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William H. Dantzler

Comparative Physiology of the Vertebrate Kidney

With 33 Figures

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Preface

This volume emphasizes the comparative approach to understanding vertebrate renal function. I am convinced that this approach is of particular value in understanding both the details of renal function at the cellular and subcellular levels and the renal role in regulating fluid volumes and solute concentrations. My exposure to this approach first occurred during a student research experience in the laboratory of Wilbur H. Sawyer, who also provided an introduction to the works of Homer W. Smith and August Krogh. The importance of this approach was reinforced by doctoral and postdoctoral research in the laboratory of Bodil Schmidt-Nielsen. It has been confirmed through years of personal experience since then.

My research and my understanding of renal function have been aided through the years by collaboration and discussion with numerous students and associates. Of particular importance in developing my views on comparative renal function, and especially on the relationship of structure to function, has been my long association with my colleague and friend, Eldon J. Braun. Donald S. Farner, who suggested the writing of this volume, provided valuable editorial assistance.

Much of my personal research in this area has been supported over the years by grants from the United States National Science Foundation and National Institutes of Health. The writing of this volume was completed while I was in Würzburg, Federal Republic of Germany, supported by a Senior U.S. Scientist Award from the Alexander von Humboldt Foundation.

I thank my fellow scientists for sometimes allowing me to note their unpublished observations. I also thank them and their publishers for permitting me to use many published figures. Finally, and most important, I thank my wife, Barbara, and my children, Amy and Kurt, for not simply enduring my absorption in my work, but, by their patient support, greatly aiding my progress.

Arizona, Autumn 1988 WILLIAM H. DANTZLER

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Chapter 1

Introduction

Kidneys play a role — usually a major role — in regulating water and solute composition of the internal environment of all vertebrates. The purpose of this book is to examine the physiological functions of the kidneys from a comparative viewpoint with emphasis on the nonmammalian vertebrates. Mammalian renal function is considered for comparison with nonmammalian renal function and as a frame of reference for some of the discussions. However, no attempt is made to consider the vast literature on mammalian renal function in detail. Even the studies on nonmammalian vertebrates are not given in complete detail. Instead, the major findings and the important questions raised are described and summarized, for this volume is not intended as an all-inclusive reference. It is intended as a reasonably comprehensive and integrated picture of comparative renal function for the biological scientist or advanced student of biology who has some knowledge of physiology and a desire to know more about renal function in vertebrates, and for the mammalian renal physiologist who wishes to obtain a broader view of renal function.

1.1 The Comparative Approach

The comparative approach to understanding renal function has a number of advantages. First, a consideration of those physiological processes that make it possible for different species to survive under diverse environmental conditions not only increases our understanding of environmental adaptations but also may suggest those scientific studies most likely to reveal important general physiological principles. Second, the comparison of renal function in a variety of species often permits the exploration of a basic physiological mechanism in an exaggerated form in a single species or through the use of a simple preparation.

The comparative approach to physiological studies has often been suggested as a means of shedding light on the evolution of physiological processes. However, the information available on renal function is so fragmented and so frequently limited to a single species or a few species that attempts to reach major conclusions about the evolution of mechanisms result only in unwarranted speculation. Therefore, although comparisons of renal mechanisms and adaptations among the major vertebrate groups are made throughout this book, particularly in Chapter 8, and the apparent independent evolution of comparable mechanisms in separate vertebrate groups (e.g., the mechanism for concentrating the urine in birds and mammals) is occasionally noted, general conclusions about the phylogenetic evolution of renal mechanisms are avoided.

1.2 Environmental Requirements in Regulating the Water and Solute Composition of the Internal Environment

Vertebrates, both mammalian and nonmammalian, survive and, indeed, thrive under a very wide range of environmental conditions. Some live in arid environments in which conservation of water is of great importance. Others live in completely aqueous freshwater environments in which excretion of excess water and retention of important inorganic solutes are essential. Still others live in completely aqueous marine environments. Here, the high osmolality of the surrounding seawater either requires osmotic conformity with the medium or the retention of water and the excretion of excess solutes, including not only sodium and chloride but also large quantities of ingested divalent ions, such as calcium, magnesium, phosphate, and sulfate. Even some of those species that conform to a marine environment, however, must adjust the solute composition of their internal environment. Many species, particularly terrestrial, tolerate variations in solute and water requirements and others, particularly aquatic species, even move from one environmental extreme to another, for example from seawater to freshwater.

1.3 Possible Roles of Kidneys in Regulating Water and Solute Composition of the Internal Environment

As noted above, kidneys generally play a role in the regulation of solute and water composition of the internal environment. Some information on this role is available for species from the major vertebrate classes: cyclostomes (Agnatha), both the myxini (hagfishes) and petromyzones (lampreys); elasmobranchs (Chondricthyes); bony fishes (Osteichthyes), teleosts, primarily, but also the lungfish; amphibians (Amphibia), both urodeles and anurans; reptiles (Reptilia), primarily the Testudinea, Squamata, and Crocodilia, but also the Rhynchocephalia; birds (Aves); and mammals (Mammalia). However, only among mammals is the kidney essentially of sole importance in regulating the solute and water composition of the internal environment. Among all other vertebrate groups, extrarenal routes for the regulation of solute and water movements, or postrenal modification of ureteral urine, or both are also important. For example, among the cyclostomes, the role of the kidney in the regulation of solute and water balance appears to be substantially greater in the lampreys than in the hagfish, but in both groups regulation of solute and water movements across the gills and, possibly, the integument or within the gastrointestinal tract may be significant. Renal function is clearly important in solute and water balance in marine elasmobranchs, but a specialized extrarenal route (rectal gland) for sodium chloride excretion also exists and ion and water movements across the gills may be regulated. Among the euryhaline teleosts, the kidneys are

particularly important in adaptation of the animals to freshwater or seawater, but regulation of renal function is clearly coordinated with regulation of ion and water movement across the gills. Although renal function is very important in regulating solute and water excretion in amphibians, it is also coordinated with postrenal ion and water movements across the bladder or cloaca and with ion and water movements across the integument. Among reptiles and birds, renal function, although highly significant, again must be coordinated with the regulation of postrenal transport of ions and water across the bladder, cloaca or colon and, in some species, with the regulation of ion excretion by extrarenal salt glands. Unfortunately, the exact quantitative relationships among the various routes of solute and water excretion are not really known for any species of nonmammalian vertebrate. Nevertheless, it must be kept in mind that regulation of renal function in nonmammalian vertebrates is always carefully integrated with the regulation of these other routes of water and solute movement, as well as with the behavior of the animals, in their total adaptation to their environment.

For renal function alone, many variations exist among vertebrates. Present knowledge is insufficient to permit a complete description of these variations or the mechanisms underlying them. Nevertheless, this volume attempts a description and analysis of all those renal functions for which there is some information available on nonmammalian vertebrates. For this purpose, it begins with a brief description of both gross and fine structure of the kidneys. It then moves to a consideration of the initial process in urine formation, primarily ultrafiltration of the plasma at the renal glomerulus but also secretion of fluid by the renal tubules. It then covers transport of inorganic ions, fluid, and organic substances by renal tubules and regulation of these transport processes. Renal aspects of acid-base balance are considered only in terms of tubular transport. Following these discussions, the processes involved in producing urine hypoosmotic or hyperosmotic to plasma, their relative importance and their regulation, are discussed. Contrasts and comparisons of renal mechanisms among the vertebrates are made throughout the book. However, a final chapter (Chap. 8) is devoted to an integrated (although, by necessity, somewhat simplified) summary of renal function for each vertebrate group and summary comparisons of several major renal functions for which adequate information is available among the vertebrate groups.

1.4 Useful Reviews on Comparative Renal Physiology

The present volume covers all major aspects of vertebrate renal physiology from a comparative viewpoint. Other reviews consider specific renal functions from a comparative viewpoint, renal function in specific animal groups only, or comparative renal function in nonmammalian vertebrates only. Because a number of these provide excellent coverage of these topics, a selection is

presented here. References to many other shorter reviews on specific aspects of renal function are given throughout the following chapters.

Two major textbooks on renal function, *The Kidney* (Brenner and Rector 1986) and *The Kidney: Physiology and Pathophysiology* (Seldin and Giebisch 1985) contain detailed chapters on all aspects of renal function. Although these are written primarily from a mammalian viewpoint, many experimental data, particularly on various aspects of tubular transport, are derived from studies on nonmammalian vertebrates, particularly amphibians. In addition, in the volume edited by Seldin and Giebisch, there is a chapter on comparative renal function in nonmammalian vertebrates only (Dantzler 1985). Similarly, in the *Handbook of Physiology,* the volume on *Renal Physiology* (Windhager 1989) contains specific chapters covering most aspect of renal function in detail. Much of the detailed information at ther cellular and subcellular level is based on studies with nonmammalian vertebrates. This volume also contains a chapter on comparative renal function in nonmammalian vertebrates (Dantzler 1989).

A number of older review chapters are available on renal function in single major groups of vertebrates. These include reviews on fishes (Hickman and Trump 1969), reptiles (Dantzler 1976a), and birds (Skadhauge 1973). A recent issue of *Renal Physiology* (1985) was devoted entirely to the comparative aspects of renal function. This contains reviews on glomerular filtration (Yokota et al. 1985a), transport in the proximal nephron (Beyenbach 1985), transport in the distal nephron (Stoner 1985), urinary concentrating process (Braun 1985), excretion of nitrogenous compounds (King and Goldstein 1985), and endocrine control of renal function (Nishimura 1985).

Chapter 2

Renal Morphology

2.1 Introduction

Similarities and differences exist among vertebrate classes in gross external morphology, internal organization, and cellular structure of kidneys. Since these similarities and differences are related to similarities and differences in renal function, some knowledge of structure is important for understanding function. However, this volume is not a treatise on renal morphology and only those known morphological features that appear most important for an understanding of comparative renal function are discussed.

2.2 Gross Internal and External Morphology

2.2.1 General Considerations

Most vertebrate nephrons consist of a glomerulus followed by a neck segment, a proximal tubule, an intermediate segment, a distal tubule, and, finally, a collecting tubule and duct system (Figs. 2.1, 2.2). However, variations in this sequence of nephron segments (Fig. 2.2), in the relationship of the nephron segments to their blood supply and to each other, and in the relationship of individual nephrons to each other occur within and between vertebrate classes. These relationships also help to define the gross external features of the kidney. The most significant variations in these structures and their relationships are considered below and later will be considered in more detail in relation to function.

2.2.2 Fishes

2.2.2.1 Cyclostomes

Among the cyclostomes, the adult Myxini (hagfishes), which conform to their marine environment in terms of the osmolality and sodium and chloride concentrations of their body fluids, retain paired apparently nonfunctional pronephric kidneys as well as paired functional mesonephric kidneys. Each of the paired mesonephric kidneys contains 30–35 very large, oval glomeruli arranged segmen-

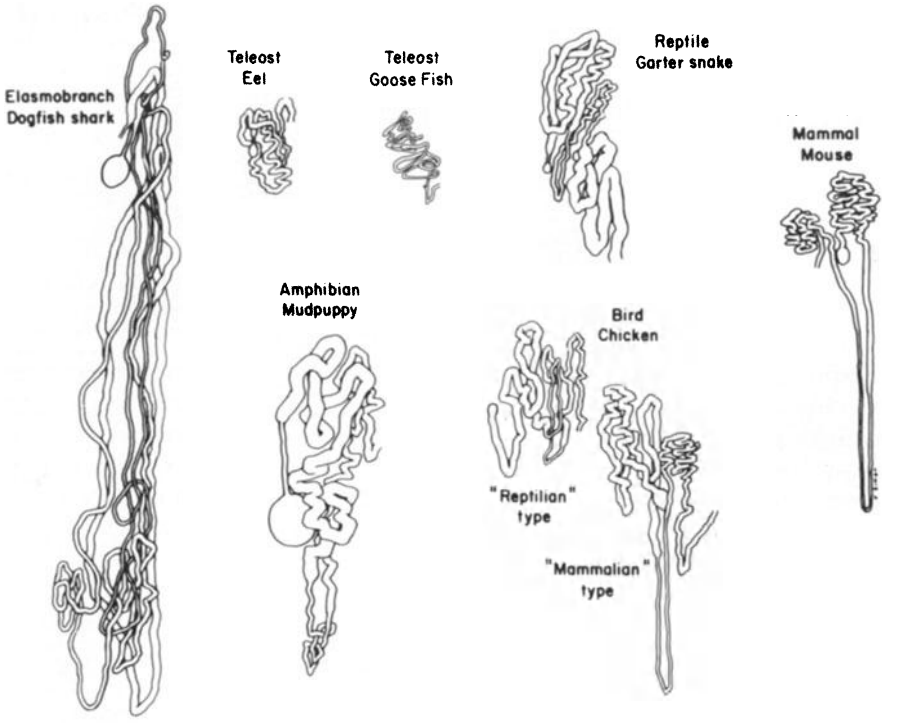


Fig. 2.1. Representations of fish (elasmobranch, *Squalus acanthias;* glomerular teleost, *Anguilla rostrata;* aglomerular teleost, *Lophius piscatorius*), amphibian (*Necturus maculosus*), reptilian (*Thamnophis sirtalis*), avian (*Gallus gallus*, domestic fowl), and mammalian (*Mus flavicolis*) nephrons drawn to a single scale. (After Long and Giebisch 1979 and Marshall and Grafflin 1928; reproduced from Dantzler 1985

tally on the medial side of a primitive archinephric duct (ureter) (Fig. 2.2). Each glomerulus is connected to the archinephric duct by a short neck segment (Fig. 2.2; also Hickman and Trump 1969). There are no other nephron segments.

The adult Petromyzones (lampreys), which apparently do not conform to their environment, thus maintaining body fluids hypoosmotic to a marine environment and hyperosmotic to a freshwater environment, have nephrons similar in gross structure to more advanced vertebrates (Hickman and Trump 1969; Logan et al. 1980b). Each nephron has a glomerulus, a ciliated neck segment, a proximal tubule, an intermediate segment, a distal tubule, and a collecting duct (Fig. 2.2). Although early evidence suggested that the urinary space of adjacent glomeruli might be continuous in some species (Regaud and Policard 1902; Vinnichenko 1966; Youson 1975), the most recent evidence indicates that this is not so (Logan et al. 1980b). Each nephron apparently has a major loop segment arranged parallel to its own collecting duct (Fig. 2.2; also Logan et al. 1980b), but the functional significance of this loop is unknown. The nephrons do not appear to be arranged in a manner that permits them to function in concert to produce a hyperosmotic urine (vide infra; Chap. 7). In the sea lamprey, *Petromyzon marinus,* the loop consists entirely of distal tubule (Youson and

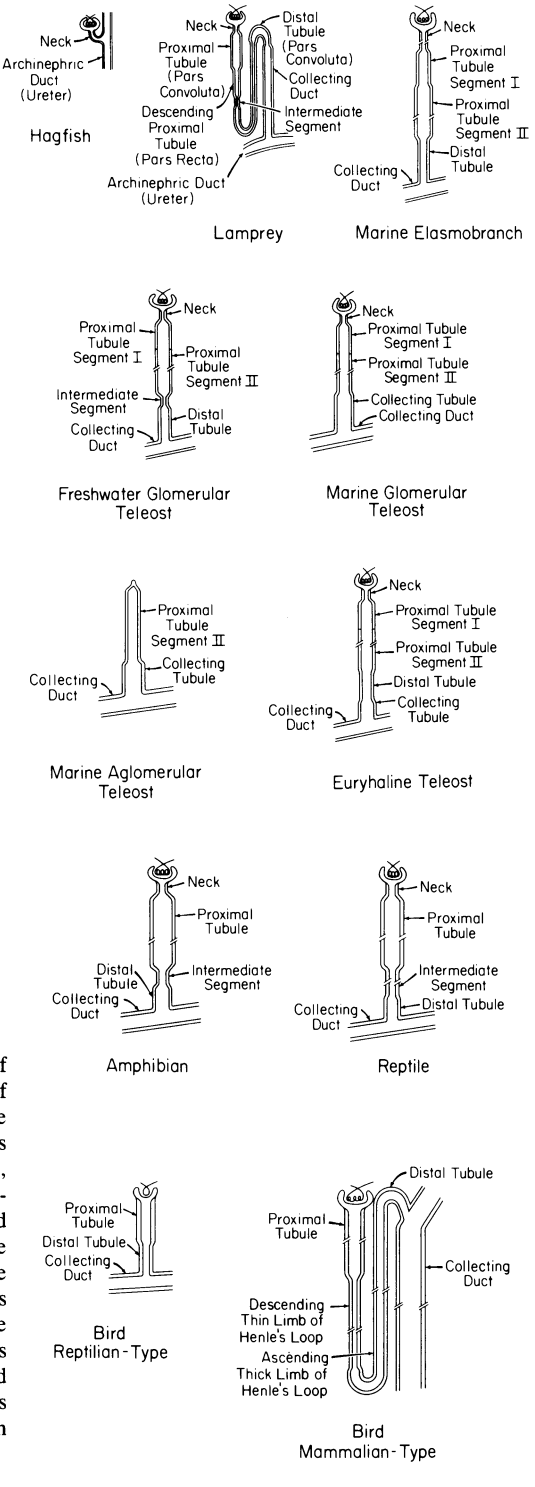


Fig. 2.2. Diagrammatic representations of nephrons from the major classes of nonmammalian vertebrates to show the major nephron segments. No attempt was made to draw nephrons to an exact scale, although some attempt was made to indicate relative sizes for fish and bird nephrons. Breaks in the nephrons indicate that the lengths of those segments may be much greater relative to other segments than actually shown. Except for those nephrons in which a loop structure was parallel to the collecting ducts (lamprey and avian mammalian-type), no attempt was made to show the shape of the nephron segments. (Dantzler 1985)

McMillan 1970b), whereas in the river lamprey, *Lampetra fluviatilis,* the descending limb of the loop consists of proximal tubule (Fig. 2.2; also Morris 1972).

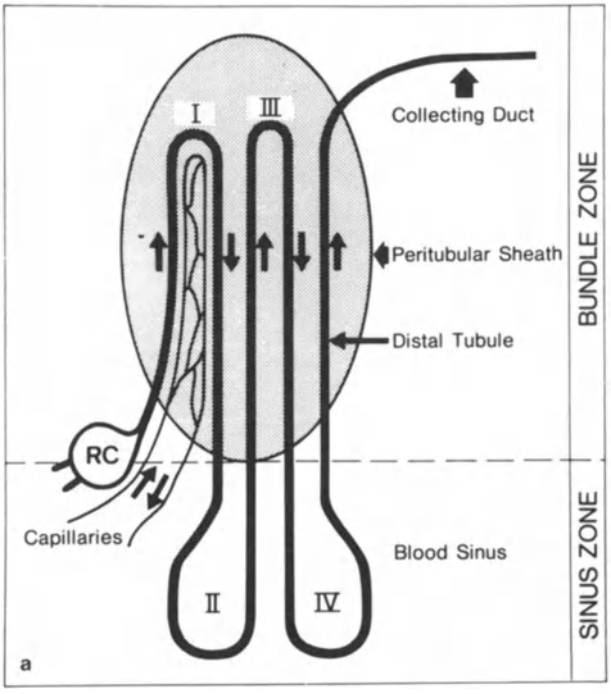
2.2.2.2 Elasmobranchs

Marine elasmobranchs, like the hagfishes, conform to the osmolality of their environment, but, unlike hagfishes, much of the osmolality of the extracellular fluid is determined by the concentrations of urea and trimethylamine oxide (TMAO). Nephrons in these animals contain all the standard vertebrate components noted above (Figs. 2.1, 2.2; also Hickman and Trump 1969), but the arrangement of the nephrons is highly complex. Although the elasmobranch kidney is not organized into discrete cortical and medullary regions like the mammalian kidney, the nephrons are arranged to permit countercurrent flow within the dorsal region of the kidney (Fig. 2.3; also Boylan 1972; Deetjen and Antkowiak 1970; Lacy et al. 1975, 1985). Moreover, the five nephron segments so arranged and the peritubular capillaries arranged in a countercurrent fashion among them are encapsulated in a peritubular sheath that may serve to create a microenvironment in which some form of countercurrent exchange or, possibly, countercurrent multiplication can operate (Fig. 2.3). Such a process may be very important in the retention of urea by the kidneys of marine elasmobranchs (vide infra; Chap. 6).

