
allocation system designed to provide reduced waiting time for children by giving them substantial preference on the waiting list (41).

Parents comprise 80% of living donors for pediatric kidney transplants. Mothers comprise the majority of parent donors; fathers account for 44%. Since there are more boys than girls who receive kidney transplants, it should not be surprising that fathers donate to sons 63% of the time and mothers to sons 59%. A recent analysis of 23,064 1-haplotype matched living related donor transplants in the SRTR database showed that mother-to-child transplants had the worst outcome of all possible groupings (HR = 2.61, $P < 0.0001$), which argued against any advantage of fetal-maternal microchimerism (42). One possible exception is that infants less than 1 year of age seem to have fewer rejections if the mother is the donor (22, 43). Due to the fact that children most often have siblings who are too young to be living donors (less than 18 years), the NAPRTCS registry has recorded only 382 transplants between siblings. Of these, 182 grafts were from donors less than 21 years of age. A review of the NAPRTCS registry identified only 15 living donors under 18 years of age, of which 13 were transplants between siblings and one was from an emancipated-minor parent to child. The majority of these young donors were 2-haplotype matches to the recipients. It is quite clear that most programs seem reluctant to use young donors (44, 45). However, a review of UNOS data revealed that out of approximately 40,000 living donors in the US between 1987 and 2000, 60 were from donors less than 18 years of age (46). Twenty-four of the recipients were children and 36 were adults; only 7 of the transplants were between identical twins. Subsequent to that report, the international transplant community recommended that minors not be used for living organ donation (47). In recent years there has been a substantial interest in living-unrelated donation in adult transplant literature since the outcome of these grafts has been shown to be equivalent to most living related donors and better than that of deceased donor kidneys (48). NAPRTCS has identified 232 instances of living-unrelated donation between 1987 and 2007. In a preliminary analysis of the first 38 living- unrelated recipients, 23 (61%) were male, 30 (79%) were Caucasian, eight were less than 6 years old, and 20 were older than 12 years (49). This was the primary transplant for 29 of the 38 recipients. Of the 38 donors, 22 were non-biologic parents, and a family friend was the donor in 10 of the cases.

About 70% of deceased donor kidneys transplanted into children are recovered from adult donors (*Fig. 75-3*) (17). In the 1980s there was a tendency to preferentially place kidneys recovered from infants into young recipients, with disastrous consequences for patient and graft survival (50). As a result of widespread dissemination of these data (51, 52), there has been a marked change in that practice. From 1987 through 1990, the percentage of deceased donors younger than 10 years ranged from 32 to 41%. From 1991 through 1994, these percentages

Figure 75-3

Numbers of pediatric and adult organ donors and recipients. In general, the number of pediatric organ donors exceeds the number of recipients for all organs transplanted (17).



ranged from 12 to 22%. Prior to 1991, children less then 2 years of age comprised 3.2% of deceased donors. In 1991, no pediatric recipient received a kidney from a deceased donor less than 2 years of age; and in 1995 and 1996, there were no such kidneys utilized in children (53). Between 1991 and 2000, less than 1% of deceased donors for children were <2 years of age (36) and between 1991 and 2007, there have been only 28 such donors (0.8%). This change in allocation of kidneys from young donors corresponded to improvement in graft survival (50). Some specialized pediatric programs have reported good results with young donors (54, 55), but many programs reserve grafts from very young donors for en bloc or single kidney transplantation into older recipients (56–58). It is also important to note that the majority of organs recovered from pediatric deceased donors are transplanted into adults (*Fig. 75-3*) (17).

Demographics of Pediatric Kidney Transplantation

Age, Gender and Race at Transplantation

Kidney transplantation prior to 6 months of age or below a recipient weight of 6 kg is exceptional. From 1987 to 2007, NAPRTCS has recorded 94 transplants performed in children younger than 12 months (59). Of these, seven transplants were performed in children between 3 and 5 months, 22 were performed in children between 6 and 8 months, and 63 were performed in children between 9 and 11 months of age. Seventeen infants have been reported to NAPRTCS since 2000 and UNOS has recorded 26. As is true for older infants, the majority of kidney transplants in infants <1 year of age have come from living donors. In general, the number of kidney transplants performed in infants <1 year seems to be declining, whereas the number in children 1–5 years has increased slightly. Since infants and adolescents have different risk factors for both patient and graft survival, children have been grouped into multiple age categories. NAPRTCS uses 0–1, 2–5, 6–12, 13–17 and 18–21 years of age for analyses. In 1987, 25% of all pediatric transplants were performed in children 0–5 years of age (20), whereas in 1995 the figure was 17% (53) and it currently is 18% (59). It had been suggested that children 0–5 years were at higher risk for graft failure (21, 60) and that was thought to account for poor outcomes in young kidney transplant recipients years ago. It is important to note that excellent results had been reported in very young patients in some individual centers even then (37, 61). The concept of a heightened immune response in young recipients was called into question (62–64). Thus, the unique problems associated with transplantation in young recipients may have been related to correctable conditions, such as infections, technical issues and differences in pharmacokinetics (22, 23, 65–67) rather than their immune response. Recent reports of outstanding

long-term graft survival rates for these young children demonstrate that their specific problems have been overcome successfully and now it is clear that children <10 years of age have the best living- and deceased-donor kidney transplant survival of all age groups of both children and adults (16, 17, 59) (*Fig. 75-1*).

As shown in *Table 75-1*, there is a strong relationship among age at transplant, gender, race and categories of etiology of ESRD. The most common causes of ESRD in infants and young children are renal dysplasia/hypoplasia/aplasia and obstructive uropathy, which are substantially more common in males than females. Thus, almost 2/3 of children less than 10 years of age who receive kidney transplants are males. At older ages, ESRD secondary to reflux nephropathy or to acquired disorders such as focal segmental glomerulosclerosis (FSGS) and lupus nephritis become more common and gender differences tend to disappear. Racial distribution of ESRD leading to transplant is similar to the general population in younger children. However African-Americans make up a disproportionately large percentage of adolescent and young-adult recipients of kidney transplants, and this proportion may be artificially low. Unfortunately, even in a pediatric setting, there are substantial differences in waitlist activation between black and white children (68). In a study of 3,284 children at the time of their first dialysis treatment, blacks were less likely to be waitlisted at any given time. The authors concluded that racial disparities in access to pediatric kidney transplant existed. Sadly, there are even disparities in outcomes after transplant, with blacks having worse outcome, even after controlling for confounding factors (69).

Etiology of ESRD

ESRD in children is generally due to congenital or inherited diseases. In reviewing 9,854 transplants, the most common congenital diagnoses are obstructive uropathy and aplastic/hypoplastic/dysplastic kidneys, each representing about 16% of the patients (36, 59) (*Table 75-2*). Among glomerular disorders, focal segmental glomerulosclerosis (FSGS) is the most common (11.7%), averaging about 60 transplants per year. The primary diagnosis also varies with the race of the recipient. Overall in the NAPRTCS registry, Caucasian children account for 60% of all recipients; however, Caucasian children only account for less than 50% of the children transplanted for FSGS. The data regarding the role of FSGS in leading to ESRD can be better appreciated by observations from the NAPRTCS dialysis registry, in which the two most common diagnoses are FSGS (14.4%) and aplastic/hypoplastic/dysplastic kidneys (14%). Of 933 children with FSGS on dialysis, Caucasian children account for only 34%, with African-American and Hispanic children accounting for 62% of the patients. FSGS accounts for ESRD in 12% of

Table 75-1

Age, gender, race and etiology of ESRD at time of kidney transplantation. All figures are percents (Adapted from (88))

	Age at Transplantation				
	0–1 years	2–5 years	6–12 years	13–17 years	≥18 years
Gender					
Male	68.8	66.3	58.9	56.4	55.6
Female	31.2	33.7	41.1	43.6	44.4
Race					
White	74.5	63.4	60.7	56.6	53.2
Black	8.1	14.5	14.6	19.8	24.2
Hispanic	11.0	15.6	18.0	17.2	14.8
Other	6.4	6.5	6.7	6.4	7.8
Etiology of ESRD					
Hypo-dysplasia	29.6	23.7	16.5	11.3	10.0
Obstruction	18.1	21.2	16.1	13.6	10.1
Other	51.5	46.4	54.7	62.2	63.6
FSGS	0.9	8.7	12.6	12.9	16.3

Table 75-2

Etiology of ESRD in children who receive kidney transplants. (The data have been adapted from (59))

Etiology of ESRD	N	%
Total	9,854	100.0
Aplasia/hypoplasia/dysplasia	1,564	15.9
Obstructive uropathy	1,538	15.6
Focal segmental glomerulosclerosis FSGS	1,154	11.7
Reflux nephropathy	515	5.2
Chronic glomerulonephritis	328	3.3
Polycystic kidney disease PKD	287	2.9
Medullary cystic disease	271	2.8
Hemolytic uremic syndrome	260	2.6
Prune belly syndrome	254	2.6
Congenital nephrotic syndrome	254	2.6
Familial nephritis	225	2.3
Cystinosis	201	2.0
Pyelonephritis/interstitial nephritis	173	1.8
Membranoproliferative glomerulonephritis type I (MPGN I)	171	1.7
Idiopathic crescentic glomerulonephritis	171	1.7
Lupus nephritis	150	1.5
Renal infarct	136	1.4
Berger's (IgA) nephritis	127	1.3
Henoch-schonlein nephritis	110	1.1
Membranoproliferative glomerulonephritis type II (MPGN II)	81	0.8
Wegener's granulomatosis	55	0.6
Wilms' tumor	52	0.5
Drash syndrome	52	0.5
Oxalosis	52	0.5
Membranous nephropathy	44	0.4
Other systemic immunologic disease	32	0.3
Sickle cell nephropathy	16	0.2
Diabetic nephropathy	11	0.1
Other	962	9.8
Unknown	608	6.2

Caucasian children receiving dialysis. In contrast 24% of African-American children on dialysis and 30% of those >12 years old have FSGS. The information about etiology of ESRD becomes critical in predicting graft survival as well as strategies for immunosuppression and preparation for transplantation because of recurrence of the original disease, as discussed below.

Indications for Pediatric Kidney Transplantation

End Stage Renal Disease (ESRD), which is also known as Stage 5 Chronic Kidney Disease (CKD), is the most severe form of chronic loss of kidney function, and, in general, is defined as the need for chronic dialysis or kidney transplant to sustain life. In this context, it is important to recognize that both dialysis and kidney transplantation are treatments for ESRD, but neither can be considered a cure. Currently in the United States, dialysis is rarely indicated in adults until the GFR has fallen below 15 ml/min/1.73 M^2 or the serum creatinine has exceeded 8 mg/dl. Thus, it is appropriate to consider the indications for both chronic dialysis and kidney transplantation to be the same, specifically the attainment of ESRD.

Not all patients who have ESRD can be considered candidates for kidney transplantation, however. The procedure may be too risky for some because of their co-morbidities and others may have some contraindications, such as chronic infections that would be exacerbated by immunosuppression. The general evaluation of potential recipients and the indications for transplantation have been reviewed several times (70–72). For those for whom transplantation is indicated, there is a survival advantage of kidney transplantation over dialysis, thus it appropriately should be considered a "life saving" procedure. This survival advantage has recently been shown to be true for children (5).

Virtually all children who develop ESRD are considered to be candidates for a renal transplant since they rarely have serious co-morbidities or contraindications. Thus, a significant number of children receive a pre-emptive kidney transplant without ever having been on dialysis (73). In a review of 8,990 primary kidney transplants in children transplanted from 1987 through 2006, NAPRTCS noted that 25% of the patients had never received maintenance dialysis prior to transplantation (59). Although dialysis is not necessary before kidney transplantation in children, that fact should not be confused with the indication for pre-emptive kidney transplantation. There is no benefit reported benefit to performing kidney transplantation before a patient reaches ESRD (74). In the past, growth failure was considered a unique pediatric indication for kidney transplantation; but the success of recombinant human growth hormone in overcoming this complication of CKD in children (9, 75) has eliminated growth failure as a valid indication for early kidney transplantation. A very disturbing result from an analysis of UNOS data demonstrated that African-Americans in the US are less likely to

be waitlisted for transplantation at any time after their first dialysis treatment than Caucasians (68).

Thus, the indication for pediatric kidney transplantation is permanent loss of renal function, resulting in ESRD, and the lack of any defined contraindication.

Absolute Contraindications for Pediatric Kidney Transplantation

There are few absolute contraindications to transplantation. One possible contraindication is an active malignancy, especially if has already metastasized, which has a very low likelihood of long-term survival. A recent analysis of the NAPRTCS dialysis registry revealed information on 70 children with Wilms' tumor or Drash Syndrome. Thirteen of these children did not receive a kidney transplant because of uncontrolled or metastatic disease and all 13 died (76). The registry also showed that for 86 children with the same diagnoses but without metastases who did receive a kidney transplant, none had graft loss due to recurrence and none had different outcome from transplant than other recipients. One child, however, who had only 6 months of dialysis prior to transplantation did die of Wilms' tumor within 6 months of transplantation. Thus, children with already existing metastatic disease are not considered transplant candidates and most centers will wait for 12 months after nephrectomy for Wilms' tumor prior to transplantation to assure that the disease is actually cured. Also, children with devastating neurological dysfunction may not be suitable transplant candidates, but the wishes of the parents, as well as the potential for long-term rehabilitation, must be considered in these circumstances. Since the transplant procedure and the life-long need for immunosuppression have several potential risks, there are serious ethical concerns about whether these patients will be able to balance those risks by understanding any of the potential benefits.

The concern of further immunosuppressing an already compromised host previously contraindicated transplantation of HIV+ children. Many children who developed HIV nephropathy succumbed to the systemic ravages of the virus, either before or very shortly after reaching ESRD status (77). However, marked advances of treatment of HIV+ patients, especially with protease inhibitors and other anti-retroviral therapies, makes consideration of kidney transplantation more likely (78, 79). Ironically, initial results from a few centers suggests that HIV+ patients with reasonable CD4 cell counts may have more problems with rejection rather than infection post transplantation.

Potential for recurrence of the original renal disease is of major concern but generally has not precluded at least an initial transplant for most children. Focal segmental glomerulosclerosis and atypical Hemolytic Uremic Syndrome have substantial potential for recurrence in kidney transplants, but our ability to correctly identify recipients in whom recurrence will occur is not yet accurate (see below). Oxalosis, which once was considered an absolute contraindication due to a high incidence of recurrence in the transplanted kidney, as well as extension in other organs, can be treated successfully with combined liver and kidney transplantation (80, 81), although the complication rate remains high (61, 82, 83).

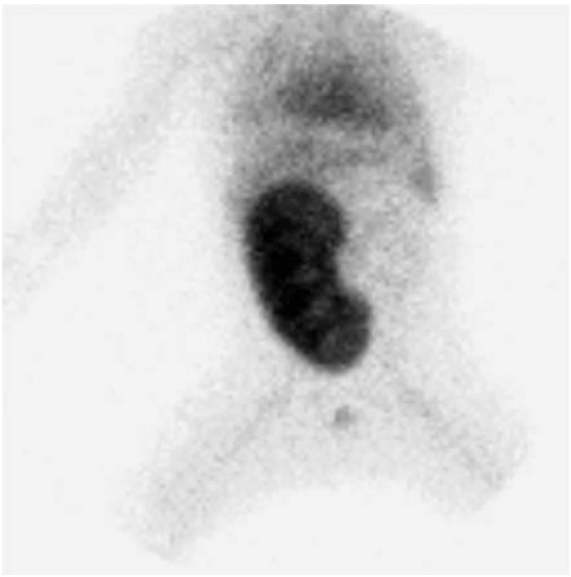
Correctable or Temporary Contraindications for Pediatric Kidney Transplantation

Pediatric kidney transplantation is generally considered an elective procedure. Because of the ready availability of dialysis treatments for children at pediatric transplant centers, even young infants can be maintained for sufficient time so that any correctable problems can be attended to before the transplant. In this setting, kidney transplants can be performed under the best possible conditions, rather than as emergencies. Importantly, donors for pediatric transplant candidates are readily available in the United States, so that they can expect to receive the transplant soon after they have been prepared for it. About half of the transplants are from living donors, who should be available when needed, and the waiting time for pediatric candidates on the deceased donor list in the United States and many other countries is much shorter than adults because of pediatric preference rules.

Most grafts for infants come from adult donors (59). All living donors are currently older than 18 years and the majority of deceased donors for children are adults (*Fig. 75-3*) (17). There is a substantial survival advantage of using adult donors for these infants (39). Thus, one of the major issues for these children is to grow big enough to accept an adult-size kidney. There is no consensus on the appropriate size, but most programs use a weight between 6.5 and 10 kg or a length of at least 65 cm as an appropriate size to meet that standard; while other programs may suggest a minimum age rather than size. *Figure 75-4* shows a Mag-3 renal scan of a small infant who has received an adult kidney transplant. Note that the graft appears to be larger than the recipient's liver and that it slightly passes the midline. Thus, most programs will maintain infants with ESRD on chronic dialysis and provide aggressive nutritional programs and growth

Figure 75-4

Mag-3 nuclear scan of adult kidney transplanted into a 9-month-old infant weighing 7 kg at post-operative day #1. Note the uptake of the tracer in the liver in the right upper quadrant of the abdomen and in the heart in the chest. There is a small amount of tracer in the bladder.



hormone to have them attain an appropriate size. This likely explains the low number of infants who receive kidney transplants prior to their first birthday.

Children with acquired or autoimmune disease often have had extended and rigorous treatments prior to the development of ESRD. It is prudent to withdraw these treatments if there is no hope of reversing or treating ESRD and allowing the child to recover nutritional and metabolic stability. For example, children with FSGS often have received extensive treatment with corticosteroids and other immunosuppressive agents and may also have had extensive protein wasting. For these children, recovery of reasonable nutritional status prior to transplantation is reasonable and this may require months of rehabilitation while receiving treatment with chronic dialysis. Some programs prefer to allow certain diseases, such as lupus nephritis, to "burn out" by maintaining the patient on dialysis for several months after they have reached ESRD rather than progressing to kidney transplantation immediately.

Other children require corrective surgery or native nephrectomy prior to kidney transplantation. For example, children with Finnish-type congenital nephritic syndrome often require native nephrectomy, despite normal renal function, to prevent protein malnutrition, sepsis and vascular thrombosis, followed by months of chronic dialysis to make them suitable for kidney transplantation (84, 85). Similarly, children with serious urologic malformations of the bladder or urinary tract may require extensive reconstructive surgery prior to transplantation to assure that the recipient will have a competent, low-pressure, functional or catheterizable internal reservoir prior to receiving a kidney transplant.

Pre-emptive Pediatric Kidney Transplantation

Since pre-emptive transplantation is an important modality for children, NAPRTCS conducted a special study to determine the frequency and outcome of this approach (73) and has updated it in the annual reports (36, 59). From 1987 through 1992, 26% of the patients were registered as having had pre-emptive transplantation. The study compared data of those recipients who had been on maintenance dialysis versus patients who had no previous dialysis. Of 2,213 primary grafts during that time period, 1,150 (52%) were from a living donor, whereas for the pre-emptive group 70% were recipients of a living donor kidney. More recently, of 8,613 primary transplants, 2,116 (24.6%) were pre-emptive, indicating that the practice has changed very little in the past 2 decades. Pre-emptive transplantation was more common in living donor (34%) than in deceased donor (13%) and in males (28%) than females (20%). The rate varies little among age groups with 19, 24, 28, 23 and 21% for the 0–1, 2–5, 6–12, 13–17 and 18–20 age groups respectively. It varies more across races with Caucasians, African-Americans, Hispanics and "other" races having rates of 31, 14, 16 and 18% respectively. As noted above, African-Americans are also less likely to be waitlisted than Causcasians following initiation of dialysis (68). A proposed objection against pre-emptive transplants has been that without the rigors of prior dialysis adherence with immunosuppression might be poor. To determine whether this hypothesis was correct, graft survival was compared between the two groups, and was determined not to be different at 1 or 4 years (73). When causes for graft loss were analyzed, the pre-emptive group did not have a higher incidence due to nonadherence. NAPRTCS also surveyed the motive for pre-emptive transplantation and determined that the primary reasons were parents' desire to avoid dialysis (34%) and the nephrologists' recommendation (18%). Desire for improved growth

was considered a contributory factor in 50% of the patients (73). A more recent analysis has even shown a survival benefit of early transplantation (5), but not before ESRD has been reached (74). The overall beneficial effects of pre-emptive renal transplantation have been well-documented (5, 73, 86, 87).

Preparation for Pediatric Kidney Transplantation

The typical components of preparation of the child with ESRD for kidney transplantation are shown in *Table 75-3*.

Donor Selection

The selection of the appropriate donor is an integral part of the transplantation procedure and may be a limiting factor in the long-term outcome of kidney transplantation for any individual child. The use of living donors has generally been much more common in pediatric kidney transplantation than in adults (17, 36, 38, 52, 53, 59, 88). In general, the choice of a living donor is a good one since, on average, graft survival can be twice as long when a living donor is used compared to a deceased donor (17, 59, 88). There are limitations to the use of living donors however, such as donor suitability, blood group incompatibility and age; and, thus, not every child may have a suitable living donor. Moreover, there is concern about using living donors when there is a substantial risk of early graft failure or recurrent disease (89, 90). When deceased donors are used for children, careful attention should be paid to utilizing low-risk donors since the mortality risk for children after kidney transplantation is low and children are expected to require the grafts for long periods of time (3, 29, 41, 91, 92).

Living Kidney Donation

The first successful kidney transplants utilized living donors and the use of living donors has been a mainstay of pediatric kidney transplantation since that time. Importantly, as the donor shortage has become more acute, considerations about donor safety have also become apparent (93). Recently, the transplant community has examined this issue and has reached consensus at national and international conferences on the appropriateness of the use of living donors, their evaluation process and the appropriate follow-up and care of those donors (47, 94).

Table 75-3
Standard preparation of pediatric renal transplant candidates

History and physical examination
Laboratory tests
Hematology (CBC, platelets, differential)
Coagulation (PT, PTT, TT)
Chemistry (serum electrolytes, BUN, creatinine, liver function, lipid profile, Ca, PO_4, PTH)
Urine volume, culture, and urinalysis
Blood bank/immunology (ABO blood type, HLA type, histocompatability testing, anti-HLA antibody screening, hepatitis profile, HIV screening)
Virology: CMV, EBV, MMR, VZV, HSV
Toxoplasmosis titers
X-Ray
VCUG[a], Renal ultrasound CXR, bone age
Consults
Audiology evaluation
Dental evaluation
Infectious disease with immunization evaluation
Social worker
Nutritionist
Pharmacist
Psychological evaluation
Vaccines[b]
DPT, Polio, MMR, HIB, Varicella, HepB, HepA, Rotavirus
Pneumococcal
Meningococcal
Human papillomavirus
Influenza (yearly)
PPD
Immunosuppression review
Opportunity to participate in clinical trials[a]

[a]For selected recipients
[b]Recommended vaccines are age-dependent

Those conferences reiterated the important principle that the donor should never be put at physical, emotional or psychological risk for the benefit of the recipient. Included in this principle was the agreement that children less than 18 years of age should not be considered as living donors, although that has not been universally observed (46, 95). The living donor pool has been increased by the use of genetically unrelated donors, which initially

included spouses, but has expanded to include other genetically unrelated family members, friends or even anonymous non-directed ("good Samaritan") donors. Importantly, the outcomes of these unrelated donors appear to be comparable to 1-haplotype matched related donors (96–103). There have even been proposals that non-directed living donors be compensated for the donation, leading to a commercial system to expand the donor pool (104, 105). Although commercial systems using paid donors have existed in many parts of the world, most countries have declared such practices illegal and the broad transplant community has condemned them (106, 107). The use of non-directed living donors have also played an important role in expanding the donor pool through the practice of donor exchange. These programs have allowed living donors to donate to an unrelated recipient in exchange for that recipient's incompatible donor to donate to the former's family member (108–110). These programs have included simple 2-recipient exchanges, multiple domino-style exchanges and exchanges with the deceased donor waiting list (111–118).

Matching

In general, all living donors are evaluated for ABO blood type, HLA histocompatibility typing and some type of cross-matching with potential transplant recipients. Parents predominate as living kidney donors for children, accounting for about 80%. Unrelated donors, siblings and occasional non-directed donors make up the balance (59). Thus, there are few 2-haplotype donors for pediatric kidney transplantation, with most donors being 1-haplotype HLA matched, or less. Although there may be some disadvantage to the lack of any HLA-B matching (59), overall the lack of histocomatibility matching for pediatric recipients, especially infants, does not seem to compromise long-term outcomes (22, 39).

Donor Evaluation

As noted above, donors are carefully evaluated in order to assure that they are not being paid, coereced or that they might be injured in any way as the result of the donation procedure. The components of an appropriate donor evaluation have been published (119, 120) and can be found on organizational websites (http://www.a-s-t.org/index2.cfm?Section=public_policy&Sub1Section=key_position_statements&content=med_eval_living_kidney_donors.cfm). Much of the evaluation is performed to assure donor safety, but also it is designed to assure that the donor does not transmit infectious or other diseases to the recipient and that the donor organ will provide sufficient function for the recipient.

Contraindications to Living Kidney Donation

In general, donor candidates are excluded if they are too young (<18 years of age), too old (as individuals age, they lose GFR and the potential for cancer, cardiovascular and other diseases increases) or have medical problems that may be exacerbated by the donation surgery or living with only one kidney (47). Conditions such as hypertension, diabetes and a history of metastatic cancer typically exclude donors. Also, chronic infections such as HIV, or hepatitis B or C are exclusionary because of the likelihood of transmission. Although CMV, HPV and EBV may also be transmitted, these are typically not exclusionary, since they can be treated in the recipient.

Laparoscopic Donor Nephrectomy

The use of a laparoscopic technique to remove the living donor kidney has become very popular recently, because it tends to shorten the hospitalization and rehabilitation time for the donors. The procedure does require substantially longer time in the operating room and the rate of delayed graft function is slightly higher. Although most programs indicate that the procedure is safe, a review of 10,000 living organ donations identified three deaths or serious complications; all three followed laparoscopic procedures (121). Single center reports show good outcomes for pediatric recipients when the donor has a laparoscopic nephrectomy (122–124); while other studies suggest more complications in the pediatric recipients (125, 126).

Deceased Donor

The majority of kidney transplants currently come from deceased donors. While living donors were more common for children in the past, recent changes in allocation policy in the United States have led to a predominance of deceased donor transplants for children there in the past 3 years (*Fig. 75-2*) (5, 38, 41). The consequences of this change in donor selection will not be known for years; but, on average, the long-term outcomes of living donor

kidney transplants in children are superior to those obtained from deceased donors (Fig. 75-1) (38, 41, 59, 88), indicating that the short term benefit of having rapid access to the deceased donor list may be counterbalanced by shortened graft survival.

Matching and Sensitization

The influence of matching on transplant outcome used to be substantial, but it has diminished significantly since the introduction of improved immunosuppression (127–130). Pediatric deceased donor kidney transplant outcomes are influenced very little by donor histocompatibility matching (41, 59, 88). ABO blood type compatibility and cross matching are still necessary, however. In general, transplant candidates who have pre-existing antibodies or who develop antibodies after transplantation are at higher risk of early rejection and should have enhanced immunosuppression protocols (131–135).

Donor Evaluation

Deceased donors are typically evaluated to assure that the graft will provide sufficient function for the recipient and that the donor does not transmit infectious or metastatic cancer diseases to the recipient. In the past, the standard for determining the donor eligibility rested on the determination of brain death. However in efforts to expand the donor supply, recent advances have been made in recovering organs from donors whose death has been determined on the basis of cardiac death, so-called donation after cardiac death (DCD). Although there is a substantially higher incidence of delayed graft function when such donors are used, the short term outcomes of kidney transplants from DCD donors is equivalent to that from brain-dead donors (100, 110, 136, 137).

Allocation of Deceased Donor organs

Most countries with national organ allocation systems have provided some sort of preference for pediatric deceased-donor transplant candidates. In the United States, a recent change of the Organ Procurement and Transplant Network allocation system gives children <18 years of age at the time of listing, preference over most other candidates, resulting in much shorter waiting times than adult candidates (41).

Donor-Defined Outcome Risks

Very young donor age was previously identified as a risk for decreased graft survival (29, 138). Since that time, specific techniques for successfully using young donor kidneys have been developed in some centers (55). Similarly, the use of elderly or extended criteria donors (ECD) has continuously been associated with poor outcomes (101, 110, 139, 140). Children rarely receive organs from elderly deceased donors or from extended criteria donors (88).

Desentitization Procedures for Sensitized Recipients

As noted above, the presence of pre-formed anti-HLA and anti-donor antibodies often precludes the use of a specific donor. Similarly, ABO blood type incompatibility will also prevent the use of specific donors for transplant candidates. Programs designed to "swap" donors may permit successful transplantation in these situations, but the chance of finding compatible donor pairs is low. Another approach would be to attempt to remove anti-donor antibodies or isohemagglutinins by desensitizing procedures. Such procedures utilizing plasmapheresis, intravenous immunoglobulins (IVIG), immunosuppression and rituximab have been described (132, 141–146). Long term outcomes of desensitized recipients is generally less successful than unsensitized recipients, however.

Growth and Nutrition

Chronic kidney disease often leads to malnutrition in children, due to anorexia, protein losses, dietary restrictions and the debilitating effects of medications used to treat autoimmune disorders. Thus, it is not unusual for the child to be malnourished when reaching ESRD. In this setting, it is reasonable to begin chronic dialysis and to provide appropriate techniques to improve nutritional status prior to transplantation (84, 85, 147–152).

Immunizations

Once children receive a kidney transplant, they will require life-long immunosuppression, which may diminish their response to subsequent killed-vaccines and prevent the use of live-virus vaccines. Thus, pediatric programs generally attempt to adequately immunize children prior

to the transplant (153–157). Varicella vaccine may be provided after transplantation, when immunosuppression levels have been reduced to low levels (158), but the response is attenuated and concern about vaccine-related disease is present.

Urologic Preparation for Pediatric Kidney Transplantation

Children with the diagnoses described in *Table 75-4* require a thorough urologic evaluation prior to transplantation and they frequently require pre-transplant reconstructive urological surgery. In a NAPRTCS report, 1,878 of 7,651 (25%) pediatric transplant recipients were identified as having lower urinary tract abnormalities (36). For all such patients, a history of voiding pattern prior to development of renal failure is most helpful. Preliminary investigations consist of measurement of urinary flow rate and ultrasound estimation of the post micturition urine volume. Urinary flow rate should be at least 15 ml per second (159), and the residual volume should be less than 30 ml. Further investigations would consist of urethrocystoscopy in patients suspected of a urethral stricture, and a voiding cystometrogram is essential for complete assessment of bladder function (160). This provides information about bladder capacity, pressure rise, and the efficiency of voiding. Still more information can be obtained by combining the urodynamic studies with radioisotope imaging. Routine voiding cystourethrogram is not indicated in older patients with no symptoms related to the urinary tract (161).

A bladder with a very small capacity may not be adequate for a functioning transplant. Occasionally a small capacity bladder may be seen in patients with prolonged oligoanuria. However if the bladder is distensible and the bladder wall compliant, such a bladder may be used safely for kidney transplantation. Other criteria for a useable bladder are an end-filling pressure less than 30 cm of H_2O, and a good flow rate. In patients with a poor flow rate, if urethral and bladder outlet obstruction are ruled out, the problem may be due to detrusor malfunction (159). When a bladder fails to empty completely, infection and obstruction are potential complications that may shorten graft survival. Intermittent, clean, self-catheterization, which is widely used in urologic practice, can be safely used post-transplantation in patients where the primary abnormality is inefficient and uncoordinated detrusor function. Most pediatric patients have a urinary bladder that will adapt to the new kidney. Although the bladder may not appear to have the capacity, especially in patients on long-term dialysis prior to transplantation, it will most often distend with usage (80). However in patients with a truly low capacity or high pressure, bladder augmentation may be necessary prior to transplantation (162–164). The goal of modern reconstructive pediatric urology is to have a competent low-pressure urinary reservoir which can be emptied by voiding or at least by intermittent catheterization. Augmentation cystoplasty consists of adding bowel or gastric wall to the bladder, whereas substitution cystoplasty is performed when most of the bladder is excised and replaced with bowel. Gastric remnants have been popular for augmentation, however they do tend to cause excessive loss of acid in the urine, leading to discomfort and metabolic alkalosis. Early attempts to reconstruct bladders with bioengineered material are ongoing. There are promising reports of "bio-engineered" bladder material, although these have not yet been tried in transplant recipients (165, 166). Urologic reconstruction, including augmentation cystoplasty, typically occurs prior to transplantation (164, 167–174); although some programs have reported successful reconstruction after transplantation also (175). In those patients in whom augmentation has been performed, long-term antibiotic therapy and intermittent catheterization may have to be carried out to prevent urine stasis and infection. In general, the incidence of urinary tract infection and other complications is higher in these recipients, their course is generally no worse than pediatric recipients without urologic abnormalities.

If native kidneys in children with ESRD are causing hypertension, chronic infections or excess losses of protein, urine or other substances, there should be serious consideration for nephrectomy prior to or at the time of transplantation (176). About 25% of children have native nephrectomies prior to index transplants (88).

Table 75-4
Lower urinary tract abnormalities of pediatric renal transplant recipients

Bladder exstrophy
Neuropathic bladder (meningomyelocele, spinal cord trauma, neurological disease)
Posterior urethral valves with dysfunctional bladder emptying
Prune belly syndrome
Vesicoureteral reflux

The Transplant Procedure

Technical Issues in Transplantation

The operative technique differs based on the weight of the child. For small children, less than 15 kg, the transplant is frequently done through a midline incision and the larger vessels are utilized for anastomosis with the donor kidney (80). After reflection of the cecum and the right colon, the anterior wall of the aorta and the inferior vena cava are exposed and dissected (177). The aorta is mobilized from above the inferior mesenteric artery to the external iliac artery on the right side. After ligating and dividing the lumbar branches, the iliac arteries and the inferior mesenteric are encircled. Next the inferior vena cava is mobilized from the left renal vein to the iliac veins. After ligating the lumber veins the iliac veins are encircled. The donor renal vein is anastomosed to the recipient vena cava in an end-to-side technique (178). The donor renal artery is then anastomosed to the recipient aorta in an end-to-side fashion. Careful attention needs to be paid to the recipient hemodynamic response upon clamping and unclamping of the major vessels, and it is desirable to maintain a central venous pressure of 15–18 cm H_2O prior to unclamping (80, 177). The filling of the transplanted kidney may be slow due to the fact that a large adult kidney will take up a significant portion of the normal pediatric blood volume. Hemodynamic studies suggest that the cardiac output of infants must double in order to perfuse the adult donor kidney adequately (179). Thus volume replacement is critical (*Fig. 75-4*). The ureteral anastomosis is done by implanting the donor's ureter into the recipient's bladder using either a Ledbetter-Politano procedure or a modification of it to assure non-refluxing anastomosis. Many surgeons now prefer a non-refluxing extravesical rather than transvesical approach for ureteroneocystostomy because it is faster, a separate cystotomy is not required and less ureteral length is necessary, thus assuring a distal ureteral blood supply (180–182).

The transplantation technique utilized in children with a body weight greater than 15 kg is similar to that employed in adults. Unlike the transperitoneal approach necessary in younger children, this transplant is extraperitoneal, with the renal vein anastomosed to the common iliac or the external iliac vein (177). The arterial anastomosis can be to either the common iliac or internal iliac artery. The ureterovesicular anastomosis is done utilizing the techniques described above.

Evaluation of Graft Dysfunction

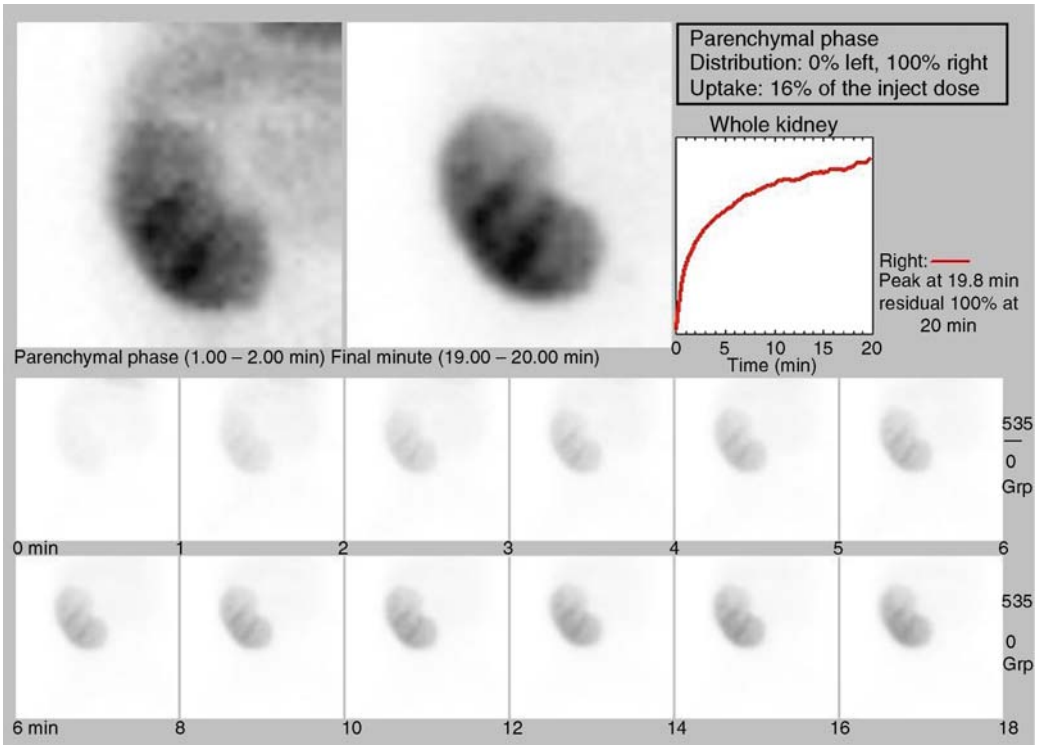
At the completion of the vascular anastomosis and release of the vascular clamps immediate function of the transplanted kidney is demonstrated by the production of urine. Various causes however, may prevent initial function, and evaluation of immediate non-function and the differential diagnosis of this condition is a critical component of the transplant physician's role.

Delayed Graft Function (DGF)

A well-function kidney graft should lead to normal renal function within 2–3 days. The lack of attainment of normal renal function, as demonstrated by a fall of the serum creatinine to normal levels, is termed delayed graft function (DGF). There is no consensus concerning the definition of DGF (183). In some settings, DGF is used only to distinguish recipients who require dialysis after transplantation, but that is a very stringent definition. Acute tubular necrosis (ATN) represents the most frequent cause of immediate graft non-function. Data from the NAPRTCS 1996 Annual Report showed that ATN was observed in 5% of living donor and 19% of deceased donor transplants (53). Since the NAPRTCS definition for ATN is stringent, requiring the use of dialysis in the first post-transplant week, these figures probably under-represent the actual incidence of ATN. The risk of early ATN in living donor kidneys is related to factors such as prior transplants and more than five pre-transplant blood transfusions. Similarly the risk factors of ATN in deceased donor kidneys include prolonged cold ischemia, absence of prophylactic antibody therapy and the use of more than five pre-transplant blood transfusions. The diagnosis is confirmed in most cases by the use of radionucleide scan (*Fig. 75-5*). If recovery of graft function is delayed, however, a transplant biopsy may be necessary since other diagnostic tests cannot distinguish between ATN and rejection (184, 185). Importantly, early acute rejection can mimic ATN or coexist with it (186). The presence of ATN does not auger well for the transplant, particularly for recipients of deceased donor grafts since graft failure and death are more common among patients with ATN (82, 187). The NAPRTCS data shows that 71% of deceased donor grafts without ATN were functioning at 4 years compared to only 51% of those with ATN (188). DGF is an independent risk factor for graft loss and death (189–191). Importantly, although the incidence of DGF is

Figure 75-5

^{99M}Tc-MAG3 radionuclide renal scan of a decease donor renal transplant in a 2-year old boy performed on the first post-op day. The warm ischemia time was prolonged due to a renal artery stenosis in the graft. The recipient was oliguric for 2 days but recovered function subsequently. Note the good perfusion followed by little excretion and "wash out" of the tracer from the graft.



increased when a DCD donor is used, the detrimental affect of DGF on long-term outcomes in this setting does not appear to be as severe as when it occurs after living donation or after transplantation from brain-dead deceased donors (183).

Graft Thrombosis

Graft thrombosis is an almost unique complication of pediatric transplantation. Although usually a major cause of immediate graft non-function, it can be seen later on in the course, and has been recorded to occur as late as 15 days post-transplant following initial engraftment and function. Graft thrombosis has been the third most common cause of graft failure in pediatric renal transplantation (36) and may rise to second if acute rejection rates continue to fall (*Table 75-5*) (59, 192). The critical nature of this complication can be appreciated from the fact that it accounts for 10% of graft failure in index transplantation and 12% in repeat transplants in the NAPRTCS registry. A dreaded event, this condition is irreversible in most cases and necessitates removal of the graft. Graft thrombosis should be suspected in cases where there has been immediate function followed by the development of oligoanuria. The diagnosis is established by a radionucleide scan using diethylenetriamine pentaacetic acid (DTPA) or MAG3 (193), which reveals a photopenic defect with no uptake by the transplant kidney (*Fig. 75-6*).

Since the outcome of graft thrombosis is uniformly dismal, numerous studies have been conducted in an attempt to understand and anticipate this complication. The etiology of graft thrombosis is multifactorial, but it is more commonly seen in young recipients (92). In a special study of 2,060 living donor and 2,334 deceased donor kidneys (194), NAPRTCS has shown that a history of prior transplantation increases the risk, whereas increasing recipient age has a protective effect for living donor kidneys. The prophylactic use of antilymphocyte antibody

Table 75-5

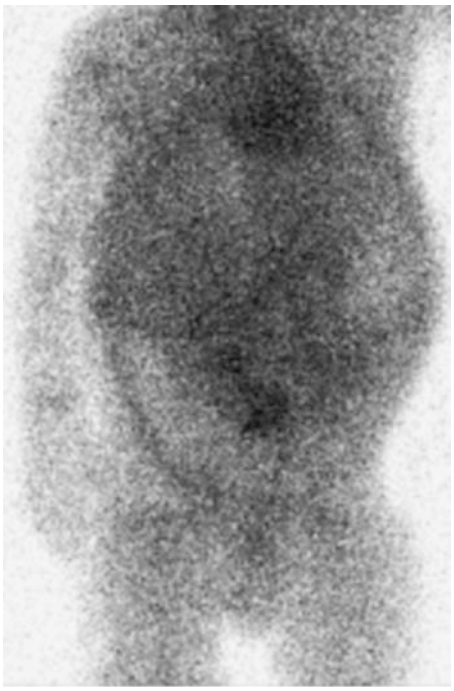
Causes of graft failure for pediatric kidney transplants. The table lists the causes of 2,556 graft losses. The number in each column represents the percent of all graft losses due to that cause (Adapted from (59))

	Index graft failure	Subsequent graft failure	All graft failures
Death with functioning graft	9.4	7.5	9.2
Primary non-function	2.6	0.7	2.3
Vascular thrombosis	10.3	12.5	10.5
Other technical	1.2	1.3	1.3
Hyperacute rejection	0.6	1.4	0.7
Accelerated acute rejection	1.5	2.6	1.6
Acute rejection	12.9	13.1	12.9
Chronic rejection	34.5	36.4	34.7
Recurrent disease	6.4	9.5	6.8
Renal artery stenosis	0.7		0.6
Infection	2.0	1.3	1.8
CNI toxicity	0.5		0.4
De novo disease	0.3	0.7	0.4
Medication discontinuation	4.6	2.6	4.4
Malignancy	1.3	0.7	1.3
Other/unknown	11.3	9.8	11.1
Total	100	100	100

also decreases the risk and this may be particularly true for the monoclonal interleukin-2 receptor antagoinists (IL2r) antibodies (195). For deceased source kidneys, a cold ischemia time longer than 24 h increases the risk of thrombosis. The use of antibody induction therapy, the use of donors greater than 5 years of age, and increasing recipient age were factors that decreased the risk of thrombosis. A heightened thrombotic state has also been implicated (151, 193, 196). One study showed that centers that performed fewer infant transplants had higher rates of graft thrombosis (35) and another suggested that pre-transplant use of peritoneal dialysis increased the risk of thrombosis (197, 198). Some centers routinely administer anticoagulants to pediatric recipients at high risk of graft thrombosis, but no clinical studies of their effectiveness

Figure 75-6

^{99M}Tc-MAG3 radionuclide renal scan in a 1-year old boy who received a living donor renal transplant 10 days previously. The recipient's IVC had been clotted and the renal vein had thrombosed 1 day post-op but had been re-anastosed to the portal vein. The graft never functioned. Note the photopenic area in the right lower quadrant that represents unperfused tissue.



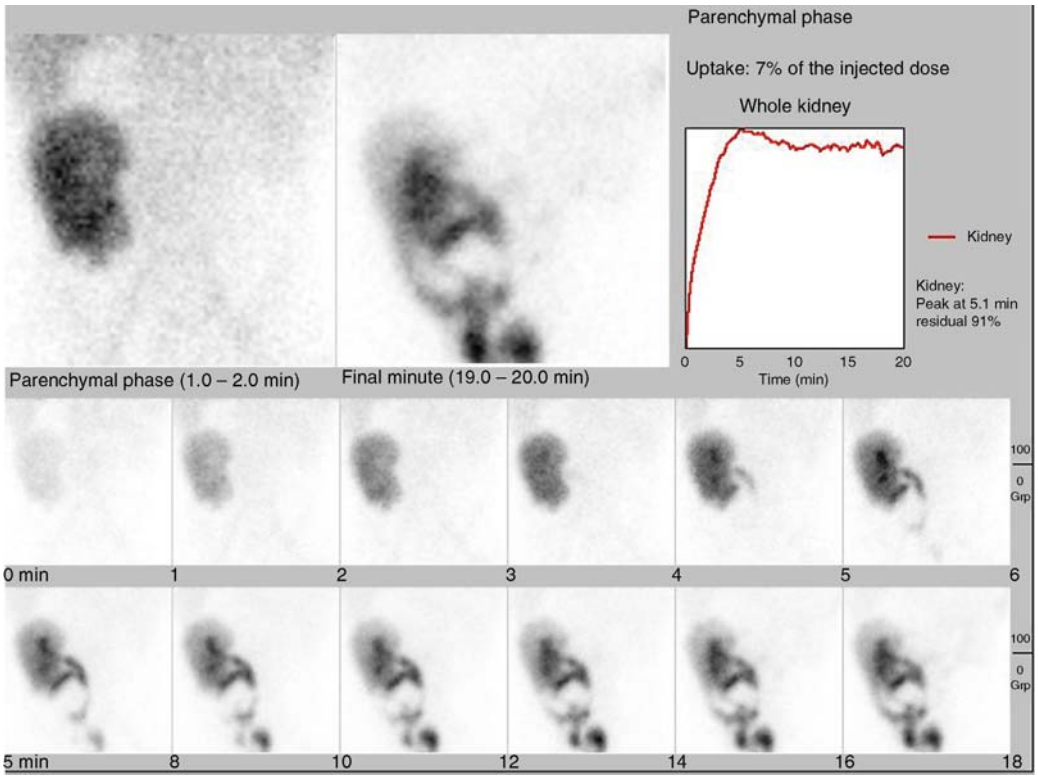
have been performed and its use is not without complications (199). This incidence of graft thrombosis had not changed over almost 15 years (36); however a preliminary report suggests that a new approach to induction therapy by using IL-2 receptor antagonists may be leading to a decrease (195).

Obstruction, Urinary Leak, Urologic Complications

An uncommon but correctable cause of immediate graft dysfunction is obstruction of the urinary flow, which presents as decreasing urine output and the development of hydronephrosis. An ultrasound or radionuclide scan with a furosemide washout enables the clinician to establish this diagnosis. Obstruction can be due to kinking of the ureter, to edema or blockage of the implantation site of the ureter or to development of a lymphocele. A more

Figure 75-7

^{99M}Tc-MAG3 radionuclide renal scan in an 8-year old girl who received a deceased donor renal transplant, performed 12 hours post-op. Note the good perfusion of the graft and the rapid concentration and excretion from the kidney. Tracer, however, rapidly accumulates in the right lower quadrant, outside of the bladder. Investigation demonstrated a traumatic bladder rupture.



ominous cause of immediate non-function is the rare case of urinary leak due to disintegration of the distal ureter or rupture of the bladder. This condition is extremely painful due to the extravasation of urine into the pelvis or peritoneal cavity and is established by radionucleide scan (*Fig. 75-7*). The appearance of the tracer in the peritoneal cavity or in the scrotal, vulvar, or inguinal area clinches the diagnosis and immediate surgical correction is necessary.

Immunosuppression Strategies

Two-thirds of pediatric kidney transplant recipients receive antibody-based induction therapy following kidney transplantation, with the majority of those receiving IL2r antibodies (59). In addition, most children receive "triple" maintenance immunosuppression with tacrolimus, mycophenolate mofetil and steroids (3, 59). Several recent multi-center research efforts have been directed to attempt to decrease the number of types of chronic immunosuppression, with corticosteroids and calcineurin inhibitors being the most common medications targeted for removal (200). Several single center (200, 201) and multi-center trials (202, 203) have reported success in avoiding or withdrawing corticosteroids, although some of these have had substantial side effects (202, 204, 205). There have also been reports of avoidance of calcineurin inhibitors in children designed to prevent nephrotoxicity. At least one of these trials resulted in excellent long-term graft function, but had a high rate of early acute rejections (206). A subsequent trial with enhanced antibody induction may have better short-term results (207).

Allograft Rejection

In the absence of tolerance the renal allograft is destined for loss by some form of rejection. Rejections are classified as hyperacute (occurring immediately upon grafting),

accelerated acute (occurring within the first week after transplantation), acute (generally occurring within the first year of transplantation), late acute (occurring after the first year), and chronic, for which the time sequence is difficult to establish since it may occur as early as three months, but generally occurs years later in the course of the transplant.

Hyperacute Rejection

Hyperacute rejection is the result of specific recurrent anti-donor antibodies against HLA, ABO or other antigens (208). Irreversible rapid destruction of the graft occurs. Histologically there is glomerular thrombosis, fibrinoid necrosis, and polymorphonuclear leukocyte infiltration. In the early years of transplantation, when the HLA matching techniques were not well developed, hyperacute rejection was more common. In most centers, it occurs very rarely. The latest data from the NAPRTCS shows the incidence of hyperacute rejection to be less than 0.7% of causes of graft failure (18 cases) over the last 20 years. The only response is surgical removal of the allograft.

Acute Rejection

A remarkable decrease in the incidence of acute rejection has occurred over the past 20 years (*Table 75-6*). In an earlier study of two cohorts of pediatric renal transplant recipient (1,469 in 1987–1989; 1,189 in 1997–1999), the rejection ratios dropped from 1.6 to 0.7/patient (209). Sixty percent of the latter group was rejection-free compared to 29% of the former and 1-year graft survival was 94% compared to 80%. Previously, over half of the patients experienced a rejection in the first post-transplant weeks. Now, fewer that 20% are expected to have an acute rejection in the first post-transplant year. Risk factors for acute rejection in living donor recipients used to include lack of induction therapy, DR mismatches, black race and age (59). With decreasing rates of acute rejections, the strongest risk factor is transplant year (there is an approximate 10% reduction with each increasing transplant year) and the only remaining statistically significant risk factors are older age (recipients younger than 2 years are less likely to have acute rejections) and the presence of DGF. Risk factors for deceased donor transplants were similar to those of living donor grafts and have undergone similar changes with dramatically lower rates in recent transplants. Currently, only year of transplant and black race are correlated with acute rejections.

In an earlier study the NAPRTCS noted that when reviewed by age groupings, rejection ratios, time to first rejection and the mean number of rejection episodes were not different; however for the initial rejection episode, recipients less than 6 years of age had significantly increased irreversible rejections leading to graft loss (210). There were conflicting data about whether infants and small children have a "heightened" immune response and an increased incidence of acute rejection episodes. Indirect evidence had suggested a more vigorous immune response especially in infants (21). Also, data from the UNOS registry demonstrated a higher rate of acute rejections in young children after both living and deceased donor transplantation, although adolescents were noted to have a higher rate of late acute rejections (22). On the other hand, data from surveillance transplant biopsies suggest equivalent rejection responses in all groups (63). But, data from one large pediatric transplant program demonstrated that infants had a lower rate of acute rejection than older children (23). A recent SRTR report demonstrated that infants and young children now have the best outcomes of all age groups (38). Thus, either the proposed heightened immune response has been overcome by improved immunosuppression or the cause previously poor outcome was related to other factors. The current acute rejection ratios by recipient age are shown in *Table 75-7*.

Table 75-6
Twelve month probability of first acute rejection episode by transplant year (Adapted from (59))

Transplant year	Living %	Donor s.e.	Deceased %	Donor s.e.
1987–1990	54.2	1.7	69.1	1.5
1991–1994	44.8	1.5	60.6	1.6
1995–1998	33.4	1.4	40.8	1.7
1999–2002	22.6	1.3	26.8	1.9
2003–2005	13.2	1.9	15.8	2.3

Diagnosis of Acute Rejection

Rejection is suspected when there is decreasing urinary outflow and a rising serum creatinine. In the past, classical signs of acute rejection included fever and graft tenderness. Under calcineurin inhibitors and prophylactic antibody therapy however, these signs are rarely seen; thus

Table 75-7

Acute rejection ratios for pediatric kidney transplants by recipient age for years 1996–2007 (Adapted from NAPRTCS 2008 Annual Report www.naprtcs.org)

	Living donor			Deceased donor		
	# of Tx	# of ARE	Rejection ratio	# of Tx	# of ARE	Rejection ratio
Total	3,043	1,374	0.45	2,440	1,530	0.63
Recipient age						
0–1 years	231	42	0.18	57	21	0.37
2–5 years	473	181	0.38	302	149	0.49
6–12 years	949	435	0.46	762	484	0.64
>12 years	1,390	716	0.52	1,319	876	0.66

early evidence of graft dysfunction even without other signs, should initiate concern. The differential diagnosis consists of ureteral obstruction, renal vascular compromise from stenosis, urinary leak and an infectious process. When rejection is suspected, a urinalysis and urine culture should be performed to assess the possibility of infection. The urinalysis is also helpful if it suggests intra-graft inflammation or immune response as evidenced by proteinuria and the presence of leukocytes and other cells in the sediment. Blood or urinary cytokine analysis may also be useful for diagnosing rejection (211, 212) and examination of the sediment may be useful in detecting other reasons for graft dysfunction. An ultrasound is performed to rule out anatomical obstruction. Obstruction can be the result of peri-renal fluid collection, a large lymphocele, hematoma, or rarely, an abscess. The ultrasound can also provide information about intragraft blood flow and pressure (184). A radionuclide renal scan, using a tracer such as MAG 3, is a very helpful tool in establishing some diagnoses (*Figs. 75-5–75-7*) (213). Rejection is suggested by rapid uptake of the tracer by the kidney but a delayed excretion. Radionucleide scans cannot distinguish among various causes of intragraft dysfunction, such as rejection, calcineurin inhibitor toxicity and ATN. Thus, a definitive diagnosis of rejection requires a transplant biopsy.

Renal Transplant Biopsy

The renal transplant biopsy procedure is very easy and safe when conscious sedation and ultrasound guidance are utilized. Recent data evaluating pediatric renal transplant biopsies, including some in intraperitoneal kidneys and many performed during the first week post-transplantation, have demonstrated a very low risk (185, 214). Special care is required for intraperitoneal biopsies. A good biopsy core should include glomeruli, tubules, interstitial tissue and vessels. This is facilitated if a dissecting microscope is used at the time of the procedure to identify glomeruli and tubules in the specimen. In acute rejection glomerular changes are restricted to an increased prominence of the mesangial stalk. However it is the presence of tubular changes that is of significance in early acute rejection, and tubulitis is considered the hallmark. Semiquantitative analysis and grading of acute rejection biopsy findings is done by the Banff criteria (215, 216). Grade I is focal interstitial lymphocytic infiltrate with mild tubulitis and normal vessels. In Grade II there is extensive interstitial infiltrate with tubulitis and vacuolation in arterial vessels, whereas Grade III shows extensive interstitial infiltration with tubulitis and lymphocytic infiltration of arterial walls with occasional fibrinoid change.

Treatment of Acute Rejection

Standard treatment of an episode of acute rejection is intravenous methylprednisolone in a single daily dose of 10–25 mg/kg (maximum dose: 0.5 – 1 Gm), for three consecutive days. Most Grade I and II rejections will respond to steroid therapy. Steroid resistant rejection episodes are treated with T-cell antibody, typically the polyclonal antithymocyte globulin, Thymoglobulin. Thymoglobulin is given in a dose of 1.5–2 mg/Kg/dose for a total of 10–14 days. It may be advisable to monitor CD3+ lymphocytes during treatment and restrict the frequency of dosing only to days when the count is greater than 20 cells/mm^3 (217). All antibodies have several side effects. Of concern are the first dose symptoms due to cytokine. This is clinically observed as fever with chills,

and rarely, as pulmonary edema. Precaution against the potential anaphylactic reaction related to polyclonal antibodies consists of using intravenous methylprednisone with the infusion of the antibody and administration of an antihistamine, such as diphenhydramine (Benadryl) and acetaminophen prior to antibody administration.

Reversibility of Acute Rejection

NAPRTCS data from the most recent era (2001–2007) observe that, among living donor kidney transplants, 53% of rejection episodes are completely reversed, 43% are partially reversed and 4% end in graft failure. Similar figures for deceased donor kidney transplants are 47%, 47%, and 6%, respectively (59). When stratified by age, young transplant recipients more frequently have irreversible rejection episodes although they also have higher rates of complete reversibility in those that are not lost. Despite decreasing rejection frequency, reversibility rates of acute rejection for pediatric recipients have not improved over the past 2 decades (53, 59) Molecular or genomic characterization of rejection biopsies may be helpful in describing different types of acute rejection, but there are no clear guidelines about its use at the present time (211, 212, 218–221).

Rescue Therapy

In those patients where neither steroids nor antibody therapy have successfully reversed a rejection episode conversion to an alternative calcineurin inhibitor or to other immunosuppressants might be warranted. There have been no controlled studies to document reversal of rejection with conversion to tacrolimus; however anecdotal reports do suggest that in some cases conversion does help to stabilize graft function (222–224). There have also been preliminary reports of the use of Rituximab for the treatment of B-cell mediated rejection (225).

Chronic Allograft Nephropathy (CAN)

The gradation from acute to chronic rejection is gradual; however many biopsies may show features of both, and some characteristic vascular changes of chronic rejection may be seen as early as 10 days post-transplant (226). The clinical picture is that of gradually declining renal function together with varying degrees of proteinuria and hypertension (227). The clinical condition may be referred to as transplant glomerulopathy, chronic rejection, chronic allograft dysfunction (CAD), chronic allograft nephropathy (CAN) or interstitial fibrosis and tubular atrophy (IFTA) (228). The succession of names reflects lack of clarity of the etiology, clinical course or treatment of this disorder. Nonetheless, this process, which will be referred to as CAN in this chapter, is the leading cause of graft loss following kidney transplantation in children (*Table 75-5*).

An ongoing controversy exists as to whether the changes seen in chronic rejection are immune mediated, secondary responses to infection, ischemic in nature, or non-immunological injury due to hyperfiltration (229–232). Data in children have shown clearly that acute rejection is a predictor of chronic rejection (19). In a study of 1,699 living donor and 1,795 deceased donor recipients NAPRTCS noted acute rejection was a relative risk factor for chronic rejection (RR = 3.1), and multiple acute rejections increased the RR to 4.3. Late acute rejections are also clinical correlates of chronic rejection (233). Even if acute rejection is the most critical element in the genesis of chronic rejection, other immune mechanisms may mediate its progression, such as antibodies directed against the donor, MICA, endothelial cells and B lymphoblasts (135, 234). Gene expression profiles in graft biopsies of patients with established CAN demonstrate upregulation of profibrotic and growth factors (235).

Symptomatic therapy is currently the only available method of dealing with CAN. Hypertension should be controlled and the proteinuria may occasionally respond to ACE inhibitors; however, renal function will generally continue to decline. In children, CAN produces an additional burden since decreased renal function will result in deceleration of growth (236, 237). It is in this context that prevention of chronic rejection by early aggressive therapy in patients who have had an episode of acute rejection may be rewarding. Since currently available immunosuppressive medications have been unsuccessful in preventing or slowing the progression of chronic rejection, the use of immunosuppressives other than those currently approved may be reasonable, such as the use of mTOR inhibitors or co-stimulation blockade rather than nephrotoxic calcineurin inhibitors (206, 238–242). Although some programs have concluded that these techniques might be beneficial after CAN is established (243), there may be a point at which substitution of non-nephrotoxic agents is not helpful (244). The presence of heavy proteinuria in recipients with CAN may also predict lack of benefit of changing chronic immunosuppression (245).

Recurrent Disease After Pediatric Kidney Transplantation

Some diseases will recur in a transplanted kidney, and the recurrent disease may lead to loss of the graft, as it had done to the native kidneys previously. Recurrence of the original disease is the cause of 6.8% of all graft losses (*Table 75-5*); and it is the cause of up to 9.5% of graft losses in subsequent transplants (59). Thus, recurrence is one of the top four causes of all graft losses. The 5-year living donor graft survival for children with FSGS is 71% and for children with glomerulonephritis it is 77%; in contrast to all other causes of ESRD in which 5-year graft survival is greater than 83%. In the deceased donor group, 5-year graft survival rates for children with FSGS, glomerulonephritis and congenital nephrotic syndrome are below 64%; HUS and familial nephritis have rates of 66%; and all other causes have a rate of 70%. Several publications have reviewed the course of recurrent disease in pediatric kidney transplantation (89, 246, 247). In some cases, recurrence of some features of the disease without affecting graft survival up to recurrence of the full disease with substantial reduction in graft survival. Unfortunately, there has been very little change in frequency of recurrent disease in pediatric grafts, despite substantial changes in immunosuppression during the past 2 decades (248).

FSGS

Focal segmental glomerulosclerosis is the most common cause of steroid resistant nephrotic syndrome leading to ESRD and is the most common acquired cause of ESRD in children. Reports of recurrence of FSGS vary from 15 to 50% and about 50% of the recurrences lead to graft loss (246–248). FSGS is a pathologic diagnosis and represents the appearance of a large number of diseases that might be due to immunologic, genetic or other causes. The genetic diseases do not seem to recur in a graft. Risk factors for recurrence include early onset of nephrotic syndrome, rapid progression to ESRD (<3 years), resistance to treatment, white or Asian race, recurrence in a previous transplant, and possible presence of a circulating "glomerular permeability factor" (248, 249). Recurrence can occur immediately after transplantation and result in massive proteinuria, acute tubular necrosis and even graft failure related to small vessel thrombosis (249). Typical FSGS lesions on pathologic examination, other than foot process fusion, may not appear early in the course of recurrence, but may follow early thereafter. In general, children with active nephrotic syndrome are not candidates for pre-emptive transplant because of the heavy proteinuria and consequent risk of graft thrombosis and delayed diagnosis of recurrence (246). Many programs will perform native nephrectomy and will maintain the children on chronic dialysis for some period of time, certainly to improve nutritional status and to normalize the serum albumin. There is no benefit to living donor transplantation in children with recurrent FSGS: although graft loss due to rejection is lower in recipients of living donor transplants, graft loss due to recurrence is higher, leading to equivalent graft survivals in living and deceased donor transplants (249, 250). Whether this result is due to a higher frequency of recurrence in living donor recipients, a more aggressive course in those recipients, or simply a higher rate of rejection in the deceased donor recipients is not known. Plasmapheresis is often used prophylactically prior to transplantation or immediately after it to attempt to prevent or treat recurrence of FSGS (251–254) and some programs report complete remission in up to 60% treated in that manner. Although no specific immunosuppression protocol has demonstrated clear efficacy in treating or preventing recurrent FSGS, there is some evidence that high dose cyclosporine may be effective in doing so (253, 254). Whether the high dose is needed to counteract the effect of high serum levels of low-density lipoprotein which binds free cyclosporine, or whether the beneficial effects are due to direct action on podocytes is not clear (89, 255). Rituximab has also been used to treat recurrent FSGS in children, with mixed results (256–259).

HUS

Hemolytic uremic syndrome in children is most commonly caused by enteropathic bacteria and the disease typically does not cause ESRD or recurrence in a kidney transplant (260). On the other hand, children with atypical or "non-Shiga toxin-associated" HUS have a much higher incidence of progression to ESRD and recurrence of the disease after transplantation (90, 261, 262). The recurrence is very infrequent after diarrhea-associated HUS, but up to 80% in atypical HUS (89). Although calcineurin inhibitors have been associated with de novo HUS in a few kidney transplants, their use in recurrent disease seems to have no effect (89). In patients with factors H, I or B mutations, the recurrence rate appears to be high and transplantation may be deferred. Some have proposed plasmapheresis with fresh frozen plasma in this setting (90) and combined kidney–liver transplantation has been proposed for some children with factor H mutations (90).

Membranoproliferative Glomerulonephritis, Types 1 and 2

Both forms of MPGN can recur in transplants, with variable frequency from 30–60% (89). Type 2 seems to be more severe, and neither form seems to be treatable after recurrence (263).

Oxalosis, MMA and Metaboloic Diseases

Primary oxalosis recurs almost immediately and universally after kidney transplantation and was once considered a contraindication to kidney transplantation. However, treatment with intensive pre- and post-transplant plasmapheresis to lower the body burden of oxalate and the use of combined kidney–liver transplantation has led to substantially better outcomes (81, 83, 264–266). If liver transplantation is being considered, however, careful consideration must be paid to determining whether the child has a variant that might be responsive to lifelong treatment with pyridoxine rather than liver transplantation (89). Methylmalonic acidaemia may be partially ameliorated by kidney transplantation, but full treatment may require liver transplantation in select recipients (89). Certain inherited diseases like insulin-dependent diabetes mellitus and sickle cell disease may recur in a kidney transplant, but this almost universally happens during adulthood, many years after the primary transplant in a child.

Other Autoimmune Diseases

IgA nephropathy, Henoch-Schonlein purpura, lupus nephritis and ANCA-associated vasculitis may recur following kidney transplantation in children, but these recurrence may be minimally apparent and less frequently lead to graft loss (89).

Cystinosis

ESRD is typically the earliest organ failure in children with cystinosis and often accounted for the bulk of deaths from this disorder. However, the use of kidney transplantation and cystine-depleting therapy with cysteamine has extended their life expectancy to the fifth decade (152, 267–269). Although cystine may accumulate in the interstitium of renal grafts, it does not cause graft failure. However, the unremitting accumulation of cystine results in substantial non-renal morbidity and mortality (268, 270).

Graft Survival

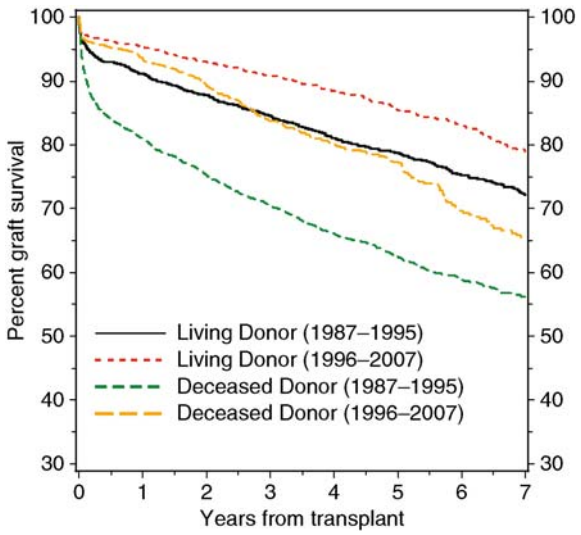
Pediatric renal centers reporting graft survival show varying results. Due to the fact that the number of patients at any one center is small, such data cannot represent the pediatric transplant population at large. Furthermore, multiple factors affect graft survival, such as donor and recipient age, histocompatability matching, recipient race, and so forth. Thus, there cannot be accurate descriptions of graft survival rates without classification of the important variables. In order to obtain a proper population mix representing gender, age, and racial diversity, multi-center registry results such as SRTR and NAPRTCS annual reports have been used (38, 59).

NAPRTCS has recorded a total of 2,556 graft failures occurred between 1987 and 2007, representing about 25% of all transplants in that time frame. Of the failures, about 9% were deaths with a functioning graft, 84% were returned to dialysis, and 7% were re-transplanted at the time of failure. *Table 75-5* provides the distribution of causes of graft failure. With increased length of follow-up, chronic rejection continues to increase in importance; it is now the most common cause of graft failure. Overall, 48% of graft failures are caused by rejection with chronic rejection accounting for 35% and acute rejection accounting for 13%. Recurrence of original disease as a cause of graft failure was observed 174 times, accounting for 7% of graft failures. The specific diseases include: focal segmental glomerulosclerosis accounted for 44% of these graft losses, membranoproliferative glomerulonephritis Type II 9%, hemolytic uremic syndrome 9%, oxalosis 5%, chronic glomerulonephritis 4%, others 28%. Vascular thrombosis remains a major cause of failure, and 361 graft failures (14.1%) were attributed to primary non-function, vascular thrombosis, or miscellaneous technical causes. These data show that such problems occur in about 4% of all pediatric transplants (35, 92, 194, 196–198, 271). Considering just transplants that have been performed since January 1, 2000, chronic rejection is the leading cause of graft loss (41.3%), followed by vascular thromobosis (8%), recurrent disease (7.9%), acute rejection (6.3%) and medication discontinuation (6.3%) (59).

Overall 5-year graft survival curves by donor source are shown In *Fig. 75-8*. Expected graft survival for index transplants performed in the last decade at 1, 3, 5 and 7 years for living donor kidneys is 95, 91, 85 and 79% respectively, and for deceased donor kidneys it is 93, 84, 77 and 65%. There has been a continuous improvement in short and mid-term graft survival rates, mostly due to marked improvements in early graft survival rates. This may be related to the decreased frequency of acute rejection rates and the decreased incidence of acute rejection as

Figure 75-8

Percent graft survival by transplant era and donor source. The graft survival has improved during the most recent era compared to previously. Living donor transplants have better 5-year graft survival than deceased donor transplants from both eras. (Adapted from 2008 NAPRTCS Annual Report (www.naprtcs.org)).



a cause of graft loss. It is notable, however, that as shown in *Fig. 75-8*, the slope of the graft survival curves have not changed significantly over the past 2 decades. These important trends in improved graft survival in pediatric living and deceased donor renal transplant outcome have been reported frequently over the past decade (16, 52, 59) and the most recent data are shown in *Figs. 75-1* and *75-8*.

Table 75-8 shows relative hazards for graft failure for selected transplant characteristics for both living and deceased kidneys. Relative risks of graft failure are derived using Cox proportional hazards regression models. For recipients of living donor grafts, the most influential prognostic variables of index graft survival are race (African American vs. non-African American; RH = 1.95, $p < 0.001$), prior transplant (RH = 1.35, $p = 0.006$), lack of induction antibody treatment (RH = 1.15, $p = 0.035$) and lack of HLA-B matches (RH = 1.40, $p = 0.008$). A linear trend in improvement of graft survival with more recent year of transplantation has also been observed ((RH = 0.95 per year, $p < 0.001$) (59). For deceased donor recipients, the important prognostic factors include: African-American race (RH = 1.56, $p < 0.001$), prior transplant (RH = 1.43, $p < 0.001$), age older than 2 years (RH = 0.59, $p < 0.001$) and male

Table 75-8

Relative hazard (RH) of individual prognostic factors for graft loss in the presence of other factors in multivariate proportional hazards models (Adapted from (59))

	Living Donor		Deceased Donor	
	RH	p-value	RH	p-value
Recipient age (>2 years vs. 0–1 year)	1.13	NS	0.59	<0.001
Prior transplant	1.35	0.006	1.43	<0.001
No induction antibody treatment	1.15	0.035	1.09	NS
>5 lifetime transfusions	1.31	0.003	1.28	<0.001
No HLA-B matches	1.40	0.008	1.16	0.014
No HLA-Dr matches	0.87	NS	1.14	0.024
African-American race	1.95	<0.001	1.56	<0.001
Prior dialysis[a]	1.16	0.052	1.23	0.040
Cold storage time >24 h	–	–	1.14	0.034
Transplant year (per year)	0.95	<0.001	0.94	<0.001
No native nephrectomy	0.87	0.051	0.96	NS
Male gender	0.87	0.036	0.85	0.005

NS not significant; *HLA* human leukocyte antigen

[a]Reported as ever having dialysis

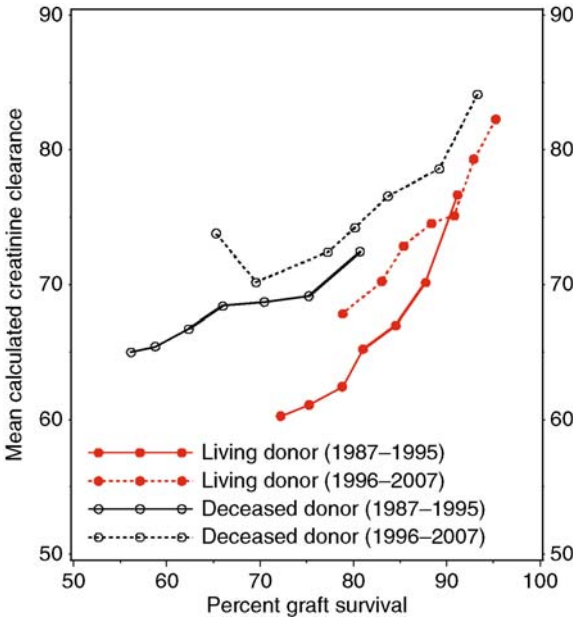
gender (RH = 0.85, p = 0.005. The same linear trend of improved graft survival rates in later transplant years is also seen (RH = 0.94 per year, p < 0.001). A history of prior dialysis may be a slight relative risk (RH = 1.23, p = 0.04. Lack of HLA-B and –DR matches also seem to be relative risks to graft survival in deceased donor transplants, as is prolonged cold-storage time. For both living and deceased donor transplants, a history of more than five lifetime blood transfusions seems to be associated with worse graft survival rates, but the significance of this finding in the modern era is not clear. Also, the interpretation of the use of induction antibody treatment is hampered by selection factors that motivate its usage; the size and direction of these biases cannot be quantified and the evaluation of this factor cannot be considered definitive. Importantly, the improvement in graft survival rates in very young recipients is strongest in the living donor recipients and the overall improvement in this age group may be related to the high percent of living donors used for them.

Another measure of long-term graft function is the calculation of graft half-life. An analysis of 8,922 pediatric and 78,418 adult renal transplants demonstrated superior long-term graft function in young pediatric recipients (22). Infants (age 0–2 years) had the worst 1-year graft survival rates (71%) compared to children (3–12 years) (83%), adolescents (13–21 years) (85%) and adults (86%). However, for all grafts that survived at least 1 year, infants had the longest projected half-life (18 years), compared to children (11 years), adolescents (7 years) and adults (11 years). A similar analysis of UNOS data showed that young recipients who received adult donor kidneys and had immediate graft function had projected half-lives >25 years, better even than HLA-indentical adult donor–recipient pairs (39).

While assessment of graft survival is a reasonable measure of transplant outcome, it does not include an accurate portrayal of the course of chronic allograft nephropathy. The usual course of a kidney transplant includes an inexorable and continuous decline in renal function over many years. Thus, many kidney transplant recipients suffer from the consequences of CKD for many years as graft function deteriorates. This decline is shown in *Fig. 75-9*. As noted above, the various causes for decrease in kidney function include immunologic, such as immune response or rejection, as well as recurrent disease; and non-immunologic, such as nephrotoxic medications, infection, perfusion injury, and so forth. Studies designed to identify the causes and ameliorate any etiologic causes are clearly indicated.

Figure 75-9

Graft survival (%) and calculated GFR (ml/min/1.73M^2) values for children with functioning grafts plotted at each annual follow-up visit for living and deceased donor recipients in two different eras. Continued decreases in graft function and survival are seen through at least the first 5 post-transplant years. (Adapted from NAPRTCS 2008 Annual Report (www.naprtcs.org).



The primary disease causing ESRD can have an effect on graft survival. Children with oxalosis used to have very bad outcomes, to the extent that the diagnosis was considered a contra-indication to transplantation. However, improvements in outcome related to combined liver–kidney transplantation have been encouraging (61, 81–83, 264–266) after the use of intensive hemodialysis prior to and immediately after transplantation, as well as performing the procedure before complications of the disease have caused multiple organ damage in the recipient. There have been reports of using the recipient liver in a "domino" procedure as the graft for another patient with liver failure due to other causes. Unfortunately, all of the complications of oxalosis quickly occur in those recipients (272). Similarly, infants with congenital nephrotic syndrome often had very poor outcomes (82, 152), but strategies designed to reduce the risk of thrombosis and improve nutrition pre-transplantation have led to marked improvements (84, 85, 151, 273). Focal Segmental Glomerulosclerosis (FSGS) can be a devastating disease that may recur very quickly following

renal transplantation, sometimes as early as the first post-transplant day (152, 274–277). Although recurrence is no more frequent in living donor transplants, the graft survival advantage of living donor transplantation is lost for children with FSGS (250). Little is known about the pathophysiology of the disorder or the cause for recurrence (278, 279). There are several proposed approaches to preventing or treating recurrence, mostly involving enhanced immunosuppression with plasmapheresis (89, 277, 280–285). Lupus nephritis surprisingly does not recur following renal transplantation to any great extent. Patients with lupus have similar outcomes compared to other patients (286, 287), except for a slight increase in mortality (287), an increase in incidence of recurrent rejections and a slight tendency to graft failure in those patients receiving deceased donor grafts following peritoneal dialysis(286). Children with sickle cell disease and ESRD can receive kidney transplants successfully (288), as can those with Down Syndrome (289, 290). Hemolytic uremic syndrome has been variably described as likely to recur or not (152, 291). After distinguish the etiologic factors, epidemic shiga toxin-associated hemolytic syndrome is unlikely to recur following renal transplantation (260, 262), whereas atypical or familial HUS may recur with devastating and irreversible consequences (262).

Growth After Pediatric Kidney Transplantation

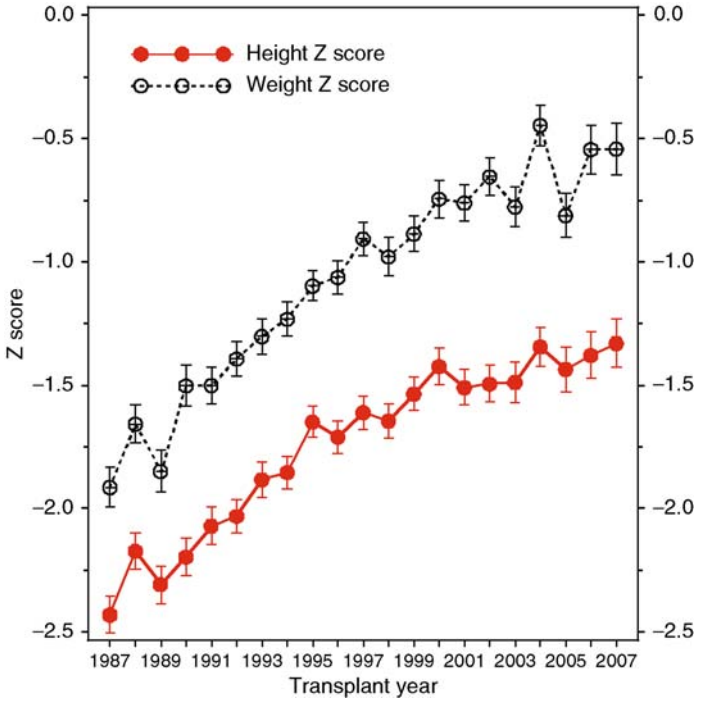
A major distinguishing feature of pediatric from adult recipients is the need for children to grow. The growth failure commonly observed in children at the time of transplantation is multi-factorial; however the most important cause is the reduced response to endogenous growth hormone (75), related to several mechanisms. Growth failure often begins insidiously early in the course of CKD. In a NAPRTCS analysis of 1,768 children with CKD (glomerular filtration rate <75/ml/min/m^2), over one third had a height deficit of more than 2 SDS. It has been amply demonstrated that chronic renal insufficiency beginning in infancy leads to permanent reduction in growth potential (292). Growth retardation continues in children on a dialysis regimen, whether the mode of dialysis is peritoneal or hemodialysis. For several years it has been suggested that a functioning transplant would enable the child to achieve catch-up growth (13). Unfortunately, long term data from registry studies has shown a more disappointing outcome.

NAPRTCS data shows that the mean height deficit at the time of transplantation is – 1.88. Males (−1.92) and younger recipients have greater height deficits at the time of transplantation (36). Younger children can show catch-up growth (7, 9, 13) with complete inversion of Z-score up to 0.60 at 2 years for those <5 years of age at transplant. Older children may grow at a normal rate, but rarely show catch-up growth. The Z-score for 19-year olds is −1.5. Final adult height for children with ESRD is improving, but all of the improvements seem to be related to the gains achieved during treatment for CKD rather than after transplantation (9). On a positive note, however, there has been an improvement in the height deficit at the time of transplantation: In 1987, children receiving their initial kidney transplant were an average of 2.4 standard deviations below average, whereas in the 2003 cohort, the deficit was only 1.5 standard deviations below average (*Fig. 75-10*) (59). As a result, the final adult height of children transplanted more recently is much better than those transplanted years ago. The Z-score for children transplanted in 1987–1991 who have reached their terminal height was −1.93 as compared to −1.08 for those in the 1997–2001 cohort.

These studies on long-term growth post-transplantation are disappointing; however they do focus on mechanisms that prevent growth despite a milieu with normal renal function. Individual center studies have adopted a variety of techniques, such as discontinuation of prednisone (293, 294), alternate-day steroid therapy (295–297), steroid avoidance (12) or the use of recombinant human growth hormone (298). It has been known for several years that steroids used for immunosuppressive therapy will inhibit growth (299). It has also been demonstrated that steroids affect growth hormone secretion (236, 300–302). Measurements of pulsatile and pharmacologically stimulated hormone release reveal that steroids play an inhibitory role (299, 303). Conversion of children to alternate-day steroid therapy has shown improvement in growth (296, 297); however the best catch-up growth is seen in patients completely withdrawn from steroids (12, 223, 304). Numerous uncontrolled studies have shown that steroids can be withdrawn from children post-transplantation (12, 223, 305); however, until recently, acute rejection tended to occur shortly afterwards in many of these patients (306), with marked detrimental long-term effects. More recent studies, using different approaches to long-term immunosuppression, have shown much better success with the avoidance or withdrawal of steroids, but the effects of these approaches on long-term growth rates is not yet known (200, 201, 203, 207). An alternative method of attaining catch-up growth post-transplantation would be the use of growth hormone. Recombinant human growth hormone (rhGH) is not approved for use in children post-transplantation;

Figure 75-10

Standardized height and weight, displayed as Z-scores, at the time of initial kidney transplant over time. There has been a substantial improvement in height and weight over the past 2 decades. (Adapted from NAPRTCS 2008 Annual Report (www.naprtcs.org).



however numerous uncontrolled studies have shown its ability to accelerate growth in this setting (307). Several complications of the use of rhGH post transplantation have been suggested (307–310) but a controlled trial demonstrated that it could be used safely and effectively (311). Although one report suggested that the pre-transplant use of rhGH may be associated with subsequent PTLD, its use post-transplantation had no such association.