2.2.2.3 Marine Teleosts

Marine teleosts maintain the osmolality of their body fluids well below that of their environment. Grossly, their kidneys are divided into an anterior head kidney containing lymphoid, hematopoietic, and glandular tissue and a posterior trunk kidney containing the renal tissue, but in many species the two kidneys are partially or completely fused and cannot be distinguished by external examina-

Fig. 2.3 a, b. Diagram of elasmobranch nephron in the bundle zone (dorsal) and sinus zone (ventral). The dorsal surface is parallel to the top of the page. a Simplified diagram showing renal corpuscle (*RC*) (Glomerulus) and four highly stylized nephron loops (*I* to *IV*). A peritubular sheath surrounds the countercurrent system of nephron segments (loops *I, III,* and the distal tubule) and anastomosing capillary loops in the bundle zone. Small arrows indicate the direction of tubular fluid and blood flow. b Schematic drawing of the pathway of the skate nephron in the bundle zone (dorsal) and in the sinus zone (ventral) showing some of the nephron complexity. The entering limbs of nephron loops I and III and the distal tubule (a) pierce the peritubular sheath near the renal corpuscle and extend to the opposite end of the sheath. Close to the renal corpuscle, the five tubular segments (loops *I, III,* and the distal tubule) located in the bundle zone are covered by the peritubular sheath and run parallel to each other (to emphasize this distinctive course, they have been drawn side by side in one plane and not assembled into a bundle as they actually are). The tubular bundle and surrounding peritubular sheath then become convoluted. The parallel course of the tubules is lost since the loops wrap around each other. For simplicity, the opposite end of the peritubular sheath, where the distal tubule emerges, has been drawn away from the renal corpuscle on the far right side of the diagrm. The distal tubule pierces the sheath at this point to join the collecting duct, whereas the two other nephron segments loop back and retrace their path, finally exiting the sheath where they entered it. Capillaries also enter and exit the peritubular sheath at its renal corpuscle terminus and form an anastomotic network around and within the tubular bundle. (Lacy et al. 1985)


I
III
Collecting Duct
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Distal Tubule
BUNDLE ZONE
RC
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Blood Sinus
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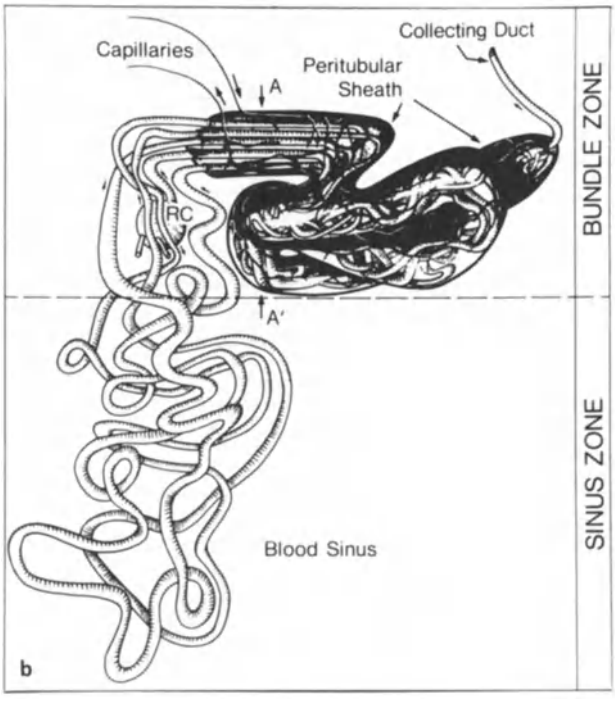
Capillaries
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Blood Sinus
SINUS ZONE
b

tion (Hickman and Trump 1969). The nephrons of glomerular marine teleosts generally contain, in addition to a glomerulus, a neck segment, two or three proximal segments that constitute the major portion of the nephron, sometimes an intermediate segment between the first and second proximal segments, and a collecting tubule emptying into the collecting duct system (Fig. 2.2). The distal tubule is absent in almost all species (Hickman and Trump 1969) and, where present, may actually indicate some degree of euryhalinity. Although the entire proximal tubule has a brush border, only the first segment is ultrastructurally similar to the proximal convoluted tubule of mammals (Hickman and Trump 1969). The nephrons of aglomerular marine teleosts typically have a proximal segment with a brush border similar to the second proximal segment of the nephrons of glomerular teleosts and a collecting tubule (Fig. 2.2).

2.2.2.4 Freshwater Teleosts

Freshwater teleosts, which maintain the osmolality of their body fluids well above that of their environment, have nephrons that differ from those of marine teleosts. Grossly, the head and trunk kidneys are fused to a large extent (Hickman and Trump 1969). The nephrons of glomerular species typically contain, in addition to a glomerulus, a ciliated neck segment, an initial proximal segment with a prominent brush border, a second proximal segment with a less prominent brush border, an intermediate segment, and a distal tubule emptying into the collecting duct system (Fig. 2.2). The glomeruli tend to be larger and the nephrons more numerous than in marine teleosts. The glomeruli of some freshwater teleosts that survive adaptation to sea water atrophy and disappear (Elger and Hentschel 1981). The few aglomerular freshwater teleosts (apparently species that evolved in seawater and later invaded freshwater) seem to have nephrons entirely like those of marine aglomerular species (Fig. 2.2) (Hickman and Trump 1969).

2.2.2.5 Euryhaline Teleosts

These animals, which can maintain the osmolality of their body fluids above that of the environment when adapted to freshwater and below that of the environment when adapted to seawater, have nephrons most similar to those of freshwater teleosts. These typically have a glomerulus (often smaller and less vascular than in stenohaline freshwater species), a first proximal segment, a second proximal segment, a variably present short intermediate segment, a distal tubule, and a collecting tubule emptying into the collecting duct system (Fig. 2.2; also Hickman and Trump 1969).

2.2.3 Amphibians

The external shape of the mesonephric kidneys of amphibians, whose habitats range from completely aqueous to arid terrestrial, varies substantially among species. They tend to be elongated with some evidence of segmentation in the urodeles and relatively short and compact in the anurans. However, basic

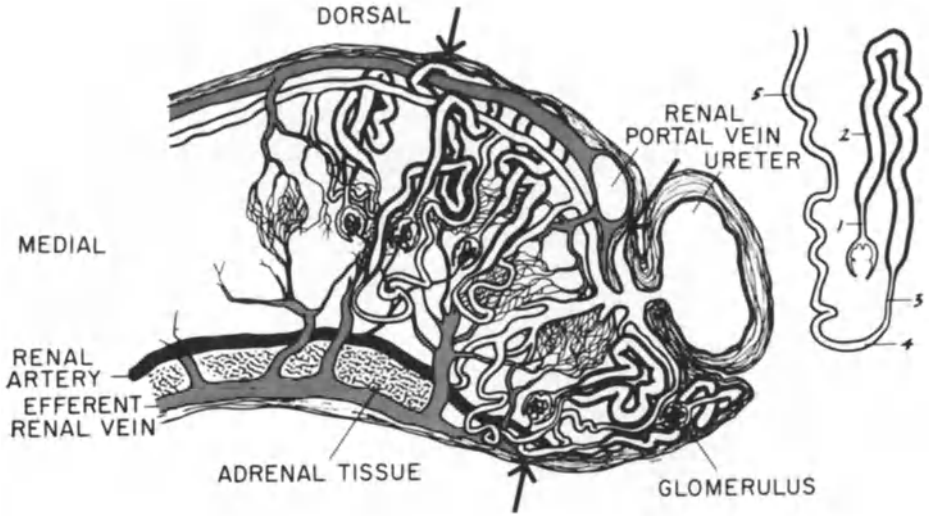


Fig. 2.4. Cross-section of frog kidney, adapted from W. von Mollendorf (1930). *1* neck segment; *2* proximal tubule; *3* intermediate segment; *4* distal tubule; *5* collecting duct. *Arrows* designate typical micropuncture sites. (Long 1973)

internal renal organization is rather similar for all amphibians and can be illustrated by that of the frog kidney shown in Fig. 2.4. All nephrons contain a glomerulus, ciliated neck segment, proximal tubule, ciliated intermediate segment, and distal tubule emptying into the collecting duct system (Figs. 2.1, 2.2, and 2.4). Of particular note, as in most other nonmammalian vertebrates, the nephrons empty at right angles into the collecting ducts; there are no discrete cortical and medullary regions (Fig. 2.4).

2.2.4 Reptiles

Like the mesonephric kidneys of amphibians, those of reptiles, whose habitats also range from completely aqueous to arid terrestrial, show marked variation in their external morphology. This is undoubtedly due to the extreme variation in body form within the class Reptilia, as exemplified by the ophidians on the one hand and the chelonians on the other. Again, however, although the external shape of the kidneys varies within the Reptilia, the internal organization of these organs is reasonably uniform. This basic organization is illustrated diagrammatically for the snake kidney in Fig. 2.5. The nephrons are composed of all standard components — glomerulus, ciliated neck segment, proximal tubule, ciliated intermediate segment, and distal tubule (Fig. 2.2) — and they empty at right angles into the collecting ducts (Fig. 2.5). Again, there are no discrete cortical and medullary regions (Fig. 2.5).

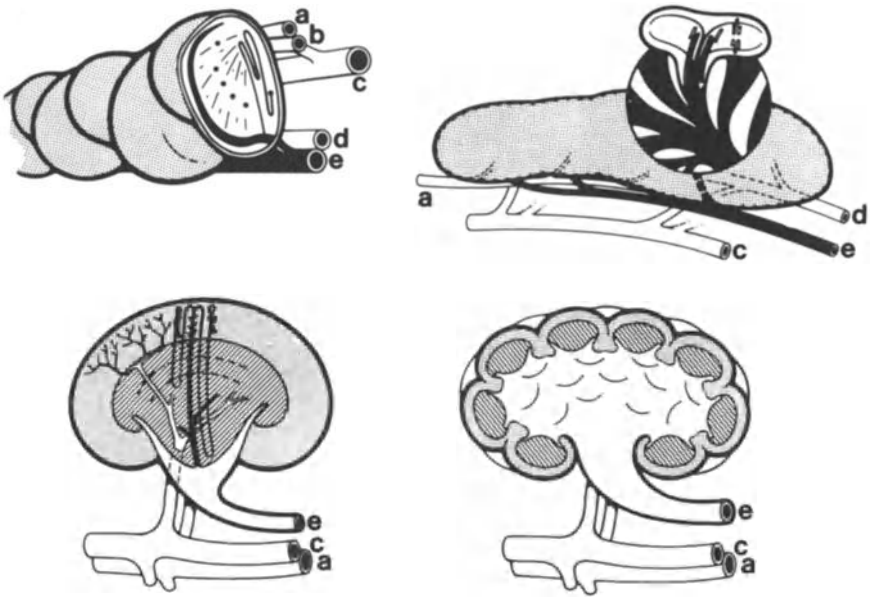


Fig. 2.5. Diagram of reptilian (snake) kidney (*upper left*), avian kidney (*upper right*), unipapillary mammalian kidney (*lower left*), and multirenculated mammalian kidney (*lower right*). Letter notations are consistent for all four figures and are as follow: *a* renal vein; *b* renal artery (reptilian kidney only); *c* aorta; *d* renal portal vein (reptilian and avian kidney); *e* ureter. Note that nephrons and collecting ducts (*solid black*) in reptilian and avian kidneys are continuous with ureters (also shown in *solid black* in these kidneys). (Dantzler and Braun 1980)

2.2.5 Birds

The kidneys of birds vary less from species to species in gross external appearance than those of amphibians, reptiles, or even mammals. They are prominent, flattened, retroperitoneal organs (Figs. 2.5, 2.6), deeply recessed into the bony synsacrum (fused lumbar, sacral, and caudal vertebrae). The kidneys are crossed by major nerve trunks and blood vessels securing them tightly in place. Each kidney usually consists of three divisions — cranial, medial, and caudal — which may or may not be apparent superficially, depending on the species (Johnson 1968).

The avian kidney can be divided into cortical and medullary regions, but the boundary between these regions is not as sharp as it is in most mammalian kidneys. Moreover, it contains nephrons resembling those found in the kidneys of most other nonmammalian vertebrates and nephrons resembling those found in the kidneys of mammals (Figs. 2.1, 2.2, 2.5, 2.6). Most nephrons (e.g., about 90% in Gambel's quail, *Callipepla gambelii;* about 70% in European starlings, *Sturnus vulgaris*) are of the type found in other nonmammalian vertebrates (Braun 1978; Braun and Dantzler 1972). These nephrons (referred to as reptil-

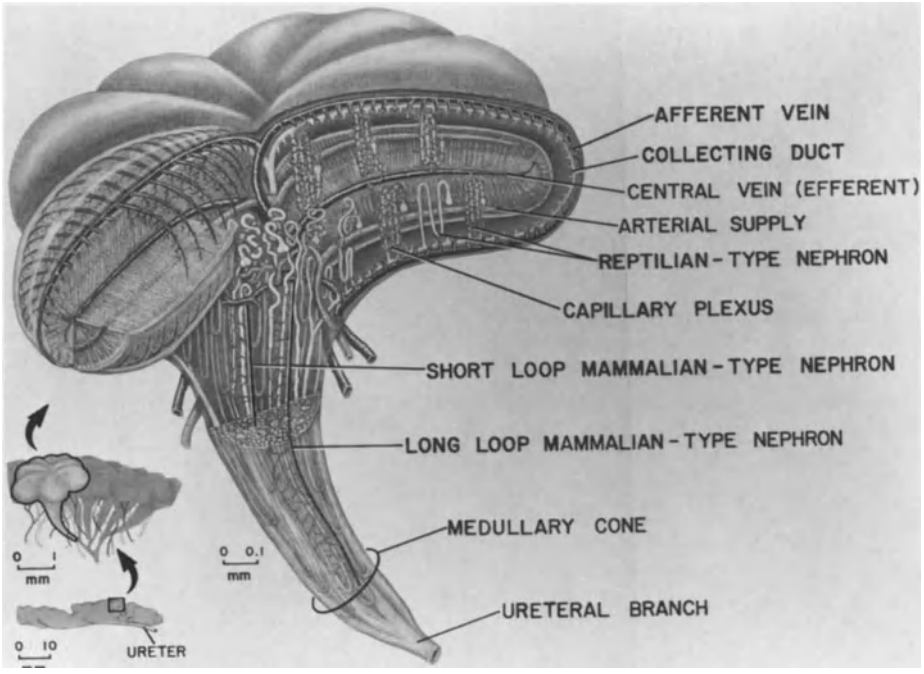


Fig. 2.6. A three-dimensional drawing of a section of avian kidney (Gambel's quail, *Callipepla gambelii*) showing types of nephrons present, their relative positions in the kidney, and their relationship to other renal structures. (Braun and Dantzler 1972)

ian-type nephrons) are located in the superficial or cortical region of the kidney (Figs. 2.5, 2.6). They are very simple, even simpler than most true reptilian nephrons. There is a glomerulus, no neck segment, a proximal tubule with a few simple folds, and then abrupt transition to an even simpler distal tubule with only one main loop before it empties at right angles into a collecting duct; there is no intermediate segment (Figs. 2.1, 2.2, 2.6, 2.7; also Wideman et al. 1981). In some, but not all nephrons, there is a short connecting tubule between the end of the distal tubule and the collecting duct (Wideman et al. 1981). Like the nephrons in the teleostean, amphibian, and reptilian kidneys, these reptilian-type nephrons are not arranged in a manner that would be expected to permit them to contribute directly to the production of urine hyperosmotic to the plasma. Instead, these nephrons are arranged in a radiating pattern about the long axis of a vein (central efferent vein) to form cylindrical cortical units (cortical lobules) (Figs. 2.6, 2.7). The cortical lobules are themselves grouped in radiating patterns from central points over the entire surface of the kidney (Fig. 2.6).

Deep to these cortical lobules containing the small reptilian-type nephrons without loops of Henle are those larger mammalian-type nephrons in which the intermediate segments are lengthened into loops of Henle (about 10% of the nephrons in Gambel's quail; about 30% in starlings) (Fig. 2.6) (Braun 1978; Braun and Dantzler 1972). Like true mammalian nephrons, these nephrons

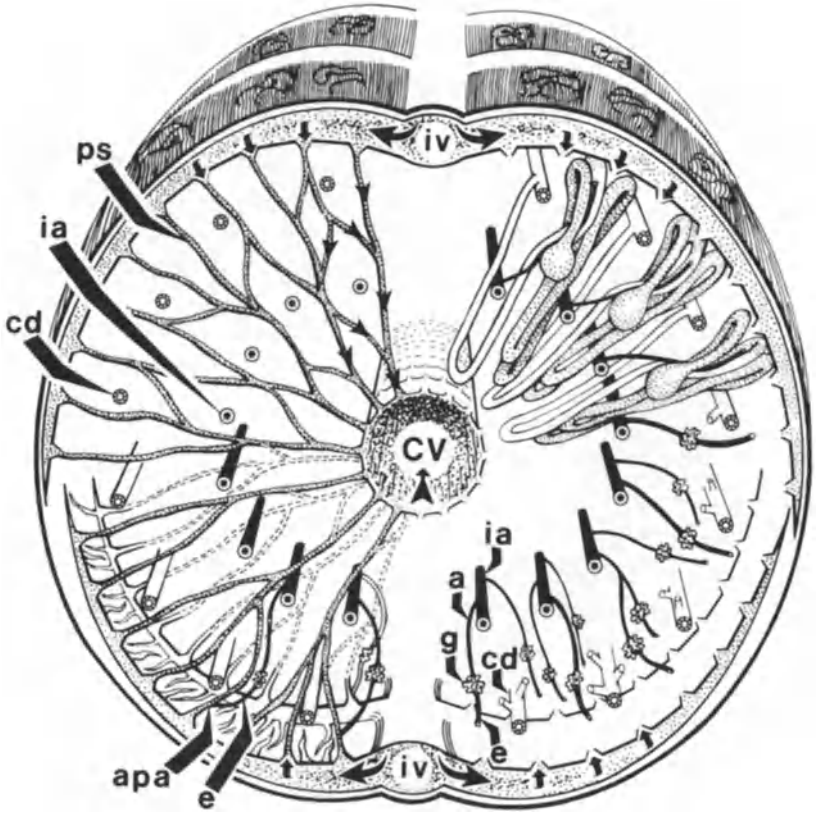


Fig. 2.7. Schematic representation of the avian renal cortex. Renal portal blood (*arrows*) flows through the interlobular veins (*iv*) and empties directly into the freely anastomosing spaces between tubules (peritubular sinuses (*ps*) to be re-collected by the central vein (*cv*). Other abbreviations: *a* afferent arteriole; *apa* arterial-portal anastomosis; *cd* collecting duct; *e* efferent arteriole; *g* glomerular capillaries; *ia* intralobular artery. (Wideman et al. 1981)

have, in addition to a larger glomerulus than the reptilian-type nephrons, a convoluted proximal tubule, a straight proximal tubule, a thin descending limb of Henle's loop, a thick ascending limb of Henle's loop that always begins before the bend, a distal convoluted tubule, and possibly a terminal collecting tubule or connecting tubule (E. J. Braun personal communication) (Figs. 2.1, 2.2). However, the transition from the reptilian-type nephrons to the mammalian-type is gradual, not abrupt, so that there are nephrons of intermediate size and relatively short loops of Henle in the area between the superficial cortical lobules and the deeper medullary region (Fig. 2.6).

The loops of Henle of the mammalian-type nephrons, the vasa recta, which arise from the efferent arterioles of glomeruli of mammalian-type nephrons, and the collecting ducts, which drain both the reptilian-type and the mammalian-type nephrons from each radial group of cortical lobules, are arranged in parallel and bound together by a connective tissue sheath into a tapering structure called a medullary cone (Figs. 2.5, 2.6). This arrangement, as in the mammalian kidney,

would be expected to permit the avian kidney to produce a urine hyperosmotic to the plasma (vide infra; Chap. 7). As in the reptilian and amphibian kidneys and in contrast to the mammalian kidney, however, there is no renal pelvis. The nephrons, collecting ducts, and ureter are continuous, and, therefore, each medullary cone terminates as a branch of the ureter (Figs. 2.5, 2.6). The number of medullary cones per kidney is relatively constant for a given species but varies markedly from species to species (Johnson 1968).

The general gross organizational pattern of many cortical areas with their associated medullary cones, the real lobes of the kidney, resembles the pattern of the extreme multirenculated kidney found in cetacean and some bovine mammals (Fig. 2.5). In both birds and cetaceans there are many lobes at varying depths throughout the kidney, but in the cetaceans these lobes are separated by connective tissue whereas in birds they tend to be fused into a single mass (Johnson 1968). In addition to this general similarity of the organization of the avian kidney to the cetacean kidney, the individual lobes of the avian kidney resemble the unipapillary kidneys of small mammals (Fig. 2.5).