Long-Term Outcomes of Pediatric Kidney Transplantation

Complications of Pediatric Kidney Transplantation

There are multiple complications of the transplant surgery and of chronic immunosuppression. These complications tend to diminish the success of kidney transplantation. Specific information about these complications are provided in the chapter, Complications of Renal Transplantation. The complications include multiple types of infections, cardiovascular complications, including hypertension and vascular problems, metabolic abnormalities such as hyperglycemia and dyslipidemias, obesity, growth deficiency, and complications of orthopedic, gastrointestinal, neurologic, pulmonary and hematologic systems.

Adherence to Chronic Immunosuppression Treatment

Non-adherence is often cited as a cause of long term graft loss in pediatric renal transplant recipients, especially adolescents (312). A major reason for non-adherence is thought to be the alteration in appearance that accompanies immunosuppressive medications, including the cushingoid facies and growth retardation related to long-term daily corticosteroid administration and the hirsuitism and gingival hypertrophy associated with cyclosporine. However, the true incidence of non-adherence is unknown. Non- adherence rates of 22% (313), 43% (314) and as high as 64% in adolescents (315) have been reported. Some factors, such as young age, adolescence, poor socio-economic status, and family stress have been

associated with increased levels of non- adherence (313, 315–317). Importantly, however, health care workers are not able to identify a significant proportion of non-adherent patients (318). Treatments such as educational programs (314) and family-based therapy (319) have been proposed, but these types of programs have not been universally successful in changing motivation (312). An alternative proposal for improving non-adherence would be to change the type or frequency of immunosuppressive medications so that the recipients do not have to adhere to rigid schedules; but these proposals are currently only hypotheses (201, 320).

Rehabilitation, School Function, Psychosocial Consequences

Organ transplantation typically results in dramatic improvement of all aspects of physical, emotional and social functioning. Importantly, cognitive skills improve after successful renal transplantation (6), suggesting stabilitzation of neurophysicolgic functioning. Health related quality of life measures are generally good, especially in older children and adolescents, although all ages reports some problems with usual activities (321). Interestingly, the perceived emotional status of the children was actually better than controls, especially during and after adolescence (321).

Long-term survival is generally excellent (322) and measures of quality of life have demonstrated excellent rehabilitation in long-term survivors (323, 324). Over 90% have rated health as good or excellent and most did not feel that health interfered with normal functioning. Most of them were full-time students or were employed. The majority was below normal height and up to a third was dissatisfied with their body appearance. In one report, only a small minority of long term survivors were married (325), but in another, 50% were married and half of those had children (324).

Mortality

Infection is generally the major cause of death, particularly in the first post-transplant years (36). Other major causes include cancer/malignancy, cardiopulmonary causes and dialysis-related complications. The best patient survival results are found in older pediatric recipients and in recipients of living donor transplants (59, 82). Risk factors for excess mortality include young recipient age, graft dysfunction (ATN) at day 30 following transplant and certain underlying renal diseases (oxalosis, congenital nephrotic syndrome, Drash Syndrome) (82). Mortality after 10 years post transplant seems to be related primarily to cardiovascular causes (59, 322), which may be linked to the hyperlipidemia and hypertension associated with chronic immunosuppression. The mortality rate of children, except for the very youngest, is very low and is much better than what is found in adults. Current 1- and 5-year patient survival rates for pediatric living donor kidney transplants are 98% and 96% and for pediatric deceased donor transplants they are 97% and 93%. Although the survival rates for deceased donor grafts are statistically worse than for living donor, they have also improved more dramatically, with 5-year survival rates rising from 91 to 96% over the past 2 decades (59). Young infants tend to have slightly worse survival than older children, but they have also shown marked improvement over the years. During the 1987–1995 era, the 3-year patient survival rates were 90 and 79% for infant living and deceased donor kidney transplant recipients respectively; and, for the most recent era those rates have improved to 95 and 93% (59).

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76 Immunosuppression in Pediatric Renal Transplantation

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Introduction

The major advance allowing prolonged graft survival has been the use of immunosuppressive drugs that down-regulate the immune response. The immunosuppression that is used varies among centers and evolves with the development of new medications and therapeutics.

A variety of factors influence the choice of immunosuppressive therapies. With the dramatic improvements in short-term graft survival in the past decade, the transplant community has started to focus on improving long-term graft survival. Current research is attempting to find the best combination that will optimize graft survival while limiting the side effects. As such, many centers now consider patient specific factors in choosing which medication regimen the transplant recipient will receive.

Induction Therapy

The goal of induction therapy is to prevent T-cell activation. This can be achieved either through depletion of the T-cell pool with monoclonal or polyclonal antibodies or by specifically inhibiting the action of the cytokine, interleukin–2 (IL-2). Retrospective data from the North American Pediatric Renal Trials and Collaborative Studies (NAPRTCS) consistently had shown a beneficial effect of prophylactic anti-T-cell antibody in pediatric renal transplant graft survival (1). However, these were retrospective studies and were subject to confounding variables such as center effect and that several different types of T-cell antibodies were used. To date, there have been no controlled trials in pediatric renal transplant recipients that have verified the benefit of using induction antibody (2). In fact, a large controlled trial of OKT3 induction versus induction with intravenous cyclosporine in pediatric kidney transplantation showed no benefit to antibody induction (3).

The choice of induction therapy is center and patient dependent. Considerations in choosing the appropriate agent include the efficacy in that patient population and the side effect profile. For instance, a center may use an IL-2 receptor antagonist for induction in the unsensitized recipient but use T-cell depleting antibody therapy in a highly sensitized patient or a patient who has had a previous failed transplant. Trends in induction therapy have changed over time with a shift away from the polyclonal and monoclonal antibody preparations towards the IL-2 receptor antagonists.

Polyclonal Lymphocyte Depleting Antibodies

Antithymocyte globulins have been used as induction therapy in kidney transplantation with the goal of decreasing the incidence of acute rejection, delaying the introduction of nephrotoxic calcineurin inhibitors, and avoiding or minimizing the use of corticosteroids (4). The primary immunosuppressive effect is mediated through the depletion of circulating T-lymphocytes through complement mediated lysis as well as initiating blockage of T-cell proliferation (5). There are two polyclonal lymphocyte depleting antibodies currently available. Anti-thymocyte globulin [equine] (Atgam®) is a polyclonal horse-derived antilymphocyte globulin. Due to the sclerosing nature of the preparation, Atgam® is given intravenously through a central catheter for up to 15 days. The recommended dose is 15 mg/kg and calcineurin inhibitors are generally withheld during the administration of the antibody. Thymoglobulin® is a rabbit derived antithymocyte globulin. Thymoglobulin® is provided through a peripheral vein at a dose of 1.5–2.0 mg/kg/dose. A recent report suggests daily monitoring of CD3 + subsets to guide therapy with the daily dose is given only when the CD3 + count exceeds 20 cells/mm^3 (6). Pre-medication with acetaminophen, corticosteroids, and diphenhydramine can decrease the incidence of administration side effects which include fever, chills, arthralgia, and dyspnea. As with other depleting antibody preparations, calcineurin inhibitors are frequently withheld during the course of treatment with Thymoglobulin®.

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Comparison of efficacy of removal of circulating T-cells suggested that Thymoglobulin® may have some benefit over Atgam® (7). A randomized, double blinded trial compared Thymoglobulin® to Atgam® for induction immunosuppression in adult renal transplant recipients found a lower incidence of rejection in the Thymoglobulin® group (4% vs. 25%, p = 0.014) (8). Although prolonged lymphocyte suppression was observed in the Thymoglobulin® group, there was no increase in infection or malignancy at 1 year post-induction (9). A multicenter study comparing Thymoglobulin® induction versus no induction showed similar rates of patient and graft survival but did show a significantly lower rate of biopsy-confirmed acute rejections with the Thymoglobulin induction group (p = 0.001) (4). This group also showed higher rates of adverse events, specifically cytomegalovirus infections and bone marrow suppression. Although there have been no controlled comparisons of the polyclonal agents on clinical outcomes in pediatric renal transplant recipients, Thymoglobulin® has been studied in small groups of this population and was found to be effective (10). In a historical comparison of Atgam® and Thymoglobulin® in pediatric kidney transplant recipients at a single center, the incidence of acute rejection was noted to be lower in the Thymoglobulin®-treated recipients (11), but this result may also be due to the fact that it was provided to the more recent cohort and the outcomes of kidney transplantation have generally improved with time (2).

Monoclonal Lymphocyte Depleting Antibodies

OKT3

Muronab-CD3 (Orthoclone, OKT3) is a monoclonal antibody that binds to the lymphocyte-CD3 complex resulting in rapid cell lysis. OKT3 is effective at reversing acute rejection but often, subsequent treatments are limited due to the production of anti-OKT3 antibodies (12). It is administered as a bolus injection into a peripheral vein daily for 10–14 days at a dose of 5 mg for older children and 2.5 mg for children weighing <30 kg. Calcineurin inhibitors are withheld during the use of OKT3. Especially problematic with OKT3 is the associated cytokine-mediated response caused by an increased amount of T-cell derived cytokines such as tumor necrosis factor, IL-2, and gamma-interferon released with cell lysis. This usually occurs approximately 30–60 min after starting therapy. Side effects include fever, chills, hypotension, nausea, diarrhea, and potentially life-threatening anaphylaxis. Pre-medication with acetaminophen, corticosteroids, and diphenhydramine can help temper side effects. Pulmonary edema is another serious complication that can occur with OKT3 therapy and careful monitoring of the patient's fluid status is necessary to minimize this possibility. Neurologic complications from mild headaches to aseptic meningitis, infection, and increased risk of malignancy are all possible complications of OKT3 therapy (13, 14). A humanized anti-CD3 antibody with a low affinity for Fc receptors may be associated with fewer and less severe adverse reactions (15). Currently, there are a number of humanized anti-CD3 agents that are in various stages of pre-clinical development and in phase I clinical trials. These newer agents include HuM291, HuOKT3-gamma1, Aglycosyl gamma 1 CD3mAB, and Anti-CD3 Immunotoxin.

Although retrospective analysis of pediatric kidney transplantation continued to show a clear benefit to the use of prophylactic induction antibody (3), a recent prospective randomized trial of OKT3 induction showed no clear advantage (3). Currently, very few pediatric transplant recipients receive OKT3 for induction therapy (16).

Alemtuzumab (Campath-1H)

Alemtuzumab (Campath-1H) is a humanized anti-CD52 antibody. CD52 is the most prevalent cell surface antigen on lymphocytes but its function is currently unknown (17). After administration, alemtuzumab quickly depletes T and B lymphocytes, monocytes, and natural killer cells with its effects lasting for months (18). It is currently undergoing clinical trials as a lymphocyte depleting agent in both pediatric and adult renal transplantation to eliminate the use of steroids and minimize other immunosuppressants, specifically calcineurin inhibitors (17). There is no recommended dose for pediatric renal transplant recipients but 0.3 mg/kg/dose has been the most common dose used in earlier intestinal, multivisceral and recent pediatric studies (19). The number of doses ranged from 1 to 4 doses during the first week with 2 doses being the most common. Due to infusion related reactions, premedications with methylprednisolone, acetaminophen and diphenhydramine are recommended with doses of antiemetic as needed for nausea and vomiting. Profound lymphopenia is alemtuzumab's major limitation with potential side effects including infections, autoimmune complications, and malignancies (19).

There have been multiple uncontrolled pilot trials of Alemtuzumab in adult renal transplant recipients.

In 1999, Calne et al. reported the use of 2 doses of Campath combined with low-dose cyclosporine monotherapy in 31 consecutive renal transplant recipients (20, 21). Cyclosporine was begun 48 h post transplantation and doses were adjusted to maintain levels of 75–125 ng/ml. Of the 31, 1 died of heart failure with a functioning graft and 1 had recurrent IgA nephropathy. Twenty-nine grafts were functioning at the time of the report and 6 of these had rejection episodes (19%). All rejections responded to intravenous steroids, 3 of the patients had prednisone and azathioprine added, the rest remained on cyclosporine monotherapy. One patient had systemic CMV and one had reactivation of abdominal tuberculosis. In 2003, Knechtle et al. reported on 29 primary renal transplant recipients who received Campath induction therapy (23 LD, 6 DD) (22). Sirolimus was started on day 1 and adjusted to a level of 8–12 ng/ml. All patients were alive at the time of the report and 28 had functioning grafts. One patient lost the graft due to rejection at 2 months post transplantation. There were no systemic infections and no malignancies. Eight patients had rejection episodes (28%) and 5 of these were acute humoral rejection with C4d-positive staining. Biopsies of long-term survivors showed no evidence of chronic allograft nephropathy. Twenty-two patients remained on sirolimus monotherapy. In 2003, Kirk et al. reported results of 7 living donor kidney recipients who received perioperative Campath and no subsequent immunosuppression (23). All 7 patients had early acute rejection episodes (between days 14 and 28) which were successfully treated. All eventually weaned to sirolimus monotherapy, except one patient who had oral prednisone reinstituted for recurrent FSGS. There were no serious infections in the patients and all 7 had normal renal function at the time of the report.

There have been other uncontrolled studies of Campath in kidney and pancreas transplantation in adults and it generally has been well tolerated without an increase in serious infections. In one small series of 3 high-risk pediatric kidney transplant recipients, Campath was well tolerated, but acute rejection was reported (17). Campath induction has been used more extensively in pediatric small bowel transplantation. In a review of intestinal transplantation at the University of Miami, Tzakis et al. reviewed 54 children receiving grafts between 1994 and 2000 (24). Although there were multiple protocols and this was not a clinical trial, they concluded that the introduction of Campath was associated with improved patient and graft survival and was not associated with an increased rate of opportunistic infections. The Cooperative Clinical Trials in Pediatric Transplantation (CCTPT) program of NIAID has sponsored a multicenter pilot trial of Campath induction in pediatric renal transplant recipients with initial maintenance immunosuppresson of tacrolimus and MMF. Patients free of clinical and subclinical rejection at 2 months, have a substitution of sirolimus for tacrolimus.

Monoclonal Non-Depleting Antibodies to Interleukin-2 Receptor

There are two high-affinity antibodies that act on the inducible alpha-chain of the interleukin-2 receptor (IL-2r) on the surface of the activated lymphocyte, basilizimab (Simulect®) and daclizumab (Zenapax®). The precise mechanism of the antibodies is not known, but is presumed to be saturation of the IL-2 receptor and subsequent competitive antagonism of IL-2-dependent proliferation Basiliximab is a chimeric human/mouse monoclonal antibody to the alpha chain of the IL-2 receptor. Generally, the dosing regime includes 2 doses administered within 2 h of transplant and on post-operative day 4. The dosing recommendation is 10 mg in patients who weigh less than 35 kg and 20 mg in all other patients. Pharmacokinetic studies demonstrate that the clearance is reduced by approximately half in children (age 1–11 years) and did not seem to be altered by weight or body surface area (25). Saturation of IL-2 receptors was studied in 14 children with the mean duration of saturation being 42 ± 16 days.

Daclizumab (Zenapax®) is a humanized monoclonal antibody to the alpha chain of the IL-2 receptor. The dosing regimen is typically 5 doses of 1 mg/kg every 2 weeks. It is thought that 5 doses provides IL-2 receptor blockade for up to 3 months. A comprehensive safety and efficacy review of 67 pediatric patients showed that the 1 mg/kg dosing in all age groups provided adequate levels to successfully saturate and block the IL-2 receptor (26). It also showed that a reduced dose in older children would most likely translate into reduced efficacy. Overall, both are well tolerated with a side effect profile that is similar to placebo. Severe acute hypersensitivity reactions have been observed in patients with both the initial and re-exposure dose. This acute reaction may include hypotension, tachycardia, cardiac failure, bronchospasm, pulmonary edema, respiratory failure, and rash. Analysis of NAPRTCS pediatric transplant recipients demonstrated that use of IL-2 receptor antagonists was associated with a decreased risk of thrombosis (27).

Both antibodies have been studied extensively in children and have been shown to be safe and effective (28–33). A novel 6-month dosing schedule of daclizumab

has been reported as part of a steroid-avoidance pilot study and appears to be well tolerated (29). This steroid-avoidance protocol is currently undergoing a controlled trial against conventional immunosuppression, under the auspices of the Cooperative Clinical Trials in Pediatric Transplantation (CCTPT) program of NIAID, in order to define the risks and benefits of this approach. The NAPRTCS 2008 annual report shows that IL-2 receptor antibody is the most common induction therapy used (34).

Co-Stimulation Blockade

T cells require two distinct signals for full activation (35). The first signal, called allorecognition, is provided by the engagement of the T cell antigen receptor (TCR) with the major histocompatibility plus peptide complex on antigen presenting cells (APCs). The second "costimulatory" signal is provided by engagement of one or more T cell surface receptors with their specific ligands on APCs (36–38). The best characterized and perhaps most important costimulatory signal is that provided by interaction of CD28 on T cells with either B7–1 or B7–2 surface ligands on APCs (35). Cytotoxic T lymphocyte-associated antigen 4 (CTLA4), expressed on the cell surface only after initial T cell activation, also binds B7–1 and B7–2 resulting in an inhibitory signal which terminates the T cell response (39, 40).

Recent evidence suggests that CTLA4 negative signaling pathway may be required for induction of acquired tolerance in vivo (41, 42). Binding of CD28 to B7–1 or B7–2 is blocked by CTLA4Ig, a recombinant fusion protein that contains the extracellular domain of CTLA-4 fused to an IgG heavy chain tail. The administration of CD28-B7 blockade prevents acute allograft rejection and induces donor-specific tolerance in several animal models (43–47). In addition, CD28-B7 blockade prevents development (48, 49) and interrupts progression (50, 51) of chronic allograft rejection in transplant models.

CTLA4Ig is currently undergoing human phase I–II testing in autoimmune diseases. A phase I trial with CTLA4Ig in patients with psoriasis vulgaris has been recently reported (52–54). Other trials in rheumatoid arthritis and multiple sclerosis are underway.

A high affinity variant of CTLA4-Ig, called LEA29Y (belatacept), has been developed. In trials, belatacept is typically administered intravenously once a month, a strategy that potentially could improve adherence in the high risk adolescent transplant population. A calcineurin inhibitor sparing trial using belatacept in adult kidney transplant recipients reported encouraging preliminary results (55). In this study, 218 patients who received induction with basiliximab, MMF, and corticosteroids were randomized into three groups; one group received intensive belatacept, another received less intensive belatacept and the control group received cyclosporine as primary immunosuppression. At 6 months, the incidence of rejection was 6–8% in all groups but at 12-months GFR was significantly higher (62–66 ml/min/1.73 m^2) and the incidence of chronic allograft nephropathy was significantly lower (20–29%) in the belatacept groups than in the cyclosporine group (53 ml/min/1.73m^2 and 44%, respectively). Of concern, was the incidence of three cases of post-transplant lymphoproliferative disorder (PTLD) in the belatacept group, two of which were related to primary EBV infection. Although two of the episodes occurred after the subjects had been changed from belatacept to other immunosuppressive agents, the concern about its use in children who are at higher risk of PTLD should be balanced against its potential benefit.

Maintenance Immunosuppression

Steroids

Steroids have broad anti-inflammatory effects on cell-mediated immunity but leave humoral immunity relatively intact (56). The preparations commonly used are prednisolone, its 11-keto metabolite prednisone, and methylprednisone. Although the half-lives of these preparations are very short they can be administered once daily because their effect on inhibition of lymphocyte production persists for 24 h (57). The dosage is usually high initially; up to 10 mg/kg as induction, starting maintenance at 2 mg/kg/day, with a gradual reduction to approximately 0.12–0.16 mg/kg/day within a 6–12 month period.

Unfortunately steroids have multiple adverse effects including growth retardation in children. Other side effects include increased cushingoid habitus, susceptibility to infection, impaired wound healing, aseptic necrosis of bone, cataracts, glucose intolerance, hypertension, hyperlipidemia, gastic ulcer disease, obesity, and acne (58). In addition, the negative impact steroids has on appearance may play a role in poor adherence, especially in the body image conscious adolescent.

Because of the multiple side effects of maintenance steroid therapy, attempts have been made to withdraw steroids altogether, reported both in adult and pediatric kidney transplantation (59). The use of alternate-day

steroid therapy, which appears to reduce the growth inhibiting effect without unduly increasing rejection episodes, seems reasonable (60, 61). A CCTPT multi-center double-blind, randomized controlled trial of corticosteroid withdrawal in pediatric kidney transplantation enrolled 274 subjects before it was halted due to a high incidence of PTLD using maintenance immunosuppression of calcineurin inhibitor and sirolimus (62). However, those children who had corticosteroids successfully withdrawn did not have higher rates of late rejection and their long-term graft survival was equivalent to the control group who were receiving chronic low-dose daily corticosteroids as part of their maintenance therapy.

An uncontrolled pilot trial of prolonged IL-2r antibody administration to permit steroid avoidance has shown very promising results with low acute rejection rates and low incidence of complications (63). This has prompted a multicenter randomized controlled trial through the CCTPT consortium comparing this combination to conventional triple immunosuppression, in order to define the risks and benefits of this approach. Although the benefits of using steroid free protocols in pediatric patients shows great promise, further study is needed to determine the impact on long term graft function.

Calcineurin Inhibitors

Cyclosporine

Cyclosporine A (Sandimmune®) changed the face of transplantation by providing a more potent alternative to azathioprine and corticosteroids, significantly improving 1 year graft survival after its introduction in 1978 (64). It is a cyclic peptide of fungal origin that inhibits T cell response by inhibiting calcineurin. It binds to cellular proteins called cyclophilins and this complex inhibits the movement of transcription factors into the nucleus, blocking IL-2 production. This cascade of events ultimately results in inhibition of T cell proliferation and differentiation (56). For induction cyclosporine can be started intraoperatively as a continuous 24 h infusion in a dose of 165 mg/m^2 daily for children under 6 years of age, and 4.5 mg/kg daily in children over 6 years. Conversion to oral CSA should be done by 48 h. The recommended starting oral dose of CSA is 500 mg/m^2/day divided every 8 h in children aged 6 years and under and 12–15 mg/kg/day divided every 12 h in children older than 6 years for patients on combination therapy with steroids and/or purine synthesis inhibitors. CSA doses are higher than those prescribed in adults because the drug appears to have a more rapid metabolism in children (65). The maintenance dosage decreases over time with average CSA doses at 1 year varying from 4.36 to 8.4 mg/kg in the NAPRTCS database (66) with higher maintenance doses being associated with lower chronic graft rejection (65). A calcium channel blocker is typically given with cyclosporine to reduce nephrotoxicity (67).

The major side effects of CSA are nephrotoxicity, hepatotoxicity, hypertension, susceptibility to infection, neurotoxicity including tremor and convulsions, hyperlipidemia and increased risk of malignancy. The impact of CSA on renal tubular handling of electrolytes can lead to hyperkalemia (68), hyperuricemia (69) and hypomagnesemia (70). A major concern, especially in children, is the hirsutism, facial dysmorphism (71) and gingival hyperplasia (72) which may have an impact on adherence especially in the image conscious adolescent. The use of the microemulsion form of CSA, (Neoral®) has essentially replaced Sanimmune. Studies comparing Sandimmune and Neoral® have found the new formulation to result in both improved and more consistent CSA absorption, with fewer dose adjustments being required (73). Generic forms of CSA are available. Comparison studies with Neoral® demonstrate equivalent bioavailabilities with similar safety and tolerability profiles in adults (74, 75).

The importance of following CSA levels closely cannot be over-emphasized. There are several techniques available to measure CSA levels but high pressure liquid chromatography (HPLC) and fluorescence polarization immunoassay (FPIA). Appropriate drug levels range from 100 to 200 ng/ml HPLC or 200 to 450 ng/ml TDX whole blood trough level after 3 months with higher levels in the immediate post-operative period (76). Matas et al studied the impact of early CSA levels on the incidence of rejection and found that CSA levels less than 100 ng/ml in the first 6 months were associated with a significantly higher incidence of rejection (77).

Unfortunately, there is considerable intra-patient and inter-patient variability in both the area under the curve measurements and the peak and trough blood concentrations for CSA. AUC_{0-4} monitoring is a very accurate way of measuring the total body exposure to CSA, however, there limitations include numerous blood draws and mathematical calculations (78). The single point that had the best correlation to AUC_{0-4} is the CSA level measured 2 h after administration (C_2) ($r^2 = 0.85$), compared with C_3 ($r^2 = 0.70$) or C_0 ($r^2 = 0.12$) (79). To date, abbreviated AUC calculations remain controversial but many transplant centers advocate following C_2 when monitoring cyclosporine therapy in children (80–82). A C_2 target of 1700 ng/ml by HPLC appears to be the

appropriate levels immediately post-transplant to minimize rejection and side effects (83).

Calcineurin inhibitors require close monitoring not only because of the narrow therapeutic window but also due to numerous drug and food interactions (84–86). The majority of these interactions deal with chemicals that inhibit or induce the cytochrome P450 system 3A4 (CYP3A4). Agents such as rifampin, nafcillin, phenobarbital, phenytoin, and carbamazepine are examples of CYP3A4 inducers causing decreased CSA and tacrolimus levels. In contrast, macrolides, azole-antifungals, and some calcium channel blockers, including diltiazem and verapamil, are examples of CYP3A4 inhibitors causing increased levels. The one drug-food interaction that is critical to inform patients about is that grapefruit and grapefruit juice cannot be given with CSA. Not only does grapefruit contain substrates that inhibit CPY3A4 causing increased levels but it is also a P-glycoprotein inducer, a membrane transporter that also affects intestinal absorption and tissue distribution of CSA, tacrolimus, and possibly sirolimus (87).

The most recent data from the NAPRTCS registry shows that less that 15% of renal transplant recipients are currently receiving cyclosporine as initial immunosuppression (34). Cyclosporine has been used in combination with all other immunosuppressants except tacrolimus. However, because of the potential of increased risk of PTLD, the use of the combination of a calcineurin inhibitor, rapamycin and corticosteroids should probably be avoided, especially in high risk children (88).

Tacrolimus

Tacrolimus (Prograf®, FK506) a calcineurin inhibitor derived from the fungus *Streptomyces tsukubaenis* was introduced as an immunosuppressant for kidney transplantation in the mid 1990s (89–91). Although it is a macrolide like cyclosporine, it differs in its chemical structure and cytosolic binding site. Tacrolimus interacts with the FK binding protein and inhibits T-cell derived lymphokines including interleukin-2, -3, and -4, gamma interferon, as well as inhibiting the clonal expansion of helper and cytotoxic T cells (56). There is no established dosage regimen for tacrolimus in the pediatric renal transplant population. The trend has been away from intravenous use in the early period and rather starting with oral or naso-gastric tacrolimus at 0.1–0.15 mg/kg/dose (92). Because of the similar mechanism of action, virtually all of the side effects of CSA therapy are also seen with tacrolimus (90), except for the hypertrichosis and dysmorphic features (66). Neurological side effects are common and may be seen more frequently than with cyclosporine (93, 94). A concern for the use of tacrolimus in pediatric renal transplantation was the development of post transplant diabetes mellitus (PTDM) possibly related to a diminished insulin secretion in association with the insulin resistance related to steroid use (95). The incidence PTLD was much higher with the use of tacrolimus than with other immunosuppresants during early experience (91, 96). However, a more recent retrospective analysis showed that the use of tacrolimus was not a risk factor for development of PTLD, likely due to the lower doses more recently utilized (97).

Blood monitoring is necessary as with CSA and recommended target whole blood trough levels, measured by an enzyme linked immunosorbent assay (ELISA), range between 5 and 20 ng/L. Blood levels can also be measured by HPLC and the resulting levels are about 10% lower than with the more commonly used ELISA methods. The goal tacrolimus level is center dependent and often depends on whether it is used in conjunction with other immunosuppressive agents. Because of concern with dose-related complications, especially the incidence of PTLD, tacrolimus levels are more commonly maintained at the lower level of recommendations, with some recipients even lower after the first post-transplant year. Diarrhea, which is common particularly in infants, may lead to increased tacrolimus levels (98). In addition, there are genetic differences in metabolism, illustrated by the need for black patients to have higher doses than in whites partially due to the fact that the CYP3A*51 allele is expressed in 90% of black patients but only 5% of white patients (99). Other factors that can effect the bioavailability and clearance includes co-administration with food, medications (such as corticosteroids and antibiotics), hypoalbuminemia, anemia, hepatic dysfunction and length of time post-transplant (100). The role of using AUC monitoring is not as well established as it is for patients receiving cyclosporine. At least one report in pediatric renal transplant recipients showed that trough levels correlated well with calculating a patient's AUC from two levels collected at 2 and 4 h ($r = 0.85$) (101). However, 85% of the patients had at least once occasion where discrepancy between the AUC and the trough lead to incorrect dosing adjustments and of all of the dosing adjustment decisions, 33% were incorrect.

Recent data from the NAPRTCS shows that more than 65% of children are being maintained on tacrolimus at 31 days post-transplantation (34). Tacrolimus has been used in combination with all other immunosuppressants except cyclosporine. However, as noted about with

cyclosporine, the combination of a calcineurin inhibitor, rapamycin and corticosteroids should be used with caution in children at high risk for developing PTLD (88). There have been several centers reporting the use of tacrolimus as a rescue agent in the setting of both acute and chronic rejection (102–104).

Choice of Calcineurin Inhibitor

Calcineurin inhibitors have been mainstays of immunosuppression for pediatric transplantation for the past decade and likely account for the continuing improvement in graft survival rates (76, 105, 106). The choice between the two calcineurin inhibitors has often been based on a center preference and on availability in different countries.

In a retrospective analysis of NAPRTCS data of the two drugs given with MMF and steroids, there was no difference in early rejection rate (29%), risk of rejection, or risk of graft loss (66). At 2 years, graft survival was not different (tacrolimus 91%, cyclosporine 95%). Tacrolimus-treated patients were less likely to require anti-hypertensives and had higher GFR at 2 years. An open-label randomized trial of the two drugs with steroids and azathioprine in pediatric renal transplant recipients was recently completed (107). Tacrolimus-treated patients had a lower rate of acute rejection (37%) than cyclosporine-treated patients (59%), although both rates in that study were higher than current standards and not all episodes were biopsy-proven (16). One-year graft survival rates were similar although the GFR was higher in the tacrolimus group. Hypomagnesemia, diarrhea, and PTDM were higher in the tacrolimus group and hypertrichosis and gingival hyperplasia were higher in the cyclosporine group. In a randomized trial comparing tacrolimus to cyclosporine in 196 pediatric patients, the tacrolimus group had a significantly lower incidence of acute rejection and corticosteroid-resistant rejection than the cyclosporine group (108). First year data did not show a difference in patient or graft survival but the tacrolimus group did show a significantly higher GFR that was sustained through the 4 years of follow-up. Further data demonstrated that at the 4 year follow-up, patient survival was similar but graft survival was significantly in favor of tacrolimus ($p = 0.025$). There was no difference in the incidence of PTLD between the two groups. Early concern about the higher risk of PTLD associated with tacrolimus use (91, 97) seems no longer to be true, probably related to lower levels of tacrolimus used in current practice (109).

As noted above, the majority of pediatric kidney, liver and intestine transplant recipients are currently receiving tacrolimus because of its efficacy and lack of cosmetic side-effects, which is particularly important for adolescent transplant recipients. One unfortunate consequence of chronic calcineurin inhibitor use is nephrotoxicity and chronic renal insufficiency (CRI). One recent review demonstrated an incidence of CRI of 11.8% and an incidence of ESRD of 4.3% 10 years after pediatric heart transplantation (110).

Antiproliferative Agents

Azathioprine

Azathioprine (AZA) was the first immunosuppressive agent approved for organ transplantation use. For several decades, virtually all organ transplant recipients received azathioprine, but other agents have supplanted its use during the past decade (2). Azathioprine (Imuran®), a pro-drug of the chemotherapeutic agent 6-mercaptopurine, acts by directly inhibiting the growth and differentiation of immune cells. After metabolism in the liver, derivatives of the pro-drug inhibit purine synthesis, preventing gene replication, and cell division. In addition to blocking cell-mediated immunity, it inhibits primary antibody synthesis and decreases circulating monocytes and granulocytes (56). The usual dose is 1–2 mg/kg/day with close follow-up, reducing the dose in the setting of myelosuppression. The major side effect of AZA is myelosuppression with leukopenia, thrombocytopenia, and megaloblastic anemia. Other side effects include susceptibility to viral infection, hepatotoxicity, pancreatitis, alopecia, and neoplasia, most notably skin cancer (111). Azathioprine has been used in combination with all other immunosuppressants except MMF.

In 1989 and 1990, 80% of pediatric patients in the NAPRTCS registry were receiving azathioprine, but as more familiarity is established with MMF the use of azathioprine has diminished substantially and is currently in less that 5% of pediatric kidney transplant patients in the United States (34).

Mycophenolic Acid

Mycophenolate mofetil (Cell-Cept®) has recently gained acceptance as a first line agent as a replacement for AZA as part of the standard triple therapy. It is an anti-metabolite agent that interrupts purine metabolism in B and T lymphocytes. In vivo, it is rapidly metabolized to mycophenolic acid which blocks conversion of inosine IMP to

guanosine IMP in the purine biosynthetic pathway. The net result is a decrease in the number of functional B and T lymphocytes and inhibition of the response of human lymphocytes to antigen challenge (56). The current recommended dose for pediatric patients is 1200 mg/m^2/day divided twice a day, adjusting the dose in the setting of myelosuppression. It is currently available in intravenous, capsule, and liquid form. MMF is relatively well tolerated with hematologic and gastric side effects being the main concerns. Practitioners should follow blood counts looking for neutropenia as well as thrombocytopenia. If hematological problems are noted, decreasing the dose is recommended until values normalize. Granulocyte colony stimulating factor (GCSF) may be required to deal with neutropenia. Current data concludes that reducing and more importantly, holding doses of MMF will lead to impaired graft survival (112). A major difficulty in widespread use of the drug in children has been the gastrointestinal disturbance especially in young children (113). Both nausea and vomiting are common, but in some patients the drug has to be withdrawn due to intolerable diarrhea. If the patient has difficulty tolerating MMF, the dose can be split into 3–4 smaller doses throughout the day. Switching the patient to the liquid form can also help with gastric complaints. Frank esophagitis and gastritis with occasional gastrointestinal hemorrhage occur in approximately 5% of patients (114). The goal of the enteric coated mycophenolic acid (EC-MPA) (Myfortic®) is to decrease gastric side effects while at the same time decreasing the amount of time needed to reach mycophenolate therapeutic exposure. In a 12-month study of 423 de novo renal transplant patients comparing MMF to enteric coated mycophenolate, both groups had similar side effect profiles (115). When looking specifically at gastro-intestinal intolerance, 9.4% of patients in the enteric coated group had dose reductions or temporary interruptions while the MMF group had 13.8% of patient reduce or stop the medication; but the difference between the two groups was not significant. A small cross over study was performed in 10 pediatric renal transplant patients to evaluate the impact of converting stable patients from MMF to enteric coated MPA (112). A gastrointestinal symptom rating scale was used looking at 5 criteria; reflux, abdominal pain, constipation, indigestion, and diarrhea. Scores dropped significantly 4 weeks after switching and 9 out of 10 patients reported to still have positive effects from the switch 6 months after the conversion. In a smaller case report, 8 pediatric patients, all patients were switched to the EC-MPA with all of the patients reporting no gastrointestinal disorders. They also noted an increase in MPA levels from a mean level of 0.8 ± 0.3 μg/ml to a mean of 3.2 ± 1.7 μg/ml (target range of 2–4 μg/ml) (116). One limitation of EC-MPA is that it cannot be crushed or made into a suspension forcing practitioners to only use this in patients who can swallow pills whole.

MMF does not have any nephrotoxicity but should not be used in combination with azathioprine for fear of increased risked of neutropenia and infection. MMF is not metabolized by any of the cytochrome P-450 isoenzymes. Some evidence indicates that patients who receive MMF in combination with tacrolimus have higher drug levels of MMF (117). As such, patients on tacrolimus are usually on lower doses of MMF at 600 mg/m^2/day.

In the adult transplant population, MMF has been shown to decrease the incidence of acute rejection episodes by as much as 50% (118, 119). A study in pediatric renal transplant recipients comparing those who received AZA versus MMF along with the standard CSA and prednisone therapy found no significant differences in the incidence of acute rejection episodes, patient, or graft survival (120). The Pediatric MMF study group has reported that MMF in combination with CSA and prednisone provides effective immunosuppression for up to 3 years with excellent graft survival rates of 98% with a low rate of acute rejection (121). Another difference between MMF and AZA is cost; MMF is six to seven times more expensive than AZA. Seikaly argues that a decrease in the number of acute rejection episodes and a potential reduction of CSA dose with subsequent reduction in CSA toxicity under the more potent MMF therapy would help balance its cost (122). Further study of MMF is warranted to assess long-term outcome with respect to incidence of PTLD and other viral infections.

There has been considerable debate regarding the utility of measuring mycophenolic acid levels (MPA). Evidence to support the use of MPA monitoring comes from the association of systemic MPA concentrations with both efficacy in preventing rejection (123–126) and adverse reactions (123, 125, 127). A multicenter study conducted in France showed significantly fewer treatment failures and acute rejection episodes in the monitoring arm with no significant difference in side effects (128). Within this study, the MPA exposure and MMF dosing were higher in the monitoring arm based on three levels measured over the first 3 h post-dose. However, results from the FDCC (Fixed Dose vs. Concentration Controlled) study being performed within 69 international centers have shown no benefit in the monitoring group, but there was no increase in dose (129). The OPTCEPT trial being performed in 65 centers in the United States also did not show any benefit within the monitoring group compared

to the fixed dose group (130). However, there was no difference in trough levels between groups As with CSA, AUC monitoring of MPA levels appears more sensitive than trough levels. However, finding a limited sampling strategy has been challenging due to the second peak level caused by intrahepatic circulation of MPA (131).

Sirolimus

Sirolimus (Rapamune®, rapamycin) is an immunosuppressive agent approved by the FDA for use in solid organ transplantation in 2000. It is a natural fermentation product of *Streptomyces hygroscopicus*, discovered on Easter Islant (Rapa Nui) in 1969. It was first investigated for anti-fungal properties and its immunosuppressant properties were first discovered in 1988. Sirolimus is classified as a TOR inhibitor. TOR is a cytosolic enzyme that regulates differentiation and proliferation of lymphocytes. TOR is activated as a result of the cascade of reactions in lymphocytes by the proliferation of cytokines. It initiates production of messenger RNA that trigger cell-cycle progression from G_1 to S phase. The TOR inhibitors bind to the immunophilin FKBP12 and inhibit the actions of TOR (132–136). The TOR inhibitors may be particularly important in long term immunosuppression because they stimulate T-cell apoptosis. They also inhibit mesenchymal proliferation, which may prove to be important in graft vascular disease (137, 138). Also, since the mechanism of action of rapamycin is different from other currently available immunosuppressive agents, it can be used in combination with all of them. A similar compound that may be an analogue, SDZ-RAD is currently undergoing clinical trials but the pediatric component of that study was halted due to concern about potential complications in immature subjects. Sirolimus is available as an oral preparation, either as a solid or liquid. Sirolimus was shown to have a prolonged half-life in adults that allowed a single daily dose in adults (139–141). However, pharmacokinetic studies in children have demonstrated a much shorter half life, as short as 12 h (142, 143). Thus, children may require twice-per-day schedules in order to maintain therapeutic levels. Retrospective analysis of early trials of sirolimus have suggested a relationship between blood levels and risk of rejection (144). Current suggestions for therapeutic levels remain speculative and range between 25 ng/ml in the early post-transplant period without calcineurin inhibitors (142, 145) and 5–10 ng/ml later in the course of transplantation.

Sirolimus has its own unique side effect profile which can limit its use including poor wound healing, gastrointestinal and oral ulcerations, hyperlipidemia, skin rash, myelosuppression, thrombocytopenia, anemia, and its association with proteinuria (140, 146–148). Studies have shown intolerance leading to dropout rates as high as 42–46% (149, 150). Some side effects can be minimized with dose reduction and delayed wound healing may require suspension of the drug until the wound heals completely. Sirolimus associated proteinuria requires that the drug be discontinued entirely. A side effect that is gaining more attention is the occurrence of interstitial pneumonitis in patients receiving sirolimus. Reports have linked that this correlation appears to be dose related and resolves with withdrawal of sirolimus within 14–28 days (151, 152). One center reported good outcomes in switching some patients to low-dose sirolimus with only one out of six patients relapsing after 5 months (152). Although lower dosing of sirolimus seems to be an option, discontinuation of sirolimus continues to be the safest treatment option. Another complication is the association of sirolimus therapy with prolonged delayed graft function (DGF) in comparison to patients who did not receive sirolimus (153). Within the experimental model, sirolimus has been shown to prolong acute renal failure in animals that had acute ischemic tubular injury, leading to the potential delay in recovering from acute tubular necrosis (ATN) (154). In the setting of acute tubular damage, a balance between proximal tubular cell death and the ability of sublethally injured tubular cells to enter the cell cycle and proliferate occurs (155, 156). Sirolimus is shown to inhibit growth factor induced proliferation of proximal tubular cells and to promote apoptosis by interfering with the survival effects of the same growth factors (154). Therefore, the delay of recovery of DGF from ATN may result from a direct effect of sirolimus by inhibiting normal cell growth (157). Sirolimus is associated with impaired spermatogenesis and may be associated with reduced male fertility (158).

Sirolimus has been found to be effective in combination with calcineurin inhibitors (139–141, 159, 160), in a calcineurin-inhibitor sparing protocol (145), and in a steroid free protocol (161). In renal transplantation, calcineurin inhibitor nephrotoxicity is a key risk factor for reduced long-term allograph survival (162). In no population group is this more important than in children due to the potentially lifetime consequences of immunosuppression. In phase I and II trials in adults, sirolimus was well tolerated and exhibited no apparent nephrotoxic properties or deleterious effects on blood pressure. Because sirolimus has little to no nephrotoxic effects, its use is an attractive alternative to the calcineurin inhibitors.