2.2.6 Mammals

The general external appearance of the paired, retroperitoneal kidneys of mammals is strikingly different from the external appearance of the kidneys of nonmammalian vertebrates (Fig. 2.5). However, as noted above, the internal organization of the mammalian kidney does bear a distinct resemblance to that of the avian kidney (Fig. 2.5). Mammalian nephrons have glomeruli, proximal convoluted and straight tubules, loops of Henle with thin and thick limbs, distal convoluted tubules, connecting tubules, and collecting ducts (Figs. 2.1 and 2.8). Although the cortical organization of the mammalian kidney differs from that of the avian kidney, the very simple cortical nephrons (nephrons with all their segments in the cortex) of multirenculated mammalian kidneys (Sperber 1944) do resemble the larger reptilian-type or transitional nephrons of the avian kidney. Moreover, the mammalian and avian kidneys resemble each other in their possession of loops of Henle arranged in parallel with vasa recta and collecting ducts in renal papillae and medullary cones (Figs. 2.5, 2.6 and 2.8). This similarity appears to result from parallel evolution as birds and mammals adapted to life on dry land and suggests that there may be a unique general way for the kidneys to eliminate solutes efficiently in a solution more concentrated than the plasma.

Two basic structural types of mammalian kidneys exist: the unipapillary type (one renculus) and the compound or multirenculated type in which this basic unipapillary unit is repeated within the kidney (Fig. 2.5; also Oliver 1968). The repeating unipapillary units in compound or multirenculated kidneys can be discrete or combined into a single compound mass enclosed by a continuous cortex (Oliver 1968). Modifications of the unipapillary kidney, such as the crest kidney, are also observed.

Which one of these general structural types is found in a given mammalian species is related not to phylogeny but to body mass (Oliver 1968, Sperber 1944).

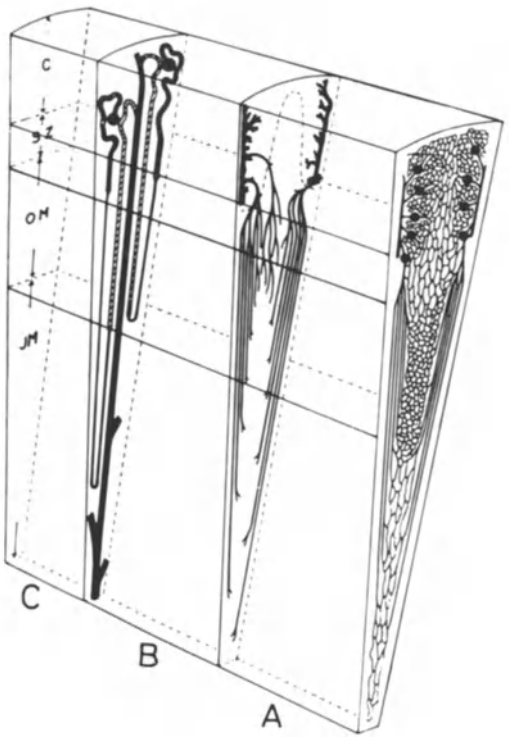


Fig. 2.8. Schematic representation of three sections of the rat kidney in which three components are illustrated separately. *A* The arterial vessels and the capillaries; *B* the venous vessels; *C* collecting duct with nephrons with long and short loops of Henle attached. Labels for the zones of the kidneys are as follows: *C* cortex; *SZ* subcortical zone; *OM* outer zone of medulla; *IM* inner zone of medulla. (Kriz 1970)

Small mammals have unipapillary kidneys. In mammals larger than rabbits the unipapillary kidneys are superceded by crest kidneys. The crest kidney, in turn, tends to disappear in animals larger than kangaroos, although animals as large as giraffes, camels, and horses still have crest kidneys. The multirenculated kidney first begins to appear in animals of the size of otter and beaver and is present in a number of very large animals, including the hippopotamus, elephant, and whale.

The change in form from unipapillary to multirenculated, i.e., many small, unipapillary units, may be related to the physical force required to move fluid along the nephrons and the need to absorb filtered solutes and water (Calder and Braun 1983; Dantzler and Braun 1980; Sperber 1944). The hydrostatic pressure of the arterial blood in the glomerular capillaries provides the driving force for the flow along the tubules, but mean arterial blood pressure in mammals does not increase proportionately with body size (Calder 1981). Because resistance to flow along a nephron will increase as nephron length increases, nephron length also would not be expected to increase proportionately with body size (Dantzler and Braun 1980; Calder and Braun 1983). Indeed, proximal tubule length does not increase continuously with increasing body and kidney mass; rather, it tends to become constant at about the same body and kidney mass at which the unipapillary kidney is replaced by the crest kidney (Oliver 1968; Sperber 1944). In very large mammals with multirenculated kidneys, the proximal tubule length is even shorter than in small mammals. Although an increase in tubule diameter

would reduce resistance to flow, it would interfere with absorption of filtered solutes and water by increasing diffusion distances (Calder and Braun 1983). And, indeed, tubule diameter does not increase continuously (Calder and Braun 1983). Apparently, to accommodate the absolute increase in metabolism and corresponding increase in excretory end products with an increase in body size, the number of nephrons increases. However, there also appears to be an optimal number of nephrons (the number contained in the largest unipapillary kidney) that can function together appropriately in the concentrating and diluting process. As the number of required nephrons exceeds this optimum, the unipapillary kidney gives way to the multirenculated kidney.

2.2.7 General Blood Vessel Patterns

Kidneys of all vertebrate species with glomerular nephrons receive an arterial blood supply. However, the number of renal arteries supplying the kidneys varies substantially among classes, species, and even individual animals. The arterial divisions within the kidney also vary substantially among vertebrate classes and species depending on the gross internal architecture discussed above. In all cases, however, each glomerulus is supplied by an afferent arteriole which breaks up into a glomerular capillary network. In the case of the primitive lampreys, each afferent arteriole apparently supplies more than one glomerulus and each glomerulus apparently contains capillaries arising from more than one afferent arteriole (Hentschel and Elger 1987), but in all other vertebrate species examined, each glomerulus is supplied by only one afferent arteriole.

The complexity of the glomerular network varies markedly among the vertebrates, as demonstrated by Bowman (1842) nearly 150 years ago. In general, the capillary network of most fishes, amphibians, reptiles, and mammals consists of throughfare channels that anastomose freely (Fig. 2.9). However, the extent of the anastomoses and, thus, the complexity of the network, is much greater in the glomeruli of mammals than in the glomeruli of nonmammalian vertebrates. Interestingly, the glomeruli of birds, homeotherms like mammals with a high sustained mean arterial blood pressure, have the simplest glomerular network (Dantzler and Braun 1980). The glomerular tuft of the smallest, reptilian-type nephrons may consist of a single capillary loop with no cross-branching or anastomoses (Dantzler and Braun 1980; E. J. Braun personal communication). The glomerular tuft of the larger, mammalian-type nephrons may consist of a single unbranched capillary coiled around the periphery of the renal capsule although occasional anastomoses or cross-branching also may occur (Dantzler and Braun 1980; Braun personal communication).

In all glomerular species, the glomerular capillaries unite into a single efferent arteriole that leaves the glomerular capsule (Fig. 2.9). The efferent arterioles draining the glomerular capillaries of nephrons in glomerular fishes, amphibians, reptiles, the reptilian-type nephrons of birds, and the superficial and midcortical nephrons of mammals break up into a network of sinusoids or capillaries surrounding the tubules. The pattern of capillaries or sinusoids, which varies among the classes and species, is particularly complex in mammals

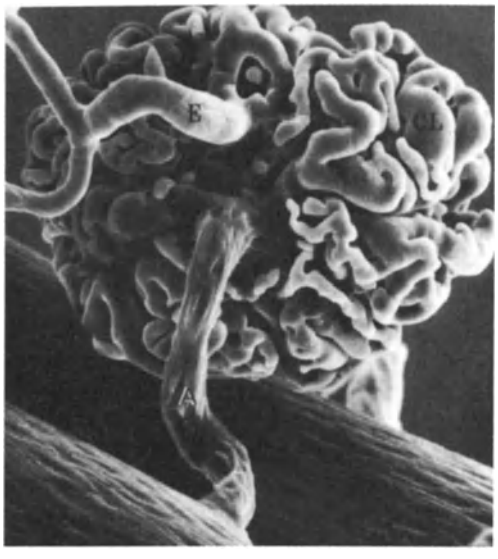


Fig. 2.9. Scanning electron micrograph of a cast of a mammalian (rat) glomerulus with its many capillary loops (*CL*) and adjacent renal vessels. The afferent arteriole (*A*) takes origin from an interlobular artery at *lower left. The efferent arteriole (E)* branches to form the peritubular capillary plexus (*upper left*). (Tisher and Madsen 1986)

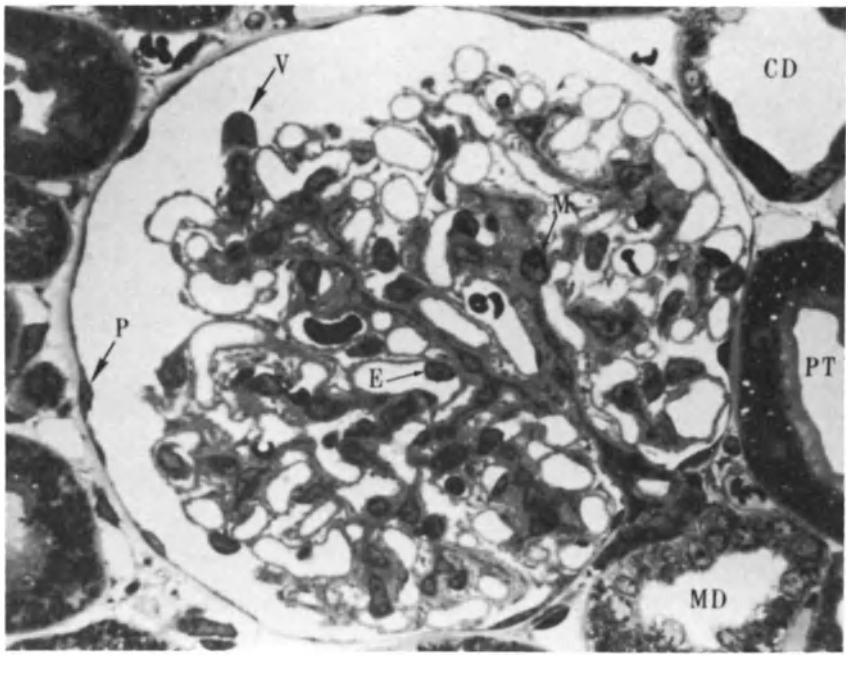


Fig. 2.10. Light micrograph of a normal glomerulus from rat, demonstrating the four major cellular components: mesangial cell (*M*), endothelial cell (*E*), visceral epithelial cell (*V*), and parietal cell (*P*). Other abbreviations: *CD* collecting duct; *PT* proximal tubule. (750). (Tisher and Madsen 1986)

(Beeuwkes and Bonventre 1975). Of special importance for tubular function, the kidneys of all amphibians, reptiles, birds, marine teleost fishes, and, probably, euryhaline teleost fishes have a renal venous portal system that contributes blood to the peritubular capillaries or sinusoids. As already indicated (vide supra), the efferent arterioles from the glomeruli of mammalian-type nephrons in birds and from the inner cortical or juxtamedullary nephrons of mammals give rise to the vasa recta that supply the medullary cones or medulla (Figs. 2.6 and 2.8). The medullary cones of birds receive no venous portal blood (Wideman et al. 1981). Finally, the aglomerular nephrons found in some teleosts receive only a renal portal blood supply (Beyenbach 1985).

2.3 Fine Internal Structure

2.3.1 General Considerations

The fine structure of kidneys, particularly ultrastructure, although extensively studied in mammals, has received only sporadic and inconsistent study in nonmammalian vertebrates. Studies of ultrastructure in nonmammalian vertebrates have been performed primarily on species that have been studied most extensively physiologically, but few descriptions are complete. Nevertheless, a few general structural descriptions for nonmammalian vertebrates, primarily in comparison to those for mammals, are given here. More detailed descriptions, where available and where significant, are mentioned later in conjunction with descriptions of specific physiological processes.

2.3.2 Glomerulus

The renal glomerulus in all vertebrates examined is composed of the capillary network described above, a central region of mesangial cells with their matrix material, the parietal layer of Bowman's capsule composed of the basement membrane that covers the outside of the capillaries and the podocytes, and the visceral layer of Bowman's capsule which is continuous with the epithelium of the proximal tubule (Fig. 2.10). The ultrastructure of the total filtration barrier — the capillary endothelium, the basement membrane, and the visceral epithelial cells or podocytes — is similar for all glomerular species as illustrated in cross-section in Fig. 2.11 and described in the figure legend. One ultrastructural variation among vertebrates in this total filtration barrier is in the number of fenestrae or pores in the capillary endothelial cells. Such fenestrae are few or absent in hagfish (Heath-Eves and McMillan 1974), lampreys (Youson and McMillan 1970a), elasmobranchs (Bargmann and von Hehn 1971), and teleosts (Bulger and Trump 1968), considerably more numerous in reptiles (Anderson 1960; Peek and McMillan 1979b), and very common in mammals (Tisher and Madsen 1986; also Fig. 2.12).

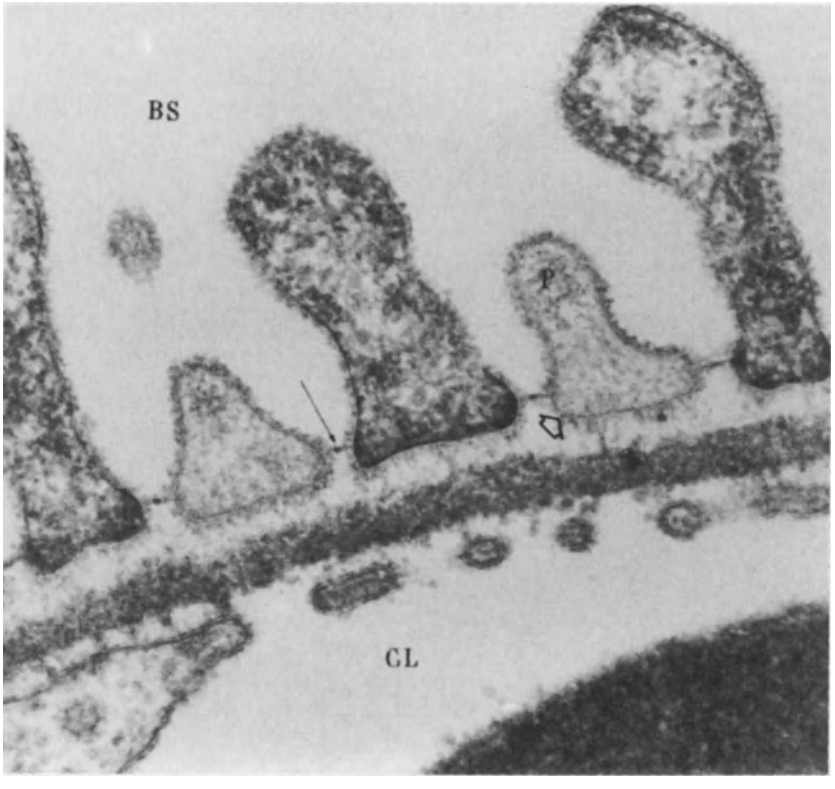


Fig. 2.11. Electron micrograph of normal rat glomerulus fixed in 1% glutaraldehyde solution containing tannic acid. Note the relationship between the three layers of the glomerular basement membrane and the presence of the pedicles (*P*) of the visceralepithelial cells embedded in the lamina rara externa of the basement membrane (*thick arrow*). The filtration slit diaphragm with the central dense spot (*thin arrow*) is especially evident between the individual pedicles. The fenestrated endothelial lining of the capillary loop is shown below the basement membrane. Other abbreviations: *CL* capillary lumen: *BS* Bowman's space. (120,000). (Tisher and Madsen 1986)

On one side of the capillaries, the endothelial cells actually rest on the mesangial cells rather than on the basement membrane and the covering podocytes, but it is not clear that filtration occurs across this region. However, in all species examined (Kriz and Kaissling 1985; Peek and McMillan 1979b; Tisher and Madsen 1986), myofibrils are found in the mesangial cells which may permit them to play a role in regulating the area available for filtration (vide infra; Chap. 3). The number of such myofibrils may vary among the vertebrate species, which may account for some variation in the regulation of filtration. The amount of extraglomerular mesangial tissue also varies among species and this too may have important physiological consequences in the regulation of the glomerular filtration rate (vide infra; Chap. 3).

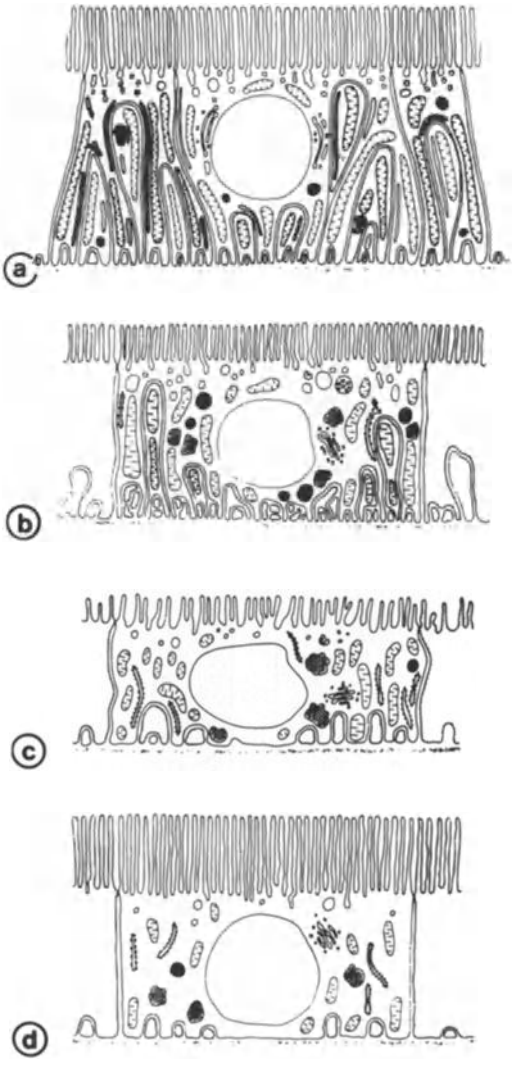


Fig. 2.12 a–d. Drawings of the proximal tubule ultrastructure in mammalian kidneys. To demonstrate the cellular interdigitation, neighboring cells and their processes are covered by a stippled texture. a P1 segment. The cellular interdigitation is most extensive and extends to the apical ends of the cells. The brush border is dense and high. b P2 segment. The cellular interdigitation is decreased; apically the cells have a smooth outline. The brush border is less dense and reduced in height. Peroxysomes (*cross-hatched profiles*) are numerous. c and d P3 segments. The cellular interdigitation is drastically reduced. Regarding the brush border, considerable interspecies differences occur. In rabbit (c), as in most species, microvilli are scanty and short. In contrast, in rat (d) the brush border of P3 is the highest among all proximal segments. (Kriz and Kaissling 1985)

2.3.3 Proximal Tubule

Ultrastructural studies have demonstrated three cell types (designated P1, P2 and P3) along the mammalian proximal tubule (Fig. 2.12; also Kriz and Kaissling 1985; Tisher and Madsen 1986). These are not intermixed but change from P1 to P2 to P3 serially along the length of the tubule. They are differentiated primarily by height of the brush border, amount of interdigitation of the basolateral membrane, and number and arrangement of the mitochondria among the interdigitations (Fig. 2.12). Although all true proximal tubules of nonmammalian vertebrates have a brush border and numerous mitochondria, there are no clear, consistent changes in cell type along the tubule even where functional differ-

ences exist (Dantzler 1974a; W. H. Dantzler and R. B. Nagle unpublished observations; Hickman and Trump 1969; Maunsbach and Boulpaep 1984). The deep interdigitations of the basolateral membrane with the linear arrangement of the mitochondria between them are absent from the proximal tubule cells of nonmammalian vertebrates (Figs. 2.13, 2.14) (Dantzler et al. 1986; W. H.

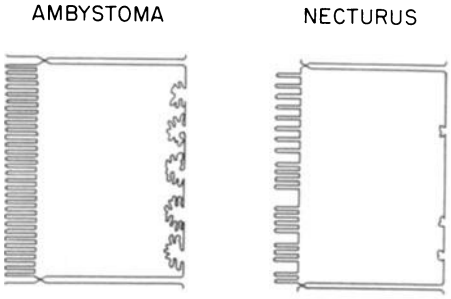


Fig. 2.13. Schematic drawings comparing proximal tubule cells in the urodele amphibians, *Ambystoma tigrinum* (tiger salamander) and *Necturus maculosus* (mudpuppy). Note the higher, more uniform, and more dense brush border of the apical membrane and greater amplification of the basal membrane in *A. tigrinum* than in *N. maculosus*. Neither species has the large amplification and intercellular interdigitation of the lateral cell membranes observed in mammals (see Fig. 2.12). (After Maunsbach and Boulpaep 1984)

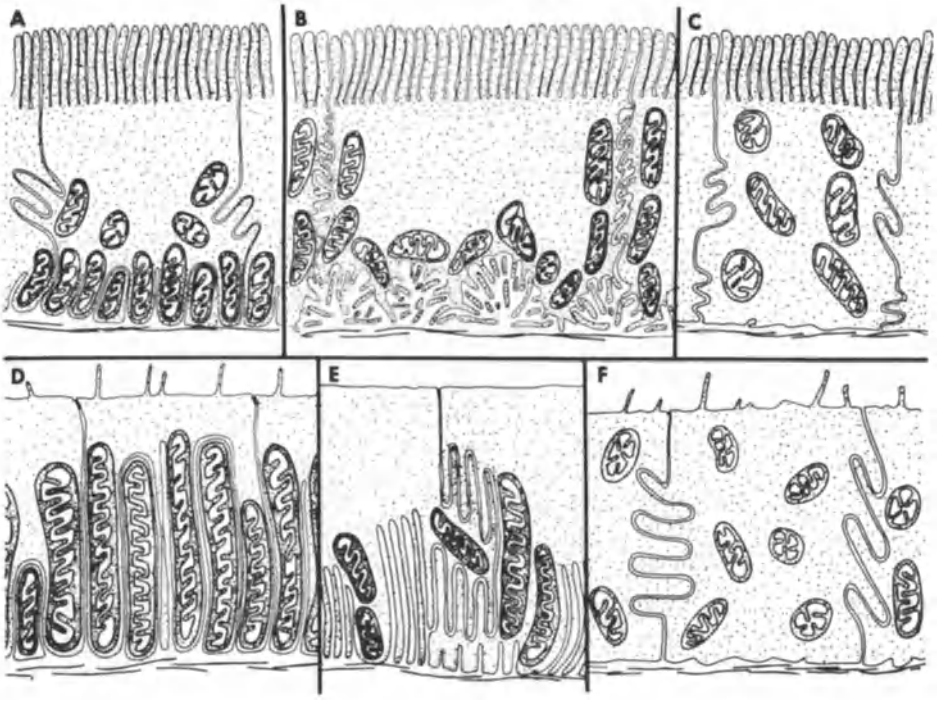


Fig. 2.14 A–F. Schematic drawings showing characteristics of surfaces of proximal and distal tubule cells of mammals and reptiles. A Mammalian proximal cell type (less detailed than in Fig. 2.12). B Gecko (*Hemidactylus* sp.) proximal cell type. Note amplification of basal surface area. C Horned lizard (*Phrynosoma cornutum*) and Galapagos lizard (*Tropidurus* sp.) proximal cell type. Note lack of amplification of basal surface area. D Mammalian thick ascending limb or early distal convoluted cell type. E Gecko (*Hemidactylus* sp.) distal cells type. Note deep basolateral infoldings with elongated mitochondria within them similar to those in mammalian cells. F Horned lizard (*Phrynosoma cornutum*) and Galapagos lizard (*Tropidurus* sp.) distal cell type. Note lack of deep basal infolding, but presence of extensive lateral interdigitations. (Roberts and Schmidt- Nielsen 1966)

Dantzler and R. B. Nagle unpublished observations; Hickman and Trump 1969; Maunsbach and Boulpaep 1984; Roberts and Schmidt-Nielsen 1966). However, there is often significant amplification of the basal cell membrane only, the degree of which can vary substantially among species of the same class (Figs. 2.13, 2.14) (Dantzler et al. 1986; W. H. Dantzler and R. B. Nagle unpublished observations; Maunsbach and Boulpaep 1984; Roberts and Schmidt-Nielsen 1966). Finally, the tight junctions between cells are generally very short; complex lateral intercellular spaces are apparent in the proximal tubules of all vertebrates.

2.3.4 Early Distal Tubule

Cells of the first portion of the distal tubule ("early distal tubule" in many renal papers and texts), beginning after the macula densa in mammals and after the end of the thin intermediate segment in most nonmammalian vertebrates, have common ultrastructural features in freshwater teleosts (Hickman and Trump 1969; Anderson and Loewen 1975), amphibians (Hinton et al. 1982; Stanton

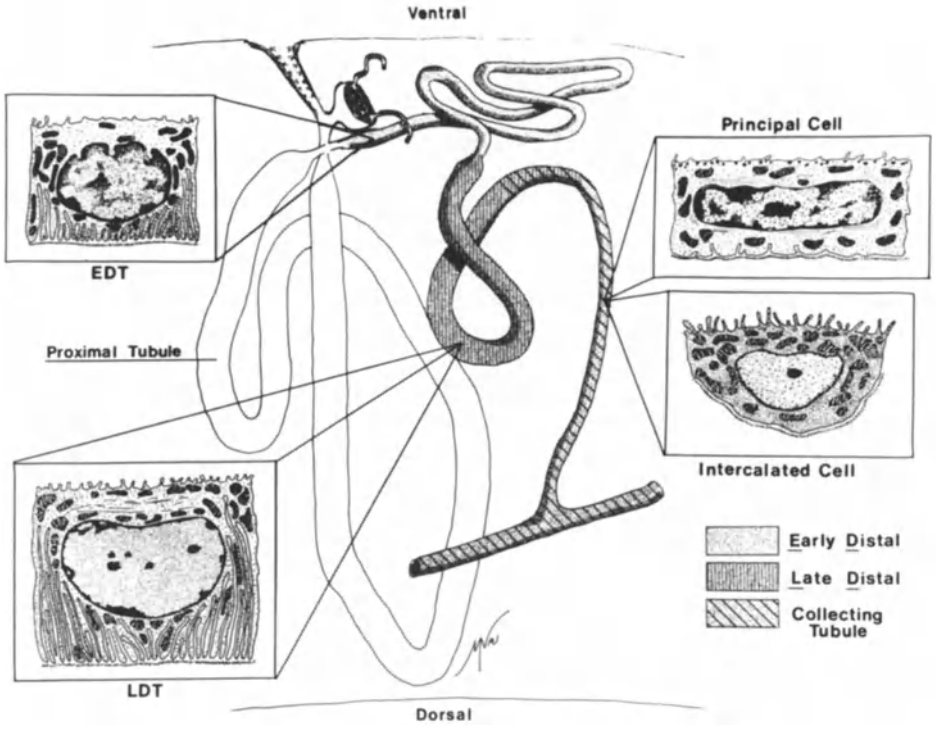


Fig. 2.15. Nephron of *Amphiuma means* (Congo eel). Glomerulus and proximal tubule drawn for reference. *Shading* indicates distal nephron segments. *Lines* from schematics to tubule segments indicate cellular composition of each segment. Abbreviations: *EDT,* early distal tubule of "diluting segment"; *LDT* late distal tubule. (Stanton et al. 1984a)

et al. 1984a), birds (Nicholson 1982), and mammals (Kaissling and Kriz 1979). The primary common features are deep basolateral infoldings with elongated mitochondria occupying the cytoplasm within these infoldings (Figs. 2.14, 2.15). These features are also characteristic of cells of the thick ascending limb of Henle's loop in mammals (Kaissling and Kriz 1979) and, presumably, in birds (Nicholson 1982).

Although the distal tubule cells of a few reptiles (e.g., gecko, *Hemidactylus* sp.) also have these common features (Fig. 2.14), those of most reptiles (e.g., horned lizard, *Phrynosoma cornutum,* Galapagos lizard, *Tropidurus* sp.; blue spiny lizard, *Sceloporus cyanogenys;* crocodile, *Crocodylus acutus;* garter snakes, *Thamnophis sirtalis*) do not (Davis and Schmidt-Nielsen 1967; Davis et al. 1976; Peek and McMillan 1979a; Roberts and Schmidt-Nielsen 1966). Instead, these cells have extensive lateral interdigitations, a large, often irregular nucleus, and ovoid or spherical mitochondria (Fig. 2.14). The type of distal cell found in reptiles does not appear to be related to the ability of a given species to produce a dilute urine (vide infra; Chap. 7).

2.3.5 Late Distal and Collecting Tubules

There is considerable confusion about the structural distinctions between the distal portions of the distal tubule ("late distal tubule" in many renal papers and textbooks) and the collecting tubule or collecting duct into which it empties in nonmammalian vertebrates (Stoner 1985). In fact, except for fishes (Hentschel and Elger 1987) and amphibians (Stanton et al. 1984a), a clear distinction may not exist. The distal tubule cells of most species studied are of the type discussed above throughout the length of the distal tubule ("early" and "late" portions). However, the cells of the late distal tubule of amphibians are much taller than those of the early distal tubule and have very large nuclei and very deep basal infoldings (Fig. 2.15; also Stanton et al. 1984a). The cells of the late distal tubule of some fishes (e.g., *Polypterus sengalus*) and of some saurian reptiles (e.g., *S. cyanogenys*) are also taller than those of the early distal tubule and have less marked lateral interdigitations (Davis et al. 1976; Hentschel and Elger 1987). The amphibian collecting tubule, like the mammalian cortical collecting tubule or duct, consists primarily of light principal cells with some dark, mitochondria-

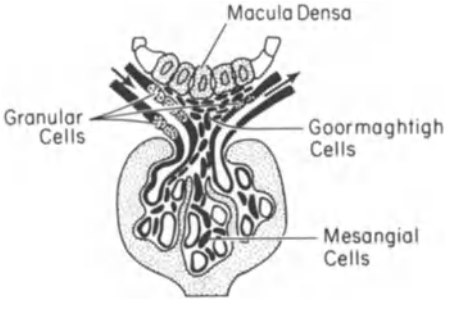


Fig. 2.16. Schematic of juxtaglomerular apparatus. Smooth muscle cell walls of afferent and efferent arterioles are shown in *solid black.* Goormaghtigh cells and mesangial cells are shown by their nuclei in *black.* (After Kriz and Kaissling 1985)

rich intercalated cells (Fig. 2.15; also Stanton et al. 1984a). The transition from the distal tubule into the collecting tubule is gradual with increasing numbers of intercalated and principal cells occurring toward the end of the distal tubule (Fig. 2.15; also Stanton et al. 1984a). Such a gradual transition from the distal tubule through the connecting tubule also occurs in the mammalian kidney. Among fishes, intercalated cells are found only in the collecting tubules of primitive lampreys, Polypteri, and lung fish (Hentschel and Elger 1987). The late distal tubule and connecting tubule of some reptiles (e. g., *S. cyanogenys*) also contain light and dark cells that may correspond to the principal cells and intercalated cells described in other species (Davis et al. 1976). However, the light cells appear to be mucus-secreting (Davis et al. 1976). Dark or intercalated cells also begin to appear at the end of the distal tubule of birds, apparently marking the transition into the collecting tubule or duct (Nicholson 1982). However, the avian collecting tubule or duct contains mucin-secreting cells as well as these dark cells; the mucin-secreting cells become the prominent type in the more distal portions of the collecting-duct system (Nicholson 1982).

2.3.6 Juxtaglomerular Apparatus

Distal nephrons are closely associated with their corresponding glomerular vascular pole, particularly the afferent arteriole, in amphibians, reptiles, birds, and mammals (Kriz and Kaissling 1985; Morild et al. 1985; Stanton et al. 1984a; S. D. Yokota, R. A. Wideman, and W. H. Dantzler unpublished observations), an arrangement that may be of functional significance in glomerular regulation (vide infra; Chap. 3). However, the development of a juxtaglomerular apparatus appears to be complete only among mammals and, possibly, birds. The principal components of the juxtaglomerular apparatus of mammals are the macula densa of the distal nephron, the renin-producing granular cells of the afferent arteriole (and sometimes the efferent arteriole), and the Goormagtigh or extraglomerular mesangial cells (Fig. 2.16; also Kriz and Kaissling 1985). The macula densa consists of an area of specialized cells situated within the wall of the cortical thick ascending limb of Henle's loop where it passes through the angle between the afferent and efferent arterioles of its own glomerulus (Fig. 2.16). These cells are taller than the surrounding cells of the distal nephron, are densely packed with small mitochondria not associated with the basolateral membrane, and have variable numbers of slender microvilli on the luminal membrane. The tight junctions are not significantly different from those of the surrounding cells, but there are no interdigitations of the lateral membranes and the intercellular spaces are normally dilated (Kriz and Kaissling 1985). The basal surface of these macula densa cells abuts the Goormaghtigh cells and variable portions of the afferent and efferent arterioles (Fig. 2.16).

Although renin-producing granular cells are present in the glomerular arterioles of amphibian, reptilian, and avian kidneys (Nishimura 1980), macula densa cells are found only in avian kidneys (Sokabe and Ogawa 1974; Morild et al. 1985). Even for avian kidneys, the presence of a macula densa has been questioned (Wideman et al. 1981). However, all studies indicate the presence of

extensive extraglomerular mesangial tissue (Goormaghtigh cells) in the avian kidney (Morild et al. 1985; Wideman et al. 1981), and the most recent anatomical investigation provides good evidence for a macula densa in both reptilian and mammalian-type nephrons (Morild et al. 1985).

Chapter 3

Initial Process in Urine Formation

3.1 Introduction

The initial process in urine formation consists of the delivery of water and plasma solutes into the lumen of the proximal tubule. In glomerular nephrons, this process involves the production of an ultrafiltrate of the plasma. The rate of formation of such an ultrafiltrate at the individual glomerulus is the primary, if not always the sole, determinant of the rate at which fluid is delivered to the corresponding proximal tubule and therefore is the initial determinant of the volume and composition of the final urine. The filtration process, which relies on arterial hydrostatic pressure maintained for other functions, is well suited to the rapid elimination of large volumes of fluid without high energy costs. Since it also can be altered quickly in some species, it can play an important role in the regulation of excretory water losses.

In the aglomerular nephrons of teleost fishes, secretion of fluid by the proximal tubule is the initial process in urine formation and is thus the initial determinant of the volume and composition of the final urine (Berglund and Forster 1958; Forster 1953; Hickman and Trump 1969). Since this process is not well suited to the rapid elimination of large volumes of fluid, it is not surprising that aglomerular nephrons evolved initially in marine teleosts. However, secretion of fluid by the proximal tubules apparently can contribute to the initial process of urine formation in some glomerular nephrons as well (vide infra; also Beyenbach 1982; Beyenbach 1986; Beyenbach and Fromter 1985; Forster 1953; Hickman 1968; Schmidt-Nielsen and Renfro 1975; Yokota et al. 1985a).

3.2 Filtration of Fluid by Glomeruli

3.2.1 Process of Ultrafiltrate Formation at Renal Glomeruli

The glomerular ultrafilter produces an essentially protein-free filtrate of the plasma with which it is in Donnan equilibrium (Navar 1978; Renkin and Gilmore 1973). If the filtration process by the individual glomerulus is modeled by considering the total capillary network as a cylinder of equivalent surface area, the single nephron glomerular filtration rate (SNGFR) may be described by the following equation containing the major factors involved in the process (Yokota et al. 1985a):

$$SNGFR = \int_{x=0}^{x=1} L_pA \left[(P_{c_x} - P_{bs}) - P_{cop_x} \right] \qquad 3.1$$

In Eq (3.1), l is the length of the cylinder. P_c, the capillary hydrostatic pressure, appears to decrease very little along the length of the capillaries and in the simplest treatments of the above relationship is considered to be constant throughout the length of the tube. The hydrostatic pressure gradient is then equal to the difference between P_c and the pressure in Bowman's space, P_{bs}, which is also considered to be constant. Because the protein that is filtered and enters Bowman's space is negligible and can be ignored, the colloid osmotic pressure of the plasma, P_{cop}, is equivalent to the gradient for osmotic pressure that opposes the hydrostatic pressure gradient driving filtration. As filtration progresses along the length of the capillaries, the protein concentration in the capillaries increases reciprocally with the fraction of fluid remaining in them and the P_{cop_x} increases as an exponential function of the protein concentration (Landis and Pappenheimer 1963). Filtration will continue only as long as the hydrostatic pressure gradient exceeds the opposing osmotic pressure gradient. The difference between these two opposing pressure gradients is the net ultrafiltration pressure.

The hydraulic conductivity, Lp, or the "leakiness" of the capillaries to water, determines the rate of filtration per unit area of filtration surface for any given net ultrafiltration pressure. The rate of filtration will be directly proportional to both the hydraulic conductivity and the surface area available for filtration, A, as indicated in Eq. 3.1. Under steady-state conditions, if filtration equilibrium is reached (i.e., if the opposing transmural hydrostatic and osmotic pressure gradients become equal), filtration will cease and, if other variables remain constant, the fraction of plasma flow filtered (filtration fraction) will remain constant. As long as filtration equilibrium is reached somewhere along the tube (the glomerular capillaries), the rate of filtration is a linear function of the plasma flow. However, if the rate of plasma flow is great enough that filtration equilibrium is not attained somewhere along the tube (the glomerular capillaries), then the rate of filtration will be dependent on hydraulic conductivity, area of the filtering surface, and magnitude of the net ultrafiltration pressure.

As described above (Chap. 2), the glomerular capillaries, with the apparent exception of the simplest avian ones, are not simple tubes but complex branching networks. The total area available for filtration is a function of the diameter, length, and number of the capillary branches. In addition, the specific morphology of the capillary network, including the branching pattern and the capillary dimensions, and the microrheological properties of the blood determine the distribution of blood flow, and thus, the area used for filtration, and the hydrostatic and colloid osmotic pressure profiles within the capillary network. Because the area available for filtration is not generally known, the surface area, A, and the hydraulic conductivity, Lp, in Eq. (3.1) are usually treated together as their product, the ultrafiltration coefficient, K_f.

The factors discussed above determine the SNGFR for any given glomerulus, but there are many glomeruli in each kidney. The whole-kidney glomerular filtration rate (GFR) is, of course, the sum of all single nephron glomerular filtration rates at any moment in time. Although the whole-kidney GFR is readily

measured by clearance methods, it is not readily determined by summing the SNGFR's. There are several reasons for this. First, the number of nephrons varies among species and even from kidney to kidney and is not easily determined accurately. Second, the morphological characteristics of individual nephrons and their corresponding functional characteristics vary among species and within individual kidneys. Therefore, the SNGFR for a given glomerulus cannot be considered representative of all other glomeruli even in that kidney. Third, the micropuncture method of measuring SNGFR only applies to those nephrons with glomeruli or tubule loops available on some renal surface. And, although it is theoretically possible to measure the SNGFR's in all nephrons in one kidney at a given time by the constant infusion sodium ferrocyanide technique of deRouffignac et al. (1970), in fact this is not practical. Despite the heterogeneity of glomerular structure and function, the glomeruli within a kidney are often grouped into general functional categories based on their size, morphological complexity, or location within the kidney and the SNGFR's of a few nephrons in each group used as an average for the entire group (vide infra).

In addition to the morphological differences among glomeruli and the resulting functional differences, individual glomerular filtration rates may change over time. Not only may glomeruli have different rates of continuous filtration, but filtration within a single glomerulus may vary with time and may even cease transiently. Therefore, in some species, the percent of nephrons filtering at any given time may vary depending upon the physiological state of the animal (vide infra).

The production of a true ultrafiltrate of the plasma and the roles described above for the glomerular capillary hydrostatic and colloid osmotic pressure and the intracapsular pressure in this process were first demonstrated by micropuncture studies on amphibians (frogs, *Rana pipiens*; mudpuppies, *Necturus maculosus*; Hayman 1927; Wearn and Richards 1924; H. L. White 1929). Since those initial studies, ultrafiltration has been demonstrated by direct micropuncture measurements in primitive cyclostome fishes (petromyzonta, river lampreys, *L. fluviatilis*; McVicar and Rankin 1985), reptiles (snakes, *Thamnophis sirtalis, Storeria occipitomaculata,* and *S. dekayi*; Bordley and Richards 1933), and mammals (Brenner et al. 1971). Curiously, in one group of cyclostome fishes (Myxini, Pacific hagfish, *Eptatretus stouti*) direct micropuncture measurements indicate that an apparent ultrafiltrate, at least a colloid-free fluid, is produced at the glomerulus in the absence of a positive net ultrafiltration pressure (when the colloid osmotic pressure apparently equals or exceeds the capillary hydrostatic pressure) (Riegel 1978, 1986a, b). This apparent paradox has yet to be resolved, but the rates of apparent filtration at single glomeruli in hagfish are very high, approaching those of mammals (vide infra).

No direct collections from Bowman's capsule have been made in other fishes or birds, and no measurements of the forces involved in ultrafiltration have been made in reptiles or birds. Nevertheless, the excretion by the kidneys of teleost and elasmobranch fishes, birds, and reptiles, of molecules that are only filtered by mammalian and amphibian nephrons has led to the general acceptance of the process of ultrafiltration and of the forces involved in that process for the glomerular nephrons of all vertebrates.