When used in combination, there appears to be a synergistic effect of the sirolimus-CSA combination allowing for a decreased CSA dose with subsequent reduction in CSA side effects (110). Data directly comparing sirolimus *versus* CSA used in combination with steroids and AZA show no significant difference in the incidence of acute rejection, graft survival, or patient survival at 1 year post-transplant (147). However, an open label phase II study in adult recipients of primary renal allografts receiving CSA and corticosterioids demonstrated that all treatment groups to which sirolimus was added had a lower rate of acute rejection (7–11%) when compared to placebo (32%), during the first 6 months following transplantation (163). In a cohort of 66 pediatric renal transplant patients where sirolimus was added to calcineurin inhibitors plus corticosteroids, acute rejection occurred in 10.6% of patients and graft survival was 98% at 6 months (164). In an attempt to reverse the nephrotoxic effect of calcineurin inhibitors, a retrospective analysis of 17 pediatric patients who underwent calcineurin inhibitor withdrawal and converted to sirolimus and MMF showed a decrease in creatinine clearance at 6 months and 1 year after conversion. Using sirolimus as rescue therapy for chronic graft injury needs to be applied with caution in pediatric patients, especially patients who have advanced graft dysfunction. There seems to be a point where graft injury is severe enough that withdrawing calcineurin inhibitors results in no significant clinical improvement (148). In the adult literature, Wu et al showed no benefit for patients with serum creatinine greater than 4 mg/dL (165) supporting the idea that rescue of patients early in the process of chronic graft injury is likely more beneficial than later. Current research is being undertaken to determine when is the optimal time of switching patients from calcineurin inihibitors to help improve and preserve renal function. One such study was done on 30 pediatric renal transplant patients who were converted from tacrolimus to sirolimus at 3 months post transplant (166). Incidence of acute rejection after conversion was low at 10% but as a group, the average renal function did not improve during follow-up.

The drug interaction with CSA and sirolimus is greatly related to administering both drugs at the same time. The drug interaction may be due to competitive interactions of both drugs as substrates for P-glycoprotein and for the cytochrome P450 isoenzymes (167). This can result in sirolimus trough levels that are 67–85% higher than when given alone (168). The initial recommendations were that sirolimus should not be given within 4 h of CSA. Some institutions give sirolimus at the same time as CSA knowing of the increased levels but start with a lower dose and follow levels. Unlike the CSA/sirolimus combination, sirolimus and tacrolimus pharmacokinetic profiles and adverse effects were not altered with simultaneous administration. Because of this, sirolimus and tacrolimus can be administered at the same time (169). Pharmacokinetics of sirolimus differs substantially in children and adults. In general, the half-life is much shorter in children. The half-life of sirolimus ranges from 4.7 to 6.1 h with tacrolimus and 8.2–11.9 h in children who received cyclosporine. This requires pediatric patients to be dosed twice a day compared to once a day in adults (170). One of the largest pediatric studies using basiliximab showed the combination of a calcineurin inhibitor, sirolimus and steroids did decrease the rejection rate over historical data but also did increase the rate of PTLD (171). Thus, it is highly likely that the combination resulted in over-immunosuppresion in high-risk pediatric patients and should not be used.

Immunosuppression Combinations

Most pediatric renal transplant recipients are currently treated with triple immunosuppression. When the number of drugs was limited, the number of possible combinations was small. However, there are at least 20 possible combinations of the 6 currently available drugs and when the induction antibodies are added, there are over 60 possible reported protocols (172). No "best" protocol for children has been established, although most clinical trials are currently directed at eliminating either steroids or calcineurin inhibitors, or both.

There are many possible targets for immunosuppression strategies for children (173). One promising new protocol of steroid avoidance has been recently reported (29). This approach consists of 6 months of anti-IL2r antibody, tacrolimus, and MMF. Short-term patient and graft survival rates have been excellent and growth rates have been very good. Major complications have included bone-marrow suppression and nephrotoxicity. A randomized, controlled trial of steroid withdrawal was successful in preventing acute and delayed rejections (62) but the initial immunosuppression of IL-2r antibody induction, followed by initial immunosuppression of corticosteroids, sirolimus and a calcineurin inhibitor was too robust, leading to an unacceptably high rate of PTLD in young children (174). Other protocols currently under investigation include calcineurin inhibitor avoidance or withdrawal (175) or co-stimulation blockade (55) with the eventual goal of avoiding both corticosteroids and calcineurin inhibitors (176). One other approach is to

use robust induction therapy with Campath, followed by eventual monotherapy with tacrolimus (177, 178). Clearly, there is no single defined approach to immunosuppression for children, but the eventual goal is to permit long-term graft acceptance with the fewest possible chronic medications.

Treatment of Rejection

There have been no controlled trials of treatment of acute organ transplant rejection in children. In general, the initial treatment of a rejection episode is with pulse intravenous corticosteroids, typically lasting 3 consecutive days with a dose of 10–30 mg/kg for each dose. This regimen may be followed by a slow taper of oral prednisone, but some centers do not routinely use the oral taper. High dose oral prednisone for rejection has been reported in children with equivalent results to intravenous therapy in terms of the rate of rejection reversal (179). Severe, recurrent or steroid resistant rejection episodes are most often treated with lymphocyte-depleting antibodies, including OKT3, Atgam or Thymoglobulin, although Campath is also being studied for this indication (180, 181). To date, there are no studies of IL-2 receptor antagonists for use in rejection (182).

Antibody-mediated rejection may be treated with plasmapheresis, infusion of IVIG, or Rituximab in addition to these other treatments, but the efficacy of these approaches is unknown (183–186). Another novel and unproven approach is the use of photopheresis (187, 188).

Re-evaluation of maintenance immunosuppression is indicated following a rejection episode. One may consider switching to an alternative calcineurin inhibitor and/or from MMF/azathioprine to sirolimus to take advantage of the synergism between sirolimus and the calcineurin inhibitors. In addition, assessment of the patient's adherence to the medication regimen is always important.

Conclusion

Advances in immunosuppressive therapy over the past decade have led to dramatic improvements in graft survival. Children currently have excellent outcomes however, long term success has been limited by the complications of lifetime immunosuppression. The current medications used to prevent graft loss are toxic and have significant complications. With the development of new agents, the focus of the transplant community is to establish regimens which maintain excellent graft survival rates but with fewer toxicities including infection, nephrotoxicity, malignancy, and cosmetic effects. In addition, the need to continue to use these medications on a continuing daily basis requires adherence that few recipients, especially adolescents, can accomplish. The future of immunosuppression for children depends on our ability to develop non-toxic long-term medications that are safe, effective and simple to administer. However, the ultimate goal of transplant immunosuppressive therapy is the induction of tolerance. As we learn more about immune function from basic and clinical research, tolerance to allografts seems an ever more reachable goal.

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77 Complications of Renal Transplantation

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Introduction

The two preceding chapters have provided a description of the process of pediatric kidney transplantation. There has been tremendous successes achieved in this field over the past few decades. Patient and graft survival have improved markedly with each successive decade from the 1960s to 1990s (1). Early acute rejection rates continue to fall (2, 3).

Yet this most recent decade has seen a leveling-off in terms of improvements in graft and patient survival (3, 4). While acute rejection rates have fallen, the community has seen the emergence of newer infection complications such as post-transplant lymphoproliferative disease (PTLD) and BK virus allograft nephropathy (BKVAN) (5–7). The infection-hospitalization cumulative incidence is much higher at 47% in children in the first 3 years post-kidney transplant than while on dialysis or in comparison to adults post-kidney transplant (8). The total cumulative infection incidence in children did not drop in recent years (9), as it has in adults. With longer patient survival, systemic complications of transplantation such as accelerated cardiovascular disease have become more prominent (10, 11). As the medical community has developed more potent immunosuppression drugs and regimens, transplant recipients have paid a price in terms of more infectious and non-infectious complications (*Table 77-1*), especially long-term complications. In this chapter we discuss the various intermediate and long-term complications associated with pediatric kidney transplantation. Early complications such as delayed graft function and vascular thrombosis as well as chronic allograft nephropathy are discussed in Chapter 83.

Special Considerations in Pediatric Transplantation

In general, organ transplant recipients of any age are at greater risk for infections due to the non-specific nature of the currently used immunosuppressive agents (5). Organ transplant recipients are also at greater risk of disease related and medication-induced systemic complications, due to the severe nature of the underlying disease (kidney failure) and the need for multiple long-term medications. In addition, *pediatric* recipients of organ transplants have some unique and additional features that add to their risk for complications. By virtue of their younger age, these recipients are exposed to disease complications and medication toxicities for a longer period of time. The risk for some infections, especially viral infections, is higher if the child had not been exposed to that organism while still immunocompetent. Thus, a primary infection in a host who is immunosuppressed tends to be more severe. The kidney allograft often acts as the source for the primary infection, since most children receive an adult donor kidney, which harbors latent viruses. Children may not have had the opportunity to receive all vaccinations prior to transplant, thereby preventing development of immunity to certain organisms. Finally, certain complications, such as growth failure, are unique to children.

Bacterial Infections

Urinary Tract Infection

Urinary tract infection (UTI) is the most common bacterial infection in kidney transplant recipients, both in adults (12, 13) and children >5 years (14). The percent of renal transplant recipients who will experience a UTI ranges from 20–65% in the first year post-transplant (12, 15) and 40–80% by 3 years post-transplant (16–20). UTI is not only a cause for morbidity but is also associated with higher rates of graft loss and patient death (16, 21). In a recent analysis of United States Renal Data System (USRDS) data, early UTI (within 6 months of transplant) elevated the risk for graft loss in children, while late UTI did not (22). The urogenital tract is the most common entry point for the development of sepsis (23). Risk factors for UTI in children include bladder

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Table 77-1
Typical non-infectious complications

Type	Complication
Cardiovascular	Hypertension
	Left ventricular hypertrophy
	Coronary artery disease
	Renal artery stenosis
Metabolic	Dyslipidemia
	Obesity
	Diabetes mellitus
Musculoskeletal	Growth failure
	Osteopenia
Gastrointestinal	Esophagitis
	Gastritis
	Vomiting/diarrhea
Hematological	Leucopenia
	Neutropenia
	Anemia
Neurological	Tremors
	Headache/stroke
	Peripheral neuropathy
Miscellaneous	Hair/skin: alopecia, hirsuitisim, skin cancers
	Hyperuricemia

abnormalities, the need for chronic or prolonged bladder catheterizations and placement of a ureteral stent (17, 24). In adults, diabetes mellitus and cardiovascular disease are comorbid factors that also associate with a higher incidence (16). UTI risk is highest in the first few months post-transplant but some risk remains at later time points. The organisms implicated are usually the same as in immunocompetent individuals, such as *E. Coli* and *Klebsiella*. A higher percentage of UTIs in transplant recipients are due to unusual organisms such as *Pseudomonas* (25). Clinical symptoms include fever, dysuria, graft tenderness and cloudy urine. In some patients, due to immunosuppression, symptoms may be masked. A rise in serum creatinine often occurs and can mimic acute rejection. UTIs can also precipitate an acute rejection. The diagnosis of UTI is usually confirmed by urine culture, though patients on trimethoprim-sulfa prophylaxis for pneumocystis may not demonstrate positive cultures. In that case, radionuclide scanning or clinical judgment may be necessary. Treatment is with anti-microbial agents. Initially, the anti-microbial prescribed should cover the common Gram negative organisms. Once the organism is known, the most specific and cost-effective anti-microbial can be prescribed. Treatment route and total duration vary somewhat by severity of infection, recipient age and other risk factors present. Shorter oral courses such as used in immunocompetent people can be used for milder cystitis episodes in older children. In more severe acute pyelonephritis in a younger child who was recently transplanted, intravenous antimicrobial therapy for 10–14 days is preferred. Not all centers prescribe routine prophylaxis for UTI. Trimethoprim-sulfa, if given daily, may serve as both UTI and pneumocystis prophylaxis.

Other bacterial infections, such as wound infections, line sepsis and pneumonia are seen with significant frequency in kidney transplant recipients. Wound infections and line sepsis are commonly due to Gram positive staphylococcus and streptococcus. Pneumonia can be due to multiple etiologies (bacterial, viral or fungal); bacterial pneumonia comprises approximately 44% (26). In adult transplant recipients, cellulitis and bacterial abscesses are frequent problems, largely due to co-morbid diabetes mellitus. In general, children are less likely to develop cellulitis and abscesses and are more likely to develop viral infections than adults. The detection and treatment of these infections generally is not different from standard investigation and treatment in immunocompetent hosts, though duration of therapy may be longer.

Bartonella henselae infection (also known as cat-scratch disease) has been reported in pediatric organ transplant recipients, including kidney transplants (27). This infection typically presents as fever and lymphadenopathy, thus becoming a differential diagnosis for PTLD. However, unlike PTLD, this infection is curable with anti-microbial therapy.

In third world countries, *Mycobacterium tuberculosis* infection occurs more commonly than in the West. The incidence is <2% in Western countries but ranges from 5 to 15% in Asian and African countries (28, 29). Use of cyclosporine, co-existing diabetes mellitus, and chronic liver disease increase the risk for post-transplant tuberculosis (30). This infection presents with myriad symptoms, including weight loss, cough, fever and lymphadenopathy, and thus should be part of the differential diagnosis for PTLD. However, pulmonary involvement is more common than lympadenopathy in the post-transplant setting. Early diagnosis is best achieved by staining for acid-fast bacilli or PCR DNA amplification from sputum, bronchoalveolar lavage or gastric aspirate. Four drug therapy with isoniazid, pyrazinamide, ethambutol and either streptomycin or ofloxacin is preferred because of drug resistant strains (29). Rifampicin can also be used but

the dosages and frequency of tacrolimus/cyclosporine/steroids have to be markedly increased to account for enhanced liver metabolism of those drugs.

Cytomegalovirus Infections

Cytomegalovirus (CMV) is a DNA virus of the herpes virus family. When considering all organ transplants as a group, this virus may be the single most important pathogen. In pediatric kidney transplantation, the incidence of CMV infection has been highly variable, ranging from 6 to 40% (31–34). The variation is partly due to differing definitions (e.g., viremia versus disease) and partly due to different detection methods (33, 35). A very recent retrospective study using pp65 antigenemia reported a viremia rate of 21% and disease rate (viremia plus organ involvement) of 9.7% (33). CMV is especially important because of its dual effects: it causes significant morbidity by direct infection, yet its immunomodulatory effects can predispose to other infectious complications (*Table 77-2*). CMV infection post-transplant can be as a primary infection, a reactivation infection or a superinfection. Of these, primary infection in the immunocompromised host is the most likely to lead to disease and often occurs when a seronegative pediatric recipient receives a kidney transplant from a seropositive donor (33, 34). Both CMV viremia and disease usually occur within the first few months post-transplant, though routine anti-viral prophylaxis has led to later appearance, with more CMV cases appearing after prophylaxis ends (35). Viremia may be silent or can manifest as CMV syndrome, characterized by fever, myalgias, malaise, leukopenia and thrombocytopenia, or CMV disease, in which there is clinical evidence of active infection. The transplanted kidney is at higher risk for CMV infection than are the native organs, but pulmonary, liver and gastrointestinal tract involvement are common, regardless of the organ transplanted.

In addition to causing direct infection, CMV has significant indirect effects including an increase in the overall state of immunosuppression leading to a greater risk for opportunistic infections. CMV infection also increases the risk of EBV-associated post-transplant lymphoproliferative disorder (36). In addition, CMV and acute rejection are interrelated. The study by Kranz et al. showed that antecedent CMV infection is a risk factor for acute rejection, while rejection is known to release tumor necrosis factor, triggering the process that ultimately leads to CMV replication (33).

Prevention of CMV infection can be accomplished with either (1) universal prophylaxis: the administration of anti-CMV therapy to all patients except seronegative recipients of an organ from a seronegative donor; or (2) preemptive treatment: the administration of anti-CMV therapy to patients at first sign of CMV infection. There is some controversy as to the optimal strategy, as both methods have advantages and disadvantages. In one recent study of pediatric liver transplant recipients, pre-emptive therapy was superior (37). Most guidelines recommend universal prophylaxis for high risk patients (e.g., seronegative recipients of an organ from a seropositive donor), with preemptive therapy reserved for patients at low or intermediate risk. Although several agents are available for prophylaxis, ganciclovir or valganciclovir have largely supplanted either oral acyclovir or CMV immune globulin (CMVIG) as the recommended treatment for prevention of CMV infection. Many protocols recommend intravenous ganciclovir, followed by oral ganciclovir. Oral ganciclovir has poor bioavailability and must be taken in large doses three times daily. Valganciclovir has superior bioavailability to oral ganciclovir, with a pharmacokinetic profile similar to intravenous ganciclovir. The standard duration of prophylactic therapy is 100 days, although many centers have extended prophylactic therapy to the first 6–12 months post-transplant given data suggesting significant reduction in late CMV-infection with this strategy.

Table 77-2

Typical infectious complications, by organism type

Type	Complication
Bacterial	Urinary tract infections: usually Gram negative rods
	Wound infections: usually Staphylococcus/Streptococcus
	Central line infections
	Pneumonia
	Mycobacterium tuberculosis
	Bartonella henselae
Viral infections	Cytomegalovirus
	Herpes simplex virus
	Epstein Barr virus
	Varicella virus
	BK virus
Other	Pneumocystis jurevicii
	Candida
	Other fungi
	Parasitic

For preemptive therapy, intravenous ganciclovir is generally recommended, but recent studies have demonstrated good results with valganciclovir. In order for preemptive therapy to be successful frequent monitoring with reliable diagnostic assays must be performed. Detection of CMV DNA or RNA by polymerase chain reaction (PCR) and the pp65 antigenemia assay are both rapid and have reasonable predictive ability for use in preemptive therapy. Serum and whole blood DNA correlate well in case of CMV (38).

The widespread use of preventative therapy has greatly reduced the incidence of CMV disease, especially in the early post-transplant period. When CMV disease does occur, treatment with intravenous ganciclovir is the treatment of choice. Some guidelines suggest that valganciclovir, at high doses, may also be as used. The optimal duration of treatment has not yet been defined, and current protocols recommend continuing treatment till viral load becomes undetectable and perhaps for a short time thereafter.

Epstein-Barr Virus Infections and PTLD

Epstein-Barr virus (EBV) is another DNA herpes virus that causes significant morbidity post-transplantation. Like its cousin CMV, this virus commonly infects immunocompetent people sometime in childhood and establishes a prolonged latency in reticulendothelial cells. Thus, the patterns of infection are identical to CMV: primary infection (often from the graft of a seropositive donor), reactivation or superinfection. Again, like CMV, the primary infection in an immunosuppressed transplant recipient is more virulent. Unlike CMV, EBV infection does not seem to have many indirect effects except for the development of PTLD, which is an uncontrolled proliferation of immune cells (typically B cells) in the setting of post-transplant immunosuppression (39). In pediatric cases especially, most PTLD is due to Epstein-Barr virus. This condition is not a true malignancy since the immune system can regain control of B-cell proliferation if extrinsic immunosuppression is reduced and EBV-directed CD8+ T cells regain function.

EBV infection can be asymptomatic or present as a non-specific viral syndrome, similar to CMV infection. Routine PCR monitoring in recent studies suggest that the rate of EBV infection in EBV seronegative pediatric transplant recipients (as defined by systemic viremia) ranges from 50 to 80% (40–42). PTLD incidence in pediatric kidney transplantation was previously low at <1%, but then climbed over the last 10 years, concomitant with the introduction of more potent immunosuppression protocols to more than 4% (43–45).

EBV viremia, in contrast to PTLD, can be asymptomatic or associated with mild non-specific viral symptoms. In the past, the diagnosis was made by documenting seroconversion (EBV IgM positive or significant rise in IgG titer). Unlike with CMV, in the case of EBV, testing can be performed against EB nuclear antigen or viral capsid. The former turns positive early, after recent infection and the latter in more distant infection. More recently, in western countries, PCR amplification of viral DNA is the method most commonly used (46). The reader should note that PCR techniques for EBV DNA amplification vary greatly based on the type of sample and laboratory standards. Thus, PCR values from peripheral blood leucocytes and whole blood generally correlate with each other but not with PCR values from plasma (47, 48). Plasma EBNA PCR may be the best single assay for diagnosing and monitoring PTLD, while the complete PCR panel is superior for ruling out its presence (49).

PTLD can present clinically in many ways (50). Most often the patient has lymphadenopathy, fever or symptoms related to pressure on internal organs from an internal lymphoid mass. Diagnosis is made histologically, though imaging can help with appropriate tissue acquisition and staging. Monoclonal or monomorphic PTLD is generally more severe than polyclonal or polymorphic. The most severe forms are indistinguishable from lymphomas.

There is no universally accepted treatment for EBV infection post-transplant. Reduction in immunosuppression is one modality. The anti-viral agents ganciclovir and valganciclovir have activity against EBV but acyclovir is not effective against EBV. The duration and route of anti-viral treatment is not standardized but most centers will treat until the EBV PCR has turned negative. Whether all patients with acute EBV seroconversion need reduction in immunosuppression or anti-viral treatment is a controversial question in pediatric transplantation (51, 52). Children in particular can develop a chronic high load carrier state without ever progressing to PTLD (50). Nevertheless, the majority of reports indicate that higher EBV PCR values are associated with a greater risk for subsequent PTLD (53–55).

Oral ganciclovir or valganciclovir are also used for prevention of EBV disease. These agents seem to delay the onset of infection rather than reduce the incidence. IVIg does not add any benefit (56). An alternative concept is pre-emptive therapy, i.e., the initiation of anti-viral agents at treatment doses as soon as the EBV PCR load

rises. An EBV vaccine, directed against an EBV-glycoprotein, is under testing in the United Kingdom.

Treatment of PTLD usually involves a sequential process (57, 58). Immunosuppression reduction is typically the first intervention, followed by anti-CD20 antibody or interferon-alpha. Chemotherapy is usually reserved for higher grade cases, though some centers will use rituximab with low dose chemotherapy.

Varicella Infections

Varicella-zoster virus (VZV) is the most infectious of the human herpesviruses. Primary infection with VZV results in chickenpox. Following primary infection, the virus remains in the body in a latent state from which it may be reactivated, resulting in cutaneous herpes zoster, or shingles. Incidence of all VZV infections in adult kidney transplant recipients was 7.4% in one study (59). Children are morel likely to be VZV seronäive at the time of transplantation and primary infection is a significant cause of morbidity and mortality (60, 61). Routine VZV vaccination has been documented to reduce the incidence of primary VZV infection post-transplantation (62). All transplant candidates over 9–12 months of age should receive immunization with the VZV vaccine (63). Studies in children with chronic kidney disease and those who are being treated with dialysis suggest that two doses, rather than one, may be necessary to elicit protective antibody levels. Therefore, antibody levels be obtained at least four weeks following immunization, and a second dose be given if necessary (63, 64). Although some studies have evaluated the use of this vaccine in post-transplant patients, both American Academy of Pediatrics Committee on Infectious Diseases and the Centers for Disease Control and Prevention's (CDC) Advisory Committee on Immunization Practices (ACIP) advise against the use of this live-viral vaccine in immunocompromised patients. Where possible, such vaccination should thus be provided, and protective antibody levels documented, prior to transplant (65, 66).

Patients who are varicella-näive at the time of transplant (not immunized or did not respond to immunization), should receive prophylactic therapy if they are exposed to varicella. Varicella zoster immune globulin (VZIG) is no longer manufactured as of October 2004. In February 2006, the FDA approved expanded access to an investigational VZIG product, VariZIG (Cangene Corporation, Winnipeg, Canada) which became available under an investigational new drug application. The ACIP recommends that use of this product may be requested in immunocompromised patients exposed to varicella infection (67). If this product is not available, intravenous immune globulin (IVIG), which contains some antivaricella antibody, may be given (67). Any prophylactic therapy should be given as soon as possible, up to 96 hours after exposure (67). Prophylactic acyclovir may be used after exposure, especially if the window for VariZIG has passed, though data are limited to a few healthy children only (American Academy of Pediatrics Red Book 2006). Patients who develop infection, either primary or secondary, should receive treatment with intravenous acyclovir (68, 69). Oral acyclovir has limited bioavailability in children, so should not be the first option in immunocompromised children.

BK Virus Infection

The BK virus (BKV) was first isolated from the urine of a renal transplant recipient in the 1970s (70). However, this virus did not represent a significant complication of kidney transplantation until the late 1990s (6, 71). Though BKV is a polyoma virus, it shares some characteristics of the herpesviruses: (1) infecting most immunocompetent people during childhood; and (2) establishing a prolonged latency. Unlike the herpesviruses, the BK virus does not establish in the reticuloendothelial cells but prefers the uroepithelium. This propensity for the uroepithelium is responsible for the clinical manifestations: hemorrhagic cystitis in bone marrow transplant recipients and allograft nephropathy in kidney transplant recipients. The incidence of BKVAN in pediatric renal transplantation appears to be the same as in adult kidney transplant recipients at 3–8% (7, 72). Risk factors from adult kidney transplant data include the intensity of immunosuppression (73, 74), recent treatment for acute rejection (75) and placement of a ureteral stent (76), though the data implicating specific immunosuppressive agents is conflicting. In a recent multicenter NAPRTCS special study, use of antibody induction and zero mismatch kidney allografts were the only two risk factors for BKVAN in multivariate analysis (7).

BKV infection and BKV disease are two separate entities. Serial PCR surveillance has shown that urine PCRs turn positive earlier and much more often than peripheral blood PCR. Currently the diagnosis of BKVAN depends on demonstration of the virus in blood or kidney tissue (either by PCR or immunostaining) and the presence of nephropathy (raised serum creatinine or tissue inflammation). Urine positivity without the other features represents infection without disease, as discussed in the recent

guidelines for study monitoring by the American Society of Transplantation (77). Generally viruria is not associated with renal dysfunction (78).

BKVAN represents a diagnostic challenge because the condition may resemble acute rejection. Symptoms are often minimal or absent. Serum creatinine elevations are found on clinical laboratory monitoring. Since the treatment of acute rejection (intensifying immunosuppression) is the opposite of the treatment of BKVAN (reduction in immunosuppression), making the correct diagnosis is critical.

Simple BK viremia, without nephropathy, may not need treatment or may be manageable by reduction in immunosuppression. Like the CMV and EBV infections, reduction of immunosuppression is usually the first step in the treatment of full-fledged BKVAN (71, 79, 80). However, by the time BKVAN is diagnosed, the serum creatinine is significantly raised and subsequent graft survival is worse. Thus, two different approaches are advocated, much as with EBV disease: pre-emptive monitoring and therapy; or simultaneous anti-viral therapy while maintaining some immunosuppression (79). There are virtually no randomized controlled trials to test any of these strategies head to head for most of the viral infections. Anti-viral therapy against BK virus is more complicated than for CMV or EBV, since acyclovir, ganciclovir nor their analogues have any activity against BK virus. Cidofovir is one anti-viral drug that has been tried with some success (81, 82). Higher doses of cidofovir can be very nephrotoxic. Probenecid combination with the higher dose cidofovir or intermediate dose cidofovir ameliorates the nephrotoxicity (83). Some centers switch mycophenolate mofetil to leflunomide (84, 85). This agent is unique in possessing both anti-viral and immunosuppressive properties.

There is no vaccine against BK virus. A preventive strategy that has been suggested is serial urine/blood BK PCR monitoring and pre-emptive reduction in immunosuppression.

Pneumocystis

Infections with *pneumocystis carinii* (now renamed *pneumocystis jiroveci*) have fortunately become rarer with the widespread use of cotrimoxazole prophylaxis in the immediate post-transplant period. The diagnosis is established by demonstration of pneumocystis in lung secretions obtained from bronchoalveolar lavage or in tissue from lung (86). Gomori stain or toluidine blue staining will demonstrate the cysts and Giemsa staining will identify the sporozoites. CMV infection is the major differential diagnosis. Many children may have dual infection, in which CMV infection predisposed to superadded pneumocystis pneumonia (PCP) infection. The antimicrobial agents cotrimoxazole and pentamidine form the mainstay of treatment. Cotrimoxazole may be given either orally or intravenously and is less toxic, whereas pentamidine is only available as a parenteral preparation and has more side effects. Chemoprophylaxis with three times per week oral cotrimoxazole (5 mg/kg trimethoprim component/dose) reduced the incidence of PCP disease from 3.7 to 0% (87). This prophylaxis is now recommended in all transplant recipients during the periods of high risk, typically the first six to twelve months post-transplant. Some centers also advocate its use after anti-rejection therapy, particularly with anti-T cell antibodies. Some centers suggest a lower dose given daily at bedtime (88).

Parasitic

Reports of parasitic infections in pediatric kidney recipients are few. *Strongyloides stercoralis* is an intestinal nematode with worldwide distribution, endemic in tropical/sub-tropical regions, and infects tens of millions of people. The southeast states have the highest incidence within the United States (89). Similar to viruses, *S. stercoralis* may initially remain in the human intestinal tract without symptoms, and then disseminate with the introduction of immunosuppressive medication post-transplant (89). In addition strongyloidiasis transmission has been reported through the allograft kidney in an adult recipient (90). Among calcineuirn inhibitors, cyclosporine (but not tacrolimus) has effects against *S. stercoralis* and may reduce the risk for disseminated strongyloidiasis (91, 92). Active infection manifests with cutaneous, gastrointestinal and pulmonary symptoms as well as eosinophilia (93). Fever, hypotension, and central nervous system symptoms suggest dissemination (93). In uncomplicated infections, diagnosis is made by detection of larvae in stool, although 25% of infected patients may have negative stool examinations (94). In disseminated disease, larvae may be found in stool, sputum, bronchoalveolar lavage fluid, peritoneal and pleural fluid (89, 95). Serologic testing using ELISA may also be of value, although may be falsely negative in immunocompromised hosts (95). Ivermectin has replaced thiabendazole as the treatment of choice for *S. stercoralis,* with albendazole as an alternative (96).

Other parasitic infections that have been transmitted by the renal allograft include Chagas' disease (97) and post-transplant malaria (98). Chagas' disease (caused by

Trypanosoma cruzi) is found only in the southern United States, Mexico and Central and South America. The manifestations of Chagas' disease classically include megaesophagus, megacolon, and cardiac disease, although CNS involvement has been reported in kidney transplant recipients (97). The diagnosis is serological and treatment typically consists of benznidazole or nifurtimox. Post transplant malaria can be frequent in high-endemic areas, so suspicion clinically should based on local region or travel to such a region (98). Screening recipients and donors for these and other parasitic infections may be worthwhile, based on the presence of risk factors including residence in or travel to an endemic area.

Fungal Infections

In general, serious invasive fungal infections such as aspergillosis are less common in pediatric kidney transplant recipients than thoracic organ recipients. Two studies reported a retrospective incidence of 4–10% overall (including milder candidial infections) in adult kidney transplant recipients (20, 99). In studies restricted to the intensive care unit, the incidence is higher at 16.5% (100). Candida is the most common organism affecting kidney transplant recipients, either as oral thrush, vaginitis, nail infection, UTI, or rarely as systemic fungemia (101). The diagnosis of thrush is made by clinical examination or demonstration of hyphae on a smear. Candidial UTI is diagnosed by urine culture. Treatment for topical candida is by topical nystatin or clotrimazole. Prophylactic measures include nystatin liquid or clotrimazole lozenges. True invasive fungal disease, though rare, increases the risk of mortality substantially (100) and needs systemic treatment with drugs such as amphotericin. Among pediatric recipients, hospitalization for fungal infection represented 0.2–2.7% of all infection-related hospitalization in the NAPRTCS registry, but was associated with a 1.64-fold higher risk for graft loss (102).

Cardiovascular Complications

Cardiovascular disease (CVD) is a major obstacle to improving long-term outcomes after transplantation. In pediatric kidney transplant recipients, death due to CVD is uncommon. However, many of the cardiovascular risk factors are already present in pediatric transplant recipients and if not addressed will likely lead to CVD in later life. Changes in the intima-media thickness of the carotid arteries have been observed in pediatric kidney transplant recipients as young as 8 years of age. Although here has been a reduction in cardiovascular related mortality over the last several years in adult kidney transplant recipients, (103) the prevalence of CVD is still high and accounts for 40% of late mortality after adult kidney transplantation (104). In children, cardiopulmonary disease accounts for 15.4% of mortality after kidney transplantation (NAPRTCS 2007 Annual Report), which is dramatically higher than the incidence in otherwise healthy children.

Cardiovascular events in transplant recipients are the result of a combination of causes which may begin pre-transplant and before clinical findings are present. The prevalence of conventional atherogenic risk factors such as hypertension (HTN), diabetes, dyslipidemia, and obesity are much higher in the transplant population. Additional pre-kidney transplant risk factors include renal insufficiency and dialysis, hyperhomocysteinemia, chronic anemia and disorders of calcium and phosphorous metabolism. The accelerated atherosclerosis and coronary artery disease leading to CVD precipitates changes in the blood vessel walls. These alterations occur early in the course of chronic renal failure and are directly related to duration of chronic renal failure and particularly treatment with dialysis (105). Transplant-specific risk factors are primarily related to the exposure of immunosuppressant medications, which are discussed in Chapter 84.

Hypertension

The prevalence of HTN is very high, occurring in over 70% of adult kidney transplant recipients (106). Data from the 2007 Annual NAPRTCS Report indicates that in the immediate post-transplant period, 79% of live donor recipients and 85% of deceased donor recipients were receiving anti-hypertensive medications. At 5 years post-transplant, the rates decreased similarly, but were still significant for both groups, to 59 and 69% (NAPRTCS 2007 report). Other studies in children with kidney transplants have shown that anti-HTN drug use is a risk factor for earlier graft loss (107). In children, casual blood pressure and ambulatory blood pressure monitoring (ABPM) have been used to establish the prevalence of post-transplant HTN (108). Usually, a higher prevalence of HTN has been observed when using ABPM criteria. Giordano and Calzolari found HTN was present in 43–50% of patients by casual measurements (109, 110). However, the prevalence rose to 62–75% when using ABPM. ABPM is now accepted as the method of choice for evaluating HTN in adult and pediatric patients.

Left ventricular hypertrophy (LVH), an early marker of hypertensive cardiomyopathy, is present in 56–82% of

children post-transplant. One study (111) observed a correlation between left ventricular mass index (LVMI) with the mean 24-hour systolic blood pressure. A prospective study by Mitsnefes et al. studied 23 children with echocardiogram while on dialysis and at least 6 months after transplant (112). The prevalence of LVH was similar, occurring in 52% during dialysis and 56% after transplant. However, in 61% of patients, LVMI decreased post-transplant indicating that patients with good blood pressure control had regression of LVH. A recent study by Silverstein et al. reported a lower prevalence of abnormal echocardiography changes (19%) when compared with the above studies (113), suggesting that the lower rates of LVH may reflect a growing awareness of the potential risks for CVD in pediatric kidney transplant patients.

Treatment should include non-pharmacologic interventions, which include weight reduction, dietary sodium restriction and exercise. However, simultaneous pharmacologic treatment should be considered. There are no randomized controlled trials of anti-hypertensive medications in children and all classes of anti-hypertensives have been used after transplantation. The best medication choice will depend on the presence of drug interactions and coexisting conditions such as proteinuria, diabetes and heart disease.

The calcium channel blockers are commonly used as first line therapy. Calcium channel blockers have been shown to counteract the vasoconstrictive effects of CNIs (114) and when used with angiotensin converting enzyme inhibitors (ACEi) have an added antiproteinuric effect (115). The non-dihydropyridine classes of calcium channel blockers interfere with the cytochrome P450 system and CNI dosage reduction is required. A retrospective study of adult transplant patients found that calcium channel blockers were associated with an increased risk of ischemic heart disease (116). However, this increased risk was not observed in a prospective randomized controlled trial in adult hypertensive patients (117). In children, the relationship of ischemic heart disease and calcium channel blockers has not been studied, but calcium channel blockers have been used in adolescent patients after myocardial infarction (118).

Beta blockers may be used as initial agents as well, particularly if there is a preexisting cardiovascular disease. However, they may contribute to dyslipidemia and insulin resistance. The ACEi and ARBs have antiproteinuric and cardioprotective effects. They are not widely used post-transplant because they may decrease renal blood flow and glomerular filtration rate in patients treated concomitantly with a CNI. Still, in adult transplant recipients, ACE and ARBs were found to be generally effective in controlling blood pressure and were not associated with a rise in serum creatinine concentrations (119). Diuretics are useful in patients with sodium and fluid excess especially if allograft function is reduced. However, diuretics may contribute to dyslipidemia and insulin resistance.

Adjustment of immunosuppressive medications can be a reasonable alternative when hypertension is associated to such therapy. Corticosteroid reduction and withdrawal studies in adults and children have reported short-term efficacy and safety with beneficial impact on post-transplant blood pressure profiles (120–122). However, the potential side effects of these medications must be weighed against the risks of acute rejection and chronic graft dysfunction when minimizing or eliminating corticosteroids.

The choice of CNI appears to affect the prevalence of hypertension, with tacrolimus (TAC) seemingly having a more favorable cardiovascular profile over cyclosporine A (CsA) (123, 124), though prospective studies haven not substantiated this obervation (125). Also, since mycophenolate mofetil (MMF) and mTOR inhibitors are devoid of intrinsic nephrotoxicity and hypertensive effects attention has been directed towards avoiding or withdrawing CNI by using these newer agents. However, mTOR inhibitors are associated with dyslipidemia, so the reduction in a cardiovascular risk factor is replaced by increased incidence of another. The combination of MMF/mTOR is associated with diarrhea and overall poor tolerability which may lead to suboptimal compliance. Overall, the risks and benefits of CNI-free regimens have to be considered since studies have indicated variable results and long term follow up data are lacking.

Transplant Renal Artery Stenosis

Transplant renal artery stenosis is a relatively less common cause of post-transplant hypertension and accounts to 1–5% of cases (126). It usually appears between 3 months and 2 years after transplantation, but it can present at any time (127). Renal artery stenosis arising early and involving the surgical anastomosis is likely the result of trauma to the vessel during harvesting (128). If a stenosis occurs several years post-transplant it may arise as a result of atherosclerosis. The mechanism of hypertension is volume-mediated with suppression of the renin-angiotensin system, similarly to what occurs in the Goldblatt one kidney model. However, the transplanted kidney is denervated and does not elicit sympathetic activation.

Doppler ultrasound is an excellent screening test (87–94% sensitivity and 86–100% specificity), which can asses the severity of the renal artery stenosis by evaluating

the peak systolic velocity at the stenotic site and the resistive index in the post-stenotic arteries (126, 129). In small children, this modality may be challenging due to patient movement. CT and MR angiography can provide superior images of the vasculature and may be used if sonography is inconclusive (130). However, both are more costly and nephrotoxicity can occur even with the newer non-ionic contrast media used with CT. Arteriography provides the definitive diagnosis of renal artery stenosis (131). Carbon dioxide can be used as a negative contrast agent in order to avoid nephrotoxicity (132). For hemodynamically significant stenoses or uncontrolled hypertension, percutaneous transluminal angioplasty (PTA) is the treatment of choice. Surgery is indicated for restenosis or lesions that cannot be treated with PTA.

Post-Transplant Diabetes Mellitus

Abnormalities in glucose homeostasis may develop for the first time after transplant and occur as a result of insulin resistance, increased insulin metabolism or diminished insulin secretion. The incidence of post-transplant diabetes mellitus (PTDM) in adult transplant patients is high. Kasiske et al. determined from a retrospective large scale analysis of the USRDS database that the cumulative incidence of PTDM was 9.1, 16 and 24% at 3, 12 and 36 months post-transplant (133). The development of PTDM in the pediatric population is less common and data are limited. A retrospective analysis of the NAPRTCS indicated an incidence of PTDM at 2.6% with the majority of the cases occurring in the first 6 months post-transplant (134). African-American children were at higher risk, and contrary to adults, Hispanics were at lower risk to develop PTDM.

Epidemiologic studies have established that diabetes promotes cardiovascular disease and other long-term complications (135) in the general population and it is believed that these same risks apply to transplant recipients with PTDM. Further rationale for treatment is highlighted in a study by Cosio et al. in which PTDM correlated with an unfavorable cardiovascular profile and reduced patient survival in adult patients (136). A similar adverse effect on patient survival was also seen for patients with pre-existing diabetes and PTDM by other investigators (137). The importance of optimizing glycemic control is underscored by recent data indicating that treatment aimed at achieving near normoglycemia decreased the risk of any cardiovascular disease event by 42% (138). The impact of PTDM on survival has not been evaluated in the pediatric transplant population.

Management of PTDM requires a multidisciplinary approach which includes collaborations with nephrology, endocrinology and dietetics services. Lifestyle changes primarily involve calorie reduction by decreasing carbohydrate intake, particularly in obese individuals. The dietary changes should address and meet the individual's nutritional needs while taking into account personal and cultural preferences. Promoting weight loss through exercise is very effective in improving insulin sensitivity and physical activity should be an important component of the overall management.

Early steroid withdrawal reduces the incidence of PTDM (139). Steroid-free immunosuppression was also associated with a lower incidence of PTDM (0.4% vs. 5.4%) (140). However, acute rejection episodes are higher in the steroid-free (31.5%) and steroid withdrawal groups (26.1%) when compared to a steroid control group (14.7%), as illustrated in the FREEDOM Study (141). Of the CNI, TAC seems to have a higher diabetogenic potential (133, 142), though again not borne out in more recent pediatric studies (125). However, converting patients from CsA to TAC frequently results in a reduction of other cardiovascular risk factors including hypertension and dyslipidemia. Therefore, the choice of immunosuppression will involve a balance between the risk for rejection and the potential for complications like PTDM.

There is little published information regarding the pharmacological management of PTDM. The 2003 international consensus guidelines recommend a step-wise approach to the management of patients with PTDM, similar to that followed for patients with type 2 diabetes (143). If adequate blood glucose is not achieved with lifestyle modifications, pharmachologic monotherapy is recommended. The agent should be chosen based on safety and patient-specific factors (age, level of kidney function, level of glycemic control). Oral agent therapy can be used when hyperglycemia is not severe. However, for most oral agents, the safety and efficacy has not been established for use in children.

The sulfonylureas directly stimulate insulin secretion and should be used with caution in the setting of decreased allograft function. However, the newer generation of sulfonylureas are almost entirely metabolized by the liver (144). The meglitinides also stimulate insulin secretion and are short acting. They can be safely used in renal transplant recipients since they are hepatically metabolized (145). Thiazolidinediones promote peripheral insulin sensitivity and have a reduced risk of hypoglycemia compared to other oral agents. The biguanides decrease hepatic glucose production, but they are largely cleared by renal tubular secretion and should be avoided in renal

transplant recipients with allograft dysfunction (143). Glucagon-like peptide-1 receptor agonists are approved for clinical use in the United States. Its use in kidney transplant is limited since there are few data concerning drug interactions and dose adjustments may be needed since it has significant effects on gastric emptying (146).