Table 3.1 Examples of pressures involved in glomerular ultrafiltration

	$P_{\bar{A}}$	$P_{\bar{G}\bar{C}}$	P_{BS}	$P_{\pi_{aff}}$	$P_{\pi_{eff}}$	$P_{\bar{\pi}_{GC}}$	$P_{UF_{aff}}$	$P_{UF_{eff}}$	$P_{\bar{U}\bar{F}}$	K_f	References
	mmHg	mmHg	mmHg	mmHg	mmHg	mmHg	mmHg	mmHg	mmHg	nl $min^{-1}mmHg^{-1}$	
Fishes											
River lamprey, *Lampetra fluviatilis*											
Freshwater	21.6	16.1	3.9	8.6	9.0	8.8	5.4	4.9	3.4	1.68	McVicar and Rankin 1985
20% Seawater	18.7	12.3	3.0	8.6	9.3	9	0.7	0	0.35	—	
Amphibians											
Congo eel, *Amphiuma means*	17.3	12.6	4.8	5.3	7.8	9.1	2.5	0	0.65	7.64	Persson 1981
Mammals											
Rat, *Rattus norvegicus* (Munich-Wistar Strain)	95	45.0	10	18	35	26	17	0	9	4.8	Brenner et al. 1971 Deen et al. 1973

Values are approximate means estimated from the references. $P_{\bar{A}}$ indicates mean arterial pressure; $P_{\bar{G}\bar{C}}$, mean hydrostatic pressure within the glomerular capillaries; P_{BS}, pressure in Bowman's space; $P_{\pi_{aff}}$, colloid osmotic pressure at afferent end of glomerular capillaries (generally within afferent arteriole); $P_{\pi_{eff}}$, colloid osmotic pressure at efferent end of glomerular capillaries (generally in efferent arteriole or immediately attached peritubular capillaries); $P_{\bar{\pi}_{GC}}$, mean colloid osmotic pressure within glomerular capillaries (arithmetic mean of $P_{\pi_{eff}}$ and $P_{\pi_{aff}}$); $P_{UF_{aff}}$, net ultrafiltration pressure at afferent end of glomerular capillaries; $P_{UF_{eff}}$, net ultrafiltration pressure at efferent end of glomerular capillaries; $P_{\bar{U}\bar{F}}$, mean net ultrafiltration pressure; K_f, ultrafiltration coefficient.

In recent years, the general pressure relationships involved in glomerular ultrafiltration, first demonstrated in studies on amphibians, have been explored in more detail, not only in amphibians but also in mammals and primitive fishes. The relevant measurements are summarized in Table 3.1. Micropuncture measurements on one species of urodele amphibian (Congo eel, *Amphiuma means*) (Persson 1978a, b; Persson 1981) and on a widely studied strain of rat (Brenner et al. 1971, 1972) show that under normovolemic conditions filtration equilibrium is reached by the efferent end of the capillaries (i.e., net ultrafiltration pressure at this point is zero) (Table 3.1). As noted above, this observation indicates that the filtration rate is influenced by the plasma flow rate through the capillaries (Brenner et al. 1972). In both mammals and amphibians, the sum of the colloid osmotic pressure at the efferent end of the capillaries and the hydrostatic pressure in Bowman's space essentially equals the randomly measured hydrostatic pressure in the glomerular capillaries, suggesting that the latter pressure does, in fact, decrease little along the length of the capillary network (Brenner et al. 1972; Persson 1981). This suggestion is further supported in amphibians by the identity of the hydrostatic pressure in the afferent arteriole and the randomly measured hydrostatic pressure in the glomerular capillaries (Persson 1981). These observations support the concept that, in both amphibians and mammals, filtration equilibrium is reached because of an increase in the colloid osmotic pressure along the length of the capillaries as filtration occurs. However, no exact measurements of the pressure profile along the capillary network from the afferent to the efferent end or of the site of filtration equilibrium have been made for either amphibians or mammals.

Because filtration equilibrium is reached before the end of the glomerular capillaries in these amphibians and mammals, a unique value for the ultrafiltration coefficient cannot be obtained. However, the ultrafiltration coefficient for *A. means* estimated from the pressure data in these studies (Table 3.1) (Persson 1981) is similar to that obtained even less directly for frog and *N. maculosus* (Renkin and Gilmore 1973). And this value for *A. means* is nearly twice that determined by micropuncture measurements on hypervolemic rats during filtration disequilibrium (Table 3.1) (Deen et al. 1973). Because the ultrafiltration coefficient is the product of the area available for filtration and the hydraulic conductivity, the difference between the values for amphibians and mammals may result primarily from the apparently greater area available for filtration in amphibian than in mammalian glomeruli (Renkin and Gilmore 1973) rather than from a difference in hydraulic conductivities. Unfortunately, as noted above, there are no really accurate measurements of the area available for filtration for any species.

In the one species of fish, the primitive, anadromous river lamprey (*L. fluviatilis*), in which filtration has been evaluated by micropuncture, the attainment of filtration equilibrium depends on whether the animals are adapted to freshwater or brackish water (McVicar and Rankin 1985). In freshwater-adapted animals, filtration equilibrium is not achieved along the glomerular capillaries, i.e., there is significant net ultrafiltration pressure at the efferent end of the capillaries (Table 3.1; also McVicar and Rankin 1985). Under these circumstances, filtration is particularly sensitive to hydrostatic pressure. In addi-

tion, a unique value for the ultrafiltration coefficient can be calculated (Table 3.1; also McVicar and Rankin 1985). This value is similar to the lower estimates in mammals (Deen et al. 1983; Navar et al. 1978). In lampreys adapted to brackish water (20% seawater), on the other hand, filtration equilibrium is achieved (Table 3.1), preventing determination of a unique value for the ultrafiltration coefficient (McVicar and Rankin 1985). In addition, as noted above, under these circumstances, filtration should be influenced by the plasma flow rate along the glomerular capillaries.

The hydrostatic pressure in the glomerular capillaries of the brackish-water-adapted lampreys is about three-fourths of that in the glomerular capillaries of the freshwater-adapted animals (Table 3.1; also McVicar and Rankin 1985). This decrease in glomerular capillary pressure in the brackish-water-adapted animals appears to result both from a small decrease in the mean arterial pressure and an increase in the pressure drop across afferent arterioles, probably the result of constriction of the afferent arterioles (Table 3.1). Since the colloid osmotic pressure of the systemic plasma is unchanged when the animals are adapted to brackish water, net ultrafiltration pressure at the afferent end of the glomerular capillaries is markedly reduced (Table 3.1), resulting in a reduced SNGFR (vide infra; Table 3.2). As in mammals and *A. means,* filtration equilibrium in these brackish-water-adapted lampreys appears to result from a rise in the colloid osmotic pressure along the glomerular capillaries (Table 3.1) rather than a decrease in capillary hydrostatic pressure. However, as for other animals, no direct measurements of the pressure profile along the capillary network have been made for this species.

No measurements of pressures involved in filtration have yet been made for birds, which, like mammals, are homeotherms with a relatively high and constant mean arterial pressure. However, the simplicity of the avian glomerular capillary tuft (vide supra; Fig. 2.10) should make direct measurements of pressure profiles along the capillaries possible in those glomeruli accessible to micropuncture (Braun 1982; Dantzler and Braun 1980). Such measurements should help greatly in understanding the regulation of filtration in all vertebrates.

3.2.2 Values for Single-Nephron Glomerular Filtration Rates

Direct measurements of filtration rates of individual nephrons have been made for mammals and for a number of nonmammalian vertebrates by micropuncture methods or by the constant-infusion sodium-ferrocyanide method (deRouffignac et al. 1970). Values available for nonmammalian vertebrates and for one mammalian species are shown in Table 3.2. A number of factors may account for differences among species in the values reported. First, they may be related, in part, to the status of the animals at the time the measurements were made and to differences in the measurement techniques. Second, they may be related to differences in the population of nephrons sampled. Although, as noted above, no anatomically distinct populations of nephrons with differing filtration rates, like those found in the kidneys of birds and mammals (Table 3.2), have been

Table 3.2 Examples of single nephron glomerular filtration rates (SNGFR)

	Condition	SNGFR nl min^{-1}	References
Fishes			
Myxinoidea			
Atlantic hagfish, *Myxine glutinosa* Marine	Seawater	24.2 ± 6.8 (6)	Alt et al. 1980; Stolte and Schmidt-Nielson 1978
Pacific hagfish, *Eptatretus stouit* Marine	Seawater	20.3 ± 2.13 (71)	Riegel 1978
Petromyzonta			
River lamprey, *Lampetra fluviatilis*	Freshwater	7.02 ± 0.027 (89)	Moriarty et al. 1978
Freshwater, marine, or euryhaline	Seawater	2.9 ± 0.3 (9)	Logan et al. 1980 c
Elasmobranchii			
Lesser spotted dogfish, *Scyliorhinus canicula* Marine	Seawater	9.5 ± 1.4 (26)	Brown and Green 1987
Teleostei			
Rainbow trout, *Salmo gairdneri*	Freshwater	1.31 ± 0.20 (5)[a]	Brown et al. 1978
Euryhaline		45% filtering	Brown et al. 1980
	Seawater	3.74 ± 1.12 (3)[a]	Brown et al. 1978
		5% filtering	Brown et al. 1978
Amphibia			
Urodela			
Congo eel, *Amphiuma means* Aquatic, Freshwater	Freshwater	17.5 ± 0.75 (18)	Persson 1978 b
Mudpuppy, *Necturus maculosus* Aquatic, Freshwater	Freshwater	12.88 ± 1.56 (12)	Giebisch 1956
Anura			
Frog, *Rana pipiens* Freshwater, semi-aquatic	Control	13.44 ± 1.62 (3)	Walker and Hudson 1937 b
Reptilia			
Squamata			
Ophidia			
Garter snake, *Thamnophis sirtalis* Moist, terrestrial	Control	5	Bordley and Richards 1933
Aves			
Galliformes			
Gambel's quail,	Control		
Callipepla gambelii	Mammalian-type	14.6 ± 0.79 (27)[a]	Braun and Dantzler 1972
Arid, terrestrial	Reptilian-type	6.4 ± 0.25 (41)[a]	Braun and Dantzler 1972
	Smallest Reptilian-type	0.37 ± 0.082 (14)	W.H. Dantzler unpublished

Table 3.2 Continued

	Condition	SNGFR nl min^{-1}	References
Passeriformes			
European starling,	Control		
Sturnus vulgaris	Mammalian-type	15.6 ± 0.75 (208)[a]	Braun 1978
Moist, terrestrial	Reptilian-type	7.0 ± 0.35 (185)[a]	Braun 1978
	Smallest Reptilian-type	0.36 ± 0.040 (17)	Laverty and Dantzler 1982
Mammalia			
Rat,	Control		
Rattus norvegicus	Superficial	30.1 ± 2.55 (7)	Brenner et al. 1971
Terrestrial	Superficial	36.4 ± 3.5 (4)[a]	Trinh-Trang-Tan et al.
	Juxtamedullary	51.7 ± 6.7 (4)[a]	1981

Values are means ± SE. Figures in parentheses indicate number of determinations except in the case of the trout and rats where they indicate number of animals. [a]SNGFR's determined by constant infusion sodium ferrocyanide technique. All other determinations of SNGFR were made by micropuncture techniques.

defined for the kidneys of fishes, amphibians, and reptiles, there is enough variation in glomerular size, at least within reptilian kidneys (Yokota et al. 1985a), that some heterogeneity in filtration rates among nephrons may exist. Thus it is quite possible that some of the variation in the micropuncture measurements shown in Table 3.2 reflect inadvertent selection of accessible nephrons whose function is not representative of all nephrons in the kidney. In addition, in those species in which nephrons function intermittently (vide infra), the selection of technically acceptible SNGFR measurements may have resulted in mean values greater than true means for all nephrons. These possibilities are suggested by marked differences for some species between the mean measured values and the mean values predicted from allometric analyses (Yokota et al. 1985a).

Although the above factors may account for some interspecific differences shown in Table 3.2, the values for the hagfish and all amphibians are very high compared to other poikilothermic nonmammalian vertebrates and even to the homeothermic birds. In fact, they are not far below those observed in many mammals. Because the net ultrafiltration pressure in amphibians is low, especially compared to that in birds and mammals (see, for example, Table 3.1; also Brenner et al. 1971; Persson 1981; Riegel 1978; Yokota et al. 1985a), the high SNGFR's may result from a large area available for filtration in the glomeruli of these species (Renkin and Gilmore 1973). Of course, the hydraulic conductivity of glomerular capillaries also may be greater in these species than in others. In the case of the hagfish, net ultrafiltration pressure has even been reported to be absent or negative, and although the area for filtration may be large, the mechanism involved in the high apparent filtration rate is unknown (Riegel 1978, 1986a, b). Whatever the mechanism, the physiological significance of these high filtration rates is not completely clear. The metabolic requirements for excretion in hagfish and amphibians are certainly lower than those in birds and mammals and, on that basis, the SNGFR's also should be lower (Yokota et al. 1985a). In amphibians, the high filtration rates may reflect requirements for excretion of water (Yokota et al. 1985a), but this is not the case for the marine hagfish.

3.2.3 Changes in Whole-Kidney Glomerular Filtration Rates

Clearance measurements of whole-kidney GFR's have been made for many nonmammalian and mammalian species. Among nonmammalian vertebrates, whole-kidney GRF's often change with changes in state of hydration or with osmotic stress. These functional patterns are shown for a number of species of fishes, amphibians, reptiles, and birds during acute adaptive changes in hydration or during intravenous administration of a salt load (hyperosmotic sodium chloride; usually 1 mol/l) or a water load (usually a hypoosmotic glucose solution) in Table 3.3.

For most wholly aquatic species that can be adapted to fresh water or salt water, whole-kidney GFR is much higher in fresh water than in salt water (Table 3.3). For the wholly aquatic sea snakes (*Aipysurus laevis*), however, the whole-kidney GFR, which is low under control circumstances, actually increases slightly with an acute salt load and decreases somewhat with an acute water load (Table 3.3). It does increase substantially with a chronic water load, either freshwater or seawalter. Of course, these marine reptiles do have an extrarenal route (oral salt gland) for the excretion of sodium chloride (Dunson et al. 1971); the function of this gland may be important in relation to the increase in whole-kidney GFR observed with a salt load. Data are not yet available on the simultaneous partitioning of ion excretion between salt gland and kidney in these wholly aquatic marine reptiles.

For terrestrial and semiaquatic nonmammalian vertebrates, the whole-kidney GFR tends to increase with a water load and to decrease with dehydration or a salt load (Table 3.3). As can be seen, however, some variation in this general pattern is found among animals of different species and from different habitats (Table 3.3). For example, among birds, the single passerine species studied (starling, *S. vulgaris*) does not show the same decrease in whole-kidney GFR with a salt load as the gallinaceous species studied. However, starlings cannot tolerate the same salt load that produces the decrease in whole-kidney GFR in gallinaceous birds (Braun 1978). Moreover, recent clearance studies on conscious, unrestrained starlings do indicate that moderate dehydration produces about a 60% decrease in whole- kidney GFR (Table 3.3) (Roberts and Dantzler 1986). Thus, the whole-kidney GFR in all avian species studied does show considerable lability with changes in hydration.

Although whole-kidney GFR in all terrestrial and semi-aquatic amphibians studied changes with hydration (Table 3.3), the response in a species of uricotelic, xerophilic South American tree frog (*Phyllomedusa sauvegei*) is quantitatively quite different from the others (Table 3.3). The whole-kidney GFR in these animals can increase dramatically when they are placed in freshwater (Table 3.3). However, under normal circumstances, they can endure long periods without free drinking water, maintaining a very high GFR on a diet of insects (Table 3.3). This failure of the GFR to decrease in the absence of free water, as it does in other amphibians, does not appear to be related in any simple fashion to the excretion of urates as the major end product of nitrogen metabolism (see Chap. 6; Table 6.2), because a number of lizards and snakes that are also uricotelic (Table 6.2) show a marked and rapid decrease in GFR with dehydra-

Table 3.3 Changes in whole-kidney glomerular filtration rate for some fishes, amphibians, reptiles, and birds

	Condition	GFR	References
		ml kg^{-1} h^{-1}	
Fishes			
Petromyzonta			
River lamprey, *Lampetra fluviatilis*			
Freswater, marine, or euryhaline	Adapted to freshwater	25.07 ± 2.32	Logan et al. 1980a
	Adapted to 50% seawater	4.66 ± 1.53	Logan et al. 1980c
Teleostei			
European eel, *Anguilla anguilla*			
Euryhaline	Adapted to freshwater	4.6 ± 0.54	Sharratt et al. 1964
	Adapted to seawater	1.0 ± 0.22	
Rainbow trout, *Salmo gairdneri*			
Euryhaline	Adapted to freshwater	8.6 ± 0.95	Brown et al. 1978
	Adapted to seawater	1.2 ± 0.05	
Plains killifish, *Fundulus kansae*			
Euryhaline	Adapted to freshwater	25	Fleming and Stanley
	Adapted to seawater	1.4	1965
Amphibia			
Bullfrog, *Rana clamitans*			
Freshwater, semi-aquatic	Control (in water)	34.2	Schmidt-Nielsen and Forster 1954
	Dehydration	5.1	
South American tree frog, *Phyllomedusa sauvegei*	Control (out of water 3 days) (fed cockroaches)	32.9 ± 4.6	Shoemaker and Bickler 1979
	Control (out of water 27 Days) (fed cockroaches)	27.1 ± 3.0	
Arid, terrestrial	Water Load (in water)	92.3 ± 6.0	
South African clawed toad, *Xenopus laevis*			
Freshwater, aquatic	Freshwater	30	McBean and Goldstein
	Hyperosmotic saline	12	1970
Toad, *Bufo boreas*	Control (in water)	63.1 ± 9.2	Shoemaker and
Moist, terrestrial	Dehydration	1.8 ± 0.5	Bickler 1979
Reptilia			
Testudinea			
Desert tortoise, *Gopherus agassizii*			
Arid, terrestrial	Control	4.7 ± 0.60	Dantzler and
	Salt load	2.9 ± 0.91	Schmidt-Nielsen 1966
	No urine flow when plasma osmolality increased 100 mosmol		
	Water load	15.1 ± 6.64	

Table 3.3 Continued

	Condition	GFR	References
		ml kg^{-1} h^{-1}	
Freshwater turtle, *Pseudemys scripta*			
Freshwater, semi-aquatic	Control	4.7 ± 0.69	Dantzler and Schmidt-Nielson 1966
	Salt load	2.8 ± 0.90	
	No urine flow when plasma osmolality increased 20 mosmol		
	Water load	10.3 ± 2.00	
Crocodilia			
Crocodylus johnsoni			
Freshwater, semi-aquatic	Control	6.0 ± 1.5	Schmidt-Nielsen and Davis 1968
	Dehydration	1.9 ± 0.2	
	Water load	3.3 ± 1.1	
Crocodylus acutus			
Freshwater and salt water, semi-aquatic	Control	9.6 ± 1.0	Schmidt-Nielsen and Skadhauge 1967
	Dehydration	6.1 ± 0.6	
	Salt load	7.3 ± 0.6	
	Water load	15.2 ± 2.0	
Crocodylus porosus			
Salt water, semi-aquatic	Control	1.5 ± 0.2	Schmidt-Nielsen and Davis 1968
	Salt load	2.8 ± 0.9	
	Water load	18.8. ± 2.3	
Squamata			
Ophidia			
Bull snake, *Pituophis melanoleucus*			
Arid, terrestrial	Salt load	16.1 ± 1.06	Komadina and Solomon 1970
	Water load	10.9 ± 1.07	
Freshwater snake, *Natrix sipedon*			
Freshwater, semi-aquatic	Salt load	13.1 ± 1.26	Dantzler 1967
	Water load	22.8 ± 1.75	Dantzler 1968
	No urine flow when plasma osmolality increased 50 mosmol		
Olive sea snake, *Aipysurus laevis*			
Salt water, aquatic	Control	0.78 (0.49 – 2.78)	Yokota et al. 1985b
	Salt load	2.24 (1.41 – 6.42)	
	Chronic intraperitoneal seawater load	7.05 (6.26 – 7.83)	
	Water load	0.17 (0.03 – 0.35)	
	Chronic intraperitoneal water load	5.67 (4.40 – 6.20)	
Sauria			
Blue-tongued lizard, *Tiliqua scincoides*			
Terrestrial	Control	15.9 ± 1.0	Schmidt-Nielsen and Davis 1968
	Dehydration	0.7	

Table 3.3 Continued

	Condition	GFR	References
		ml kg^{-1} h^{-1}	
	Salt load	14.5 ± 0.5	
	Water load	24.5 ± 2.0	
Horned lizard, *Phrynosoma cornutum*			
Arid, terrestrial	Control	3.5 ± 0.32	Roberts and Schmidt-Nielsen 1966
	Dehydration	2.1 ± 0.20	
	Salt load	1.7 ± 0.40	
	Water load	5.5 ± 0.54	
Sand goanna, *Varanus gouldii*	Dehydration	10.99 ± 0.88[a]	Bradshaw and Rice 1981
Arid, terrestrial	Salt load	5.51 ± 1.10[a]	
	Water load	15.98 ± 1.35[a]	
Puerto Rican gecko, *Hemidactylus sp.*			
Moist, terrestrial	Control	10.4 ± 0.77	Roberts and Schmidt-Nielsen 1966
	Dehydration	3.3 ± 0.37	
	Salt load	11.0 ± 2.18	
	Water load	24.3 ± 1.67	
Rhynchocephalia			
Sphenodon punctatus			
Moist terrestrial	Control	3.9	Schmidt-Nielsen and Schmidt 1973
	Dehydration	3.4	
	Water load	4.8	
Aves			
Galliformes			
Chicken, *Gallus gallus*			
Moist, terrestrial	Control	73.8 ± 2.40	Ames et al. 1971
	Salt load	21.0 ± 2.40	Dantzler 1966
	Water load	190.8 ± 2.40	Skadhauge and Schmidt-Nielsen 1967a
Gambel's quail, *Callipepla gambelii*			
Arid terrestrial	Control	52.8 ± 2.40	Braun and Dantzler 1972, 1975
	Salt load	9.0 ± 1.20	
	Water load	83.4 ± 13.20	
Passeriformes			
European starling *Sturnus vulgaris*			
Moist terrestrial	Control	169.8 ± 4.80	Braun 1978
	Dehydration	69.0 ± 5.40	Roberts and Dantzler 1986
	Salt load	168.6 ± 12.60	Braun 1978

Values are means or means ± SE except for sea snakes for which, because the data did not show a normal distribution, the values are given as medians and interquartile ranges. All means with SE and medians are for four or more values. The values for the birds and some of the fishes were taken from the literature and converted to ml kg^{-1} h^{-1}.

[a] Measurements of plasma levels of arginine vasotocin (AVT) were obtained simultaneously with these measurements of GFR.

tion or a salt load (Table 3.3). The maintenance of a relatively high GFR with only the water obtained from an insect diet may relate to the continued absorption of filtered water in the bladder as well as to a low evaporative water loss (Shoemaker and Bickler 1979). In any case, whole-kidney GFR even in these amphibians can change substantially with hydration (Table 3.3).

Among terrestrial and semi-aquatic reptiles, the response to a salt load or dehydration is particularly variable for the crocodilians and saurians. Since the time these studies were performed, however, each of these crocodilian species has been found to have a functional extrarenal route (lingual salt gland) for the excretion of ions (Taplin and Grigg 1981), and, as in the case of the sea snakes, its presence may account for the lack of decrease, or even increase, in GFR with a salt load. None of the other terrestrial or semi-aquatic species listed in Table 3.3 has an effective extrarenal route for excreting ions, but other reasons for the observed differences in responses are not known (Dantzler 1976a). Whatever the reasons for some of these differences, the whole-kidney GFR in nonmammalian vertebrates can change markedly, especially with acute changes in hydration, and therefore may contribute significantly to changes in the volume and composition of the final urine (vide infra).

The whole-kidney GFR in mammals, in contrast to that in nonmammalian vertebrates, is relatively stable during acute, but moderate changes in hydration (Yokota et al. 1985a). There is no increase with a water load and, although decreases do occur with dehydration, for most species these do not appear to be physiological (Yokota et al. 1985a). Therefore, in most species physiological changes in whole-kidney GFR do not contribute significantly to changes in the volume and composition of the final urine (vide infra). However, in a few mammalian species from arid habitats, the whole-kidney GFR decreases significantly during chronic dehydration that the animals tolerate easily (Dantzler 1982a). For example, the whole-kidney GFR of the spiny mouse (*Acomys cahirinus*) decreases about 55% after 14 days of acclimation to a minimal water supply (Haines and Schmidt-Nielsen 1977) and that of the camel (*Camelus dromedarius*) decreases as much as 70% after 10 days of dehydration (Yagil and Berlyne 1976).

3.2.4 Changes in Single-Nephron Filtration Rates and in Number of Filtering Nephrons

The physiological decreases in whole-kidney GFR observed in a few mammalian species (for example, the camel and spiny mouse, noted above) apparently involve a decrease in the filtration rates of all nephrons or populations of nephrons rather than a decrease in the number of nephrons actually filtering (Yokota et al. 1985a). In contrast, however, the changes in whole-kidney GFR observed in most nonmammalian vertebrates apparently result primarily from changes in the number of glomeruli filtering (Braun and Dantzler 1972, 1974, 1975; Brown et al. 1980; Dantzler 1966, 1967; Dantzler and Schmidt-Nielsen 1966; Forster 1942; Hickman 1965; Lahlou 1966; Mackay and Beatty 1968; Richards and Schmidt 1924; Schmidt-Nielsen and Forster 1954). Although

changes in the individual filtration rates of glomeruli that continue filtering also occur (Braun and Dantzler 1972, 1975; Brown et al. 1978; Brown et al. 1980; Richards and Schmidt 1924), changes in the number of glomeruli actually filtering appear to be more important for the regulation of the whole-kidney GFR in nonmammalian vertebrates. However, an exception to this generalization appears to be the glomerular function in the primitive river lamprey (*L. fluviatilis*) discussed above. Apparently, when the whole-kidney GFR in these animals decreases with adaptation to brackish water (Table 3.3), the filtration rates of all individual nephrons decrease (Table 3.2), but all continue to filter (McVicar and Rankin 1985; Rankin et al. 1980). The decrease apparently results from an increase in resistance and, therefore, a fall in hydrostatic pressure across the afferent arterioles (vide supra; Table 3.1).

The concept that changes in the whole-kidney GFR result from changes in the number of glomeruli filtering in most nonmammalian vertebrates is supported by a number of lines of evidence. The earliest evidence was the observation that for representative species of most major groups of the nonmammalian poikilotherms — teleost fishes, amphibians, and reptiles — the maximum rate of transport (Tm) of glucose or para-aminohippurate (PAH) by the renal tubule cells varies directly with the whole-kidney GFR (Brown et al. 1980; Dantzler 1967; Dantzler and Schmidt-Nielsen 1966; Forster 1942; Lahlou 1966; Mackay and Beatty 1968). If changes in the whole-kidney GFR result from changes in the filtration rate of each glomerulus (or a population of glomeruli) but all continue to filter, the Tm for glucose or PAH transport should not change because the mass of tissue transporting these substances and contributing to the final urine would not have changed (Forster 1942; Ranges et al. 1939). Although this evidence for glomerular intermittency is indirect, it agrees rather well with direct visual observations of the activity of the glomerular circulation in amphibians and reptiles (Garland et al. 1975; Grafflin and Bagley 1952; Richards and Schmidt 1924; Sawyer 1951). Moreover, histologic studies of the kidneys of a number of reptiles show that the ratio of the number of open to closed proximal tubule lumina correlates roughly with the whole-kidney GFR (Schmidt-Nielsen and Davis 1968). Because a proximal tubule in most species collapses when its glomerulus ceases filtering, these observations also support the concept that changes in whole-kidney GFR reflect changes in the number of glomeruli filtering. In addition, direct quantitative measurements of the blood flow rates in single glomeruli in the kidney of a reptile (garter snake, *T. sirtalis*) now confirm the presence of intermittent blood flow, and, presumably, intermittent filtration, and indicate that the fraction of glomeruli with intermittent blood flow increases with increasing plasma osmolality (S. D. Yokota and W. H. Dantzler unpublished observations). These studies also reveal highly variable blood flow in glomeruli that are continuously perfused, suggesting that variations in the filtration rates of the glomeruli that are filtering may be more significant in reptiles than previously supposed. However, as noted above, the degree to which changes in blood flow along the glomerular capillaries influence the SNGFR depends on whether filtration equilibrium is reached along the glomerular capillaries, which may not be the case in these reptiles (S. D. Yokota personal communication).

Studies with the constant infusion sodium ferrocyanide technique (de Rouffignac et al. 1970) and with Alcian blue injections have supported the concept of intermittent glomerular function and provided additional quantitative information on single nephron glomerular filtration rates in teleost and elasmobranch fishes (Brown et al. 1978; Brown et al. 1980; Brown and Green 1987; Elger et al. 1984). The sodium-ferrocyanide infusion technique, which relies on the formation of insoluble precipitates of radioactively labeled Prussian blue, permits the measurement of the filtration rates of individual nephrons inaccessible to micropuncture at a single point in time. Such studies on a species of euryhaline teleost (rainbow trout, *Salmo gairdneri*) indicate that about 45% of the glomeruli are filtering in animals adapted to freshwater whereas about 5% are filtering in animals adapted to sea water (Table 3.2). This difference between the number of glomeruli filtering in freshwater- and seawater-adapted animals corresponds to the difference between whole-kidney glomerular filtration rates under the same circumstances (Table 3.3). Of interest but of no clear physiological significance, the few glomeruli functioning during adaptation to seawater filter at an average rate greater than that for glomeruli functioning during adaptation to freshwater (Table 3.2).

These studies and others on a marine elasmobranch species (lesser spotted dogfish, *Scyliorhinus canicula*) also indicate that during changes in whole-kidney GFR, the filtration rates of individual functioning glomeruli can change (Brown et al. 1980; Brown and Green 1987). These observations provide quantitative support for the inferences drawn from visual observations of changes in blood flow through the glomerular capillaries of amphibians and reptiles (Bordley and Richards 1933; Grafflin and Bagley 1952; Richards and Schmidt 1924; H. L. White 1929; W. H. Dantzler unpublished observations).

Finally, these sodium ferrocyanide infusion studies suggest that some of the nonfiltering glomeruli in both teleosts and elasmobranchs are still perfused with blood (Brown et al. 1980; Brown and Green 1987). Because this technique does not measure blood flow directly and distribution of the marker may sometimes be misleading, these observations require additional confirmation. If they are correct, however, they suggest that for some nephrons cessation of filtration results from a drop in capillary hydrostatic pressure slightly below the plasma colloid osmotic pressure or from an increase in the barrier to filtration, not from a complete interruption of blood flow. In this regard, recent studies show structural changes in the podocytes and their processes in the glomeruli of seawater-adapted trout (Brown et al. 1983), suggesting that there may be a change in the barrier to filtration and, thus, in the ultrafiltration coefficient.

Studies involving Alcian blue injections into stenohaline freshwater teleosts (Prussian carp, *Carassius auratus gibelio*) transferred to isosmotic seawater indicate that, during the first 2 hours of adaptation, many glomeruli are no longer perfused with blood (Elger et al. 1984). With prolonged adaptation to this medium, however, the survivors actually show atrophy and loss of many glomeruli (vide supra) (Elger and Hentschel 1981).

Regulation of whole-kidney GFR by the regulation of the number of glomeruli filtering appears to be a practical adaptation for fishes, amphibians,

and reptiles in which the nephrons empty at right angles into collecting ducts and are not arranged to function in concert to produce a urine hyperosmotic to the plasma. Moreover, most of those species in which glomerular intermittency has been well documented have renal venous portal systems that can continue to nourish the cells of nonfiltering nephrons in the absence of a postglomerular arterial supply. A rudimentary renal venous portal system is also reported for one freshwater teleost species (northern pike, *Esox lucius*) (Hickman and Trump 1969), for which some evidence for glomerular intermittence is available (Hickman 1965). There is, however, no report of even a rudimentary renal venous portal system in another freshwater teleost (white sucker, *Catostomus commersonii*) in which a change in the number of filtering nephrons with temperature appears to be documented (Mackay and Beatty 1968). The possibility of a collateral postglomerular arterial blood supply from the efferent arterioles of filtering glomeruli to the capillary network surrounding the tubules of nonfiltering nephrons in these animals has yet to be explored. However, the lamprey, which spends part of its life cycle in seawater and part in freshwater and lacks any evidence of a renal venous portal system or of a postglomerular collateral blood supply (Logan et al. 1980b; McVicar and Rankin 1985), does not exhibit glomerular intermittency (vide supra; also Rankin et al. 1980; McVicar and Rankin 1985). The absence of glomerular intermittency in lampreys also may be related to the fact that each glomerulus is supplied by more than one afferent arteriole and that each afferent arteriole supplies more than one glomerulus (vide supra; Chap. 2) (Hentschel and Elger 1987). Finally, as already pointed out above, the prolonged adaptation of the stenohaline freshwater Prussian carp to isosmotic seawater, which some animals do tolerate, leads to the atrophy and complete disappearance of many nephrons, possibly as a result of an inadequate nutrient blood supply in the absence of a renal venous portal system.

The function of the individual nephrons in birds is somewhat different from that in fishes, amphibians, and reptiles. Unlike other nonmammalian vertebrates and like mammals, birds are homeotherms with a relatively high and stable blood pressure. However, as discussed above, the avian kidney contains both reptilian-type and mammalian-type nephrons (vide supra; Figs. 2.1, 2.2, 2.6). Also, like most other nonmammalian vertebrates but unlike mammals, birds have a renal venous portal system that contributes to the sinuses surrounding the reptilian-type nephrons and the proximal and distal tubules of the mammalian-type nephrons (Wideman et al. 1981).

Because of the arrangement of the reptilian-type nephrons in a manner that should not contribute directly to the production of a concentrated urine and because of the presence of the renal venous portal system, it appeared possible that avian nephrons could function intermittently. Indeed, initial studies on gallinaceous birds demonstrated that the Tm for glucose absorption or PAH secretion by the renal tubules varies directly with the whole-kidney GFR (Braun and Dantzler 1972; Dantzler 1966). These observations suggested that changes in whole-kidney GFR resulted from changes in the number of glomeruli filtering, as in most other nonmammalian vertebrates, but this approach was too indirect to indicate whether the apparent changes in the number of filtering glomeruli involved only the reptilian-type nephrons or both types of nephrons.

Studies employing the constant sodium ferrocyanide infusion technique, however, have provided an answer to this question and quantitative information on the filtration rates for both types of nephrons (Braun 1978; Braun and Dantzler 1972, 1974, 1975). In both the gallinaceous species (Gambel's quail, *C. gambelii*) and the passerine species (starling, *S. vulgaris*) studied, the mean SNGFR for the mammalian-type nephrons is slightly more than twice that for the reptilian-type nephrons during a control diuresis (Tables 3.2 and 3.4). Moreover, the mean control values for the SNGFR's for each population are essentially the same for the two species (Tables 3.2 and 3.4). However, because there are more nephrons in the starling kidney (about 74,000) (Braun 1978) than in the quail kidney (about 47,000) (Braun and Dantzler 1972), the whole-kidney GFR for the starlings is greater than that for the quail under control conditions (Table 3.3). There is, of course, a range of SNGFR's within each nephron population in both species, apparently related to the range of nephron sizes noted above (Chap. 2), and both the sodium ferrocyanide infusion and micropuncture studies show that the smallest, most superficial reptilian-type nephrons have very low filtration rates (Laverty and Dantzler 1982, 1983b; J. R. Roberts and W. H. Dantzler unpublished observations).

When the whole-kidney GFR in Gambel's quail decreases during a salt load (Table 3.3), all reptilian-type nephrons apparently cease filtering, whereas the mammalian-type nephrons continue filtering at a slightly reduced rate (Table 3.4). Because about 90% of the nephrons in this species are of the reptilian type, the decrease in the number of these nephrons filtering accounts almost entirely for the decrease in the whole-kidney GFR (Braun and Dantzler 1972). Studies in which the renal vasculature in Gambel's quail is filled with a silicone elastomer support the concept that during a salt load reptilian-type nephrons cease filtering and suggest that this results from constriction of the afferent arterioles (Figs. 3.1 and 3.2; also Braun 1976). During a control diuresis, the vessels in the superficial areas of the kidney fill well and the afferent arterioles and glomerular capillaries of reptilian-type nephrons are clearly seen (Fig. 3.1). During the salt load that produces the decrease in whole-kidney GFR and apparent decrease in the number of filtering reptilian-type nephrons described above, few, if any, afferent arterioles or glomerular capillaries of reptilian-type nephrons in this same superficial area of the kidney are filled (Fig. 3.2).

It is also important to note that in Gambel's quail only 71% of the reptilian-type nephrons are filtering even during the control diuresis (Table 3.4). This finding is consistent with direct visual observations of the surface of the quail kidney in vivo during micropuncture experiments (W. H. Dantzler unpublished observations). It also resembles the situation in rainbow trout (*S. gairdneri*) adapted to freshwater (Table 3.2) (Brown et al. 1980).

When whole-kidney GFR in Gambel's quail increases during a water load, all reptilian-type nephrons as well as all mammalian-type nephrons appear to be filtering and SNGFR's of both types approximately double (Table 3.4). In this species, therefore, the increase in whole-kidney GFR produced by a large intravenous water load apparently results from a marked increase both in the number of filtering reptilian-type nephrons and in the SNGFR's of all nephrons (Braun and Dantzler 1975).

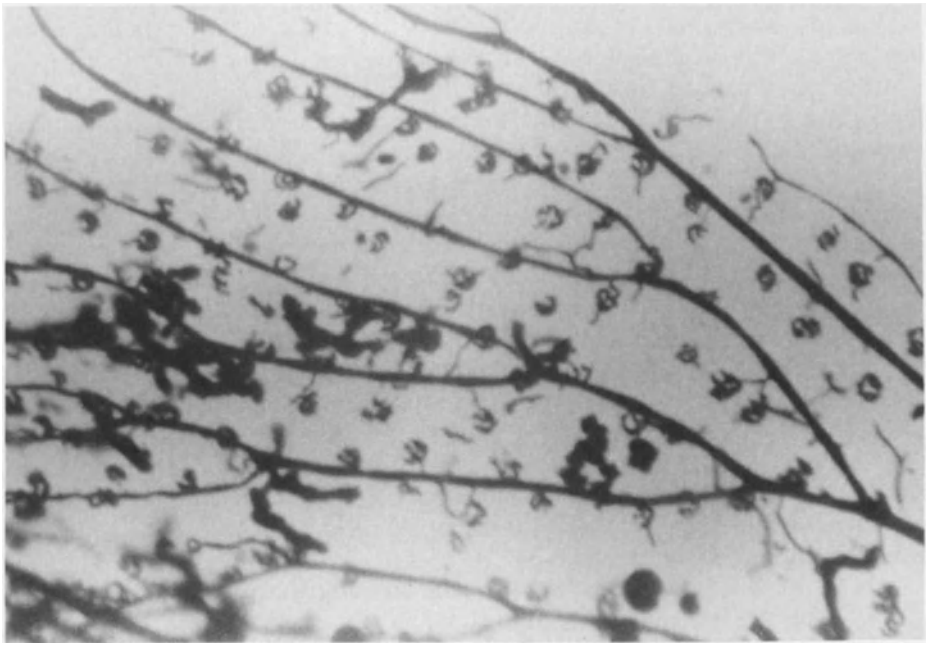


Fig. 3.1. Section of cleared tissue from superficial area of kidney from bird (*Callipepla gambelii,* Gambel's quail) that had received only control mannitol infusion. Vaculature filled with silicone elastomer (Microfil). (Braun 1976)

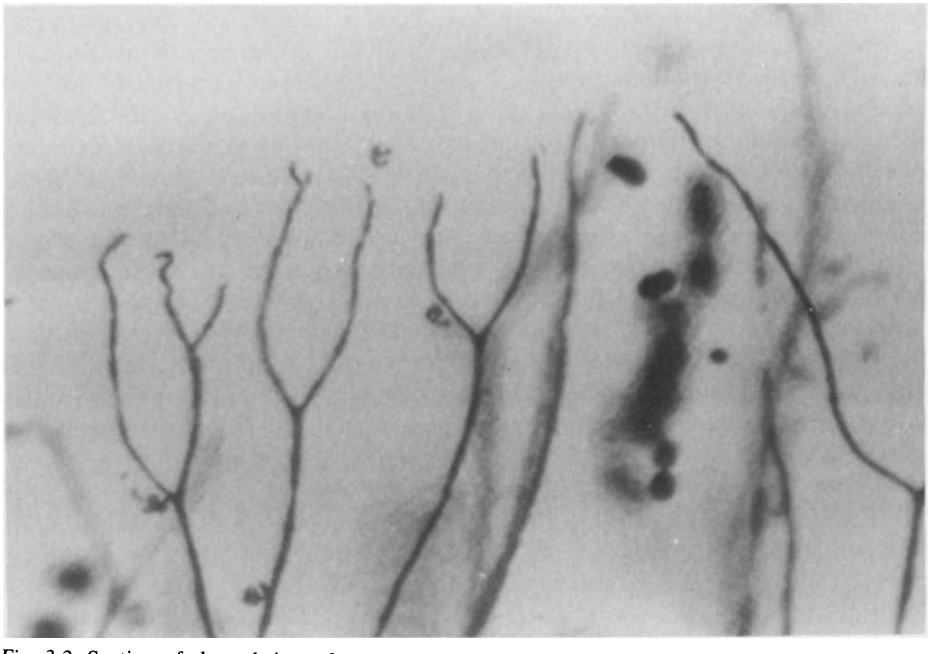


Fig. 3.2. Section of cleared tissue from superficial area of kidney from bird (*Callipepla gambelii,* Gambel's quail) that had received salt load. Vaculature filled with silicone elastomer (Microfil). (Braun 1976)

Table 3.4 Effects of salt load, water load and arginine vasotocin administration on single nephron glomerular filtration rates (SNGFR) in Gambel's quail and European starlings

Treatment	*Mammalian-type Nephrons*		*Reptilian-type Nephrons*		*References*
	SNGFR ($nl\ min^{-1}$)	Percent *filtering*	SNGFR ($nl\ min^{-1}$)	Percent *filtering*	
	Gambel's quail *(Callipepla gambelii)*				
Control (2.5% mannitol)	14.6 ± 0.79 (27)	100	6.4 ± 0.20 (41)	71	Braun and Dantzler 1972
Salt load (45 mEq kg^{-1})	12.7 ± 0.52 (70)	100	—	0	Braun and Dantzler 1972
Water load	33.2 ± 1.57 (155)	100	11.4 ± 0.75 (146)	100	Braun and Dantzler 1975
Arginine Vasotocin					
(10 ng kg^{-1})	11.3 ± 0.89 (102)	100	4.7 ± 1.05 (31)	52	Braun and Dantzler 1974
(50 ng kg^{-1})	16.5 ± 0.75 (64)	100	6.9 ± 0.42 (26)	26	Braun and Dantzler 1974
	European starling *(Sturnus vulgaris)*				
Control (2.5% mannitol)	15.6 ± 0.75 (208)	? 20	7.0 ± 0.35 (185)	? 45	Braun 1978
Salt load (34 mEq kg^{-1})	14.6 ± 0.51 (28)	? 72	—	0	Braun 1978

Values are means ± SE. Numbers in parentheses are sample sizes.