Insulin is the safest agent in the pediatric transplant population. Insulin does not interact with immunosuppressive medications. However, insulin metabolism occurs in the kidney, and dosage adjustments may be needed with changes in allograft function (147). The modern insulin regimens use a long acting agent to provide basal glycemic control and rapid-acting insulin analogues to be given at the time of meals (148).

Dyslipidemia

Dyslipidemias play a role in the development of atherosclerosis and cardiovascular disease and are becoming a greater concern for children. Earlier studies have indicated that fatty streaks in vessels are present in individuals as young as 10 years (149). More recent data have confirmed that atherosclerotic lesions were present in more than half of the right coronary arteries of adolescent individuals (150). In children, the extent of atherosclerotic lesions correlated significantly with serum total cholesterol concentrations, serum LDL cholesterol concentrations and serum triglyceride concentrations (151). In transplant patients, many factors such as genetic predilection, age, gender, medical conditions (such as proteinuria), and medications influence the prevalence of dyslipidemia.

Dyslipidemia is common after kidney transplantation. Total cholesterol has been found to be elevated in 51–66% of patients and LDL cholesterol in up to 84% of patients in several studies (152). More recent studies indicated hypercholesterolemia in 20% of patients and elevated triglycerides in 45–50% of patients (153, 154). In this report, about one third of the study population was receiving corticosteroid free immunosuppression. The prevalence of dyslipidemia post-transplant is thought to decrease over time and may reflect changes in immunosuppression. At 10 years post-transplant, Milliner et al. found that the prevalence of hypercholesterolemia decreased from 70.4 to 35% and hypertriglyceridemia from 46.3–15% (155). However, the mean lipid values were not different over the course of 20 years and the decreased prevalence was the result of increasing normal values with advancing age.

The National Kidney Foundation Kidney Disease Outcomes Quality Initiative (K/DOQI) Work Group created guidelines for the management of dyslipidemias in kidney transplant patients (152). Since there are few studies of dyslipidemia in children, the Work Group considered that adolescents be included in the guidelines and that children before the onset of puberty be managed according to the National Cholesterol Expert Panel on Children (NCEP-C) (156). The K/DOQI guidelines recommend that a fasting lipid profile (total cholesterol, LDL, HDL and triglycerides) be measured during the first 6 months post-transplant, at 1 year after transplant and annually thereafter. A lipid profile should also be measured if 2–3 months after stopping or starting an immunosuppressive medication known to affect lipid levels. If dyslipidemia is detected, evaluation and if indicated modification/treatment of secondary causes (medications, nephrotic syndrome, hypothyroidism, diabetes) is recommended.

For adolescents with adequate nutrition, treatment with therapeutic lifestyle changes (TLC) should be considered if fasting triglycerides are $\geq$500 mg/dL. These include changes in diet, physical activity and habits. Pharmacological therapy with fibrates or nicotinic acid may be warranted if isolated hypertriglyceridemia persists after 6 months. However, the safety and efficacy of these medications has not been well established in adolescents so their routine use is not recommended. For LDL levels between 130–159 mg/dL, TLC should be the first line therapy. Treatment with a statin should be considered if after TLC the LDL level remains above 130 mg/dL, if the initial LDL is above 160 mg/dL or if there is hypertriglyceridemia and an elevated non-HDL cholesterol level. If the desired target is not achieved, a bile acid sequestrant can be considered as long as triglyceride levels allow its use.

The NCEP-C guidelines are recommended for the management of dyslipidemia in younger children. However, they are not specific for kidney transplant recipients or patients with chronic kidney disease. The NCEP-C recommends diet therapy as the primary approach for treating dyslipidemia in children. If LDL levels are >130 mg/dL, a Step 1 AHA diet is prescribed, followed in 3 months by a Step 2 AHA diet if the target levels are not achieved. Pharmacological therapy is recommended if LDL level is >160 mg/dL and there is a positive family history of CVD or if two or more CVD risk factors are present in the child.

Obesity

The prevalence of obesity in children has increased over the last decade and is a major concern for health care

providers. Obesity is a recognized cardiovascular risk factor and its impact post-transplant is significant. Weight gain after transplant occurs as a result of multiple factors including, increased appetite from corticosteroids and elimination of uremia, lack of physical activity, genetic predisposition, age, gender and race (157).

Data collected from the United Network of Organ Sharing (UNOS) described the prevalence of overweight and obesity in individuals undergoing kidney transplantation (158). Between 1987 and 2001, the number of patients classified as overweight (BMI 25–29.9 kg/m^2) increased by 32% and those obese (BMI $\geq$30 kg/m^2) rose 116%. Furthermore, nearly 60% of current kidney transplant recipients are classified as overweight or obese. In children, a similar trend has been observed. Data from the NAPRTCS indicated that 9.7% of children were obese at the time of transplantation (159). Of importance, is that the percentage of children with obesity increased from 8.2% between 1987 through 1995 to 12.4% between 1996 to 2002. After transplant, the incidence of overweight and obesity doubles (160).

The negative role of obesity on outcome of renal transplant has been documented by several investigators. Meier-Kriesche et al. found that an elevated BMI was associated with increased mortality, particularly for those with a BMI; >36 kg/m^2. However, this association held true even for patients with mild obesity (BMI >25 kg/m^2) (161, 162). In a NAPRTCS report, obese children ages 6–12 years had a higher risk for death than non-obese transplant recipients (159). In this age group, death from cardiopulmonary disease was more common in obese recipients (27%) as compared with non-obese individuals (17%).

The treatment of obesity generally involves TLC (dietary modifications and exercise) and requires a multidisciplinary approach. Corticosteroid avoidance has been associated with a lower incidence of obesity by some investigators (163). However, other studies indicated that body weight increase following transplantation occurs even in the absence of corticosteroids (164). In children, the combination of TLC and pharmacological therapy to achieve weight loss has not been studied. In adults, limited data on chronic kidney disease patients have shown a positive impact on weight loss (165).

Musculoskeletal Complications

The skeletal complications of transplantation include impairment of linear growth, loss of bone mass, fractures and avascular necrosis. Factors such as chronic illness and renal osteodystrophy have a negative impact on bone before transplantation, so that at the time of transplant, a significant preexisting metabolic bone disease may exist. During childhood, skeletal growth and the development of peak bone mass occur, both of which are affected by renal failure and subsequent post-transplant medications.

Growth

Growth retardation in children with chronic kidney disease occur as a result of inadequate caloric intake, metabolic acidosis, renal osteodystrophy and abnormalities of the growth hormone (GH)- insulin-like-growth factor (IGF) hormonal axis (166). GH and IGF levels are normal, but the IGF bioactivity is low as a result of excess IGF binding proteins. At the time of transplantation, the mean height deficit for children is 1.79 standard deviations (SD) below the appropriate age and adjusted height level (NAPRTCS 2007 Annual Report). In 1987, the mean height deficit was 2.4 SD, so height pre-transplant has improved, but not by much. It was initially anticipated that a successful renal transplant would improve growth velocity and induce catch-up growth. Data from the NAPRTCS indicates that improvement in height delta *z*-score does occur in children younger than 6 years of age at the time of transplant. However, recipients between 6 and 12 years of age exhibited linear growth that is consistent with that of the normal population, and adolescents fail to show improvement in height delta *z*-score (NAPRTCS 2007 Annual report).

The major risk factors affecting linear growth post-transplant are age at the time of transplant, level of allograft function and corticosteroid therapy. The reason why younger children experience a greater improvement in linear growth post-transplant is not very clear. However, children with renal insufficiency undergoing transplantation have a diminished pubertal growth spurt. In prepubertal children, the height velocity increases in both boys and girls after transplant, surpassing the values in normal children. The onset of pubertal growth spurt is delayed, but the duration is shortened by 1.6 years compared to normal children, reducing the total pubertal height gain by 20% (167). Mean adult height is lower than mid-parental height by 5.2 cm in boys and 13 cm in girls. However, for children transplanted after the onset of puberty, mean adult height is below mid-parental height by 12.6 cm in both boys and girls (167).

Tejani et al. showed that renal allograft dysfunction has an adverse effect on growth. The investigators found that each increase in serum creatinine concentration of

1 mg/dL was associated with -0.17 decrease in the Z-score (168). A more recent, retrospective analysis of the NAPRCTS by Fine et al. confirmed that allograft dysfunction over time as well as well as renal function at baseline (pre-transplant) are important predictors of linear growth (169).

A firm relationship exists between corticosteroid exposure and growth retardations following transplantation. Corticosteroids suppress linear growth through various mechanisms which include abnormalities in GH, gonadotrophin secretion as well as increased IGFBP-3 levels (170). Also, corticosteroids reduce bone formation leading to bone turnover cycles in which bone resorption exceeds bone formation (171).

Maximizing growth and minimizing renal osteodystrophy pre-transplant is an initial first step in addressing growth retardation. The above data indicate that performing a kidney transplant at a younger age could have a positive effect on growth. Furthermore, minimizing time on dialysis before transplantation or pre-emptive transplantation may also confer benefits. Recombinant human growth hormone (rhGH) has been effectively used to improve linear growth. Fine et al. performed a randomized, multi-center, double-blind, placebo-controlled study that confirmed the efficacy and safety of rhGH in children with chronic renal failure (172). A subsequent review of the NAPRTCS database indicated that side effects from rhGH were infrequent and similar in treated and untreated children (173). However, a recent report evaluated the association between PTLD and rhGH use pre and post-transplant (174). The investigators indicated that the use of rhGH during CRI pre-dialysis was associated with a higher risk for PTLD post-transplant (odds ratio 1.88, 95% CI = 1.00–3.55). Hence, further research and careful monitoring is indicated to confirm these findings. Recombinant growth hormone has been effectively used post-transplant to improve linear growth (172). Further trials confirmed the efficacy of rhGH and indicated a significant increase in growth velocity in the treated group compared with the untreated group without adverse effects on graft function (175, 176).

Reducing or avoiding corticosteroid treatment will have a beneficial impact on linear growth. Alternate day corticosteroid dosing has revealed improvement in height delta *z*-score when compared to daily therapy (177). Furthermore, steroid avoidance protocols have demonstrated improvement in linear growth (122).

Post-transplant allograft function may also predict growth, so strategies aimed at maximizing allograft function may be another form of therapy towards improving growth. Of the CNIs, TAC seems to be associated with a more favorable kidney function when compared to CsA. An analysis of the NAPRTCS indicated that patients treated with TAC/MMF/corticosteroids had a significantly higher GFR at 1 and 2 years post-transplant when compared to those treated with CsA/MMF/corticosteroids (123).

Bone Loss and Fracture Risk

Chronic kidney disease and transplantation have significant effects on bone and future fracture risk, since peak bone mass is achieved following sustained childhood growth. Using dual energy X-ray absorptiometry (DEXA), several investigators have identified a decreased bone mineral density (BMD) during the first few months and in some cases years after transplant (178, 179). However, in these studies, osteopenia may have been overestimated, since measurements of BMD were compared to age-matched controls, and children undergoing transplant are usually shorter. When adjusting for height, post-transplant osteoporosis is not a prominent finding in pediatric renal transplant recipients (180, 181). However, height-adjusted DEXA yields inappropriately elevated *z*-scores since older transplant subjects may be compared to younger and skeletally less mature subjects. Alternative methods such as quantitative computed tomography and magnetic resonance imaging may allow for more accurate measurements of bone density following renal transplant but have other limitations.

Across all age groups, a United States Renal Data System (USRDS) analysis indicated that the relative risk of hip fracture is four times greater in dialysis patients independent of age and gender (182). Children post-kidney transplant have approximately a 2% risk of suffering a fracture. Corticosteroid use post-transplant is a major factor contributing to bone disease. Corticosteroids decrease intestinal calcium absorption, increase renal calcium excretion, decrease osteoblast number and activity, increase PTH concentration and increase the number of remodeling sites (183). Predominantly, corticosteroids result in a reduction in bone formation early post-transplant. The CNIs have been also associated with osteopenia mostly by increasing bone resorption (184). However, the mTOR inhibitors do not appear to have significant clinical effects on bone. Other factors that play a role in bone disease include inactivity, poor allograft function, hyperparathyroidism and the primary disease (cystinosis and oxalosis).

Adequate nutrition post-transplant is essential for bone health and calcium should be supplemented if necessary. Exercise is beneficial for bone strength and should be encouraged. Vitamin D stores should be adequate and the administration of calcium and active vitamin D may

be necessary if secondary hyperparathyroidism persists. Studies in adult transplant recipients have confirmed the value of bisphosphonates in the treatement of osteopenia and osteoporosis, but data on children are scarce (185, 186). In one study of pediatric kidney transplant recipients, bisphosphonate therapy improved BMD and was safe, tolerable and easy to administer (187). Clearly, more data are needed, particularly since the long term effects of these therapies in children are uncertain. Reduction of corticosteroids may be considered in selected subjects while assessing the potential risk of rejection and to overall outcome.

Avascular Necrosis

Avascular necrosis (AN) is a reported complication of kidney transplantation and occurs in about 1% of patients at 3–4 years post-transplant (183). Reports with longer follow up have indicated higher prevalence rates, but overall the incidence of AN is decreasing as a result of better control of renal osteodystrophy prior to transplant and lower corticosteroid dosing after transplant (188).

AN most commonly affects the epiphysis of long bones such as the femur, but may affect the humerus, talus or hand. Occasionally, individuals may be asymptomatic and have abnormal radiographic studies. However, most patients with abnormalities on MRI or bone scan have symptoms and will eventually require surgical intervention.

Conservative management with restriction of activity is used in early cases. Core decompression, were the internal bone pressure is relieved is used in selected cases. If the previous treatments fail or if pain persists, then total hip replacement is performed.

Gastrointestinal Complications

Gastrointestinal complaints in renal transplant recipients are common and all levels of the gastrointestinal tract may be involved (189). Most gastrointestinal complications including oral lesions, esophagitis, gastritis/peptic ulcer, diarrhea and abdominal pain, are either due to infections or medication toxicity.

Oral and Esophageal Lesions

Apthous ulcers may be single or multiple and can involve all areas of the oral mucosa with the exception of the hard palate, gingival and vermillion border. They are typically caused by viral infections such as CMV and HSV. HSV may also cause gingivostomatisis. Oral ulcers have also been seen in a significant number of patients receiving steroid free immunosuppression with a combination of SRL/MMF (190). Therapy consists in reducing immunosuppresion or discontinuing the offending drug, topical steroids and antiviral therapy. Histological examination of the lesions may be required if there is no response to therapy.

Oral candidiasis usually occurs early post-transplant, when immunosuppression is more intense, or after aggressive antibiotic therapy. *Candida albicans* and *Candida tropicalis* are the most common organisms involved. Nystatin or clotrimazole are effectively used for prophylaxis during the first months post-transplant and after treatment of a rejection episode.

Esophagitis may be secondary to chemical irritation of the esophageal mucosa from medications or may occur with infections such as Candida, CMV and HSV. Most cases present during periods of most intense immunosuppression, like after the treatment of acute rejection. The most common complaint is dyspepsia, but dysphagia, odynophagia and cough may also occur. Endoscopic examination is required to confirm the diagnosis. Therapy will depend on the etiology. Generally, mild cases of candidial esophagitis will respond to nystatin, but some cases require oral triazoles or amphotericin B (191). The treatment of herpetic esophagitis includes acyclovir or ganciclovir if there is a concomitant CMV infection.

Gastrointestinal Disorders

Gastric discomfort with nausea, vomiting, diarrhea and abdominal pain are the most common side effect of several medications. In particular MMF and AZA can lead to nausea and gastric discomfort (192). Gastroduodenal lesions are frequently associated with *Helicobacter pylori* infection in pediatric renal transplant candidates (193). However, the incidence of *H. pylori* colonization post-transplant in children is not known, but occurs in about one third of adult recipients (194). Overall, the incidence of peptic ulcer is low with the common use of prophylactic H_2-receptor antagonists and proton pump inhibitors.

Since many transplant medications that are toxic to the gastrointestinal tract are used in combination, it is difficult to determine relative contribution of each medication. However, dosage adjustment of the offending agent is usually required to improve the gastrointestinal symptoms. Dyspepsia can be minimized by the concomitant use of H_2-receptor antagonists or proton pump inhibitors. Therapy targeted towards *H. Pylori* should be provided in cases where the gastroduodenal lesions are associated with this infection.

Lymphoproliferative disorders are one of the most common post-transplant malignancies and may involve the gastrointestinal tract. Gastrointestinal PTLD symptoms may be mild and non-specific initially, but can lead to bleeding, obstruction and perforation (191). PTLD is discussed with greater detail earlier in this chapter.

Hematologic Complications

The most common abnormalities in the hematological parameters after kidney transplant are anemia and leucopenia. However, all three cell lines of the hematopoietic system may be affected in various ways.

Anemia

The incidence of anemia after kidney transplant is high and has been reported to occur at rates of 30–80% at 1 and 5 years post-transplant (195, 196). Anemia tends to occur early after transplant, followed by recovery and a later increased risk in the longer term (197).

Several factors play a role in the development of anemia in the early post-transplant period. Blood loss from the surgical procedure coupled with cessation of erythropoietin administration and use of certain medications leading to bone marrow suppression will all contribute. OKT3 and anti-thymocyte globulin are induction immunosuppressive agents that cause anemia by direct bone marrow suppression. Of the medications used for maintenance immunosuppression, AZA and MMF also suppress the bone marrow by direct nucleic acid synthesis inhibition (198). Anemia has also been correlated to the use of mycophenolate sodium (199). Very rarely, TAC has been associated with anemia secondary to hemolytic uremic syndrome (200). The mTOR inhibitors may cause anemia by blocking signal transduction of the cytokines needed for maturation and proliferation of bone marrow cells and may interfere with iron homeostasis (201).

Many other medications given in the post-transplant period have also been associated with anemia including, the sulfa containing drugs, dapsone and the anti-viral agents acyclovir/ganciclovir/valacyclovir/valganciclovir. The renin-angiotensin-aldosterone system is also an important regulator of erythropoiesis. Hence, ACE inhibitors and ARB have a hematocrit lowering effect (202).

Hemolytic anemia may be seen in cases of post-transplant HUS. However, hemolytic anemia can occur as a result of an anti-rhesus immune response, when a rhesus-positive recipient receives a transplant, with transfer of plasma cells, from a rhesus-negative female donor who developed anti-rhesus antibodies during a pregnancy (203).

Other factors such as poor nutritional intake may lead to iron and vitamin deficiencies and nutritional anemia. Viral infections, particularly parvovirus B19, can cause pure red cell aplasia and chronic anemia by entering the cells via the P blood antigen receptor and destroying them (204).

A few months post-transplant, in the absence of iron deficiency and with good allograft function, the anemia tends to improve or resolve. Over time, as GFR gradually decreases, anemia once again develops with a greater prevalence when compared to chronic kidney disease subjects with similar creatinine clearance.

Prevention of anemia through adequate nutrition and erythropoietin use is important prior to transplant. Treatment of anemia post-transplant depends on the identified etiology. If the anemia is medication induced, changes in dosing or drug discontinuation should be considered. Generally, use of erythropoietin in the early post-transplant period is not effective in increasing the hematocrit since there is a systemic inflammatory response and already a large increase in erythropoietin production.

Leukopenia and Thrombocytopenia

There are two main causes of leukopenia after transplant: medication toxicity and infection. Post-transplant infections are discussed earlier in this chapter.

The polyclonal antilymphocyte antibodies are associated with leukopenia in the early post-transplant period. In a randomized, double-blinded comparison of the horse and rabbit antithymocyte globulin formulations, leukopenia was more common in those patients treated with the rabbit product (205). Thrombocytopenia also occurs commonly and usually transient by may require dose adjustments. Hematologic disturbances are less frequent with the use of IL-2R antagonists.

Of the maintenance immunosuppresants, MMF and AZA cause bone marrow suppression and the risk of leukopenia is dose related (206). Using MMF at a dose of 600 mg/m^2 twice daily, leukopenia was more common in children less than 6 years of age. Subjects receiving AZA at low doses may develop leucopenia if they have a genetic defect of thiopurine methyltransferase, one of the catabolic routes for the drug. A similar effect is seen if allopurinol is given concomitantly since it inactivates xanthine–oxidase which is important in the catabolism of the active metabolites of AZA (207).

Patients receiving maintenance therapy with SRL at 5 mg per day had a higher incidence of leukopenia and

thrombocytopenia when compared to those receiving 2 mg per day. The incidence of leukopenia is also higher among patients receiving valganciclovir from day one after transplantation, especially at higher doses (208). Leukopenia and thrombocytopenia may also be seen with trimethoprim-sulfa and the administration of parenteral pentamidine.

Neurologic Complications

Post-transplant neurological complications may occur *de novo* or be the result of a previous condition that developed during the period of kidney failure and dialysis. The neurological complications can be compiled into five different categories: (1) Medication toxicities, (2) Cerebrovascular accident, (3) Peripheral neuropathy, (4) Infections, (5) Malignancy. A review on these complications has been presented by Ponticelli and Campise (209).

Neurotoxicity from CNI can be subtle, causing tremor, paresthesias and headache. Symptoms may be severe causing hallucinations, seizures, cerebral edema, demyelination and a posterior leukoencephalopathy. Often, the symptoms of CNI neurotoxicity are reversible with dose reduction or medication withdrawal. In some cases, the complications may be permanent or even fatal.

Corticosteroids administered at high doses may cause emotional lability, while prolonged corticosteroid use is associated with brain atrophy. Both, corticosteroids and growth hormone are associated with the development of pseudotumor cerebri. OKT3 administration can lead to a cytokine release syndrome manifested by aseptic meningitis and in severe cases encephalopathy with cerebral edema.

Cerebrovascular accidents in children following transplant are rare when compared to adults and data on its prevalence are lacking. Peripheral neuropathy can occur as a result of compression of the femoral nerve, when the allograft is transplanted in the iliac fossa, and compression of the lumbosacral plexus with dual kidney transplantation (210). The symptoms usually develop within the first 48 hours after the procedure and may persist for several months.

Listeria monocytogenes, *Cryptococcus neoformans* and *Aspergillus fumigatus* account for most of the central nervous system infections post-transplant (211).

Other System Complications

Pulmonary complications of renal transplantation in children are mostly confined to drug-induced pulmonary edema or infections such as pneumocystis. Skin pathology relates mainly to higher risk for squamous cell and basal cell carcinomas, which can be minimized by sunblock protection. Viruses can lead to cutaneous warts. Cyclosporine A leads to hirsuitism with a leonine facies, especially in conjunction with corticosteroids. Conversely, tacrolimus has been associated with alopecia. Hyperuricemia and gout can occur post-renal transplant, with cyclosporine increasing the risk (212). Hyperuricemia is also prevalent in obesity and hypertension. While hyperuricemia may remain silent, gout symptoms are similar to those in the general population. Allopuinol is generally more effective than uricosuric therapy because its antihyperuricemic effect is independent of renal function.

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Pediatric Nephrology Around The World

78 Overview

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The subspecialty of pediatric nephrology has developed within the last 40–50 years. Sophisticated diagnostic and therapeutic standards have been achieved for the care of children with congenital or acquired renal diseases. The technical equipment developed for adults has been adapted to children.

A series of reports titled "Pediatric Nephrology around the World" was first presented in the second edition of this book to draw attention to the clinical spectrum of renal disease in children across various parts of the world, with particular emphasis on differences from the disease patterns seen in Europe and North America (1). Cameron (2), reviewing the historical, social, and geographic factors affecting the pattern of renal disease in children, used the challenging phrase "pediatric nephrology in an unjust world" (describing the fact that most of contemporary pediatric nephrology arose from and was relevant to only a minority of the world's children). The situation has hardly changed since that time. The only difference is that more and more pediatricians around the world have become interested in this field of patient care and clinical research (3, 4). There has recently been an impressive change in the growth of competence and knowledge in the care of children with renal problems through the establishment of different training centers around the world. The establishment of the International Pediatric Nephrology Association (IPNA) in 1971 has played a critical role in promoting knowledge and communication among those interested in pediatric kidney disease, and improving care and treatment of diseases of the kidney and urinary tract in children throughout the world. The different regional societies from around the world, such as the African Society of Pediatric Nephrology, American Society of Pediatric Nephrology, Asian Pediatric Nephrology Association, Australia/New Zealand Society of Pediatric Nephrology, European Society of Pediatric Nephrology, Japanese Society of Pediatric Nephrology, and Latin America Society of Pediatric Nephrology, are represented within the IPNA Council. More recently, the Pan-Arabic Pediatric Nephrology Association, including almost 150 members from different countries, has been incorporated within the Asian Pediatric Nephrology Association. In separate chapters, the different regional societies are represented to provide an overview of the specific geographic, ethnic, and socioeconomic aspects of pediatric renal disease around the world. The productive and efficient organization within each of them has led to rising numbers of participants engaging in regional activities (see Appendix) and to an increase in growth and expertise in the care of children with kidney disease. These activities are all signs of increasing attention to and concern for the care of children with kidney disease. Furthermore, there is a growing interest among young pediatricians in the field of nephrology prompted by the sincere desire to provide better care for children with renal disease and to achieve this goal by pursuing better and more adequate training.

Children have a right to treatments and to resources that are as sophisticated and as well developed as those available to adults; yet in countries in transition to new demographic and economic patterns, children still have difficulty competing with adults for limited resources, as the contributions from Latin America (see Chapter 83) and Africa (see Chapter 82) in "Pediatric Nephrology around the World" clearly show. Many of the old problems remain. The proper management of renal disease in children is expensive, particularly the provision of renal replacement therapy for end-stage renal disease. Poor countries are doubly deprived: not only is their gross national product low, but also a smaller fraction of it is made available for health care (5). Nevertheless, a concern about renal disease in children has developed in all regional societies and working groups. The newest regional society of IPNA, the African Society of Pediatric Nephrology, is slowly making progress in its efforts to reach pediatricians with interest in this subspecialty within the African continent, despite the presence of a wide variety of cultural and economic barriers. The extreme geographical distances as well as differences in social, economic, and political structures between South African and North African countries, and the quite different structures within Central Africa, define only some of the ongoing challenges, even independently of the difficult economic questions. Notwithstanding these obvious issues, there is an increasing awareness of the need and a willingness to deliver better care for children. Different incidences and epidemiologic characteristics of diseases in

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developing and tropical countries from those in developed countries reinforce the interest of the field of nephrology in the pathogenesis and outcome of similar diseases in various parts of the world. One can conclude that, in spite of the economic differences and difficult political situations, the pediatric nephrology societies have an increasing professional commitment to studying the clinical and pathologic differences in kidney diseases in various parts of the world (6–10).

To address the prevailing problem of economic limitations to the equitable distribution of high-quality diagnostic and therapeutic services in all parts of the world, the community of pediatric nephrologists has a growing conviction that they must help to increase the knowledge of basic care for children with renal disease (11). Sophisticated research and introduction of efficacious technical procedures are not on the main worldwide agenda and thus for the near future are not achievable. Therefore, in order to fulfill its mission, IPNA has established teaching courses around the world in which a group of pediatric nephrologists with a wide range of knowledge attend the courses in coordination with a local group of physicians to facilitate education and the future establishment and recognition of pediatric nephrology in those specific countries. In addition, IPNA has initiated a fellowship training program with the primary objective of providing basic clinical training in pediatric nephrology. The goal of the program is to disseminate knowledge of pediatric nephrology to different regions of the world. For example, in India, this fellowship program is currently the only source of funding for the training of future pediatric nephrologists.

The modern structure of the specialty can be traced to the formation of the International Study of Kidney Disease in Children in the mid to late 1960s (12, 13). Such multicenter studies are of value not only for the power that they bring to the resolution of a clinical problem, such as nephrotic syndrome, by virtue of the large numbers of cases available for study, but also for the professional relationships, understanding, and standards that they foster. This study was naturally followed by the formation of several regional pediatric nephrology societies and IPNA, which held its first congress in Paris in 1971. The European Society for Pediatric Nephrology (see Chapter 79) and the American Society of Pediatric Nephrology (see Chapter 81) have the longest tradition. Both are associated with registries that maintain databases of children on renal replacement programs: the registry of the European Dialysis and Transplantation Association and European Renal Association, and the registry of the North American Pediatric Renal Transplant Cooperative Study (14, 15), which in particular developed the best resource of actual data not only on transplantation but on other renal diseases as well. There is no other registry in the world of comparable quality.

The European Society for Pediatric Nephrology is addressing the issues that arise from the harmonization of standards within the European Community, with particular focus on the adaptation of countries in Eastern Europe. Special training courses have been implemented within the Eastern European community in Russia, Rumania, and the Baltic states. This program has been received with enthusiasm by local pediatric nephrologists.

The American Society of Pediatric Nephrology has taken the lead in defining the requirements for pediatric nephrologists in developed countries (16). The Japanese Society for Pediatric Nephrology is the oldest and largest of the regional pediatric nephrology societies (See Chapter 80). A unique feature of pediatric nephrology in Japan is that children are screened annually by urinalysis in a nationwide program (17). The screening has provided invaluable epidemiologic information and the opportunity to set up clinical trials involving children in the early stages of disease.

Asia accounts for 57% of the world's population and an even higher proportion of its children because of that region's demographic characteristics. The Asian Pediatric Nephrology Association has developed into a well-recognized society worldwide, with annual conferences that are well attended. Educational courses have been introduced to expand competence into areas of limited financial and technical resources. Because of this achievement, generous space has been allocated to this region in this section of the book (see Chapter 85). The Asian Pediatric Nephrology Association and the Latin American Association of Pediatric Nephrology (see Chapter 83) are principally concerned with the problems of delivering health care to children with renal disease in countries in transition to new demographic and economic structures. The Australia/New Zealand Pediatric Nephrology Association is a well-organized, smaller regional society, which organized the IPNA Congress in 2004 (see Chapter 84). The youngest society, the African Society of Pediatric Nephrology, had its first congress in Cairo, Egypt, in 2000, and its second congress in 2002 in Nigeria, with participants primarily from Nigeria but also from South Africa and Egypt (see Chapter 82).

IPNA provides a coordinating framework for the regional associations. The global association has nearly 1,600 members in 89 countries around the world. It promotes the specialty of pediatric nephrology by holding a triennial international congress, supporting other meetings and teaching ventures, and publishing the journal *Pediatric*

Nephrology. In the Appendix of this Overview, we highlight select research- and training-related activities that serve to clarify the IPNA mission. We encourage everyone with an interest in pediatric nephrology to visit the IPNA web site for additional information on current activities and opportunities in this field, association membership, and links to affiliate organizations worldwide:

http://ipna-online.org/

Appendix

The International Pediatric Nephrology Association (IPNA) is a nonprofit organization with the overarching goal of promoting knowledge and communication among those interested in pediatric kidney disease so as to improve care and treatment of diseases of the kidney and urinary tract in children throughout the world. To accomplish this mission, IPNA pursues the following activities in support of research and training in pediatric nephrology:

A. Publication of the monthly scientific journal *Pediatric Nephrology*, which serves both scientific and educational goals.
B. International triennial congress: All members from the basic science, clinical science, and clinical care disciplines convene to disseminate knowledge of pediatric nephrology. The last IPNA Congress took place in Budapest, Hungary, in 2007, with more than 1,100 participants from 83 countries. The next congress will take place in New York City in 2010; and thereafter in Shanghai, China, in 2013.
C. Regional medical meetings and training facilities: The goal is to disseminate information on pediatric nephrology to all areas around the world, with an emphasis on underserved regions. Examples of IPNA-supported regional meetings include the Latin American Association of Pediatric Nephrology, in Buenos Aires, Argentina, 2008; European Society of Pediatric Nephrology, in Lyon France, 2008; and Asian Pediatric Nephrology Association, in Bangkok, Thailand, 2008. In addition, IPNA has supported the annual International Seminars in Pediatric Nephrology organized by the University of Miami, including travel fellowship grants.
D. Clinical and basic science workshops: The goal is to stimulate interest in and disseminate information on new and innovative research, and provide a forum to encourage young physicians in pediatric nephrology research. For example, IPNA supports the triennial workshops on growth retardation in children with chronic kidney disease, which typically draw 150–200 participants from around the world. The next symposium will be held in Oviedo, Spain, in 2009. Summaries of the scientific presentations are published in the journal *Pediatric Nephrology*. In 2012, this meeting will take place in San Diego, California.
E. Satellite symposia: Immediately prior to each IPNA triennial international congress, there is a satellite symposium called the "developmental meeting." Prior to the 2007 congress in Budapest, the developmental meeting was held in Pecs, Hungary. The next developmental symposium will be held at the Mohonk Mountain Resort, in New York State, in August 2010, just prior to the triennial congress in New York City. The goal of the developmental meetings is to provide a comprehensive state-of-the-art exploration of issues relevant to the developing kidney and urinary tract. IPNA assembles an interdisciplinary group of scientists and clinicians, approximately 120 individuals in total, to exchange information and perspectives on new knowledge and techniques relating to the biology, physiology, and genetics of the developing kidney in health and in disease. It is important to emphasize that congenital renal anomalies are the major cause of renal failure during childhood, and that the morbidity and mortality resulting from malformation of the kidney and urinary tract are considerable. The symposium is designed to foster dynamic exchange and in-depth discussion.
F. Instructional courses: In response to specific areas of instructional need around the world, IPNA supports the development and implementation of teaching courses, in different countries around the world, for example, Morocco, Japan, Venezuela, Brazil, and Bolivia, with others in Eastern Europe. During these teaching courses, usually conducted over a period of 3–5-days, a group of 3–5 pediatric nephrologists with a wide range of knowledge in nephrology as well as outstanding teaching credentials participate in the program as well as in case discussions with the local physicians. IPNA selects specific areas of the world, mainly underserved locales, based on need. The courses are organized in collaboration with a local group of physicians, who are responsible for all of the logistics and guest accommodations. In some areas of the world, such as Eastern Europe, these courses have provided the basis for the establishment and recognition of pediatric nephrology in the country or region.
G. Fellowship training program: The primary objective of this program is to provide basic clinical training in pediatric nephrology for pediatricians with limited or no training in this specialty and who work far from a

pediatric nephrology training center. The goal is to disseminate knowledge of pediatric nephrology to different regions of the world. The period of training is a minimum of 6 months and a maximum of 12 months, with the 12 months' duration encouraged. Afterward, each trainee is expected to disseminate the new knowledge at his or her home institution. The program supports a limited number of fellows for advanced training in pediatric nephrology or research training. IPNA provides approximately US $4,000–$ 8,500 per 6 months' period, depending on the cost of living at the training site, as determined by the director of the proposed training center. IPNA also provides funds for travelling expenses to and from the training site. The trainees are asked to prepare a detailed report of their training activities upon completion of the fellowship, and to report on their activities at 1- and 5-year intervals thereafter. Each trainee receives a certificate of completion from IPNA, a copy of the current edition of the textbook *Pediatric Nephrology*, and funds for travel expenses to attend one national or regional meeting in pediatric nephrology. For example at the Asian Pediatric Nephrology meeting held in Bangkok, Thailand, in August 2008, a total of 17 fellows from different countries (e.g., India, Pakistan, and Vietnam) participated in the proceedings. As evident from their contributions and discussions, the fellowship program had significant impact in advancing pediatric nephrology in their respective countries.

The fellowship program is advertised in the journal *Pediatric Nephrology*, with application deadlines in April and October of each year. Applicants are requested to send via e-mail the following documents to the IPNA secretary: (1) a curriculum vitae, (2) a letter of recommendation from the program director of the home institution, and (3) a letter of acceptance from the director of the proposed training center. A fellowship committee reviews all completed applications and recommends acceptance or rejection to the IPNA Secretary General, who renders the final decision. Since different regions of the world have differing needs for expertise in pediatric nephrology, the regional assistant secretaries and the senior nephrologists of the region determine the minimum requirements for a training center. As of August 2008, a total of 50 fellows have graduated, from 29 different countries: Albania, Bangladesh, Belarus, Benin, Bolivia, Brazil, Chile, China, Columbia, Egypt, El Salvador, India, Iraq, Jordan, Kazakhstan, Kenya, Lithuania, Mongolia, Myanmar, Nepal, Nicaragua, Nigeria, Pakistan, Philippines, RD Congo, Slovenia, Sudan, Uganda, and Vietnam.

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79 Europe

Christer Holmberg

During the last decade, pediatric nephrology and treatment of renal diseases in Europe has greatly improved such that more countries can help their child population. This is partly due to the European Union (EU) and end of the cold war. Thus, traveling and cooperation over national borders is easier and the European Academy of Pediatrics (EAP, former C.E.S.P.) has had a major impact on standardizing specialization programs. In Pediatric Nephrology, the EAP has developed the syllabus for the training of Pediatric Nephrologists in Europe (1). Still there are differences in availability of care and treatment of end-stage renal disease between different countries depending on their economical, social, and political stability. However, these borders and differences diminish. In the more developed countries children with renal diseases are mostly taken care of by well trained pediatric nephrologists, in some countries by pediatricians with a special interest in renal diseases or by internist nephrologists. Less often, children with severe kidney disease are treated by general practitioners.

ESPN

The European Society for Pediatric Nephrology (ESPN), founded in 1967, is the representative body for pediatric nephrology in Europe and represents Europe on the council of the International Society for Pediatric Nephrology (IPNA). The Council of ESPN has representatives from the different parts of Europe, including the former east European countries and Russia. Thus, the interests of all children with renal diseases in Europe at large are taken into account in the policy of ESPN. The membership of ESPN is growing and currently includes 490 members from 42 countries. Most of the ESPN members were previously from France, Germany, UK, and the more developed central and south European countries, whereas countries like Turkey and Poland have become very active during the last decade. Their increased membership has stimulated increased research into all areas of Pediatric Nephrology, and is reflected in the number of publications emanating from these countries in journals like Pediatric Nephrology and other international publications. Turkey hosted the annual meeting of ESPN in 2005 and will serve as the host for the annual meeting of the International Pediatric Transplant Society in 2009.

Training

The annual meetings of ESPN are the major teaching occasions and they attract participants from the USA, Africa, and Asia with an increasing number of active participants. In 2008, there were over 700 attendees from more than 60 countries at the meeting held in Lyon, France. At ESPN meetings, the latest research in pediatric nephrology is presented in addition to clinical seminars and state-of-the-art lectures. Additionally, the national societies for pediatric nephrology organize annual teaching courses and many centers have annual seminars for European researchers in renal diseases. The presidents/representatives of the National societies for pediatric nephrology in Europe at large meet annually at the ESPN annual meeting and discuss common problems in health care for renal patients, the syllabus, requirements and demands for training centers, and registry matters. Thus, cooperation and harmonization has improved in Europe. To help establish pediatric nephrology in the economically weaker countries in Europe at large, ESPN has sponsored a training program where it helps local centers to organize training courses with ESPN- sponsored and local speakers. Such courses have recently been held in many parts of Russia, (Moscow, St. Petersburg, Orenburg, and Vladivostok), in the Baltic countries and in Belarus. Some of these teaching courses have been in collaboration with IPNA, ISN, and ERA/EDTA. The last such teaching course was in Morocco in 2008 and was organized together with IPNA, the African Society for Pediatric Nephrology, and the Moroccan Society for Pediatric Nephrology, who were responsible for the practical arrangements.

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Centers

In 2007, ESPN conducted a survey among 48 institutions from 11 European countries of which 13 were from the UK, 9 from Italy, and 7 from Germany. Of these 72% were in university hospitals 62% were in divisions of pediatric nephrology, and 23% were in divisions of pediatrics. Most centers had 3–6 pediatric nephrologists and 4–9 nurses. Only four centers had over nine nephrologists and over ten nurses. More than 70% had trainees both in pediatrics and in pediatric nephrology, 17% had trainees in internal medicine nephrology. The mean numbers of hospital beds were 15. Most centers had performed 40–120 renal biopsies during the last 36 months and only 7 had over 200. Sixty-seven percent of the centers performed renal transplantations but ninety-seven percent performed chronic and acute dialysis, including neonates. This gives some idea about the size of most centers within Europe but cannot be generalized for the whole continent.

Registry

The European Dialysis and Transplantation Society (EDTA) has, since 1965, gathered data on renal replacement therapy (RRT) in children and adults with end-stage renal failure (2). The pediatric registry collapsed in the late 1990s, but has been reinstituted during the last years with a half-time ESPN employee. The ERA-EDTA Registry office moved in June 2000 to the Academic Medical Centre in Amsterdam (3). Today, 26 national registries are reporting data to the registry and results from the first 12 were published in 2004 (4). The incidence of RRT increased from 7.1 per million age related population (pmarp) in 1980–1984 to 10 pmarp thereafter. The biggest increase was in the 0–4 year age group where Finland with many infants with hereditary nephropathies showed an incidence of 15.5 pmarp in 1995–2000. In the youngest patients, hypoplastic and dysplastic kidneys and hereditary diseases were most common and glomerulonephritis increased with age. HD was the first choice of treatment in the French speaking countries and Spain whereas PD was common in Finland, The Netherlands, and Scotland. Preemptive transplantation was most common in Norway. Long term survival probability was over 85% during dialysis and 95% after transplantation. The mean incidence of children under 15 years in Europe with a population of 728 million in 2005 was 19.8% of the general population in 2006, the highest being in Turkey (29%) and lowest in Spain (13%). The mean prevalence of renal replacement therapy in 2006 pmarp in the now 26 reporting countries was 23.2, but varied from 5.2 in Serbia to about 40 in most countries and 88.5 in Finland. Infants are still the group with the lowest prevalence of treatment 10.8, except for some countries with a larger number of infants with rare congenital renal diseases presenting at birth like Finland with the Finnish type of congenital nephrosis (prevalence 66.3 pmarp in the 0–4 year old) (5).

Development

Today, there are many excellent training and teaching centers in Europe. Urinary tract infections and their long term effects are today well handled in most European countries and do not constitute a major risk in the long term for the child. Renal stones and infection related glomerulopathies are still a major problem in the poorer countries. Renal biopsies are done more commonly in Russia and the east European countries are improving diagnostics and classification of renal diseases. Many congenital diseases have been characterized during the last decades and their genes isolated. This has helped us to understand the pathophysiology of these diseases and helped us to select those patients who are helped by specific therapeutic regimens. Idiopathic nephrotic syndrome, especially FSGS still constitutes a major challenge. Dialysis, nutrition, and ESRD care has been developed and guidelines on PD, HD, nutrition, growth, and osteodystrophy have been produced (http://espn.uwcm.ac.uk). Renal transplantation has today a patient survival of over 95% and a graft survival of over 90% in every age group in the short term with most of the modern immunosuppressive protocols. Still, the youngest patients should be handled by the most specialized centers.

Cooperation with other transplant centers that perform liver transplantation has become more important with the finding that some inherited diseases with primary liver defects also cause renal pathology. Further, certain diseases with liver and kidney involvement can be cured by combined liver and kidney transplantation. Examples are atypical HUS with factor H mutations, oxalosis, and autosomal recessive polycystic kidney with congenital hepatic fibrosis. As more and more younger children are treated with dialysis and renal transplantation and subsequently with life-long, stronger immunosuppression, the side effects and the long-term effects on the child and his or her quality of life becomes more important. Short-term survival is similar with many protocols, but those regimens with the least long-term side effects and with good graft survival should be chosen. Multi-center studies

are now being planned in Europe, and data will be entered in the newly re-established and functioning registry.

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80 Japan

Takashi Igarashi

Most of the pediatric nephrologists in Japan are academic pediatricians who work in academic pediatric departments in University Hospitals, pediatric nephrology departments in Children's Hospitals, and less commonly, in pediatric departments of general hospitals or private clinics. They advocate for pediatric patients with renal diseases and their families.

The Japanese Society for Pediatric Nephrology

The Japanese Society for Pediatric Nephrology (JSPN; http://www.jspn.jp/) was founded in 1967 to promote optimal care for children with renal disease and to spread advances in the clinical practice and basic science of pediatric nephrology to all the members and pediatricians in Japan. JSPN aims to develop guidelines for the diagnosis and treatment for renal diseases. They are (1) the revised guidelines for the diagnosis and treatment of hemolytic uremic syndrome (HUS) following enteropathogenic *Escherichia coli* gastrointestinal infection published in 2000, (2) treatment guidelines for idiopathic nephrotic syndrome in children published in 2006, and (3) treatment guideline for IgA nephropathy (IgAN) in children published in 2007. They are accessible on the JSPN homepage (1).

JSPN was composed of 1,100 active members as of April 2009. JSPN organizes an annual national scientific meeting where pediatric nephrologists and other health care providers learn new advancements in the care and knowledge of pediatric nephrology and related fields. In 2007, the 43rd annual scientific meeting of JSPN was held in Yokohama. Over 800 pediatric nephrologists attended the meeting. The 44th annual scientific meeting took place in Fukuoka in 2008.

JSPN promotes clinical and basic scientific research in pediatric nephrology and supports training of pediatric nephrologists. JSPN and the members surveyed the methods and complications of percutaneous renal biopsy in Japanese children. They demonstrated that the overall complication rate of renal biopsy was 5.8%; gross hematuria was 2.7% and perirenal hematoma was 0.9%. They also showed that the use of the automated biopsy needle with ultrasound guidance provided a safe method for pediatric renal biopsies in Japan (2). JSPN and the members surveyed the clinical features and outcome of antineutrophil cytoplasmic autoantibody (ANCA)-associated glomerulonephritis in Japanese children. They identified that 87% of the patients were female and median age of onset was 12 years old. 84% of the patients achieved remission with immunosuppressive treatment and serum ANCA titers correlated with the response to the treatment and disease activity (3).

JSPN has satellite study groups in eight local areas in Japan. They have scientific and clinical study meetings once or twice a year. JSPN financially and educationally supports their activity. JSPN started a special educational program for trainees and young pediatric nephrologists in 2008. JSPN collaborates with other related pediatric nephrology societies including: International Pediatric Nephrology Association (IPNA), Asian Society for Pediatric Nephrology (ASPN), Japanese Society of Nephrology (JSN), Association of Pediatric Renal Failure, Study Group of Pediatric Peritoneal Dialysis, Study Group of Chinese Herbs for Pediatric Kidney Diseases, Association of Developmental Nephrology and Association of Pediatric Hypertension. JSPN also belongs to Science Council of Japan.

JSPN has supported a Japanese-Korean Pediatric Nephrology Seminar once a year since 2003. The Fifth Seminar was held in Sakai-city in 2007. The Sixth Korean-Japanese Pediatric Nephrology Seminar was held during the period of JSPN meeting in Fukuoka in 2008.

Chronic kidney disease (CKD) is common and affects about 10% of the adult population worldwide. CKD is known to increase the risk of heart disease, vascular disease, stroke and early mortality (4). JSPN has had an active collaboration with the Japan Association of CKD Initiative (JCKDI) since 2006 (http://j-ckdi.jp/index.html).

JSPN publishes two issues of Journal of JSPN (in Japanese) a year. JSPN developed guidelines for handling potential conflicts of interest for authors in 2007.

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School Urine Screening Rogram

Urinalysis is one of the most effective methods to achieve early detection of glomerulonephritis (5). Urinary screening programs for elementary and junior high school children started nationwide in Japan in 1974. School children bring their first-voided early morning urine to school, where they are collected and sent to centralized examination centers. Proteinuria and hematuria are detected by a simple dipstick method. When a urine test results in positive (proteinuria; +/− 15 mg/dL or more, hematuria: + or more), children with a positive finding received a second urine test in the same manner. The goal of the program is to identify school children with chronic glomerulonephritis and permit early therapeutic intervention.

Table 80-1 summarizes the result of the first urine examination by the school urinary screening program for school children in Tokyo in 2005. This revealed that elementary school children aged between 6 and 11 years had proteinuria, hematuria, or both abnormalities at prevalence of 0.86, 1.73 and 0.11%, respectively. The corresponding prevalence was 2.33, 3.99 and 0.41%, respectively in junior high school children aged between 12 and 14, and 1.88, 2.30 and 0.36%, respectively in high school children aged between 15 and 17. The second urine examination for school children with positive urine findings at the first urine examination revealed that the prevalence of proteinuria, hematuria, or both abnormalities was 0.19, 0.67, and 0,05% respectively in elementary school children, 0.62, 0.87, and 0.15% respectively in junior high school children, and 0.43, 0.40, and 0.12% respectively in high school children (*Table 80-2*). This school urine screening program revealed that chronic glomerunephritis was found in 61.2% of the children with hematuria and proteinuria, 1.0% of the children with isolated proteinuria and 2.2% of the children with isolated hematuria.

Now, urinary screening programs are routine for school children in Korea, Taiwan, Philippine and Singapore (6–9). However, this urinary screening program has not been adapted by other countries, as its cost-effectiveness is not proven. We could not disclose the distinct evidence that the urine screening program for school children reduced the incidence of childhood or adult end stage renal failure (ESRF). However, the urine screening program for school children permits early detection of children with severe IgAN or membranoproliferatiove glomerulonephritis (MPGN) and permits controlled therapeutic trials to ameliorate disease development.

IgAN is the most common form of chronic glomerulonephritis in the world (10). IgAN has been generally regarded as a benign form of glomerulonephritis, with approximately 25–30% of patients reaching ESRF after 10 years (11). However, IgAN is the leading cause of ESRF due to chronic glomerulonephritis in Japan; 40% of all ESRF is due to IgAN (12). Despite its prevalence and clinical importance, there is no consensus for the treatment of IgAN. Recently, immunosuppressive therapies showed a dramatic impact on renal survival. A controlled study demonstrated that combination treatment with prednisolone, azathiopurine, heparin-warfarin, and dipyridamole for two years early in the course of the IgAN reduced the severity of immunologic renal injury and prevented any increase in the percentage of sclerosed glomeruli in patients with severe IgAN that showed diffuse mesangial proliferation (13). However, treatment with prednisolone alone for 2 years increased the percentage of sclerosed glomeruli in patients with severe IgAN (14). Despite aggressive and prolonged steroid therapy for

Table 80-1

Result of the first urine examination in Tokyo in 2005

Age (year)	Sex	Number of children	Number of children with hematuria	Number of children with proteinuria	Number of children with hematuira and proteinuria
6–11	Male	113,874	572	1,142	62
	Female	111,322	1,362	2,754	186
			(0.86%)	(1.73%)	(0.11%)
12–14	Male	46,412	1,154	600	94
	Female	48,562	1,061	3,194	300
			(2.33%)	(3.99%)	(0.41%)
15–17	Male	5,262	112	43	13
	Female	12,634	225	369	51
			(1.88%)	(2.30%)	(0.36%)

Table 80-2

Result of the second urine examination in Tokyo in 2005

Age (year)	Percentage of children with hematuria	Percentage of children with proteinuria	Percentage of children with hematuira and proteinuria
6–11	0.19	0.67	0.05
12–14	0.62	0.87	0.15
15–17	0.43	0.40	0.12

Table 80-3

Disease categories that cause end stage renal failure

Year	Number of patients	Disease categories	
		Glomerulonephritis (%)	Congenital renal disease (%)
1968–1979	720	81.6	7.5
1980–1986	710	60.6	14.7
1998–2003	347	29.1	50.4

36 months, approximately 15% of patients experienced a recurrence of proliferative IgAN within 3 years (15). This treatment intervention, if adopted widely, might reduce the incidence of ESRF caused by IgAN in Japan. IgAN is an immune complex glomerulonephritis that involves intense deposition of dimeric and polymeric forms of IgA1 within the mesangium of the glomerulus. Although the true pathogenesis of IgAN is unknown, aberrant glycosylation of IgA or IgG anti-IgA1 autoantibodies may have a role in the development of the disease (16). Those mechanisms and natural history of IgAN suggest a genetic background in the pathogenesis of IgAN. Therefore, lifelong effective therapy that is acceptable for the patients must be established.

The outcome of MPGN is poor in children, irrespective of type, with 50% losing renal function by 10 years and 90% by 20 years in western countries (17). The presentation of MPGN generally falls into three categories; nephrotic syndrome, acute nephritic syndrome, or asymptomatic hematuria and proteinuria discovered by chance. However, this is not the case in Japan, where many children have been identified by urine screening at an early stage of the disease. Only one patient progressed into ESRF after the treatment among 41 patients for 9-year follow-up period (18). Superior outcome of the patients with MPGN in Japan may be the result of earlier detection by urine screening and treatment.

In addition, a population-based study suggested that the number of new patients with ESRF due to glomerulonephritis reduced after the school urinary screening program started (19). This study also showed that the proportion of Japanese patients with glomerulonephritis who are less than 45 years old decreased after urinary screening program for school children started. A similar decrease has not been observed in Western Europe and the U.S., where screening is uncommon. The reasons for these differences in data are not clear, but may suggest benefits of mass urinary screening. Dent disease is an X-linked renal tubular disorder characterized by low molecular weight proteinuria, hypercalciuria, nephrocalcinosis/nephrolithiasis and progressive renal failure. Almost all of the Japanese patients with Dent disease are school children. They are identified by the school urine screening program every year. Dent disease is due to the mutations in *CLCN5* or *OCRL1*. Molecular abnormalities were dentified in more than 60 families with Dent disease in Japan (20, 21).

JSN and JSPN collaboratively developed guidelines for patients with hematuria in 2006 (22). The Study Group for Early Detection, Diagnosis, and Treatment of Intractable Renal Diseases developed a school urine screening guidebook for doctors and health care providers in 2007 (23).

National Registry Data for Children Undergoing Peritoneal Dialysis

The Japanese Pediatric Peritoneal Dialysis Study Group has produced an annual report on pediatric end stage renal disease (ESRD) since 1987. This provides valuable information on epidemiology, types of therapy, complications, morbidity, and mortality in pediatric patients with ESRF (24). The 1998 report was published in English in 2002 (25). 105 patients with ESRD were introduced to renal replacement therapy (RRT) in Japan in 1998. The prevalence rate of the ESRD patients already on the treatment was 22 per million of age-related population (pmarp) aged between 0 and 19 years in 1998. The annual incidence of RRT was 3 pmarp in Japan. In contrast, it was 10 in U.S.A and 7 in Europe (26, 27). Older patients had a higher prevalence rate than younger ones. The major disease categories causing ESRF were congenital renal diseases and glomerulonephritis. The proportion of congenital renal diseases is increasing (*Table 80-3*). Renal hypoplasia/dysplasia and focal segmental glomerulosclerosis are predominant causes of ESRD during childhood.

Peritoneal dialysis (PD) was used more frequently than hemodialysis under the age of 15. 46.9% of the patients with ESRD received renal transplantation and 96% of the transplanted kidneys were from living kidney donors. 83.1% were transplanted by the third dialysis year. The transplant rate in 1998 was 10 per 100 dialysis patient-years in patients aged between 0 and 19 years. Preemptive renal transplantation for children with ESRD started in Japan in 1998 and by 2005 totaled 46 children. Preemptive renal transplantation comprised 15.4% of all renal transplantation for children with ESRD in 2005. The death rate was 15.6 per 1,000 dialysis patient year in patients with ESRD aged between 0 and 19 years. This rate was similar to that in Europe (27). The leading causes of death were cardiovascular diseases and infections.

The ultimate goal of the care provided to children with ESRD is the achievement of normal growth and development. Attention to dialysis adequacy, control of osteodystrophy, nutrition, and correction of anemia is mandatory to attain this goal (28, 29). The Association of Pediatric Renal Failure and Study Group of Pediatric Peritoneal Dialysis developed guidelines for PD in children in 2004, in collaboration with JSPN.

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81 Pediatric Nephrology Around the World – North America

Lisa M. Satlin · Sharon P. Andreoli · H. William Schnaper

Introduction

Pediatric nephrologists in North America care for children who have a wide variety of acute and chronic kidney diseases. Two registries provide insight into the current state of chronic kidney disease in pediatric patients: the North American Pediatric Renal Trials Cooperative Study (NAPRTCS), previously called the North American Pediatric Renal Transplant Cooperative Study; and the United States Renal Data System (USRDS), which produces an annual report on end stage renal disease that includes pediatric patients.

NAPTRCS was organized in 1987 (1). Scientific objectives included capturing information about current practice and identifying trends in immunosuppressive therapy, with an ultimate goal of improving the care of pediatric renal allograft recipients. In 1992, the registry was expanded to include pediatric dialysis patients and in 1994 it was further expanded to include pediatric patients with chronic kidney disease (CKD) (estimated GFR less than 75 ml/min/1.73 m^2 as calculated by the Schwartz formula). The goal is to register, follow and study the large majority of the children receiving dialysis and/or renal allografts and to determine the clinical course and natural history of patients with renal dysfunction as they progress to end stage renal disease. This voluntary collaborative effort has recorded data from over 16,000 patients in 130 pediatric renal centers in the United States, Canada, Mexico and Costa Rica.

In the three NAPTRCS registries, renal dysplasia with or without obstructive uropathy is the most common cause of CKD or the need for dialysis or a kidney transplant. Focal segmental glomerulosclerosis is a common acquired cause of CKD and is particularly prevalent in the pediatric dialysis population. The NAPTRCS data base also reports graft survival, morbidity, and the relationship that these end points have to patient characteristics such as race/ethnicity, sex and primary renal disease; and to transplant characteristics such as age at transplantation, donor source, immunosuppressive treatment, and HLA mismatches. Analogous patient and event characteristics are described in the CKD and dialysis populations.

Since 1988, the USRDS report has provided valuable information on the demographics, epidemiology, modes of therapy, morbidity and mortality in patients with end stage renal disease. The 2007 USRDS Annual Report describes underutilization of growth hormone in pediatric CKD patients with growth failure, as well as overutilization of percutaneous catheters for hemodialysis access; since catheters are associated with infectious complications and increased morbidity this was noted as a major concern (2). Alarmingly, the 2007 USRDS reported that there has been no progress in the 5-year survival of pediatric end stage kidney disease patients. In fact, in some populations the likelihood of survival declined slightly between the 1991–1995 and 1996–2000 periods. Children with a transplant have the best chance of surviving 5 years at 0.93 while children who are maintained on hemodialysis or peritoneal dialysis have 5-year survival rates of 0.79 and 0.82, respectively (2). Detailed reports of USRDS data are published yearly.

Organization of North American Pediatric Nephrologists

North American pediatric nephrologists are affiliated with the International Pediatric Nephrology Association (IPNA) through the American Society of Pediatric Nephrology (ASPN). The ASPN presently has approximately 700 members. One-fifth of these are trainees and another 15% are emeritus members. The Society conducts an annual scientific meeting each May in association with the Pediatric Academic Societies meeting. Standing committees of the Society address such issues as policy, research, workforce needs, clinical matters, and education and training.

One of the major problems confronting pediatric nephrology in the United States is the limited number of pediatricians choosing to enter careers in pediatric nephrology. The median age of pediatric nephrologists who are certified by the American Board of Pediatrics® exceeds that of the next "oldest" subspecialty by approximately 2 years. In recent years, efforts to attract trainees into the

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field have yielded some success, with 40–50 new Fellows entering training each year. However, some of these candidates either do not complete their training or do not sit for the certifying examination. The causes of this attrition are myriad, and include lifestyle issues, debt incurred during the course of education and training, and the relatively lower salaries received by academic physicians compared with those in the private, general practice of pediatrics. It should be noted that the median age of pediatric nephrologists has not increased over the past few years, indicating that the situation has been stabilized and may be improving.

Another major concern is the relative lack of funding for research in pediatric nephrology in the United States, reflecting a trend in overall Federal funding for research over the past 7 years. Research funding in the U.S. is primarily dependent upon support from the National Institutes of Health and the National Science Foundation, which support mostly research programs proposed by individual investigators. Limitation of this funding in recent years has meant that many promising young academicians have struggled to establish or continue their careers. Alternative sources, such as charitable foundations and industry, have mitigated this problem to some extent, but the extent of available funds does not equal that previously derived from Government support for the academic biomedical research enterprise. One positive aspect of Federal funding is that the ASPN and other organizations have collaborated with the National Institute of Diabetes, Digestive and Kidney Diseases (NIDDK) to develop initiatives to support national collaborative studies of kidney disease in childhood. These are described in more detail below.

Public Policy

The ASPN has evolved into a proactive organization regarding public policy and legislative issues. The ASPN advocates for policies and programs that advance the interests of pediatric nephrologists, patients and their families. The Public Policy Committee (PPC) and the Washington representatives of the ASPN represent the interests of the ASPN, patients and their families before the U.S. Congress and federal agencies, in coalition with similar organizations.

In its public policy activities, the ASPN interacts extensively with other nephrology and pediatric organizations, with government agencies and with industry. Coalitions and caucuses have been formed to speak with a stronger voice to advocate for patient care, research, and other issues important to our members. Collaborators include the American Society of Nephrology (ASN), the Renal Physicians Association (RPA) and the National Kidney Foundation (NKF). As an example of promoting public policy and patient care issues, the ASPN, NKF, RPA, and ASN each submitted testimony to the U.S. Congress related to the safety concerns of Erythropoiesis Stimulating Agents (ESA) dosing in June 2007 following the warnings issued by the FDA for ESA. ASPN also joined with the RPA, American Association of Kidney Patients (AAKP), and the Renal Support Network (RSN) in submitting testimony to the FDA related to appropriate use and labeling of ESA and assuring that "black box" warnings are patient- and population specific. These joint efforts among the nephrology societies have been very successful in advocating for adult and pediatric patients with kidney disease.

ASPN, ASN, NKF, and RPA joined together to recognize World Kidney Day, 8 March 2007, with an editorial authored by the current president of each of the organizations, to disseminate the message that chronic kidney disease is common, harmful and treatable (3). ASPN joined the ASN and NKF in promoting World Kidney Day, 13 March 2008, with several representatives from each of the organizations visiting lawmakers and staff to discuss the growing epidemic of kidney disease and to advocate for expansion of NIH-funded kidney disease research and surveillance and prevention programs.

Political issues in Canada differ significantly from those in the United States. Health care in Canada is funded by the provinces, consistent with federally established, overriding principles involving universal access to health care. Given that the political/governmental systems are quite different, the role of public lobbying as a contributor to governmental decisions also is very different.

Research in Pediatric Kidney Disease in North America

A major mission of the ASPN is to promote clinical and basic science research activities in the pediatric nephrology community and to participate in the development of new strategies for encouraging and supporting research by pediatric nephrologists. In the late 1990s, the U.S. House of Representatives and the Senate encouraged the NIDDK to develop and implement plans to address the special research needs of children with kidney disease. A task force comprised of NIDDK and ASPN representatives who are experienced in basic, clinical and translational research analyzed the research needs in pediatric nephrology and produced a document ("Research Needs in Pediatric Kidney Disease, 2000 and Beyond") that has served as a blueprint for implementing several NIH-supported,

multicenter observational studies, clinical trials and registries related to pediatric nephrology.

Widespread participation in these studies is critical to their success, given the limited number of patients with a specific disease at any single center. Accordingly, the NIDDK has sought to expand support of investigator-initiated, multicenter clinical studies to systematically investigate the causes, early diagnoses, improved treatment and, where possible, prevention of kidney diseases endemic to the pediatric population. A successful mechanism that has evolved to support such research is that of the multicenter clinical cooperative agreement (U01). This cooperative agreement is characterized by substantial NIH programmatic involvement with the awardees in a partnership role. The UO1 consortia generally include Clinical Centers (CC), responsible for subject recruitment and development and implementation of a common protocol, and a Data Coordinating Center (DCC), which organizes the Clinical Centers, specifically focusing on the biostatistical analyses and data management aspects of the clinical trials. NIDDK encourages investigator-initiated research project applications for ancillary studies to these ongoing, Institute-supported clinical trials, epidemiological studies and disease databases.

The NIDDK has supported three pediatric multicenter studies in recent years, including the (1) Clinical Trial in Children and Young Adults with FSGS (FSGS-CT); (2) Prospective Study of Chronic Kidney Disease in Children (CKiD); and (3) Clinical Study of Vesicoureteral Reflux in Children (RIVUR). Preliminary findings from the observational studies over the next several years will likely inform the designs of clinical trials in pediatric kidney disease patients in the near future.

The *FSGS-CT* (http://fsgstrial.org) began as a Phase III, randomized clinical trial of therapy of biopsy-proven focal segmental glomerulosclerosis (FSGS) in children and young adults. This ongoing study, aimed at comparing the efficacy of treatment with cyclosporine (CsA) to treatment with mycophenolate mofetil (MMF) combined with oral pulse dexamethasone in patients with steroid-resistant FSGS, is being conducted at 65 participating sites in North America which are divided among three core networks with principal investigators, study coordinators, and a central DCC. Eligible patients are randomly assigned to one of the two active treatment arms. The target period for each therapeutic intervention is 12 months. Both study groups are also treated with either lisinopril or losartan for 18 months and low dose, alternate-day steroids for 6 months. The primary outcome to be measured is attainment of partial or complete remission of proteinuria and the main secondary outcome is the persistence of remission in patients who achieved a complete or partial remission after 12 months on therapy, 6 months following withdrawal of immunosuppressive agents. Patient enrollment began in November 2004 and ended in May 2008; the total randomized sample size is 138. To date, 17 ancillary studies have been approved, of which 11 have received NIH support. It is expected that the results of this study, the largest FSGS randomized trial ever to be performed, will establish a standard of therapy for steroid-resistant primary FSGS. Additional benefits of the trial are the establishment of an infrastructure for the study of FSGS, the creation of a national repository of biospecimens for investigations on the pathogenesis of FSGS and the role of histological subclassifications of FSGS in the response to therapies, and the evaluation of the efficacy of withdrawing immunosuppressive drugs while maintaining ACE inhibitors/ARBs. A logical extension of the trial is the initiation of a Pediatric Nephrology Clinical Trials Group to facilitate new investigations, innovations and faculty development in translational research.

The *CKiD* study (http://www.statepi.jhsph.edu/ckid/), begun in October 2003, is a prospective observational cohort study aimed at defining risk factors for chronic kidney disease (CKD) progression and the effects of CKD progression on cognition, behavior, cardiovascular risk factors and growth (4, 5). This study, being conducted at 44 participating sites in North America led by an East Coast and Midwest Clinical Coordinating Center, a Data Coordinating Center, and a Central Biochemistry Laboratory, has enrolled 574 children ranging in age from 1 to 16 years, all with moderately impaired renal function (GFR 30–90 ml/min/1.73 m^2); the enrollment period, which began in April 2005, ended in March 2008. The study protocol consists of annual study visits; as of May 2008, 548 baseline studies and 442 6-month, 333 1-year-, 131 2-year-, and 6 3-year follow-up visits have occurred. Using iohexol plasma disappearance and a newly developed GFR-estimating equation (6, 7), CKiD has demonstrated improved precision and accuracy in GFR measurement and estimation in children. Associations between known risk factors for CKD progression (e.g., proteinuria) and level of GFR have been demonstrated, as have associations between low levels of GFR and known complications of CKD in children, including growth failure, anemia (8), hypertension, abnormalities of ambulatory blood pressure monitoring, left ventricular hypertrophy, and deficits in attention and quality of life. The collection of biological samples for banking at study entry and annually at follow-up will provide a valuable resource to explore other risk factors. To date, 14 ancillary studies have been approved of which four have received NIH

support. It is expected that the extension of CKiD (CKid II) will allow for an analysis of additional novel risk factors for CKD progression and associated morbidity, including low birth weight, inflammation, environmental factors, and nutritional Vitamin D deficiency.

The most recent study to be launched is the *RIVUR* study (http://www.cscc.unc.edu/rivur/). This multicenter, randomized, double-blind, placebo-controlled trial is designed to determine whether daily antimicrobial prophylaxis with trimethoprim and sulfamethozazole (TMP/SMZ) is superior to daily placebo in children less than 6 years old with grade I–IV vesicoureteral reflux (VUR) in reducing the frequency of urinary tract infection (UTI) and renal scarring (9). Patients are randomly assigned to treatment for 2 years with daily TMP/SMZ or placebo. The study is designed to recruit 600 children (approximately 300 in each treatment group) ranging in age from 2 months to 6 years over an 18–24 month period. The primary endpoint is recurrence of UTI. In addition, patients will be evaluated for secondary endpoints related to renal scarring and antimicrobial resistance. Scarring will be determined based on renal scintigraphy by 99mTc dimercaptosuccinic (DMSA) scans. Dysfunctional voiding symptom score, constipation surveys, quality of life, compliance, safety parameters, utilization of health resources, and change in VUR will be assessed periodically throughout the study. Currently there are a total of 15 clinical sites in North America participating in the study and these include five core sites. The patient recruitment started in late 2007 and about 120 patients have so far been enrolled in the study. The study involves pediatric nephrologists, pediatric urologists and primary care pediatricians, and the patients are being recruited from private offices, hospital admissions, emergency room visits, and nephrology or urology consult referrals.

The NIH has also fostered research related to pediatric kidney disease by supporting three Interdisciplinary Research Centers of Excellence. Two new Centers focus largely on polycystic kidney diseases (PKD), the most common renal genetic diseases of adults and children. The Research Center of Excellence in Pediatric Nephrology at the Medical College of Wisconsin (P.I. Ellis D. Avner, MD; http//:www.chw.org/research) focuses on delineating of the pathophysiology of PKD, with an ultimate goal of developing new therapeutic targets for clinical trials. Because events in the pediatric age range are critical for determining the outcome of kidney disease, investigators are examining early cellular and molecular abnormalities in PKD which may be amenable to therapeutic intervention. The genetic and metabolic determinants of glomerular and systemic hemodynamics in ischemic renal disease and PKD are being studied in an effort to understand how acute and chronic renal injury in childhood progress to end-stage disease. The ultimate goal of these studies is to identify mechanisms to delay or prevent the loss of kidney function in genetic and acquired renal diseases. A unique feature of this Center is a robust Pilot and Feasibility grant program, which was specifically developed to draw new investigators into childhood kidney disease research. At the University of Alabama at Birmingham (UAB) Recessive Polycystic Kidney Disease (RPKD) Core Center, (P.I. Lisa Guay-Woodford, MD; http://www.rpkdcc.uab.edu/), the main objective is to stimulate interdisciplinary research into the diagnosis, pathogenesis, and therapeutics of ARPKD. To stimulate such research, the Core makes PKD research technologies and methodologies, intellectual resources, and research infrastructure available to local and national investigators. These resources may be applied to new multidisciplinary projects, interactions, and collaborations. It further extends an observational study of ARPKD initiated by the North American ARPKD Database, provides a mechanism for genetic evaluation of unusual recessive PKD phenotypes, and provides educational tools for physicians and patients regarding the natural history, pathogenesis, and genetic testing in ARPKD. A unique product of this Center is the development of a HIPAA-compliant, web-based portal for data entry, which makes use of a unique data collection instrument that could serve as a model for other databases once its effectiveness has been proven. This instrument makes use of an IRB-approved algorithm for data entry that obviates the need for the contributing physician to obtain local IRB approval. The Research Center of Excellence in Pediatric Nephrology at Vanderbilt University is now in its 17th year of funding, and is focused on the Fibrotic Sequelae of Childhood Renal Disease (P.I. Agnes Fogo MD; http://www.mc.vanderbilt.edu/research).This Center focuses on the basic pathophysiology of various aspects of CKD, including tubulointerstitial fibrosis, podocyte injury and repair, atherosclerosis, and chronic inflammation. Such studies continue to provide novel insights about progressive childhood renal injury and potential therapeutic interventions.

In addition to its registry components, *NAPTRCS* initiated randomized, prospective clinical trials in the mid 1990s. These include a study of OKT3 induction therapy in children and adolescents undergoing renal transplant, trials to investigate the use of growth hormone in dialysis patients, transplant patients and children and adolescents with chronic kidney disease, and recent randomized trials designed to evaluate potential complete withdrawal of steroid therapy in transplant patients. NAPRTCS has served to document

and influence practice patterns, clinical outcomes, and changing trends in renal transplantation and the care of children with CRI. Information about NAPTRCS, including a bibliography of the numerous publications generated from this cooperative study, can be found at https://web.emmes.com/study/ped/announce.htm.

Each year, the ASPN Research and Public Policy Committees generate, with input from the general membership of the Society, legislative language summarizing priorities for research related to the care of children with kidney disease. The statement for fiscal year 2009, inserted into the *Congressional Record* by members of Congress, identifies the urgent need to promote translational and clinical research aimed at understanding the mechanisms involved in kidney injury and progression, a crucial step to develop and test new interventions and therapies in children. Additional areas of high interest for the pediatric nephrology community of North America are understanding factors that contribute to, or impede, patient adherence to treatment regimens; transition from pediatric to internal-medicine caregivers; and developing a stable infrastructure for clinical research in pediatric kidney disease.

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82 Africa

Felicia U. Eke

Africa remains the world's poorest and most underdeveloped continent. The continent includes 53 countries including all the island groups. Despite many similarities, African nations are heterogeneous, with varying cultural and socioeconomic differences.

The African Pediatric Nephrology Association, AFPNA is the official representative of the International Pediatric Nephrology Association in Africa. It has currently 71 members from 29 African countries. There are no members from 24 countries. AFPNA was born in 1999, and affiliated with IPNA in 2000. Its first congress was in Cairo in March 2000, with the subsequent congresses in Nigeria (2002), and South Africa (2006). Dr Bahia Moustafa, Director of Pediatric Nephrology, Cairo University Egypt was the initial Secretary General of AFPNA, with Egypt, Nigeria, Libya, Sudan, Mali, Cameroon, and South Africa constituting the founding countries which developed AFPNA's Constitution. Through 2000–2006 Prof. Moustafa represented AFPNA at IPNA council and two additional African IPNA councilors were elected to represent Central Africa (Prof Felicia Eke; who was also elected Secretary General in 2006) and South Africa (Dr Mignon McCulloch). AFPNA supports a newsletter, workshops, and training programs. Communication with members and global colleagues, and updates of activities are regularly reported via AFPNA's website (http://www.afpna.org). AFPNA maintains three regional training centers in Cairo, Cape Town and Johannesburg. With regular recruitment of new members, it is hoped that all 53 countries of Africa will be represented in AFPNA by the next IPNA International Congress in 2010 (New York, USA).

Many children with kidney disease in Africa do not receive appropriate medical care, and there are critical shortages of Pediatric Nephrologists (PN) and resources in many African countries. In Egypt there is one PN per 500,000 child population, in Nigeria there is one PN per 4.5 million children and in South Africa there is one PN per 1.5 million. Therefore pediatricians and general practitioners commonly treat children with kidney disease (1). In the last two decades pediatric nephrology units have developed throughout the continent. They are concentrated mainly: (1) in the northern region in Egypt, Tunisia, Algeria, Libya, and Morocco; (2) in the eastern region in Kenya, Sudan, and Ethiopia; (3) in the western region in Nigeria and Cameroon; and (4) in the southern region in South Africa. Most of these units provide primary and secondary renal care. Some provide tertiary care (i.e., comprehensive dialysis and transplantation). In North Africa dialysis and transplantation programs are well developed; however, they are run by internist nephrologists in many areas because of the high ratio of internist nephrologists to pediatric nephrologists. This situation is slowly changing as more PNs are being trained. In Egypt, university-related pediatric nephrology units act as regional centers for training and treatment. There are pediatric hemodialysis units in national hospitals. National medical insurance covers the costs for patients treated in university or national hospitals.

In the east, only Kenya has organized renal services including peritoneal dialysis, hemodialysis, and renal transplantation. In the western and central zones, Nigeria is the largest and richest country. At this writing, there are 13 PNs serving a child population of approximately 80 million. Six hundred of these children are expected to develop end-stage renal failure per year. There are 20 adult hemodialysis units, with variable experience in treating children. There are three transplantation centers, but no child has received a transplant to date. Children requiring hemodialysis are dialyzed in adult units; peritoneal dialysis is available for children with acute renal failure (ARF). Continuous ambulatory peritoneal dialysis (CAPD) is not undertaken. There are no national medical insurance programs which cover the expense of end stage renal therapy. Children with chronic renal failure (CRF) whose parents can afford the costs travel to other regions for dialysis and transplantation. In South Africa, despite the shortage of trained pediatric nephrologists, there are several centers that provide secondary levels of renal care, and most have facilities and expertise for peritoneal dialysis. Care for children with end-stage renal disease (including dialysis and transplantation) is centralized in three major centers: Cape Town, Johannesburg, and Durban. In Durban, renal replacement therapy is limited to older children, but with the recent commissioning of the Inkosi Albert Luthuli Central Hospital, tertiary and quaternary renal care will be offered to all age groups.

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The policy of the country is to centralize quaternary care to limited designated sites for the entire country, such that provision of renal replacement therapies will remain limited to a small number of centers.

Childhood Renal Disease in Africa

The profile of renal diseases prevalent in Africa is unique with respect to etiology and clinical presentation. Endemic infections are a major cause of many renal diseases. The low socioeconomic status in many areas, coupled with the lack of clean water (secondary to poor sanitation and irrigation systems), and overcrowded housing promote endemic infections such as schistosomiasis, malaria, tuberculosis, hepatitis, streptococcus, salmonella, shigella, and filarial. A number of social and political factors, including poor control of drug abuse and prostitution, contribute to a high rate of HIV and other infections. Other factors contributing to the unique pattern of renal diseases in Africa includes use of alcohol, drugs of abuse, herbal and traditional medicines, toxins from bites, and local environmental pollutants. Obviously the dry environment and tropical climate with inadequate water supply leads to a high incidence of nephrolithiasis in the population. Racial differences are also seen particularly in South Africa where blacks are more prone to severe renal diseases.

Clinically, infection and malnutrition commonly complicate the presentation and treatment of renal disorders. Infection either may be the primary cause of kidney disease or may be acquired during treatment. The higher morbidity and mortality observed in African children with renal disease can often be attributed to late diagnosis and late referral to specialized units. Moreover, the poor intradialyic care children receive because of limited resources or insufficient staff explains the poor quality of life compared to that of similarly treated children in developed countries. The most prevalent renal disorders in Africa include nephrotic syndrome (NS), urinary tract infections (UTIs), acute renal failure (ARF) and chronic renal failure (CRF), (*Fig. 82-1*).

Nephrotic Syndrome (NS)

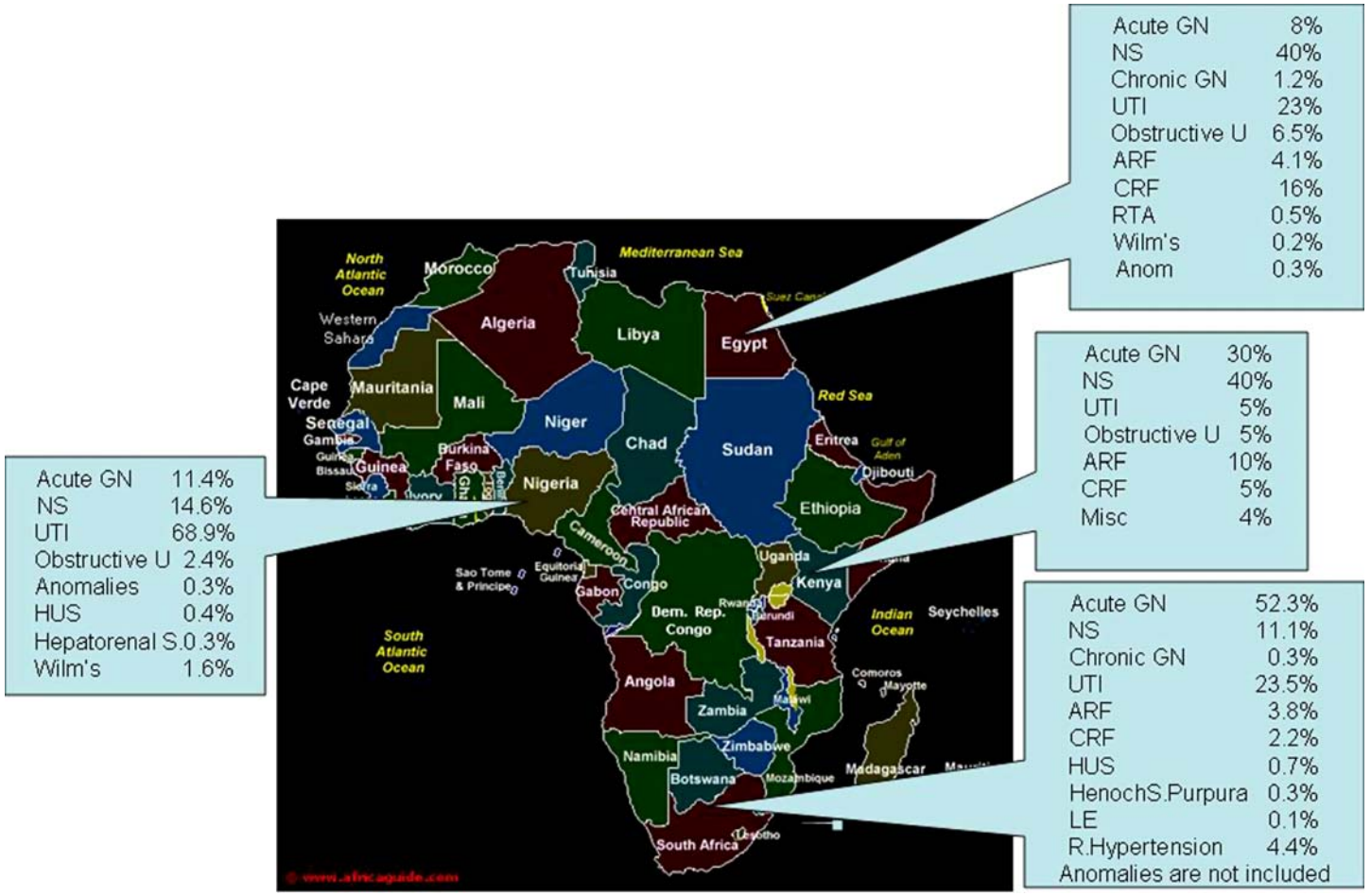
NS (see Chapters 27–28 Nephrotic Syndrome) is the most common childhood renal disorder in Africa. It accounts for 14.6% of renal disorders in eastern Nigeria (2), 40% in Sudan (3), and 40% in Egypt. In North Africa etiologies resemble those in western countries: minimal change NS (MCNS) constitutes 88% in Egypt, 85% in Libya (4), and 37% in Sudan (3). Steroid responsiveness is common in Egypt. Frequently relapsing nephrotic syndrome (FRNS) and steroid-dependent nephrotic syndrome (SDNS) occur in 42% and 36% of MCNS in Cairo and 21% and 15% in Mansoura, respectively. Relapse is often associated with noncompliance with steroid therapy or superimposed infection. Mortality, often from infections, is approximately 2%. In Egyptian children with other pathology underlying NS, focal segmental glomerulosclerosis (FSGS) occurred with equal prevalence as mesangial proliferative glomerulonephritits (MPGN) (22.7%) in 394 biopsies analyzed over 10 years but FSGS predominated in the 1980s (5).

Secondary forms of NS are uncommon in North Africa accounting for 6.6% of cases (4). The most common is postinfectious schistosomal nephropathy, which is endemic in the Nile delta in Egypt, Ethiopia, and Sudan. The causative parasite is Schistosoma haematobium which together with Schistosoma mansoni is also found in East, West, and Central Africa. Such patterns correlate with prevalence of the snail host and poor hygiene. Infected children present with haematuria, glomerulonephritits, NS, or renal failure. Hepatosplenomegaly with the presence of bilharzial ova in urine or stools commonly point to the diagnosis. Patients demonstrate unselective proteinuria, marked reduction in total serum levels of proteins with a characteristic high gamma globulin fraction, normal serum cholesterol level, normal to low serum complement profile, low urine osmolality, and normal to low glomerular filtration rate (GFR). Many such patients are chronic Salmonella carriers with intermittent bacteriuria, especially those with refractory anemia and persistent fever. Urinary tract imaging may reveal bladder calcification and/ or obstructive uropathies and vesicoureteral reflux (VUR); renal biopsies show diffuse MPGN (20%), membranous nephropathy (12%), focal MPGN (7%), or mesangiocapillary glomerulonephritits (18%). Different immune mechanisms have been implicated in the pathogenesis of these lesions (6). The recent aggressive use of chemotherapy among school children and extensive snail eradication have led to a fall in schistosomal infections. There are hopes for the development of a schistosomal vaccine (7).