Unfortunately, no direct determination of the number of nephrons filtering in the starling kidney under any conditions is available. However, if all the mammalian-type and reptilian-type nephrons were filtering at the rates determined during the control diuresis (Table 3.4), the whole-kidney GFR would be far greater than that actually measured in these animals (Table 3.3). Because about 70% of the nephrons are of the reptilian type and about 30% are of the mammalian type in this avian species, the control whole-kidney GFR shown in Table 3.3 could be attained if 45% of the reptilian-type nephrons and 20% of the mammalian-type nephrons were filtering at the control rates shown in Table 3.4 (Braun 1978). Of course, other combinations are also possible. It is not known whether any mammalian-type nephrons that are not filtering are small, transitional nephrons with short loops of Henle or larger nephrons with long loops. During the maximum intravenous salt load tolerated by starlings, all the reptilian-type nephrons apparently cease filtering, but the SNGFR's of the filtering mammalian-type nephrons are unchanged from the control values (Table 3.4). Because the whole-kidney GFR does not change at this time (Table 3.3), about 72% of the mammalian-type nephrons must now be filtering.

As discussed above for other nonmammalian vertebrates, regulation of the whole-kidney GFR in birds by altering the number of filtering reptilian-type nephrons appears practical because these nephrons empty at right angles into collecting ducts and apparently cannot function in concert to contribute directly

to the urine-concentrating mechanism. However, such changes in the number of filtering reptilian-type nephrons may still influence the operation of the concentrating mechanism in less direct ways (vide infra; Chap. 7). The apparent changes in the number of filtering mammalian-type nephrons in the starling kidney may have direct effects on the operation of the concentrating process (vide infra; Chap. 7).

In the avian kidney, the viability of the proximal and distal tubules of the nonfiltering mammalian-type nephrons as well as all portions of the nonfiltering reptilian-type nephrons can be maintained by the renal portal system. However, the renal portal system of birds differs from that of other nonmammalian vertebrates in having a unique smooth muscle valve at the juncture of the external iliac vein and the efferent renal vein that determines whether blood from the posterior extremities bypasses the kidney and flows directly into the central circulation (open valve) or flows first through the renal portal system and supplies the peritubular sinuses (closed valve) (Sperber 1948). The degree of flow in either direction, of course, depends on the degree to which the valve is opened or closed. Although this valve is subject to autonomic neural control (Akester and Mann 1968) and, possibly, humoral control, it is not yet clear how this control is coordinated with changes in the number of filtering nephrons (vide infra).

3.2.5 Regulation of Single-Nephron Filtration Rates and Number of Filtering Nephrons

3.2.5.1 Hormonal Regulation

3.2.5.1.1 Neurohypophysial Peptides

These hormones apparently are important in regulating both the individual nephron filtration rates and the number of filtering nephrons. Among the mammals, the naturally occurring antidiuretic neurohypophysial peptide, arginine vasopressin, is a powerful vasoconstrictor, but there is no convincing evidence that it influences glomerular filtration rate under physiological conditions. It is still possible that it plays a role in the apparent physiological decrease in whole-kidney GFR observed in some desert species (vide supra), but this has yet to be adequately studied.

Arginine vasotocin (AVT), which has been identified in the neurohypophyses of all those nonmammalian vertebrates examined (Follett and Heller 1964a, 1964b; Heller and Pickering 1961; Munsick 1964, 1966; Sawyer et al. 1959, 1961), clearly has a role in glomerular regulation. In fact, among the nonmammalian vertebrates, it may be the most important physiological regulator of the number of filtering nephrons and of the SNGFR with changes in hydration or the salinity of the aqueous environment. However, the details of this regulation can vary greatly among nonmammalian vertebrate classes and species. For example, among the fishes, the administration of small, apparently physiological doses of AVT produces an increase in whole-kidney GFR and urine flow in the African lungfish (*Protopterus aethiopicus*) (Sawyer 1970), and initially this appeared to be true for teleosts as well. However, later work on euryhaline teleosts (Euro-

pean eel, *Anguilla anguilla*) adapted to freshwater showed that small, nonpressor doses of AVT cause a decrease in whole-kidney GFR and urine flow whereas higher, vasopressor doses cause an increase in whole-kidney GFR and urine flow (Babikir and Rankin 1978; Henderson and Wales 1974). The renal function of seawater-adapted eels responds only to the higher, vasopressor doses of AVT (Babikir and Rankin 1978). These observations suggest that the renal vessels, probably the afferent glomerular arterioles, are more responsive to the vasoconstrictor action of AVT than are the peripheral systemic arterioles. With sufficient AVT to produce an increase in systemic arterial pressure, however, the constriction of the afferent glomerular arterioles is overridden (Nishimura and Imai 1982; Nishimura 1985). If the hormone does function in this manner, then the seawater-adapted animals may already be responding maximally to low levels of AVT. Thus, AVT may play a physiological role in the glomerular adjustment of euryhaline teleosts to seawater or freshwater (Table 3.3), probably largely by regulating the number of functioning nephrons (Table 3.2).

Arginine vasotocin also appears to have an important physiological role in the control of the glomerular filtration rate in amphibians and reptiles. Injections of small, apparently physiological doses of AVT that do not alter mean arterial pressure produce decreases in whole-kidney GFR that mimic those observed with a salt load or dehydration (Table 3.3; also Bradshaw and Rice 1981; Butler 1972; Dantzler 1967; Jard and Morel 1963). Moreover, the plasma AVT level and osmolality in the one lizard species in which they have been measured (sand goanna, *Varanus gouldii*) increase with dehydration or a salt load as the whole-kidney GFR decreases, and decrease with a water load as the whole-kidney GFR increases (Table 3.3; also Bradshaw and Rice 1981; Rice 1982). In addition, glomerular blood flow, and presumably filtration, in the one reptilian species in which it has been measured quantitatively (garter snake, *T. sirtalis*) decreases following the administration of small doses of AVT and ceases entirely following the administration of larger, but probably still physiological doses of AVT (S. D. Yokota and W. H. Dantzler unpublished observations). Thus, AVT appears to be physiologically important in amphibians and reptiles for the regulation of the whole-kidney GFR, primarily by regulating the number of glomeruli filtering but also by regulating the filtration rates of those glomeruli that are filtering.

Arginine vasotocin is also important physiologically in the regulation of glomerular filtration in birds. Small, apparently physiological doses that do not alter systemic blood pressure produce significant decreases in whole-kidney GFR in chickens and Gambel's quail (Ames et al. 1971; Braun and Dantzler 1974). In Gambel's quail these decreases are explained quantitatively by decreases in the number of reptilian-type nephrons filtering without any significant changes in the average SNGFR of either nephron type (Table 3.4; also Braun and Dantzler 1974). The decreases in the number of filtering reptilian-type nephrons with the administration of AVT, as in the case of the administration of a salt load, appear to result from constriction of the afferent glomerular arterioles (Dantzler and Braun 1980). Furthermore, in chickens, a salt load comparable to that used in Gambel's quail produces a marked increase in the plasma level of AVT (Koike et al. 1979).

The role of AVT in regulating the glomerular filtration rate and the number of filtering nephrons in birds is further supported by the results of acute neurohypophysectomy in Gambel's quail (Dantzler and Braun 1980). Following this procedure, which is assumed to remove endogenous AVT, the mean systemic blood pressure is reduced, the whole-kidney GFR and the SNGFR's for both types of nephrons decrease, but all the reptilian-type as well as all the mammalian-type nephrons are filtering. These observations suggest that AVT not only helps regulate the number of filtering reptilian-type nephrons by altering the resistance at the afferent arterioles but also helps maintain normal systemic blood pressure and, therefore, the normal renal blood flow and SNGFR. Along with these general vasopressor effects, it would be particularly significant physiologically if AVT also stimulated the smooth muscle of the renal portal valve (vide supra) to contract. If the portal valve had a sensitivity to AVT similar to the afferent glomerular arterioles of the reptilian-type nephrons, this vasoconstrictor response could be very important in directing more venous blood through the peritubular sinuses at the time that the number of filtering nephrons was reduced.

Although mesotocin (8-isoleucine oxytocin) is present in the neurohypophyses of many nonmammalian vertebrates, its function is not well understood. Preliminary studies suggest that it may have a physiological role as a glomerular diuretic agent in some anuran amphibians (Uchiyama et al. 1985). Small doses of mesotocin that have no effect on systemic blood pressure produce an increase in whole-kidney GFR and urine flow without affecting the relative free water clearance in amphibious bullfrogs (*R. catesbeiana*) and terrestrial Japanese toads (*Bufo bufo japonicus*). It appears most likely that the increase in whole-kidney GFR reflects an increase in the number of filtering nephrons, although there also may be some increase in the SNGFR, but the mechanism involved, possibly dilation of afferent arterioles, is unknown.

3.2.5.1.2 Renin-Angiotensin System

Renal renin activity and granular cells apparently first appeared in primitive bony fishes and are present in teleosts, lungfishes, and all tetrapods (Nishimura 1980). However, renal renin activity is low in the holocephalians and absent in the cyclostomes and elasmobranchs (Nishimura 1980). Any renal effects of the renin-angiotensin system could result from effects on systemic blood pressure, the delivery of angiotensin II formed outside the kidney to renal structures, or the action of angiotensin II formed within the kidney on renal structures. Although angiotensin II could play a physiological role in regulating glomerular filtration in those nonmammalian vertebrates with a renin-angiotensin system (Sokabe 1974), presumably by regulating the number of filtering glomeruli, the few studies on this problem have generally been negative or equivocal. Most experimental effects of angiotensin II on the glomerular filtration rate appear to be pharmacological in nature (Nishimura 1985). However, preliminary data on bullfrogs (*R. catesbeiana*) do indicate that during perfusion of the renal arteries under constant pressure even a nonpressor dose of angiotensin II reduces the whole-kidney GFR (Nishimura 1985). Also, a more complete study on anesthetized trout (*S. gairdneri*), in which the systemic arterial pressure was main-

tained by an infusion of norepinephrine, indicates that infusions of angiotensin II can reduce whole-kidney GFR in freshwater-adapted animals by reducing the number of filtering glomeruli and in seawater-adapted animals by reducing the SNGFR of the few filtering nephrons (Brown et al. 1980). However, an additional, although less extensive, study on this same species in the absence of norepinephrine (see below for effects of catecholamines on GFR) suggests that regulation of GFR by angiotensin II in freshwater-adapted animals is accompanied by a systemic pressor effect (Gray and Brown 1985). Thus, although angiotensin II is a naturally occurring peptide in frogs and teleosts and its glomerular effects in trout suggest that it may play a physiological role in the renal adaptation to seawater, it is still not clear whether the observed effects in either species are primarily physiological or pharmacological. The pharmacological effects of angiotensin II on the glomerular filtration rate of mammals are clearly documented (Brenner et al. 1981), and physiological regulation, if it exists, may involve the distal-tubule — glomerular feedback system (vide infra).

3.2.5.1.3 Prolactin

The role of prolactin, if any, in regulating vertebrate renal function is unclear. However, a few studies suggest that it could have a physiological role in determining the number of filtering nephrons in nonmammalian vertebrates. A histological study of the kidneys of one euryhaline teleost species (the stickleback, *Gasterosteus aculeatus*) indicates that the administration of prolactin to prolactin-deficient, seawater-adapted animals increases the number of filtering glomeruli in a manner similar to adaptation to freshwater (Lam and Leatherland 1969). Also, the administration of prolactin to some freshwater turtle species (at least, *Chrysemys picta* and, possibly, *Pseudemys scripta*) produces a significant increase in whole-kidney GFR produced by hypophysectomy (Brewer and Ensor 1980). Such changes in whole-kidney GFR in turtles are presumed to reflect changes in the number of filtering glomeruli (vide supra). These studies indicate that prolactin could help to determine the increase in GFR observed with adaptation to freshwater in teleosts or with the intake of a water load in some reptiles. However, much more information on the possible physiological versus pharmacological significance of this apparent glomerular action and on the mechanism involved is certainly required.

3.2.5.1.4 Epinephrine

The physiological role of circulating epinephrine, released from the adrenals under stress, in the direct regulation of glomerular filtration is unclear. However, recent studies on a marine elasmobranch species (lesser spotted dogfish, *S. scyliorhinus*) indicate that infusions of epinephrine markedly increase whole-kidney GFR (Brown and Green 1987). Measurements of the number of filtering nephrons by the sodium-ferrocyanide infusion method and of single nephron glomerular filtration rates by micropuncture during this epinephrine infusion indicate that the increase in whole-kidney GFR results primarily from an increase in SNGFR with a decrease in the number of filtering nephrons (Brown and Green 1987). This pattern is different from that expected, but the exact effects on the local vasculature, probably the afferent arterioles, as well as the

balance of local and systemic effects have yet to be evaluated. Moreover, it is not clear that such an infusion of epinephrine adequately mimics the physiological effects.

3.2.5.2 Neural Regulation

Neural regulation of glomerular filtration has long appeared to be of little physiological significance, but recent studies suggest that it may be important for fishes, amphibians, and reptiles. Varicosities of unmyelinated adrenergic nerves are found in the vicinity of the glomerular arterioles in rainbow trout (*S. gairdneri*) (Elger and Hentschel 1981), and the administration of α-adrenergic blockers, bretylium and phentolamine, to these animals partially prevents the reduction in whole-kidney GFR observed with adaptation to salt water (Elger and Hentschel 1983). These observations suggest that α-adrenergic nerves play a role in controlling the resistance of afferent glomerular arterioles, and, thus, the number of functioning glomeruli, during adaptation to waters of varying salinity.

Similarly, the autonomic nervous system may be important in controlling the number of filtering glomeruli in amphibians during changes in hydration. A glomerular antidiuresis occurs during water deprivation in bullfrogs (*R. catesbeiana*) in the absence of any increase in circulating AVT and following the destruction of the hypothalamus, the source of AVT production (Gallardo et al. 1980). This glomerular response is eliminated when the animals are pithed or when an α-adrenergic blocker, phenoxybenzamine, is administered; it is mimicked by the arterial administration of norepinephrine to pithed animals (Gallardo et al. 1980). Neural elements appear to exist in close proximity to the glomerular vessels (Gallardo et al. 1980), and, as in the case of the teleosts, it appears that α-adrenergic nerves may be involved in the control of the resistance of the afferent glomerular arterioles in amphibians. How, under normal, physiological conditions, this neural control may be integrated with control by AVT in the glomerular adaptation of teleost fishes or amphibians to waters of varying salinity or to varying degrees of hydration is completely unknown.

Perliminary data indicate that nerve endings exist near the glomerular arterioles of garter snakes (*Thamnophis* spp.) (S. D. Yokota, R. A. Wideman, and W. H. Dantzler unpublished observations) and that α-adrenergic inhibitors, phenoxybenzamine and phentolamine, block the decrease in whole-kidney GFR observed with high plasma concentrations of potassium in sea snakes (*A. laevis*) and garter snakes (Yokota et al. 1985b; S. Benyajati, S. D. Yokota, and W. H. Dantzler unpublished observations). These data suggest that α-adrenergic agonists may be released by high plasma potassium levels and may play a role in regulating the resistance of the afferent arterioles (Yokota et al. 1985b; S. Benyajati, S. D. Yokota, and W. H. Dantzler unpublished observations).

3.2.5.3 Renal Portal Influence

Renal portal flow contributes to the blood supplying the renal tubule cells of nonmammalian vertebrates. There is no evidence that it contributes directly to glomerular filtration under physiological conditions. However, for the avian

kidney in which the amount of portal blood flow is regulated by the valve discussed above, there is some evidence that the amount of portal flow may indirectly influence the glomerular filtration rate. When the portal blood flow in chickens is manipulated experimentally so that one kidney is completely perfused and the other kidney is completely bypassed, the whole-kidney GFR of the perfused kidney always exceeds that of the bypassed kidney (Braun and Wideman 1979). The mechanism underlying this observation is unknown. The method by which the manipulation is performed does not lead to backperfusion of the glomeruli themselves in the perfused kidney. However, it is possible that some increase in the pressure in the peritubular sinuses in the perfused kidney could lead to an increase in resistance at the efferent arterioles in that kidney. Other explanations also may be possible. The important point, however, is that the amount of portal blood flow may have an influence on the filtration rate without contributing directly to the blood flow in the glomerular capillaries.

3.2.5.4 Autoregulation

In mammals, the renal blood flow and whole-kidney GFR are relatively independent of the mean systemic blood pressure over a wide range of such pressures. This relative independence of renal blood flow and filtration rate from mean systemic blood pressure is termed "autoregulation" because it does not depend on systemic neural or humoral influences but is intrinsic to the kidney itself (Brenner et al. 1981). The control appears to involve primarily the resistance of the afferent arterioles (Brenner et al. 1981), but the actual mechanism involved in this control of resistance is not understood.

Little is known about possible autoregulation among the nonmammalian vertebrates. Only the homeothermic birds with their high, stable systemic arterial pressures seem likely to exhibit significant autoregulation. However, there is too little information available to know whether this is the case. The poikilothermic nonmammalian vertebrates with their highly variable mean systemic blood pressures and frequently changing glomerular filtration rates appear unlikely to exhibit significant autoregulation. Indeed, in the one nonmammalian vertebrate in which this question has been addressed, a urodele amphibian (the Congo eel, *A. means*), micropuncture measurements show that the glomerular capillary pressure and SNGFR vary directly with the mean arterial pressure and that there is no autoregulation (Persson 1981).

3.2.5.5 Distal-Tubule — Glomerular Feedback Regulation of SNGFR

A feedback mechanism from the distal tubule helping to control the filtration rate in individual mammalian nephrons is well supported by experimental evidence (Schnermann and Briggs 1985). Apparently some element of the fluid composition in the early distal tubule (perhaps sodium chloride) is sensed as this fluid flows past the region of the macula densa and the filtration rate of the corresponding glomerulus is adjusted appropriately: an increase in the concentration of the sensed element as a result of above normal flow leads to a decrease in SNGFR; a decrease in the concentration of the sensed element as a result of

below normal flow leads to an increase in SNGFR. However, the system appears poised in the direction of producing a decrease in SNGFR with an increased delivery of the sensed element (Schnermann and Briggs 1985). There is also considerable evidence, but certainly not incontrovertible evidence, that the signal sensed involves transport, most probably of sodium chloride, by the macula densa cells (Schnermann and Briggs 1985). Although the data clearly suggest that the changes in SNGFR result from alterations in the resistance of the afferent and, possibly also, the efferent arterioles, the mediating mechanism is far less clear. It very likely involves the renin-angiotensin system but may also involve prostaglandins, adenosine, and calcium (Schnermann and Briggs 1985).

The possibility of such a single nephron feedback system in nonmammalian vertebrates is particularly intriguing because of the rapid changes that occur in the number of functioning nephrons and in the single nephron filtration rates and because a macula densa appears to be present only in avian nephrons (vide supra; Chap. 2). This possibility has yet to be examined in birds. However, it has been examined in a urodele amphibian (the Congo eel, *A. means*) (Persson and Persson 1981) in which nephrons show no evidence of a macula densa (Stanton et al. 1984a). Surprisingly, microperfusion studies in these animals demonstrated a feedback system similar to that in mammals (Persson and Persson 1981). A depression in SNGFR in an individual nephron occurs when the distal tubule is perfused with amphibian Ringer's solution at rates of 25 or 50 nl/min but not at 10 nl/min. The depression in SNGFR appears to result from an increase in resistance at the afferent arteriole. The lowest distal perfusion rate at which a decrease in SNGFR occurs is not known and the rates above appear somewhat high even for the high SNGFR's (Table 3.2) and relatively low proximal fluid absorption (Table 5.1) in these animals. Moreover, the variable or variables sensed and the effector substance or substances that produce the change in afferent arteriolar resistance and reduction in SNGFR are unknown. Therefore, the physiological significance of this feedback process in these urodele amphibians is far from clear. However, its very presence in the absence of a macula densa raises questions about the precise mechanism involved and the structural requirements even in mammals.