Hepatitis B accounts for 44% of secondary forms of NS in Egypt; Hepatitis C is uncommon. Tuberculosis and filariasis are now less prevalent in the northern region. Lupus erythematosus nephropathy is found in 8%. Sickle cell nephropathy is rare in North Africa although the disease is not rare. Ochratoxins and aflatoxins, which are

Figure 82-1

Renal disorders in northern, eastern, western and southern Africa. *Anom.* anomalies; *ARF* acute renal failure; *CRF* chronic renal failure; *GN* glomerulonephritis; Henoch S. Purpura, Henoch-Schonlein purpura; Hepatorenal S. hepatorenal syndrome; *HUS* hemolytic uremic syndrome; *LE* lupus erythematosus; *Misc.* miscellaneous; *NS* nephrotic syndrome; *R.* renal; *RTA* renal tubular acidosis; *U.* uropathy; *UTI*, urinary tract infection (Data are collected from most developed pediatric nephrology units in Africa, Cairo University Children's Hospital, Egypt; Kenyatta National Hospital Nairobi, Kenya; King Edward VIII Hospital Durban, South Africa; University of Port Harcourt Teaching Hospital, Nigeria.



common constituents of food in Africa, were detected in urine, serum, and kidney biopsy specimens of children with NS or renal failure in Tunisia and Egypt (8). Cadmium and aluminum which are common environmental pollutants in Africa were recorded as toxic causes for NS in Africa. Although specific data are not available, it is a clinical impression that HIV nephropathy is a largely undiagnosed cause of NS and renal failure in African children as noted below.

In west and Central Africa the profile of NS is unlike that of the Western world. Idiopathic NS is not common. When it affects black children it is often associated with a high rate of steroid unresponsiveness. This is true in Togo, West Africa (9), western Nigeria (10), northern Nigeria (11), Uganda (12), and Zaire (13). In Port Harcourt, South Eastern Nigeria (14), and Yaounde, Cameroon (15), however, steroid responsiveness is not uncommon. Infections play a major role in the pathogenesis of NS in west and Central Africa (11). Quartan malarial nephropathy, first described in Ibadan, western Nigeria, was detected in up to 81% of renal biopsy specimens in children (10) in the 1970–1980s. Quartan malaria; nephropathy is predominantly associated with *Plasmodium malariae* and also with *Plasmodium falciparum* infection. Typically there is focal and segmental glomerulonephritits with thickening of capillary walls but without endothelial cell proliferation. Finding of P. malariae or P falciparum antigen in the glomeruli by, immunofluorescence is diagnostic. Malaria induced acute renal failure is an important complication of malaria infection. Recent studies however have questioned a dominant role of a chronic malaria glomerulopathy (16). In eastern Nigeria, MCNS is the most common pathologic diagnosis in cases undergoing biopsy. In Nigeria the recent upsurge of HIV infection has been associated with HIV nephropathy mainly from vertical transmission but manifesting with NS or CRF around the age of 7–10 years (17). Other infections reported in Zimbabwe, West and Central Africa include hepatitis B and C with membranous nephropathy (18). NS often complicates post streptococcal glomerulonephritits. Schistosomiasis, filariasis, and leprosy are also recorded.

In eastern Nigeria, sickle cell nephropathy is common and sickle cell anemia is common with a heterozygous carrier rate of 25%. FSGS and renal papillary necrosis are explained by endothelial damage caused by occlusion by sickled cells and microinfarcts. There are numerous cases of unexplained renal pathology, with NS possibly caused by toxins found in herbal remedies or environmental contamination.

In South Africa the epidemiology of NS disease has undergone considerable change since first reported during the 1970s and 1980s, (*Table 75.E1*). Black children had a paucity of minimal change disease and the majority was steroid resistant (19). Indian, white and children of mixed race had MCNS similar to that reported from developed countries with a high rate of steroid responsiveness. Membranous nephropathy with a strong male predominance was seen in 40% of black children in Durban; FSGS in up to 50% in Johannesburg; MCNS in black children accounted for only 14% of all cases of N.S (20–22). The introduction of hepatitis B vaccine in April 1995 dramatically reduced the incidence of Hepatitis B virus associated nephropathy.

Another interesting finding is the distinct difference in the response to therapy in black and Indian children with steroid resistant nephrotic syndrome. While over 80% of Indian children with steroid resistant nephrotic syndrome responded to a trial or oral cyclophosphamide and prednisone therapy, none of the black children achieved remission. Black children also showed a poorer response to other forms of intensive therapy (intravenous methylprednisolone, intravenous cyclophosphamide, cyclosporine and tacrolimus) (23). Steroid resistance in none of the nonblack children predicted FSGS as 50% had MCNS on biopsy. Seventy percent of Indian children with SRNS responded to oral cyclophosphamide whereas none of those with focal segmental glomerulosclerosis on histology responded to therapy.

Table 82-1

Histologic pattern of nephrotic syndrome before and after 1995 in Durban, South Africa

Histologic findings	1976–1994	1995–2000
Minimal change disease	95	11
Focal segmental glomerulosclerosis	41	43
Membranous nephropathy	44	6
Membranous nephropathy (hepatitis B associated)	63	24
Diffuse mesangial proliferative glomerulonephritits	26	1
Focal mesangial proliferative glomerulonephritits	13	2
Mesangiocapillary glomerulonephritits	4	1
Total	286	88

Urinary Tract Infections (UTI)

UTIs (See Chapter 54 UTI) are common in Africa, in both rural and urban areas and among neonates, preschool children, and school – aged children. Symptomatic UTIs show a prevalence of 22% in Egypt; while the presence of asymptomatic bacteruria is 4.2% in girls and 2.8% in boys among schoolchildren in urban Egypt and 11% in girls and 3.6% in boys in rural Egypt (24). In Central and West Africa, UTIs have an incidence from 8.2 to 72% in symptomatic children (25) and 48% in an asymptomatic rural community. In Cape Town, approximately 1,000 cases of UTIs are seen yearly. Because of the nonspecific complaints in neonates and young children with UTIs, many cases are missed, especially in rural areas. The microbiology of UTIs in Africa is consistent over the continent (26). In Nigeria the predominant organism is *Klebsiella* resistant to amoxicillin and cotrimoxazole (26); in Egypt *Escherichia coli* is found in 70%, *Proteus* 10% *Klebsiella* 5%, and *Pseudomonas* 5% (27). Schistosomiasis is considered a common predisposing factor for UTIs in Egypt, Senegal (28) and Cameroon (29). Stones, developmental anomalies of the urinary tract, and VUR are common underlying common structural lesions which predispose children to UTIs with prevalences of 2.5, 12, and 17%, respectively in Egypt. VUR is rare in West and Central Africa whereas posterior urethral valves and meatal and urethral strictures are reported to have an incidence of 29.3% among children with UTIs (26). In South Africa, local predisposing factors include malnutrition, congenital anomalies, immunodeficiency states, and VUR (29). The latter is rare in black children but common in whites (30). Renal growth and GFR are commonly affected in African children with UTIs because of late diagnosis and referral, as well as patient noncompliance with treatment. For example, impaired renal growth was reported in 36% of cases referred to the Cairo University unit in a recent unpublished analysis. *E. coli* strains resistant to amoxicillin and co-trimoxazole are commonly reported in Africa. For example, 88 and 86% resistance to these drugs, respectively, is seen in Cape Town.

Acute Renal Failure (ARF)

ARF (see Chapter 64–66 ARF) has unique epidemiology in African children. Infection is the major cause. Bacterial infections commonly reported include streptococci, cholera, salmonellosis, shigellosis, leptospirosis, tetanus, and diphtheria. Viral infections include rotavirus, HIV infection, hepatitis A, B and C, and cytomegalovirus infections. Common parasitic infections are malaria and schistosomiasis. Among all pathogen-related causes, diarrhoeal diseases, schistosomiasis, and malaria remain the most common. Infection precipitates ARF through immune mechanisms and alteration of kidney hemodynamics. Toxins after snakebites, scorpion or other insect stings, or following ingestion of herbal medicines or illicit drugs are also unique causes of ARF in Africa. *Table 82-2* summarizes causes of ARF in Egypt, Nigeria (31), and South Africa (32). Septicemia, gastroenteritis, and hemolytic uremic syndrome (HUS) are common causes in the three regions. Epidemic forms of ARF after use of native herbal medicines and "holy water" were reported in Nigeria (33). In Sub-Saharan Africa an epidemic of shigella dysentery type1 occurred in 1994–1996 starting in Burundi and progressing to the Cape (32). One hundred and fifty-nine cases of HUS occurred in black children during this epidemic. Among 81 cases of post-Shigella-induced HUS in Durban, complications included ARF in 90.1%, encephalopathy in 37%, convulsions in 14.8%, hemiplegia in 2.3%, intestinal perforation in 9.9%, protein-losing enteropathy in 32.1%, toxic megacolon in 4.9%, rectal prolapse in 6.2%, hepatitis in 13.6%, myocarditis in 6.2%, disseminated intravascular coagulation in 21%, CRF in 32.1%, impaired renal function in 9.9%, ESRD in 1.2%, and death in 17.3% (32). Since 2000, *E. coli*-induced HUS, especially coexistent with HIV infection, has been increasingly reported in Cape Town.

Chronic Renal Failure (CRF)

The pattern of CRF(see Chapter 68–71 Chronic Renal Failure) in African children is unique with respect to its etiology, clinical presentation, and management. Causes of CRF in Egypt, Nigeria (2), and South Africa are summarized in *Table 82-2*. Obstructive uropathies and reflux nephropathy are prevalent causes in Egypt; glomerulonephritis in Nigeria (34) and South Africa. Some uncommon causes of CRF such as Takayasu arteritis (35) and cystinosis are found predominantly in South African blacks. The recent pandemic of HIV/AIDS in sub Saharan Africa has resulted in increasing cases of CRF from HIV nephropathy (17). Late referral of cases is common. Signs of CRF in African children are aggravated by four distinct elements: infection (whether causing CRF or acquired during dialysis), malnutrition (especially deficiencies of iron, vitamin D, and trace elements), late diagnosis and poor management (which aggravate anemia, growth retardation, and bone disabilities).

Table 82-2

Causes of acute and chronic renal failure in selected locations in Africa

	Egypt (%)		Nigeria (%)		Cape Town (%)		Durban blacks (%)	
Acute renal failure								
Septicemia and urinary obstruction		21		16		24.1		–
Gastroenteritis	27		34		7.4		–	
Poststreptococcal glomerulonephritis		29		12				–
Lupus erythematosus		12				–		–
Hemolytic uremic syndrome		6		11		9.3		–
Nephrotoxins	3		–				–	
Unknown	2		5		–		–	
Acute glomerulonephritis	–				–		–	
Malaria	–		10		–		–	
Birth asphyxiation	–		12		–		–	
Post cardiac surgery		–		–		16.7		–
Myocarditis	–		–		11.1		–	
Rapidly progressive glomerulonephritis		–		–		9.3		–
Necrotizing enterocolitis		–		–		7.4		–
Kwashiorkor	–		–		7.4		–	
Leukemia	–		2		7.4		–	
Chronic renal failure								
Reflux nephropathy	15		–		–		–	
Glomerulonephritis	26		72		–		45	
Unknown	28		14		–		–	
Urinary obstruction	31		7		–		–	
Pyelonephritis		–		7		–		–
Focal segmental glomerulonephritis		–		–		–		
Anomalies	–		–		–		26	
Other causes	–		–		–		4	

HIV Related Chronic Kidney Disease (see Chapter 52 HIV nephropathy)

Following the onset of the HIV epidemic in the late 1980s, over three million children are now infected in sub-Saharan Africa. KwaZulu-Natal is one of the epicenters of the epidemic. To date Durban has 37 patients with HIV-related renal disorders. Renal biopsy undertaken in 13 black patients showed FSGS in 11: membranous in one. Fifteen biopsies in Cape Town showed HIV associated nephropathy, HIVAN in 13, mesangioproliferative glomerulonephritis in two and tuberculous granulomata complicating one case. In Nigeria, most cases with vertical transmission present approximately at the age of 7–10 years with NS or CRF with increased echogenicity and strikingly bright appearance on ultrasound. Histology in a recent series was predominantly collapsing glomerulonephritis and markedly dilated tubules and tubular cysts (17).

Dialysis and Transplantation (See Chapters 72–77)

Acute peritoneal dialysis is the most common type of dialysis in Africa and is more readily available than acute hemodialysis. Chronic intermittent peritoneal dialysis is the most common technique used in end stage renal failure (ESRF). The use of continuous cycling peritoneal dialysis is less common. In developed areas such as Cape Town, however, use of CAPD is predominant. Efficiency

of dialysis varies among pediatric nephrology centers. It depends on the availability of funds as well as trained staff. Many children with ESRD experience poor quality of life, dialyzing once a week. Factors that contribute to their poor outcome include deficient dialysis, malnutrition, and lack of appropriate interdialytic care due to unavailability of erythropoietin, iron, active vitamin D, and growth hormone (1).

Although renal transplantation is more cost effective than dialysis, it is available for children only in South Africa (27) Egypt, Tunisia, Morocco, Libya, Algeria, Sudan, and Kenya. Nigeria has transplanted adults since 2000 but no child has been transplanted to date. Since 2004 there have been an average of 10–15 renal, and 5–10 liver transplants yearly in children at the Red Cross Children's Hospital in Cape Town (personal communication). Ethical and religious factors in each country determine whether cadaveric or living donor grafts are used. In South Africa, cadaver organ-based programs are widely used, whereas in Egypt, only living related donor-based programs are permitted. Morbidity and mortality of patients undergoing renal transplantation is higher in Africa than in developed countries, due mainly to infections, lack of compliance with therapy, lack of funds for immunosuppressive and other therapy, and lack of trained staff.

Most African countries lack national kidney foundations as well as medical insurance systems. To date there is no national Registry though some individual centers have a Renal Registry (2). Funding for ESRD therapy remains a significant handicap for most African countries. Prevention of ESRD is the best strategy when approaching the problem of CRF in Africa. Simple measures include health education to combat unhygienic habits and inappropriate use of traditional remedies, many of which are nephrotoxic. Additional measures include infection control through antischistosomal and antimalarial campaigns; early treatment of streptococcal infections and obligatory vaccination against tuberculosis and hepatitis. Screening for renal disease among school children might identify patients early in the course of the disease and maximize appropriate intervention.

The previously described development of the African Pediatric Nephrology Association (with its affiliation to the International Pediatric Nephrology Association) affords new opportunities for upgrading the practice of pediatric nephrology in Africa. Training programs supported by IPNA exist in Cape Town and Johannesburg and more are envisaged in Northern and Central Africa to increase the number of trained pediatric nephrologists. Shortage of specialists and trained staff as well as restricted financial resources remain a major challenge in many areas. There has been a recent upsurge in the development of regional African pediatric nephrology societies such as the Egyptian Society of Pediatric Nephrology, the Nigerian Association of Paediatric Nephrology, the Moroccan Society of Pediatric Nephrology and the South African transplantation and pediatric nephrology groups. These societies as well as improved education offer promise for dramatic improvements in pediatric nephrology care in Africa over the next decade.

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83 Latin America

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Demography

The Latin America subcontinent consists of 20 nations in North, Central, South America and the Caribbean, in which the prevailing languages – Spanish and Portuguese-derive from Latin. It encompasses an extensive territory with many common characteristics and also with significant cultural and socioeconomic differences. Latin America's population is a composite of ancestries, ethnic groups and races, making the region, arguably, the most diverse in the world. Race composition varies from country to country and includes population of mixed, Amerindian, European and African ancestry. Most Latin American countries also have Asian minorities. Europeans and groups with part-European heritage make nearly 80% of the population (1).

Living conditions for the aboriginal inhabitants of the subcontinent are generally poor, while social imbalances are prevalent in urban areas. However, noteworthy features, such as hospitality, tolerance, and respect for ethnic differences, that characterize Latin America's population, come from the fusion between the ancient indigenous cultures and the European influences.

Concentrated primarily in urban areas (78%) and growing at an annual rate of 1.3%, Latin America's population reached 558 million in 2006. Life expectancy at birth is 72 years; in 2002 32% of the population was 14 years old or younger. By 2010 and 20020, however, the average age is expected to decrease by 28% and 25% respectively (2). The infant mortality rate (per thousand live births) for the 2005–2006 period is 24/1000, ranging from 7.2 in Chile to 45.6 in Bolivia (3).

Latin American Association of Pediatric Nephrology (Alanepe)

The Latin American Association of Pediatric Nephrology (ALANEPE) was founded in 1981 in Buenos Aires, Argentina, by 48 founding members. The organization's main objective is to promote the knowledge and science of pediatric nephrology and to improve the care of children affected with kidney diseases in Latin America. It also contributes, in conjunction with other similar societies affiliated to the International Pediatric Nephrology Association (IPNA), to the worldwide improvement of Pediatric Nephrology as a field of study (4).

From a practical/operational stand point of view ALANEPE is divided into three regions:

- Region 1: Mexico, Guatemala, El Salvador, Costa Rica, Nicaragua, Honduras, Panama, Caribbean islands, Cuba, Dominican Republic and Puerto Rico. (Despite being an English speaking country, Belize has been added due to its geographical situation).
- Region 2: Venezuela, Colombia, Ecuador, Peru, Bolivia
- Region 3: Chile, Argentina, Brazil, Paraguay, Uruguay.

The official Journal of ALANEPE is Pediatric Nephrology (the official journal of IPNA). However to accommodate languages spoken in the subcontinent, the publication of *Archivos Latinoamericanos de Nefrologia Pediátrica* [Latin American Archives of Pediatric Nephrology], was initiated in 2001, and is published three times a year both a printed and electronic journal. This Journal includes invited reviews, practical nephrology items, guidelines for common nephrological entities, news, and reports of meetings and other activities which take place in different countries in the region. The publication has increased the interest of pediatricians in the specialty, and has served as a continuing medical education tool for pediatric nephrologists and related professionals. Like the triennial ALANEPE congress, the Journal is a symbol of ALANEPE's commitment to its mission.

Pediatric Nephrology Development in Latin America

The development of Pediatric Nephrology in Latin America can be traced back to the 1950's, when Gustavo Gordillo created the Department of Pediatric Nephrology at Mexico City's Children's Hospital, and Carlos Gianantonio championed the specialty in the southern Argentina. Gianantonio dedicated extraordinary effort to understanding the basic pathophysiology, clinical course and

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management of hemolytic uremic syndrome (HUS), which is endemic in the region. Contemporaneously, Julio Toporovsky started the development of Pediatric Nephrology in Brazil. The influence of these pioneers promoted the development of pediatric nephrology centers in these countries that subsequently were emulated throughout Latin America. Their nephrology units became early educational centers for nephrologists from the entire region.

Additionally, the development and promotion of pediatric nephrology as a field of study in the subcontinent has been crucially influenced of other nephrologists, such as Pierre Royer, Renee Habib and Michelle Broyer (France), Juan Rodriguez Soriano (Spain), Cyril Chantler and T Martin Barrat (England), Ira Greifer (USA) and other North American nephrologists.

Currently, there are Pediatric Nephrology educational centers in Argentina, Brazil, Colombia, Chile, Mexico, Costa Rica and Venezuela. Some of them have achieved recognition from prestigious universities.

ALANEPE has grown from 48 to 500 members in the region. The emergence of Pediatric Nephrology in Latin America is well documented by the incremental number of Latin American abstracts submitted to the last six congresses of the International Pediatric Nephrology Association, from 22 in Jerusalem (1992) to almost 100 in Budapest (2007), and by the growing number of Latin American papers published over the past 20 years in Pediatric Nephrology.

Specific Areas of Clinical and Research Interest: Hemolytic Uremic Syndrome (HUS)

The world's highest incidence of HUS is reported in Argentina, with more than 14 cases per 100,000 children younger than 5 years of age. In this country, approximately 400 new cases are reported annually. Nearly all cases are secondary to infections caused by verotoxin-producing Escherichia coli O 157:H7. After Gasser's description in 1955, a study of 64 cases was published in 1964 by Gianantonio and co-workers. A seminal achievement was the implementation of peritoneal dialysis to treat acute renal failure in these children, which reduced the acute mortality from 50 to 5% (5).

The Nephrology Committee of the Argentinean Society of Pediatrics registered 7570 HUS patients in 11 public centers in Argentina between 1965 and 2001. In that period, mortality varied between 2 and 5.6% during the acute phase. There were no significant differences between November and March (summertime). The high incidence of HUS in Argentina is related to high rate of ingestion of contaminated, undercooked beef meat and its early introduction in young children's diets (6). Several studies are in progress in this region to understand aspects of HUS, such as the role of polymorphonuclear leukocytes in the pathophysiology of typical HUS (7), the functional state of neutrophils and its correlation with the severity of renal dysfunction in children with HUS (8), and the involvement of the fractalkine pathway in the pathogenesis of childhood HUS (9).

Collaborative Studies

National Collaborative Studies

Venezuela

The Pediatric Nephrology Chapter of the Venezuelan Society of Pediatrics and the Venezuelan Society of Nephrology reported epidemiological data on renal diseases in children in recent years.. Information was obtained from 14 centers with at least one pediatric nephrologist. A total of 3,624 patients were evaluated during a period of one year, either through a first outpatient consultation or at a first hospital admission.

The patients were grouped into the following categories: (1) urinary tract infection (32%), with detection of abnormalities of urinary tract in 25%; (2) metabolic disorders (28%) mainly idiopathic hypercalciuria and hyperuricosuria; (3) glomerulonephritis (9.5%), (4) urolitihiasis (7%), (5) nephritic syndrome (4.5%), (6) "primary" hematuria (4.2%), (7) acute renal failure (2.8%), with 43% of cases secondary to acute dehydration, 15% due to birth asphyxia, (14% secondary to septicemia, and 23% due to multiple factors), (8) chronic renal failure (1.6%) and (9) miscellaneous diseases (4.8%) (10, 11).

Recently a national study of renal transplantation in children has been published in Venezuela, reporting the experience of 25 years in the four centers that perform this technique in the country. Results showed that during the period 1982–2006 a total of 268 patients with end-stage renal disease received 275 renal transplants, the age of patients was 11.1± 7.3 years. 70% of the grafted kidneys were obtained from cadaveric donors and 30% from live related donors. Most important causes of mortality included: septicemia 34%, cardiovascular and acute hemodynamic complications 22%, metabolic complications 17%, hematological complications 12% and miscellaneous causes 8%. Graft lost was due to chronic rejection

in 60% of the cases, thrombosis of renal vessels 20%, recurrence of renal disease in the graft 13%, and acute tubular necrosis 7%. Actuarial survival of patients was: 98% at 6 months, 98% at 1 year, 90% at 3 years and 85% at 5 years. There was no statistically significant difference between patients transplanted from live donors and patients with kidneys from cadavers ($p > 0.05$). The first graft actuarial survival was: 97% at 6 months, 90% at one year, 80% at 3 years and 70% at 5 years. These results confirm the feasibility of kidney transplantation programs for the rehabilitation of children with end stage renal disease in Latin American countries, with satisfactory actuarial survival (12).

Chile

Chronic Peritoneal Dialysis in Chile – A Multicenter Study

A multicenter study of continuous ambulatory peritoneal dialysis (CAPD) in five centers was carried out in Chile: One hundred and twenty-nine children younger than 18 years of age were evaluated; 97 of them, with at least 6 months of follow-up, were included in the analysis. Causes of end stage renal disease were renal hypoplasia or dysplasia (26%), glomerulopathies (17%), reflux nephropathy (15%), obstructive uropathy (15%), vascular diseases (15%), and other disorders (12%). Staphylococcus aureus accounted for the majority of episodes of peritonitis, and the incidence of this complication was 0.67 episodes per patient per year, a rate similar to that reported in the literature (13).

Argentina

National Register of Chronic Renal Disease, Dialysis, and Transplantation

In 1996, Argentina initiated a centralized Registry of pediatric patients with end-stage renal failure undergoing conservative management, dialysis and transplantation. Registry data of 710 patients, under the age of 19 years demonstrated that the etiologies of chronic renal failure were: obstructive uropathy 18%, reflux nephropathy 16%, hemolytic uremic syndrome 14%, renal aplasia/dysplasia/hypoplasia 14% and focal segmental glomerulo sclerosis 9%. The incidence of end–stage renal disease was 6.5 patients/total million inhabitants. Of the patients on dialysis treatment, 63% were undergoing hemodialysis and 37% were treated with chronic peritoneal dialysis. 16% of patients received kidney transplants, with 46% from live donors (14).

Brazil

The experience in pediatric renal transplantation in various Latin American countries is growing, and several centers in Mexico, Colombia, Venezuela, Brazil, Chile and Argentina, among others (12, 14–18) perform routine kidney transplantation in children. In certain countries pediatric transplantation is positively impacted by the adoption of a priority policy for assignment of cadaver kidneys to pediatric patients (19, 20), however, renal transplantation is limited in young children. A collaborative study from Brazil reported the results in 38 children (40 transplants), ages 1–5 years, transplanted between 1989 and 2005. Mean age at transplantation was 3.3 ± 1.3 years, and mean weight was 14 kgs (range, 5.7–25 kgs); etiology of end-stage renal disease was uropathic/vesicoureteral reflux (45%) glomerulopathies (25%), congenital/hereditary diseases (10%), and hemolytic uremic syndrome (12.5%). Prior to transplantation, 5% were on hemodialysis, 85% on peritoneal dialysis, and 10% were performed pre-emptively. All children were followed for at least 6 months post transplantation, except two who died in the first month. In 75% of cases, kidneys were obtained from living-related donors and cadaveric in 25%. Thirty-nine kidneys were extraperitoneally placed. Primary immunosuppressant therapy consisted of cyclosporine (61%), tacrolimus (39%), mycophenolate (49%), and azathioprine (51%). A steroid-free protocol was used in 17% of patients. In the last 21 cases, basiliximab or daclizumab was added. There were 13 (32.5%) graft losses (4 artery/vein thromboses, 3 chronic rejections, 3 deaths, 3 other causes). The 5-year patient and graft survival rates were 89.6% and 72.2%. These data demonstrate that renal transplantation can be performed with good long-term results in children younger than 6 years old (21).

International Latin American Collaborative Studies

Hypercalciuria and Nephrolithiasis in Children

A study on the epidemiology of Nephrolithiasis was done, including 13 centers in Latin America. Eight hundred and seventy children were recruited; 552 (63%) were boys and 318 (37%) were girls. The age at which the disease was detected ranged from 2 months to 17 years. Only 4% of the subjects were asymptomatic. The initial symptoms reported most frequently were gross hematuria in 337 (39%) patients, abdominal and/or lumbar pain in 235

(39%) patients, hematuria associated with pain in 147 (17%). Additional findings were dysuria in 17 (8%), recurrent urinary tract infection in 146 (17%), and elimination of calculi in 28 (3%). Kidney malformations were found in 78 (8.9%) (22).

Given the epidemiological data showing a high frequency of hypercalciuria and urolitihiasis in several areas of Latin America (23, 24), several studies are in progress in order to determine the normal values of urinary excretion of calcium and other electrolytes, and aspects of bone and metabolic status of these children. Such studies will determine, for example: the role of the hypocitraturia as a risk factor for reduced bone mineral density in children with idiopathic hypercalciuria (25, 26).

Epidemiologic Aspects of Chronic Renal Failure in Latin American Countries

A study on the epidemiology of CRF in several Latin American countries (Argentina, Brazil, Colombia, Mexico, Uruguay, Chile and Argentina) was performed. Data demonstrated that the incidence of CRF shows a wide variability, ranging between 2.8 and 15.8 new cases per 1 million inhabitants.

The causes of CRF were glomerulopathies in 36%, obstructive uropathies and reflux nephropathy in 31% systemic diseases in 9%, and other disorders in 15%. The histopathologic entity most frequently associated with CRF is focal segmental glomerulosclerosis. HUS is an important cause of CRF -but not the most frequent- in Argentina (27).

Antibodies against Streptococcal Zymogens in the Sera of Patients with Acute Glomerulonephritis-A Multicenter Study

In this study, one hundred and fifty patients with acute poststreptococcal glomerulonephritis from Argentina, Chile and Venezuela were studied (10); 140 were male and 49 were female. The source of infection was the skin in 84 patients, the throat in 44, and unknown in the remaining 14. Furthermore, 23 patients with streptococcal infections without glomerulonephritis and 93 healthy controls were also studies.

Antizymogen and antiproteinase titers were determined. The conclusion was that Antizymogen antibody titer is the best available marker for streptococcal infections associated with glomerulonephritis (28).

Continuous Ambulatory Peritoneal Dialysis in Children: a Collaborative Analysis of Results in Latin American Countries

Data concerning clinical practice and long-term outcome on peritoneal dialysis (PD) in Latin America are scarce, although regional registries are increasing in number and quality. The prevalence of Hemodialysis (HD) and PD replacement therapy in this region, included in the Registry of the Latin American Society of Nephrology and Hypertension (adults and pediatric patients), was 198 and 81 patients per million population respectively, corresponding to 92,875 hemodialysis and 37,732 peritoneal dialysis patients (18). By extrapolation of local data, we assume that between 20–30% of the total PD population in Latin America represents pediatric patients; in Venezuela, among 500 PD patients, 31 are children, In Argentina, among 409 dialyzed children in 32 Medical Centers, 154 of them are being treated with peritoneal dialysis. In Chile and Uruguay, for 71 of 339 and 12 of 126 PD patients are children. The main underlying renal disorders for starting PD include: renal dysplasia, reflux nephropathy, hemolytic uremic syndrome, obstructive uropathy and chronic glomerulonephritis. In some countries like Chile, most of the patients are treated with automated modalities of PD, while in Venezuela at least a half of the patients are under CAPD regimen.

Currently, most of the PD programs include routine measurement of growth, dialysis dose (Kt/V) and Peritoneal Equilibration Test (PET) according to DOQI recommendations. Some of our Centers are performing routine measurement of Protein Equivalent of Urea Nitrogen Appearance (PNA) and other kinetic measures of dialysis. To date, the recommended values for those variables in the pediatric population are under revision. In that sense, local communications in Latin America have shown that the mean 4-h D/P creatinine ratio was 0.78 ± 0.02 and 0.74 ± 0.13 initially and at 12 months of follow-up respectively, and the mean D/D_0 glucose ratio was 0.35 ± 0.11 and 0.34 ± 0.08 during the same observation period. Nutritional studies have shown that, for PD children with a positive growth, the mean PNA was 1.31 ± 0.40, and the daily protein and caloric intake were 114.6 ± 31.5 (DOQI %) and 108 ± 15 (RDA %) respectively. Latin America represents a very important region in PD, although clinical practice and dialysis approach are heterogeneous throughout the different countries. These data present an overview of the current situation of pediatric PD in Latin America (29).

Latin American Registry of Pediatric Renal Transplantation

Recently this Registry was started under the coordination of C Garcia (Brazil), A Delucchi (Chile) and N Orta (Venezuela). The reporting of data takes place every two years. The first report showed data on renal transplants in 13 countries and the main conclusions from the registry, comprising 21 centers in Latin America, have been compiled. Data from 401 renal transplants performed in pts <21 years-old, from Jan 2004 to Dec 2005, were analyzed. Mean follow-up was 18m. Mean age was 11.8y (53% female). Main etiology of ESRD was glomerulopathy (33%, of which 34% FSGS), followed by uropathy (25%). The donor was deceased (DD) in 55.4%. One year actuarial graft survival (Kaplan Meier) was 88.6% in DD and 92% in Live Donors (LD). There were 43 graft losses (23 from DD), mainly due to arterial or venous thrombosis (28%), with similar rates of thrombosis between DD and LD. Other causes of graft loss were death with functioning kidney (23.2%), acute rejection (11.6%), recurrence of kidney disease (11.6%), and chronic allograft nephropathy (9.3%). According to the recipient age at renal transplant, one year graft survival was 80% in 0–5 year-old recipients (death: 37.5%; vascular thrombosis: 11%), 90% in 6–2 year-olds (thrombosis: 31.5%) and 92% in 13–21 year-olds (thrombosis: 26.7%). One year graft survival was 91% in patients receiving steroids (n = 344), 93% in the steroid withdrawal group (n = 31) and 87% in the steroid-free group (n = 23). One year patient survival was 96.3%; 16 patients died due to infection. Although one year patient and graft survival are comparable to the international experience, there is a relatively high rate of graft loss due to vascular thrombosis (30).

The collection of data for the Registry corresponding to the renal transplants performed during the years 2006 and 2007 is in progress.

Organization and Resources

The nephrological care of children in Latin America varies; countries range from those having medical systems that provide care to all patients who need dialysis procedures to others offering no possibilities to provide dialysis or transplantation to children with end-stage renal disease. Even in countries in which hemodialysis, CAP, and kidney transplantation programs are available, such services are markedly hampered by economic problems. Health authorities appropriately give priority to prevention and treatment of common diseases. Despite the fact that this is improving, it is difficult to obtain funds for children with chronic renal disease-a small number of patients in need of expensive resources. This is worsened by the current state of devaluation of Latin American currencies, because the relative cost of the materials for treatment of end-stage renal disease, all of which are of foreign origin, constantly increases (31). To determine the situation in each of these countries, in 2002 an investigation about these topics was done. A questionnaire was sent that was answered by nephrologists skilled in these procedures. Results showed that Argentina, Brazil, Venezuela, Chile and Mexico have well-structured programs. Peritoneal Dialysis and hemodialysis programs, as well kidney transplantation programs, have and adequate infrastructure. Uruguay has adequate hemodialysis programs, was a pioneer in Chronic Peritoneal Dialysis and has a growing program of transplantation in public hospitals. Cuba and Costa Rica have adequate programs of hemodialysis and transplantation; chronic peritoneal dialysis was not used at the time of this investigation. Other countries, such as Paraguay, Ecuador, and Colombia, were developing new programs for hemodialysis, CAPD, and renal transplantation (32).

Since then, ALANEPE has decided to identify areas which need major support in Pediatric Nephrology in order to develop teaching courses and cooperation between countries. There are four areas with this profile: Central America, Bolivia-Paraguay, Ecuador-Peru and some Caribbean islands (e.g. Cuba). Several programs are under development in these specific geographical areas, including the introduction of a program of chronic peritoneal dialysis in Cuba, several teaching courses of Pediatric Nephrology in Ecuador, Bolivia, Paraguay, Panama; and the development, of important programs with support from organizations such as "Associazione Bambino Nefropatico" and the Lombardia Region, (Milan Italy). With the preliminary work developed in Nicaragua, Honduras, El Salvador, Guatemala, and Costa Rica a large program is under development to improve education and prevent pediatric chronic renal disease.

Medical Education Systems

Pediatric Nephrology residency programs exist in Argentina, Brazil, Chile, Colombia Mexico, Costa Rica, and Venezuela. Besides the residency in public hospitals, there are university Pediatric Nephrology residencies in Argentina, Chile, Mexico and Venezuela.

In conclusion, there is still a great discrepancy in the quality of care available for children with renal disease among different Latin American countries. Many challenges remain. However, dramatic improvement has been made in the care of children with renal disease over the past fifteen years in this area of the world.

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84 Pediatric Nephrology in Australia and New Zealand

William Wong · Stephen Alexander

Introduction

Pediatric nephrology programs were established in a number of Australian state capital cities in the early 1970s. In 1980, New Zealand's pediatric end stage renal failure program was formed. The Australasian Paediatric Nephrology Association was formed during the International Pediatric Nephrology Association (IPNA) meeting in Toronto in 1989 and within 2 years the name was changed to the Australian and New Zealand Paediatric Nephrology Association (ANZPNA) to recognize New Zealand's contribution to the organization. In 1993, ANZPNA became officially affiliated to IPNA as a regional society. The association has maintained representation on the Council of IPNA over the past two decades. The membership of the society has steadily grown over the past 15 years.

Pediatric Nephrology in New Zealand

The country is served by a single comprehensive pediatric nephrology service based in the largest city, Auckland. It provides the entire range of diagnostic services and renal replacement therapy. There is presently a ratio of 1 specialist per 318,000 children under the age of 15 years. The service undertakes outreach clinics to all main centers and provides long distance care to children with end stage renal disease. The increasing use of telepediatrics has helped to improve access to nephrology care around the country. A nationwide network of general pediatricians with an interest in pediatric renal diseases, assist in the management of children with renal disease. Since 1998, a regular program of biennial workshops in pediatric nephrology and transplantation has been held to assist in the continuing education of pediatricians.

Epidemiology of Childhood Renal Disease in New Zealand

Glomerular Diseases

New Zealand has a high incidence of post streptococcal glomerulonephritis affecting mainly Maori and Pacific Island children. Lennon et al. (1), published an epidemiologic longitudinal study of rheumatic fever and APSGN in Auckland and estimated the rates were in excess of 100 per 100,000 age appropriate population amongst Maori and Polynesian Pacific Islanders. She calculated the overall prevalence rate for Auckland children at 16 per 100,000 with the rates of 50.5 and 46.5 for Maori and Pacific Island children respectively. A number of retrospective studies over the past 25 years have shown this to be a continuing problem. A prospective nationwide study is presently being conducted to determine precise incidence and prevalence rates.

The incidence of chronic glomerulonephritis in adult Maoris and Pacific Islanders was found to be significantly higher than in adults of European descent (2). A retrospective biopsy series of children with nephrotic syndrome by Simpson et al. (3) showed that there was an increased incidence of membranoproliferative glomerulonephritis, post streptococcal glomerulonephritis and focal segmental glomerulosclerosis (FSGS). Maori children were more likely to have membranoproliferative glomerulonephritis followed by minimal change disease, whereas Caucasian and Pacific Island children tended to have minimal change disease followed by FSGS. It was also of interest that lupus nephritis occurred predominately in Maori and Pacific Island children.

A prospective nationwide surveillance study of idiopathic nephrotic syndrome in children showed that the incidence is 1.9 children per 100,000 under the age of 15 years with no significant difference between ethnic

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Figure 84-1

Idiopathic Nephrotic in New Zealand children compared with the general population (4).

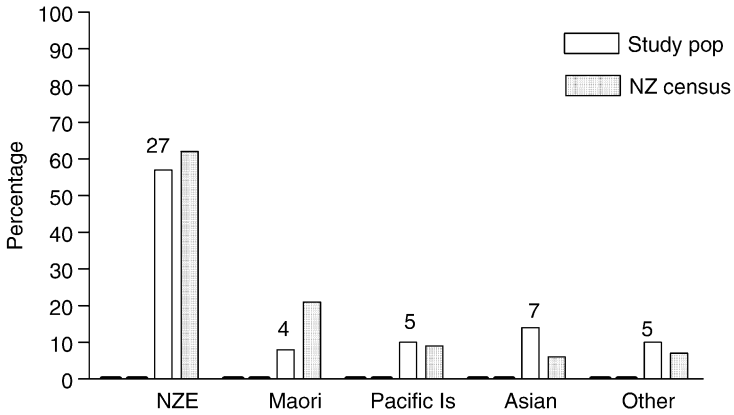
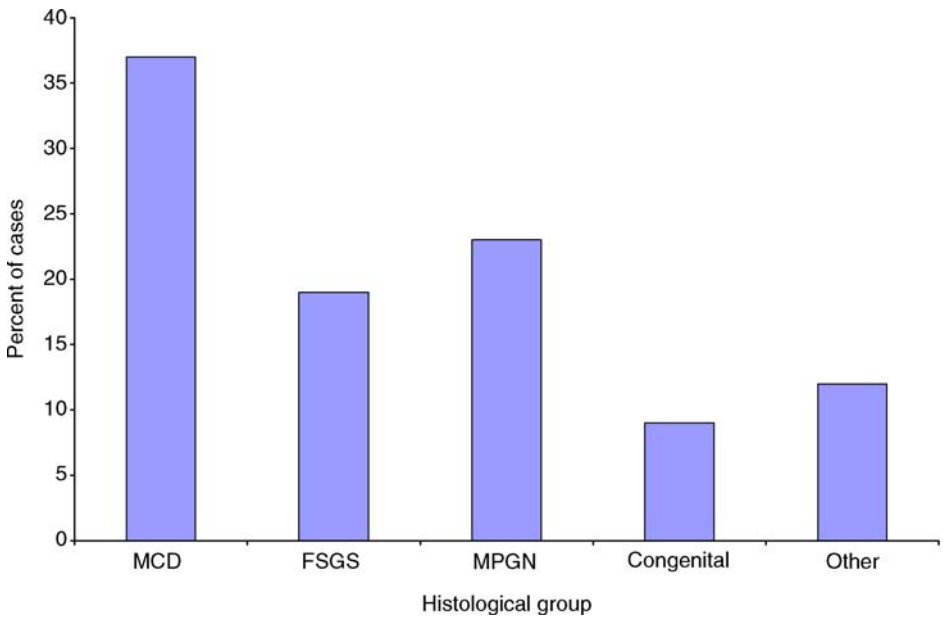


Figure 84-2

Histology at first biopsy (*n* = 57) in children with nephrotic syndrome (3). *MCD* minimal change disease; *FSGS* focal segmental glomerulosclerosis; *MPGN* membrano-proliferative glomerulonephritis; Congenital congenital nephrotic syndrome.

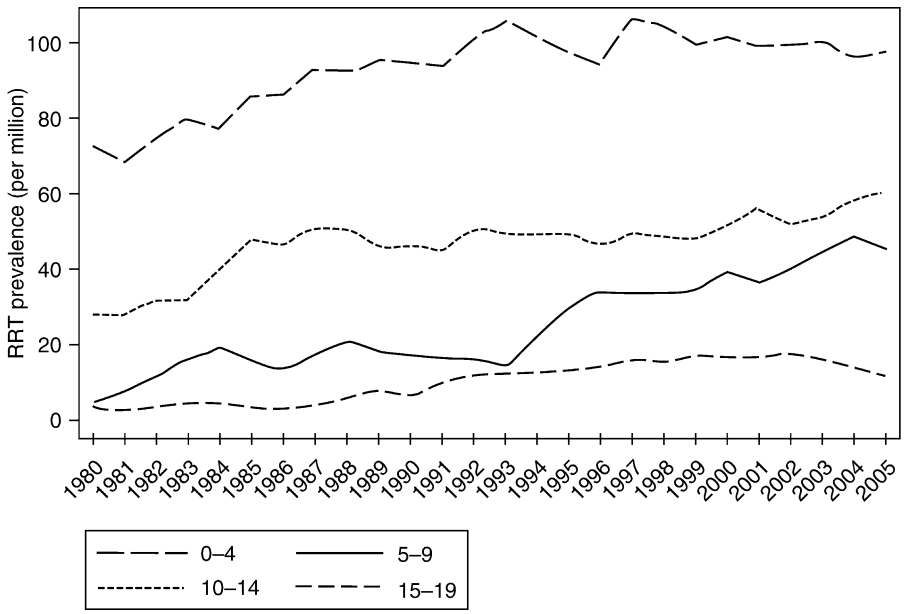


groups (4) (*Fig. 84-1*). Eighty percent were steroid responsive. Three quarter of those biopsied showed minimal change disease and 17% were focal segmental glomerulosclerosis (FSGS). The proportion of children with FSGS was the same as that reported by Simpson et al. in their retrospective biopsy study (3) (*Fig. 84-2*).

Congenital nephrotic syndrome in New Zealand also appears to have different natural history to that described in Finland and other countries in the Northern Hemisphere. Affected infants tend to become less proteinuric with increasing age with a much later onset of chronic renal failure. Recent mutation analysis of a group of eight

Figure 84-3

Prevalence of renal replacement therapy in Australia in age 0–19 from 1980 to 2005 (ANZDATA registry report 2006) (6).



children revealed that five had mutations which had not been previously described. There was however a common haplotype associated with a novel *p.R711S* mutation supporting a founder effect (5).

Acute Renal Failure in New Zealand

Beyond the first 3 months of life, hemolytic uremic syndrome (HUS) is the most common cause of acute kidney injury requiring acute renal replacement therapy (*Fig. 84-3*). A 10 year prospective study of HUS in New Zealand by the author showed 82% were diarrhea related with 75% of the cases occurring in the upper North Island of New Zealand. *E coli* 0157 was the most common serotype identified. Pneumococcal sepsis was the most common cause of non diarrhea related HUS. The annual incidence of diarrhea associated HUS was 1.1 per 100,000 in children under the age of 5 years and 0.8 per 100,000 in children under 15 years of age. No seasonal relationship was observed in contrast to the North Hemisphere. Mortality in diarrhea related HUS is 1% and pneumococcal HUS is 8% (unpublished observations).

Pediatric Renal Care in Australia

Currently there are seven pediatric renal centers in the five larger states of Australia. In addition Tasmania is served by Victoria and the Northern Territory is served by South Australia. All centers are based at the major pediatric teaching hospitals in their state and each provides comprehensive diagnostic and therapeutic services for children with acute and chronic kidney disease, including acute and chronic dialysis and transplantation. Most centers have a team including 2–4 nephrologists, renal nurses, dieticians, and social workers. There are differing models of care across the different states with on-site hemodialysis available in most units. In others, dialysis services are offered in affiliation with associated adult facilities.

Acute Care

In addition to hemodialysis and peritoneal dialysis, treatment of acute renal failure frequently involves the use of continuous veno-venous hemofiltration. This is delivered in pediatric intensive care units by intensivists and nephrologists. There has been a shift from the use of

systemic anticoagulation with heparin to local anticoagulation with citrate.

Peritoneal dialysis is delivered almost exclusively as continuous cycling peritoneal dialysis using a number of platforms and is utilized as the primary mode of dialysis in young children and for patients in remote locations. Hemodialysis is largely delivered "in center" Occasionally older adolescents, and rarely smaller children, are treated with home hemodialysis. Home therapies have been developed in some centers with increasing expertise in appropriate blood volume monitoring.

Transplantation is largely done in pediatric centers with dedicated transplant surgeons. The majority of donors are living related, with an increasing number of nonparental donors including aunts, uncles and grandparents. An increasing number of donor kidneys are harvested laparoscopically.

Transplantation Outcomes

All renal transplants in Australia and New Zealand are reported and followed six monthly by the ANZDATA registry (www.anzdata.org.au). Thus all pediatric data on renal transplants have been collected since 1963. The outcomes for children transplanted between 1963 and 2002 have been reported. The long-term survival rate among children requiring renal-replacement therapy was 79% at 10 years and 66% at 20 years. Mortality rates were 30 times as high as for children without end-stage renal disease. Dialysis was associated with a four fold risk compared to renal transplantation. Transplant outcome results have improved steadily over this time (7).

Etiology of Renal Disease

Australia and New Zealand both have a similar profile of renal disease with younger children presenting with renal dysplasia, structural renal anomalies including posterior urethral valves, and congenital renal disease, and older children presenting with glomerulonephritis secondary to systemic disease such as SLE. Increasingly there has been recognition of the growing number of cases with a genetic basis including ARPKD, cystinosis and CAKUT (8) (*Tables 84-1* and *84-2*). There is also increasing recognition of familial and genetic forms of nephrotic syndrome leading to renal failure with identification of WT-1, podocin, nephrin and LAMB-2 mutations in children in Australia and New Zealand (9–11). Like NZ, Australia has a significant proportion of postinfectious glomerulonephritis including poststreptococcal glomerulonephritis which particularly is endemic in indigenous aboriginal communities. In indigenous communities renal failure occurs at some of the highest rates in the world. and There is great interest in establishing whether unique genetic or acquired conditions in aboriginal children such as postinfectious GN may contribute to this high prevalence of chronic kidney disease. HUS has also been a major cause of ARF and has been associated with epidemics. The most severe outbreak was reported from Adelaide and associated with contaminated salami (12).

Immunosuppression

The majority of pediatric transplants receive a calcineurin inhibitor, mycophenylate mofetil, steroids and an IL-2R

Table 84-1

Causes of end stage renal disease 1996–2005 in Australia and New Zealand (ANZDATA registry report 2006) (6)

Paediatric transplant patients causes of end stage renal disease					
ESRD	1996–1997 (%)	1998–1999 (%)	2000–2001 (%)	2002–2003 (%)	2004–2005 (%)
Glomerulonephritis	19	11	17	29	24
Reflux nephropathy	25	27	20	14	14
Congenital renal hypoplasia/dysplasia	11	16	26	18	29
Medullary cystic disease	28	14	3	34	21
Haemolytic uraemic syndrome	30	20	15	20	15
Posterior urethral valves	19	19	11	16	35
Other	23	17	18	21	21
Uncetain	–	–	33	33	33

Table 84-2

Causes of end stage renal failure by age group in Australia and New Zealand, 2000–2005 (ANZDATA registry report 2006) (6)

Causes of end stage kidney disease by age group 2000–2005					
Primary renal disease	Age groups				Total
	0–4	5–9	10–14	15–19	
Glomerulonephritis	3(6)	15(21)	19(28)	74(44)	111(31)
Familial glomerulonephritis	–	–	2(3)	10(6)	12(3)
Reflux nephropathy	1(2)	6(8)	3(4.5)	32(19)	42(12)
Polycystic kidney disease	–	3(4)	3(4.5)	1(<1)	7(2)
Medullary cystic disease	1(2)	3(4)	5(7)	8(5)	17(5)
Posterior urethral valve	12(24)	6(8)	6(9)	6(4)	30(8)
Haemolytic uraemic syndrome	–	2(3)	1(1)	3(2)	6(2)
Hypoplasia/dysplasia	14(28)	14(20)	10(15)	1(<1)	39(11)
Diabetes	–	–	–	1(<1)	1(1)
Cortical necrosis	3(6)	2(3)	3(4.5)	4(2)	12(3)
Interstitial nephritis	–	1(1.5)	–	6(4)	7(2)
Cystinosis	–	4(6)	3(4.5)	–	7(2)
Uncertain	1(2)	1(1.5)	4(6)	5(3)	11(3)
Miscellaneous/other	15(30)	14(20)	9(13)	17(10)	55(15)
Total	50(100)	71(100)	68(100)	168(100)	357(100)

Values in parentheses indicate percentages

antagonist. A number of patients have been switched to Rapamycin (13). Acute rejection has become much less common. However, infectious problems including BK virus have increased (14).

Research

Throughout ANZ there has been a strong interest in clinical and basic research in pediatric nephrology and transplantation (*Fig. 84-4*). This has included research into the antecedents of renal disease in aboriginal communities, the establishment of the renal Cochrane library based in Australia and New Zealand, the development and participation in clinical trials related to CMV prophylaxis and treatment with erythropoietin and antibody prophylaxis for urinary tract infection (the PRIVENT trial). A number of studies derived from the ANZDATA registry including adolescent outcomes and the development of chronic allograft nephropathy in pediatric patients have been reported (15). In basic science there have been a number of studies related to mechanisms of fibrosis, the development of tolerance strategies involving Tregs, dendritic cells and mesenchymal stem cells as well as direct patient studies in children with ESRD, and following transplantation (16, 17). Increasingly there have been genetic studies aimed at the identification of known renal genetic defects and discovery of novel disease genes. A Kidney Gene Bank has been established to facilitate such studies. Finally work on clinical outcomes such as growth and long term outcomes such as cardiac disease in renal failure and post transplantation in children have been studied in Australia and New Zealand (18–20).

Training

Pediatric nephrology training is offered at a number of the centers and attracts both local and overseas trainees. In addition there has been a long history of nephrology training post fellowship overseas in North America and Europe, particularly in the UK.

Figure 84-4

Stars show the eight pediatric nephrology centers in Australia and New Zealand.

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85 Pediatric Nephrology in Asia

Hui-Kim Yap · Arvind Bagga · Man-Chun Chiu

Overview

Asia is the largest continent in the world with an estimated population approaching 4 billion, of which children under the age of 19 years constitute more than 1 billion (1). Moreover, Asia includes the world's most populated countries, China and India. Unfortunately, health care delivery across the Asian continent is very heterogeneous. Childhood mortality and morbidity are important indicators of the quality of health care delivery. Most childhood deaths occur in less developed countries and poorer sectors of the community in more developed countries, reflecting enormous disparities among different geographical areas and population groups. The more urgent issues in child health in many Asian countries encompass the control of infections, malnutrition and diarrheal diseases. However, with improvements in public health especially in urban communities, the pattern of childhood disorders has changed, resulting in a shift in the causes of mortality from infections to other chronic diseases. Although the major challenges in pediatric nephrology practice still include the management of children with acute kidney injury due to severe dehydration, sepsis and toxins, the prevention and treatment of children with chronic kidney disease is emerging as the new challenge for this millennium.

The first Working Group for Pediatric Nephrology in Asia was formed in 1986, and the first scientific meeting addressing the "Epidemiology and Treatment of Renal Diseases in Asian Countries" was held in Tokyo, Japan in 1988. The Asian Society of Pediatric Nephrology (AsPNA) was officially inaugurated in 1996 with members from 10 Asian countries/regions. Its mandate was to promote the development of pediatric nephrology in Asia and foster regional cooperation amongst member countries. With regional education and training as one of the main focus, quality care including both acute and chronic renal replacement therapies, as well as strategies for the prevention of chronic kidney disease, will be available more widely to the pediatric population in Asia. Currently, the AsPNA has 15 member countries from East Asia, South Asia and South-East Asia, and further expansion with representation from West Asia, in particular the Middle East, will enable AsPNA to extend its mission throughout the continent.

Data on childhood end-stage renal disease in Asia are scarce. A survey on renal replacement therapy by Chiu et al. in 2005 which included 12 Asian countries, namely, China, Hong Kong SAR, India, Indonesia, Japan, Malaysia, Pakistan, Philippines, Singapore, South Korea, Taiwan, and Thailand, reported 2,874 cases on renal replacement therapy (2). Among the 1,033 reported cases of renal transplants, 63.5% were from live donors and 36.5% were from deceased donors. Patient survival rates were 98 and 92% at 1 and 5 years respectively, while kidney graft survival rates were 92 and 81% at 1 and 5 years respectively, comparable to those of the North American Pediatric Renal Transplant Collaborative Studies (NAPRTCS) for the same period.

Children on chronic peritoneal dialysis were predominantly on continuous ambulatory peritoneal dialysis (64%), followed by automated peritoneal dialysis (30%) and intermittent peritoneal dialysis (6%). Chronic hemodialysis was more commonly practiced than peritoneal dialysis in a few countries such as Indonesia, Pakistan, Philippines, Malaysia, and Taiwan. Other than center preference and funding, this might also be related to the inclusion of adolescents. On the whole, renal replacement therapy is expensive and financial support is an important issue in the care of children with end-stage renal disease, especially in the developing countries. On the other hand, in the more developed centers in Asia, overall graft survival, peritonitis rates and provision of renal replacement therapy are quite comparable to global standards.

East Asia

China

China is a vast country with a 1.3 billion population, 28.6% of which are 19 years and below. In recent years, the country has experienced rapid economic growth and development, including that of health care. However, distribution of health resources still varies greatly, especially between urban and rural areas. Unfortunately, prevalence

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data on childhood renal diseases are lacking. A national project supported by the Ministry of Science and Technology to study the nephro-urological abnormality rates in urban children is presently underway.

Retrospective studies on hospitalized children with renal diseases are being conducted to help understand the epidemiology of childhood renal diseases in China. Some of the completed studies examined renal diseases requiring hospitalization (3), systemic lupus erythematosus, acute renal failure (4), renal biopsy findings (5), and glomerular diseases (6). A nationwide urinary screening program in children aged 2–14 years showed that 0.85% of 224,291 children screened had urinary abnormalities, including asymptomatic hematuria, nephritis, urinary tract infection, asymptomatic bacteriuria, urinary stones, hydronephrosis, and urinary malformations (7). Another urinary screening program done among school children in Shanghai showed a 1% prevalence of urinary abnormalities (8).

The Chinese Society of Pediatric Nephrology conducted a retrospective analysis of children from 91 hospitals admitted with chronic renal failure from 1990 to 2002 (9). Using a criterion of creatinine clearance less than 50 ml/min per 1.73 m^2, 1,658 hospitalized children were diagnosed with chronic renal failure during this period, giving a mean prevalence of 1.25% of hospitalized children with renal diseases. Onset of renal failure was mainly at school-age. There was a significant increase in number of cases admitted with chronic renal failure comparing the two 6-year periods from 1990 to 1996 and 1997 to 2002. The main primary renal diseases causing chronic renal failure were chronic glomerulonephritis and nephrotic syndrome (52.7%). One-third of all cases were congenital and hereditary renal diseases, with hypoplastic or dysplastic kidneys comprising the majority. Most of the patients were managed conservatively and only 15.8% received renal replacement therapy. Hemodialysis (66.5%) was the predominant dialysis modality. Only 29 of the dialyzed patients underwent renal transplantation, with a 1-year graft survival of 93.1%. The mortality of patients with chronic renal failure over the 13-year review period was approximately 10%, with cardiac failure, infections and uremia being the main causes.

A nationwide retrospective study from 33 hospitals on IgA nephropathy in 2006, reported a total of 1,349 cases of renal biopsy-proven IgA nephropathy in children 1–14 years of age (10). This accounted for 1.37% of the hospitalized nephro-urological diseases and 11.8% of renal biopsies. Family history of renal disease was present in 9.31% cases of IgA nephropathy. Renal pathology studies showed that the majority of IgA nephropathy were graded as WHO class III (41.4%) and class II (28.51%). No standard treatment regimen was utilized to treat these children.

Hong Kong Special Administrative Region (SAR)

Hong Kong SAR has a population of 6.86 million of which 1.20 million are 19 years and under. The health care system in Hong Kong is quite different from that in mainland China. About 90% of patients requiring hospitalization are in public hospitals which are heavily subsidized, with the remaining 10% in private hospitals. Children with renal diseases are cared for in most major public hospitals. A pediatric renal center for dialysis and transplantation has been established since 1999, offering children with chronic renal failure a centralized multidisciplinary end-stage renal disease program.

The Hong Kong Society for the Study of Kidney Diseases was formed in the 1980s to bring together pediatricians working on pediatric renal diseases to promote the specialized practice of pediatric nephrology. Several surveillances and a renal registry have been set up for hospitalized pediatric renal patients. It had been reported that the number of new cases per year of nephrotic syndrome, lupus nephritis, cystic kidney diseases, and hereditary tubular disorders were 57, 10, 12, and 2 respectively, and an average of seven new cases per year of chronic renal failure with glomerular filtration rate lower than 25 ml/1.73 m^2/min were seen in children under 15 years old (11). A collaborative multi-center study on primary nocturnal enuresis involving 105 Chinese children showed that the combined use of an enuresis alarm and oral desmopressin was most effective and yielded a lower relapse rate than any other regimen (12). Another multicenter study of 128 Chinese children with lupus nephritis showed that 70% of the patients had Class III and IV nephritis and this series of Chinese children had a good medium term survival rate and chronic morbidity rates which were similar to that reported in the Caucasian population (13).

The incidence of end-stage renal disease in children younger than 15 years of age was estimated to be four per million children in the early 1990s (14). The common causes were chronic glomerulonephritis (26%), hypoplastic or dysplastic kidneys (18%), chronic pyelonephritis (18%) and hereditary or familial diseases (9%). In recent years, according to the electronic Hong Kong Renal Registry, the incidence and prevalence for those less than 20 years of age with end-stage renal disease (ESRD) was

about 5 and 28 per million children respectively. ESRD was six times more common in children between 11 and 20 years of age compared to younger children (15).

Continuous ambulatory peritoneal dialysis was started in the 1980s, and since 1996, automated peritoneal dialysis has been the modality of choice to nearly all children on chronic peritoneal dialysis. In a recent review on 30 children on automated peritoneal dialysis, a relatively low peritonitis rate of 1 in 54.2 patient-months was reported (16). Chronic hemodialysis was offered to older children or those deemed clinically unsuitable for peritoneal dialysis. Although available for young children below the age of 3 years, vascular access remains a major challenge. A review of the outcome of 25 transplanted kidney grafts in 2004 showed that 1 and 3-year actuarial graft survival rates were 93.8 and 70.3% for cadaveric grafts, and both 100% for live donor grafts respectively (17). A recent review in 2007 of 61 grafts including 37 cadaveric and 24 live donor grafts, the overall 5-year patient and graft survival rates were 100% and above 90% respectively. Even the 18 transplanted adolescents (age 13–17 years) had excellent 5-year graft survival rate at 90% (18).

South Korea

South Korea has a population of 47 million, of which 24.4% are younger than 19 years of age. There are 37 pediatric nephrologists giving a ratio of approximately 1:140,000 pediatric population. A nationwide hospital discharge survey of pediatric patients less than 18 years old was conducted in 2007. Of 826,896 patients hospitalized between 2004 and 2006, diseases of the genito-urinary system accounted for 4.1%. Urinary tract disorders (45.8%), glomerulopathies (17.3%) and tubulo-interstitial diseases (10.6%) were the main underlying etiologies. The common renal presentations of glomerular diseases were nephrotic syndrome (40.6%), recurrent and persistent hematuria (33.1%) and unspecified nephritic syndrome (17.4%).

A national surveillance system for diarrhea-associated hemolytic uremic syndrome has been in operation since 2003 by the Korean Society of Pediatric Nephrology in concert with the Korea Center for Disease Control. The nationwide annual incidence is about 20 per 9.6 million children under the age of 15 years. More than 80% of the patients were younger than 6 years old, and about a quarter underwent dialysis for acute renal failure. The overall mortality rate was 6%.

The Korean Society of Pediatric Nephrology established a web-based pediatric chronic kidney disease registry in 2004. The registry includes data of patients with chronic kidney disease stages 3–5, including transplantation. More than 530 children were enrolled in the registry by the end of 2007.

In Korea, the school screening program for urinary abnormalities has been mandated by law since 1998. Over 5 million students have been screened since its inauguration. Isolated proteinuria was detected in 0.2%, occult blood in 0.8%, and glucosuria in 0.07% from January 1998 to December 2004 (19). Of the patients referred for renal biopsy, 63.1% had isolated hematuria, 10.5% isolated proteinuria and 69.9% hematuria with proteinuria. Histopathological findings included IgA nephropathy in 43.8%, mesangial proliferative glomerulonephritis in 38.4%, Henoch-Schönlein nephritis in 2.7%, membranoproliferative glomerulonephritis in 1.6%, lupus nephritis in 0.5% and Alport syndrome in 0.6%.

Taiwan

Taiwan has a population of 22,973,622 with more than 5 million in the pediatric age group 19 years and below. A biannual mass urinary screening of elementary and junior high school students has been in operation in 1990. Results from the screening of 2.6 million children showed that 0.10 –0.35 had proteinuria and 0.07–0.21 had hematuria. In a retrospective evaluation for prevalence of proteinuria and chronic renal insufficiency to identify factors related to disease progression, 10,288,630 urinary screenings of elementary and junior high-school students were reviewed (20). The 4-year prevalence of proteinuria was higher in girls than in boys, however, the disease progression to chronic renal insufficiency was higher in boys. Further analysis showed that persistent serum cholesterol greater than 220 mg/dl, serum albumin level lower than 3.5 g/dl, total protein greater than 6 g/dl, and diastolic blood pressure higher than 90 mmHg were significant risk factors for disease progression. Therefore early detection of heavy proteinuria by mass urinary screening with early appropriate treatment, and monitoring of significant risk factors were considered helpful to decrease or delay progression to chronic renal failure.

Genetic studies in Taiwan on gene polymphisms associated with severity of vesico-ureteric reflux showed that C3123A of the angiotensin-2 receptor gene was associated with both the development and severity of vesicoureteric reflux in 100 children (21). Studies on HLA class I gene polymorphisms in Taiwanese children with steroid-sensitive nephrotic syndrome showed that the frequency of A11, B39, B48, B60, B62, CW4 and CW7 were

significantly higher than controls, suggesting that the immunogenetic background of steroid-sensitive nephrotic syndrome in Taiwanese children may be different from other populations (22).

In a survey of dialysis and kidney transplantation in patients under the age of 21 years, 110 patients were on chronic peritoneal dialysis, 264 patients on chronic hemodialysis, and 161 were transplanted. For patients on chronic peritoneal dialysis, namely continuous ambulatory peritoneal dialysis and automated peritoneal dialysis, the peritonitis rate was 1 in 24.5 patient-months. The majority of patients (95.5%) on chronic hemodialysis have arterio-venous fistulae as vascular access, with the remaining (4.5%) using permanent catheters. With regards to kidney transplantion, there were 135 cadaveric transplants (83.8%) and 26 live donor transplants (16.2%). The 1 and 3-year graft survival rates were 100 and 87.7% respectively; and the 1 and 3-year patient survival rates were 100 and 94.4% respectively.

South Asia

Bangladesh

Bangladesh has a population of 153 million, with close to 66 million children in the pediatric age group 19 years and under. There are only 14 certified pediatric nephrologists in the country, or one pediatric nephrologist per 5.7 million children. During the last two decades of the twentieth century, there was an outbreak of acute renal failure with very high mortality. Epidemiological investigations incriminated ingestion of paracetamol syrup contaminated with diethylene glycol as the cause (23). Another study (1988–1992) reported a mortality rate of 77% in 1,837 children with acute renal failure (24). Diarrheal losses were the most common cause of ischemic acute kidney injury. Poverty, illiteracy and ignorance were responsible for delays in seeking medical advice and the resultant lack of adequate treatment contributed to the high mortality. Diarrhea-related hemolytic uremic syndrome was also an important cause of renal failure (25). The incidence of acute renal failure secondary to these causes has now declined.

Although there is no national registry of childhood diseases, hospital records show that nephrotic syndrome constituted the major cause of admissions to the pediatric nephrology ward during 2005–2007, with 12% having steroid-resistant nephrotic syndrome. Obstructive uropathy and hydronephrosis (7%), chronic renal failure (6%), urinary tract infection (5%) and acute glomerulonephritis (4%) comprised the other causes. The chief causes of chronic kidney disease stage 4 and 5 in 106 patients admitted to Bangabandhu Sheikh Mujib Medical University (BSMMU, Dhaka) during 2005–2007, were posterior urethral valves, unilateral hydronephrosis, neurogenic bladder, glomerulonephritis and lupus nephritis.

Dialysis facilities are available at BSMMU, Bangladesh Institute of Child Health and National Institute of Kidney Diseases & Urology, but kidney transplantation for children is only available in one facility, at BSMMU. Regulations for kidney transplantation were enacted in 1999, with provision for live-related donation.

India

India has close to 1.15 billion people in 2008, with children 19 years and below comprising about 40% of the population. A network of state-managed health centers, and district and referral hospitals has been in place for more than 45 years. The resources available at those centers are often limited, and a large proportion of the population obtains medical care through a parallel private sector. The Indian Society of Pediatric Nephrology has more than 300 active members and has published national guidelines for management of nephrotic syndrome, urinary tract infections, hematuria, hypertension and antenatally detected hydronephrosis (26–30). A Chronic Kidney Disease Registry has also been formed.

Idiopathic nephrotic syndrome is a common problem, although its exact incidence is unknown. The clinicopathological features and response to therapy are similar to those reported from developed countries. However, the incidence of serious infections, which complicate the course of the illness, is higher (31). Levamisole has been found effective and safe, and is widely used as a steroid-sparing agent for patients with frequent relapses and steroid dependence (32). Case series also support the utility of mycophenolate mofetil as an effective medication for these patients (33). The therapy of patients with steroid-resistant nephrotic syndrome is challenging and limited by economic factors. Treatment with intravenous dexamethasone, which is less expensive, has been found as effective as methylprednisolone for inducing remission in these patients (34). Therapy with intravenous "pulse" cyclophosphamide has been used with promising results (35, 36). A multicentre study examining the efficacy and safety of tacrolimus with intravenous cyclophosphamide in patients with steroid-resistant nephrotic syndrome is currently underway.

The incidence of poststreptococcal acute glomerulonephritis, high during the 1970s, has gradually declined. Acute kidney injury caused by severe hypovolemia

from acute gastroenteritis has also decreased due to the widespread use of oral rehydration therapy (37). Acute intravascular hemolysis in individuals with glucose-6-phosphate dehydrogenase deficiency, snakebite and leptospirosis in coastal regions and rural areas, and malaria are important causes of acute renal failure. While diarrhea-associated hemolytic uremic syndrome was the most common cause of acute renal failure in the 1990s (38), its incidence has also declined. Clinical and epidemiologic evidence suggests that most patients with typical hemolytic uremic syndrome are associated with infection due to *Shigella dysenteriae*; infection caused by verotoxin producing *Escherichia coli* is rare in the Indian subcontinent. Perinatal asphyxia, septicemia and injudicious use of medications are important causes of acute kidney injury in the newborn period. Peritoneal dialysis is the most commonly used dialysis modality for children with acute renal failure.

The incidence of hepatitis B nephropathy is low, although the carrier rate of hepatitis B antigenemia is 1.5–2%. The incidence of familial and genetic disorders, collagen vascular diseases, IgA nephropathy and renal tubular disorders is similar to that in developed countries. Takayasu disease (idiopathic aortoarteritis) is an important cause of hypertension in children (39).

The chief causes of chronic renal failure include obstructive uropathy, reflux nephropathy and chronic glomerulonephritis (40). The management of patients with progressive kidney disease is challenging. Although facilities for dialysis and renal transplantation are available in all metropolitan cities, their utilization is limited by socioeconomic factors (41, 42). The Human Organ Transplantation Act became law in 1994, but the cadeveric transplant program is not well developed. Most of the live donor transplants are from parents, most often the mothers (42). Neither the state nor insurance companies subsidize the costs of therapy, which are high especially in relation to per capita income.

Pakistan

The population of Pakistan is estimated as 168 million with a pediatric population 19 years and under comprising 48%. Pakistan has a large rural population (66%) that is served by inadequate medical facilities. Larger cities with university hospitals have well-equipped centers. In 1997, the Pakistan Pediatric Nephrology Group was formed under the umbrella of the Pakistan Pediatric Association. It has approximately 100 members, including pediatricians with special interest in nephrology.

Nephrotic syndrome is one of the most common renal diseases seen in the country (43). Its clinical features and response to steroid therapy are similar to those in developed countries. Recurrent infections, affecting the upper respiratory and gastrointestinal tracts, are associated with frequent relapses (44). The seroprevelance of hepatitis B virus is high in Pakistan, but its association with nephrotic syndrome has not been demonstrated. Post-streptococcal acute glomerulonephritis has shown a decline compared to that in previous studies, while renal calculi are recognized more frequently (45). Vesicoureteric reflux and posterior urethral valves are the most common structural abnormalities, accounting for 60% of congenital anomalies of the urinary tract.

Urolithiasis is an important cause of renal morbidity, particularly in southern parts of the country. The province of Sindh has the highest prevalence of stone disease in the world, attributed to multiple factors, including hot and humid climate, inadequate fluid intake, consumption of a chiefly cereal-based diet, and recurrent diarrhea in malnourished children (45). Urolithiasis is an important cause of acute and chronic renal failure (46) and was responsible for end-stage renal failure in 20% children who underwent renal transplantation (47).

Of 4,392 patients in the National Dialysis Registry, 2.3% were less than 15 years old (48). Approximately 15 centers provide facilities for peritoneal dialysis and evaluation of children with renal problems; facilities for hemodialysis are limited to five centers. The majority of pediatric kidney transplants are performed at the Sindh Institute of Urology and Transplantation (Karachi). An act governing "Transplantation of Human Organs and Tissues" became law in 2007, whereby deceased donor organ transplants were legalized and live unrelated donor transplants declared illegal.

Sri Lanka

Sri Lanka has an estimated population of 21 million, with the population 19 years and under comprising 32%. Based on hospital records and published reports, urinary tract infections are an important cause of renal disease in children. Post-streptococcal acute glomerulonephritis is common in rural areas, though the overall incidence is decreasing. The spectrum and course of nephrotic syndrome is similar to that reported in developed countries. The majority of children with nephrotic syndrome in Sri Lanka respond to corticosteroid therapy. However, the proportion of children with steroid-resistant disease has increased significantly over the past two decades.

Nephrotic syndrome due to other diseases and syndromes are uncommon. The chief cause of acute kidney injury requiring dialysis is diarrheal dehydration or shock. The incidence of diarrhea-related hemolytic uremic syndrome has also declined.

Efforts have been made to maintain a registry of patients with chronic renal failure in the country. Congenital abnormalities account for the majority of patients with chronic kidney disease. Focal and segmental glomerulosclerosis, nephrocalcinosis, reflux nephropathy and hemolytic uremic syndrome are other causes. Pediatric renal transplants are done at the Teaching Hospital, Peradeniya. Since September 2004, 27 live donor transplantations have been performed. Since facilities for dialysis are limited, most transplants are preemptive.

South-East Asia

Indonesia

Indonesia is a densely-populated nation with more than 235 million people, and a pediatric population 19 years and under of more than 88 million. The ratio of pediatric nephrologists to the pediatric population is approximately 1:3,684,210 with 19 trained pediatric nephrologists. Based on a multicentre study in 2000–2004 involving seven pediatric institutions, the top three kidney diseases affecting children were nephrotic syndrome (35%), acute post-streptococcal acute glomerulonephritis (26%), and urinary tract infections (23%). Other renal disease entities from most to least common included acute renal failure, chronic renal failure, nephrolithiasis, enuresis, and congenital renal disease. Urinary incontinence was an increasingly recognized problem. The underlying etiologies identified were spinal dysraphism, malignant osteolytic vertebral lesions, non-neurogenic neurogenic bladder and anatomical abnormalities. More than 50% of these children developed chronic renal failure (49).

Among the causes of acute renal failure in older children, jengkol bean intoxication was an important cause, accounting for 31% of children hospitalized for acute renal failure in a large general hospital in Jakarta (50). Jengkol bean is widely consumed in Indonesia, especially in rural areas. The pathophysiologic mechanism of kidney injury include obstruction of renal tubules by jengkolic acid crystals, as well as a direct toxic effect on renal tubular cells.

Studies on frequent relapsing nephrotic syndrome in Indonesia children have shown an association with certain HLA class II haplotypes, including HLA-DRB1*12 (protective haplotype) and DQB1*02 (at risk haplotype) (51). Other studies in Indonesian children on genetic variants of the Th2 cytokine genes IL-13 and IL-4 showed some association with minimal change nephrotic syndrome (52).

Malaysia

Malaysia has a population of approximately 25 million, of which more than 40% are 19 years and under. Pediatric nephrology services in Malaysia, similar to other pediatric subspecialities, are provided on a regional basis across the country. The Ministry of Health of Malaysia has set a norm of one pediatric nephrologist for every 300,000 children, which translates to 33 pediatric nephrologists for the country. In 2007, Malaysia has about 10 trained pediatric nephrologists in institutional practice.

Based on the National Renal Biopsy Registry, primary glomerular disease accounted for 51% of all biopsies and secondary glomerular disease 44%. The main primary glomerular diseases seen include focal segmental glomerulosclerosis in 62%, and minimal change disease in 26%. Lupus nephritis accounted for 58% of renal biopsies done for secondary causes of glomerular disease, followed by post-infectious acute glomerulonephritis at 30% and Henoch-Schönlein at 10%. Proliferative features (WHO class III or IV) constituted 82% of the cases of lupus nephritis.

The incident and prevalent rate of treated end-stage renal failure in patients younger than 20 years of age has been about 8 and 54 per million age-related population respectively in 2006 (53). Transplant rate was at two per million age-related population. Glomerular diseases excluding lupus nephritis accounted for 42% of all new cases of end-stage renal failure, with lupus nephritis accounting for 8%.

Philippines

Philippines has a population of approximately 93 million, of which more than 40% are children under the age of 19 years. There are 63 pediatric nephrologists per 39 million children under the age of 15 years in the country. The Philippine Nephrology Society of the Philippines study group, comprising the four training institutions for pediatric nephrology in the country, examined the distribution of kidney and urinary tract diseases referred to accredited tertiary medical centers. Unpublished data from January 1995 to December 2007

showed a total of 15,057 admissions for pediatric nephrology. The top renal causes for admissions included primary nephrotic syndrome (20%), post-infectious acute glomerulonephritis (9.5%), acute and chronic renal failure (18.5%), complicated and uncomplicated urinary tract infections (15.7%), bladder dysfunction (8.6%), secondary glomerulonephritis (6%) such as systemic lupus erythematosus, Henoch-Schönlein purpura, hypertension and renal tubular diseases (5.5%), IgA nephropathy (1.9%), tumors (1.7%), congenital or inherited disorders (1.6%), and urolithiasis (0.7%).

From 2003 to 2007, a total of 291 pediatric patients were diagnosed to have end stage renal disease. Peritoneal dialysis remained the renal replacement therapy of choice (71.4%) compared to hemodialysis (28.6%). Unpublished data from the National Kidney and Transplant Institute, Department of Pediatric Nephrology showed that from 1984 to 2007, there were 94 pediatric kidney transplants in children aged 0–15 years. The majority of these transplants was from live-related donors (65%), followed by live non-related donors (19%) and cadaveric donors (16%).

Singapore

Singapore has a population base of 4.6 million, with the pediatric population 19 years and under comprising 20%. The country has a ratio of approximately 1:360,000 pediatric nephrologists in institutional practice to pediatric population. Over the last two decades, the pattern of renal diseases in Singapore children has changed drastically. Similar to other South-East Asian countries, post-streptococcal acute glomerulonephritis was the most common type of glomerulonephritis. However, like its counterpart acute rheumatic fever, post-streptococcal acute glomerulonephritis is a disease that is known to be influenced by socioeconomic and environmental factors. Singapore's rapid urbanization in the 1970s to 1980s resulted in a decrease in significant streptococcal skin infections and a concomitant decrease in the incidence of acute glomerulonephritis (54). In fact, idiopathic nephrotic syndrome has taken on increasing prominence as a cause of morbidity in children with more than 60% developing steroid-dependency (55). Renal biopsy data showed that minimal change disease and focal segmental glomerulosclerosis were the major causes of steroid-dependent and steroid-resistant nephrotic syndrome in childhood (56).

Singapore has seen a change in the pattern and modalities of treatment of acute kidney injury in children. An 18-year study of children requiring acute dialysis showed that 91% were dialyzed for acute renal failure, while 9% had metabolic disorders (57). The main causes of acute kidney injury were ischemic injury (62.2%), hepatorenal syndrome (15.1%), acute glomerulonephritis (4.5%), hemolytic-uremic syndrome (7.6%) and obstructive uropathy (1.5%). Analysis of 146 infants and children dialyzed in the pediatric intensive care unit over the last 25 years revealed that dialysis was exclusively by the peritoneal route in the first 15 years, whereas continuous venovenous hemodiafiltration was the modality of choice over the last 10 years. This option allowed an increasing number of critically ill children to be dialyzed across all age groups. The overall mortality rate was 53%.

Data from the Singapore Renal Registry over the 10-year period from 1997 to 2006 showed that the age-specific standardized rate of end-stage renal failure in pediatric patients ranged from 4.5 to 13.5 per million population 19 years and under. The two leading causes of end-stage renal failure in pediatric patients were glomerulonephritis (33.4%), and congenital renal malformations (obstructive uropathy, renal hypoplasia/dysplasia and reflux nephropathy) (37.9%). The dialysis acceptance rate was 9.4 per million age-related population in 2006, with a dialysis prevalence rate of 28.1 per million age-related population. Peritoneal dialysis remained the preferred mode of dialysis, with more than 90% of pediatric end-stage renal failure patients on automated peritoneal dialysis. Data from the Singapore Renal Registry showed a transplant rate of 4.3 per million age-related population in 2006. The early 5-year cumulative survival of the pediatric patients with end-stage renal failure was excellent at 91% for dialysis patients and 88% for transplant patients. However, with longer periods of follow-up, survival of dialysis patients declined to 83% at 10 years as compared to a cumulative survival of 80% at 10 years for transplant patients. More than 90% of the pediatric transplants were live donor transplants, where the 1-year and 5-year allograft survival of 94 and 89% respectively.

Screening programs aimed at detecting renal disease at an early stage have been initiated in several Asian countries. Of 9,479 children screened in a pilot Singapore school screening program for urinary abnormalities, 1,048 or 11.1% were found to be positive for proteinuria on one dipstick examination. Further evaluation established that the prevalence of clinically significant proteinuria was 1.25 per 1,000 children screened, where about 90% was due to an underlying glomerulonephritis (58). Multivariate analysis looking at the predictors of proteinuria in the school population showed that low body weight, presence of persistent newly detected hypertension and lack of sports activity were significant predictors for persistent proteinuria (59). In fact, low body weight

was associated with a 1.8-fold greater risk for proteinuria after adjusting for confounding factors, suggesting that low renal mass together with additional environmental influences resulting in decreased body weight may result in earlier manifestation of renal disease.

Thailand

Thailand has a population of more than 65 million with approximately 30% in the pediatric age group 19 years and under. The country currently has 58 trained pediatric nephrologists, with an approximate ratio of 1:275,000 pediatric population. Common renal diseases seen include urinary tract infection, nephrotic syndrome, poststreptococcal acute glomerulonephritis, lupus nephritis and tropical acute renal failure. The most common histopathological cause of primary nephrotic syndrome in children is mesangial proliferative glomerulonephritis with IgM deposition. Common etiologies of tropical renal failure are dengue shock syndrome and wasp stings. The prevalence of chronic renal failure in children below 15 years of age is four per million age-related population. Congenital abnormalities of the kidney and urinary tract are the most common cause of chronic renal failure, with obstructive uropathies and renal hypoplasia or dysplasia forming almost 25% of the cases (60).

Systemic lupus erythematosus has a high prevalence in Thailand with about 150–200 new cases diagnosed each year. In a multi-center study involving 13 tertiary care hospitals between 2002 and 2006, the hospital admission rate for newly diagnosed pediatric patients with systemic lupus erythematosus was 1:754 children. Lupus nephritis was seen in 81% of these 500 patients. In a review of the clinicopathologic features of 82 pediatric patients with lupus nephritis, the age range at onset of disease was 2–12 years (61). Almost 50% had severe proliferative lupus nephritis (WHO class IV classification), with a patient mortality rate of 26% over 5 years, of whom 65% died of serious infections, 15% of cardiovascular complications, and 10% of end-stage renal disease. Cyclophosphamide was the main second-line immunosuppressive medication, either oral or intravenous administration, and infectious complications were noted in those patients who were maintained on concomitant higher doses of prednisolone (62).

Vietnam

Vietnam has a population of 86 million, of whom 36% are 19 years or under. Similar to the other South-East Asian nations, the common renal diseases in children include primary nephrotic syndrome, acute glomerulonephritis, lupus nephritis, urinary tract infection and congenital abnormalities of the kidney and urinary tract. Of the children with idiopathic nephrotic syndrome, 10% were corticosteroid-resistant. Pathologic findings frequently found in patients with corticosteroid-resistant nephrotic syndrome were focal segmental glomerulosclerosis, minimal change disease, and mesangial proliferation (63). Corticosteroid-resistant nephrotic syndrome was the main cause of end-stage renal failure. Five percent of children with nephrotic syndrome were secondary to systemic lupus erythematosus, Henoch-Schönlein purpura, hepatitis B, and malaria. Nephrotic syndrome in the first year of life is rare in Vietnam.

The most common cause of acute glomerulonephritis in childhood is poststreptococcal glomerulonephritis but its incidence appears to have declined in recent years. Other common causes of glomerulonephritis in Vietnam include IgA nephropathy lupus nephritis. Diffuse proliferative glomerulonephritis was found in 33% of renal biopsies (64–66). Methylprednisone, intravenous cyclophosphamide pulse therapy and mycophenolate mofetil have been used to treat severe cases.

The main causes of acute renal failure in Vietnam are septicemia and wasp and bee stings. Hemolytic uremic syndrome as a cause is rare. The chief causes of chronic renal failure include focal segmental glomerulosclerosis, obstructive uropathy, reflux nephropathy, and renal hypoplasia or dysplasia.

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Subject Index

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B

C

D

E

F

G

H

J

K

L

M

N

O

P

Q

R

S

T

U

W

X