3.3 Secretion of Fluid by Tubules

Secretion of fluid by the proximal renal tubules is the essential process in the initial formation of urine by the aglomerular nephrons found in certain teleost fishes (Berglund and Forster 1958; Forster 1953; Hickman and Trump 1969). However, it is now known to contribute to the initial formation of urine by the glomerular nephrons of marine teleosts (e.g., winter flounder, *Pseudopleuronectes americanus;* southern flounder, *Paralicthys lethostigma;* longhorn sculpin, *Myxocephalus octodecimspinosus*) (Beyenbach 1982; Forster 1953; Hickman 1968), marine elasmobranchs (e.g., dogfish shark, *Squalus acanthius*) (Beyenbach and Fromter 1985), euryhaline teleosts adapted to freshwater (e.g.,

American eel, *Anguilla rostrata*) (Schmidt-Nielsen and Renfro 1975), euryhaline teleosts in seawater (e.g., *Fundulus heteroclitus*) (Beyenbach 1986), and even marine snakes (e.g., olive sea snake, *Aipysurus laevis*) (Yokota et al. 1985b). The possible mechanisms involved in this secretory process are discussed in Chapters 4 and 5. However, among glomerular species, it appears to be most important when glomerular filtration is low, particularly perhaps when the number of filtering nephrons is reduced (Beyenbach 1982, 1986; Hickman 1968; Schmidt-Nielsen and Renfro 1975; Yokota et al. 1985b). In some species (e.g., flounders, dogfish sharks, and killifish) the rate of secretion of fluid by the tubules is about equal to the rate of filtration of fluid by the glomeruli (vide infra; Table 5.1; also Beyenbach, 1982, 1986), whereas in others, e.g., olive sea snake, the only tetrapod vertebrate in which fluid secretion has been demonstrated, it is apparently much lower (Yokota et al. 1985b). In a number of marine species, e.g., longhorn sculpins, American eels, and olive sea snakes, net fluid secretion appears to be most important when there is a need to excrete water (Forster 1953; Schmidt-Nielsen and Renfro 1975; Yokota et al. 1985a). Beyenbach (1986) suggests that the potential for net fluid secretion by the renal tubules may be present as a primitive characteristic in many species with glomerular nephrons, not just in those with aglomerular nephrons. At present, however, it appears to be of physiological significance only in some marine and euryhaline fishes.

Chapter 4

Transport of Inorganic Ions by Renal Tubules

4.1 Introduction

A large fraction of the inorganic ions filtered by glomerular nephrons does not appear in the ureteral urine and, therefore, must be absorbed by the renal tubules. In addition, because it has become clear that the variation in excretion of inorganic ions cannot be explained by filtration and absorption alone, tubular secretion must play a role therein. The degree to which these processes have been studied in nonmammalian vertebrates varies greatly, but some major observations can be considered and compared and contrasted among the mammals and the nonmammalian vertebrate classes.

4.2 Sodium and Chloride

4.2.1 Direction, Magnitude, and Sites of Net Transport

Absorption of a major portion of the filtered sodium and chloride occurs along the renal tubules of mammals, all nonmammalian tetrapods, and most fishes under physiological conditions (Dantzler 1976a; Hickman and Trump 1969; Jard and Morel 1963; Nishimura and Imai 1982; Sawyer 1970; Skadhauge 1973; Stolte et al. 1977a, b). However, only the renal tubules of mammals and birds are capable of absorbing almost all the filtered sodium, more than 99% (Clark et al. 1976; Dantzler 1987; Skadhauge 1973). The renal tubules of most other vertebrates — reptiles, amphibians, glomerular teleosts, lungfish, and freshwater lampreys — absorb between 35 and 97% of the filtered sodium and chloride, the largest amounts being absorbed by only a few freshwater forms (Dantzler 1976a; Hickman and Trump 1969; Jard and Morel 1963; Long 1973; Nishimura and Imai 1982; Sawyer 1970; Stolte et al. 1977b). The renal tubules of marine and, possibly, freshwater elasmobranchs apparently absorb even less of the filtered sodium and chloride (Hickman and Trump 1969; Stolte et al. 1977 a). And the archinephric ducts of the primitive marine hagfishes (e.g., *Myxine glutinosa),* which conform to their environment, absorb no filtered sodium (Stolte and Schmidt-Nielsen 1978).

It is important, however, to be aware of two factors regarding these values for fractional absorption by the renal tubules. First, they are obtained primarily from clearance studies that supply information only for the kidney as a whole. Such studies show only the net difference between the amount filtered and the

amount excreted in the ureteral urine. Although net absorption is apparent, this approach does not distinguish between simple net absorption throughout the length of the tubules and net secretion in one portion and net absorption in another. In fact, as in the case of fluid, net secretion of sodium and chloride definitely can occur along isolated, perfused proximal tubules from glomerular teleosts and elasmobranchs (Table 4.1) (Beyenbach 1982, 1986; Beyenbach and Fromter 1985). The proximal tubules of some other nonmammalian species also may secrete sodium and chloride to a lesser extent (Beyenbach 1986). In addition, the collecting tubules of marine catfish of the family Plotisidae contain cells whose ultrastructure is the same as that of chloride cells in the gills and that may secrete sodium chloride (Hentschel and Elger 1987). Secretion of sodium chloride by the tubules, when it occurs, may explain the apparent low fractional absorption of sodium by the whole kidney in many nonmammalian vertebrates. Second, the clearance determinations of fractional absorption of sodium and chloride are based solely on measurements of ion concentrations in the aqueous phase of the urine. In birds and uricotelic reptiles, significant quantities of filtered sodium may be contained in urate precipitates (vide infra) so that the actual fraction of filtered sodium absorbed may be lower than that usually measured (Dantzler 1978b).

Even considering these cautionary notes about the clearance measurements of net sodium and chloride transport, it is apparent that the fractional absorption of filtered sodium by the renal tubules of many nonmammalian species is low. Additional absorption, depending on the requirements for sodium and chloride, may occur in regions distal to the kidney — cloaca, colon, or bladder — and must be integrated with the renal absorption in the maintenance of overall ionic balance.

The major sites of sodium and chloride transport in the renal tubules, determined by micropuncture or microperfusion techniques, vary among the vertebrates. Although the proximal tubule has generally been considered the primary site for the absorption of filtered sodium and chloride, this is really only true for the mammals, 60–80% (Giebisch and Windhager 1964), and birds, 50–60% (Laverty and Dantzler 1982), among those vertebrates in which this problem has been studied (Table 4.1). Only some 20 to 45% of the filtered sodium is absorbed along the proximal tubules of those amphibians and reptiles studied (Table 4.1; also Dantzler and Bentley 1978a; Garland et al. 1975; Long 1973; Stolte et al. 1977b), and only 10% is absorbed along the proximal tubules of the primitive river lamprey even in freshwater (Table 4.1; also Logan et al. 1980a). In these nonmammalian vertebrates, much of the filtered sodium and chloride clearly is absorbed distal to the proximal tubule (Table 4.1). Some 50 to 70% of the filtered sodium (and, presumably, the chloride) is absorbed along the distal tubules or collecting ducts of those amphibians and reptiles studied and about 80% of the filtered sodium and chloride is absorbed along the collecting ducts of the river lamprey (Table 4.1).

The rate of sodium and chloride absorption per unit length of proximal tubule is about the same for most amphibians, reptiles, and birds studied (Table 4.1). It is somewhat higher for garter snakes (*Thamnophis* spp.) and considerably higher for mammals (Table 4.1). The fraction of filtered sodium and chloride

Table 4.1 Sodium and chloride transport by tubule segment

Tubule segment and species	$_JNet$ Na^+ % Filt. load	$_JNet$ Na^+ pmol min^{-1} mm^{-1}	$_JNet$ Cl^- % Filt. load	$_JNet$ CL^- pmol min^{-1} mm^{-1}	References
PROXIMAL					
Fishes					
Petromyzonta					
River lamprey, *Lampetra fluviatilis* (freshwater)	10	29.3	10	28.7	Logan et al. 1980a Moriarty et al. 1978
Elasmobranchii					
Dogfish shark, *Squalus acanthias* (stimulated)		−10.3 ± 3.3 (4)		− 8.5 ± 2.5 (4)	Beyenbach 1986 D.B. Sawyer and Beyenbach 1985
Teleostei					
Winter flounder, *Pseudopleuronectes americanus*		− 4.6 ± 0.5 (17)		− 4.5 ± 0.4 (17)	Beyenbach et al. 1986; Beyenbach 1986
Killifish, *Fundulus heteroclitus* (seawater)		− 6.1 ± 1.0 (16)		− 7.7 ± 1.3 (16)	Beyenbach 1986
Amphibia					
Anura					
Bullfrog, *rana catesbeiana*	17	35.7	17	30	Irish and Dantzler 1976; Long 1973
Urodela					
Tiger salamander, *Ambystoma tigrinum*		26.9		24.6	Sackin and Boulpaep 1981a
Mudpuppy, *Necturus maculosus*	29	39.3 ± 6.2 (47)			Boulpaep 1972 Garland et al. 1975
Reptiles					
Squamata					
Ophidia					
Garter snake, *Thamnophis* spp.	45	130.5	45	115.4	Dantzler and Bentley 1978a
Sauria					
Blue spiny lizard, *Sceloporus cyanogenys*	36.5	27.8			Stolte et al. 1977b
Aves					
European starling, *Sturnus vulgaris* Reptilian-type nephrons	56	31.4	58	27.5	Laverty and Dantzler 1982
Mammalia					
Rabbit, *Oryctolagus cuniculus*					

Table 4.1 Continued

Tubule segment and species	$_JNet$ Na^+ % Filt. load	$_JNet$ Na^+ pmol min^{-1} mm^{-1}	$_JNet$ Cl^- % Filt. load	$_JNet$ CL^- pmol min^{-1} mm^{-1}	References
Convoluted segment	65	168.1			Kokko et al. 1971
Straight segment	65	63.9		54.1	Schafer et al. 1977
EARLY DISTAL "DILUTING"					
Fishes					
Petromyzonta					
River lamprey. *Lampetra fluviatilis* (freshwater)	1–2	17.6	1–2	17.6	Logan et al. 1980a Moriarty et al. 1978
Teleostei					
Rainbow trout, *Salma gairdneri* (freshwater)				65.5 ± 20.6 (12)	Nishimura et al. 1983a
Amphibia					
Anura					
Leopard frog, *Rana pipiens*		81.1 ± 10.1 (8)		56.6 ± 6.7 (3)	Stoner 1977
Bullfrog, *Rana catesbeiana*	40				Long 1973
Urodela					
Tiger salamander, *Ambystoma tigrinum*		85.6 ± 16.4 (5)			Stoner 1977
Mudpuppy, *Necturus maculosus*	32				Garland et al. 1975
Congo eel, *Amphiuma means*				33.4 ± 1.4 (12)	Oberleitner et al. 1982
Aves					
Japanese quail, *Coturnix coturnix*				271.8 ± 32.9 (13)	Miwa and Nishimura 1986
Mammalian-type nephrons, thick ascending limb					
Mammalia					
Rabbit, *Oryctolagus cuniculus*					
Medullary TAL	25	28 – 276		19 – 94	Rocha and Kokko 1973; Fine and Trizna 1977; Stokes 1979
Cortical TAL	25	60 – 70		39 – 60	Horster 1978; Burg and Green 1973; Stokes 1979; Shareghi and Agus 1982
LATE DISTAL AND COLLECTING TUBULE					
Amphibia					
Urodela					

Table 4.1 Continued

Tubule segment and species	$_JNet$ Na^+ % Filt. load	$_JNet$ Na^+ pmol min^{-1} mm^{-1}	$_JNet$ Cl^- % Filt. load	$_JNet$ CL^- pmol min^{-1} mm^{-1}	References
Tiger salamander, *Ambystoma tigrinum*		21.2 ± 4.2 (18)			Stoner 1977
Mudpuppy, *Necturus maculosus*	22				Garland et al. 1975
Congo eel, *Amphiuma means*	25				Wiederholt et al. 1971
Late distal					
Reptilia					
Squamata					
Sauria					
Blue spiny lizard, *Sceloporus cyanogenys*	21.1	61.7			Stolte et al. 1977b
Mammalia					
Rabbit, *Oryctolagus cuniculus*	5 – 7	62 – 82			Shareghi and Stoner 1978
COLLECTING DUCT					
Fishes					
Petromyzonta					
River lamprey, *Lampetra fluviatilis* (freshwater)	80	153.3	80	150	Logan et al. 1980a; Moriarty et al. 1978
Amphibians					
Anura					
Bullfrog, *Rana catesbeiana*	47.1				Long 1973
Urodela					
Tiger salamander, *Ambystoma tigrinum*		25.9 ± 4.9 (8)			Delaney and Stoner 1982
Reptilia					
Squamata					
Sauria					
Blue spiny lizard, *Sceloporus cyanogenys*	37.2				Stolte et al. 1977b
Mammalia					
Rabbit, *Oryctolagus cuniculus*					
cortical	2 – 3	23		0	Stokes 1981, 1982
outer medullary	2 – 3	~ 0	~ 0	~ 0	Stokes 1981, 1982
papillary	2 – 3	~40	~80	~80	Rocha and Kudo 1982

Values are means, ranges, or means ± SE. They are taken directly or calculated from the references. Numbers in parentheses indicate number of determinations. J^{Net}_{Na+} indicates net transepithelial sodium transport. J^{Net}_{Cl-} indicates net transepithelial chloride transport. Negative sign in front of value indicates net secretion rather than net absorption.

absorbed in this tubule segment reflects the balance among the filtration rate, the absorption rate, and the length of the segment. The rate of sodium and chloride absorption per unit length of distal tubule and collecting duct segments is about the same for amphibians, reptiles, and mammals (Table 4.1). However, the rate of chloride absorption per unit length of early distal tubule (thick ascending limb) in the one avian species studied (Japanese quail, *Coturnix coturnix*) is considerably higher than that in amphibians and reptiles and as high as or even higher than that in mammals (Table 4.1). This high rate of absorption may reflect the primacy of sodium and chloride absorption in this tubule segment in the concentrating process in birds (vide infra; Chap. 7). Again, the fraction of filtered sodium and chloride absorbed in any of these distal segments reflects the balance among the filtered load reaching these regions, the rate of absorption per unit length, and the length of the segment. However, the rate of sodium and chloride absorption per unit length of collecting duct is much higher in the lampreys than in other vertebrates, apparently reflecting the high fractional absorption in this region (Table 4.1).

The tubule sites, the direction at a given site, and the magnitude of sodium and chloride transport are not completely clear for the glomerular teleosts and elasmobranchs. Although net absorption is said to occur along the first segment of the proximal tubule (Beyenbach et al. 1986; Bulger and Trump 1968; Nishimura and Imai 1982), only one micropuncture study on a marine elasmobranch (little skate, *Raja erinacea*) supports this concept directly (Stolte et al. 1977a). Even this study provides no quantitative data on the magnitude of the absorptive transport in the first segment of the proximal tubule. Moreover, the data suggest that, in this marine species, substantially more sodium and chloride is absorbed in the second segment of the proximal tubule than in the first segment. To complicate matters further, studies with isolated, perfused tubules clearly demonstrate that net secretion of sodium and chloride can occur along the second segment of the proximal tubule of another marine elasmobranch (dogfish shark, *S. acanthias*), a marine teleost (winter flounder, *P. americanus*), and a euryhaline teleost (killifish, *F. heteroclitus*) adapted to seawater (Beyenbach 1986; D. B. Sawyer and Beyenbach 1985). The rate of such net secretion is about one-fifth to one-third the rate of net absorption along the proximal tubules of other poikilothermic nonmammalian vertebrates and an even smaller fraction of the rate of net absorption along the proximal tubules of mammals (Table 4.1). It is not yet known whether such net secretion ever changes to net absorption in these species. However, in stenohaline glomerular marine fish and euryhaline glomerular fish adapted to seawater, it seems likely that net secretion always occurs and plays some role, in addition to extrarenal routes of ion excretion (gills and rectal glands), in eliminating excess sodium and chloride. In fact, Beyenbach (1986) suggests that it may account for the apparent production of a urine hyperosmotic to the plasma by killifish when they are first transferred from seawater to freshwater (Fleming and Stanley 1965; Stanley and Fleming 1964). For euryhaline teleosts this secretory process may become important for maintaining excretory function when the number of filtering glomeruli is reduced during adaptation to seawater (vide supra; Chap. 3; also Beyenbach 1986). It is apparently essential for aglomerular marine teleosts (vide infra; Chap. 5).

Net secretory transport of sodium and chloride can occur along the second portion of the proximal tubules of euryhaline teleosts adapted to seawater, but it is not yet known whether this changes to net absorptive transport when the animals are adapted to freshwater. Preliminary data on isolated, perfused tubules from killifish adapted to freshwater do suggest that some, but not all, tubules secrete sodium and chloride (K. W. Beyenbach personal communication). The factors that might determine the direction of transport are unknown. It is also not known whether net sodium and chloride absorption occurs along the distal tubules of euryhaline teleosts adapted to seawater although clearance data do suggest that some must occur along the distal nephrons of stenohaline marine teleosts (vide supra).

Studies of isolated, perfused early distal tubules from one euryhaline teleost species adapted to freshwater (rainbow trout, *S. gairdneri*) (Nishimura et al. 1983a) show that net chloride absorption can occur at rates similar to those that occur under like circumstances in this segment of amphibian and mammalian nephrons (Table 4.1). Nishimura and Imai (1982) suggest that such net absorption, producing dilution of the tubular fluid, occurs in the early portion of the distal tubule of all freshwater and freshwater-adapted teleosts (vide infra, Chap. 7).

4.2.2 Mechanism of Transport

4.2.2.1 Introduction

The mechanisms involved in net sodium and chloride absorption have been studied in most detail in amphibians, although many important measurements have been made in mammals. Similarly, the mechanisms involved in the net secretion of sodium and chloride have been studied only in elasmobranchs. For both transport processes, this situation results from the accessibility of the renal tubules of these animals for micropuncture and microperfusion and the suitability of the large tubule cells for microelectrode impalements. Because of these characteristics, amphibians (primarily the urodeles, *N. maculosus, A. means,* and *Ambystoma tigrinum*) have been used extensively by renal physiologists to determine the common mechanisms for the renal tubular transport of inorganic ions. Current general concepts with an emphasis on mammalian function but with information drawn extensively from amphibians have been reviewed in detail by others (Burg 1986; Greger 1985; Hierholzer 1985; Kokko and Jacobson 1985; Weinstein and Windhager 1985). Only those aspects most important from a comparative point of view, with emphasis on the nonmammalian vertebrates, are considered here. Some of the measurements of electrical potentials, electrical resistances, and ion fluxes from mammalian and nonmammalian vertebrates that are most important for a comparative discussion of transport mechanisms are summarized in Tables 4.1 and 4.2.

4.2.2.2 Proximal Tubules

Although the data on sodium and chloride transport in the proximal tubules of nonmammalian vertebrates are far from complete or comprehensive, certain general characteristics are apparent. Micropuncture and microperfusion studies

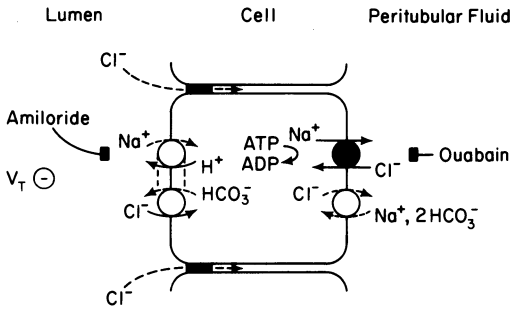


Fig. 4.1 Model for sodium and chloride absorption in nonmammalian proximal tubules based on work in amphibians. *Filled circle with solid arrows* and breakdown of ATP to ADP indicates primary active transport. *Open circles* indicate carrier-mediated transport that may involve carrier-mediated diffusion or secondary active transport. *Broken arrows* indicate movement down an electrochemical gradient. *Solid arrows* indicate movement against an electrochemical gradient. *Lines with bar at end* indicate inhibition. *Vt* indicates transepithelial potential difference with *sign enclosed in circle* indicating whether lumen is negative or positive relative to peritubular fluid

reveal a lumen-negative transepithelial potential during sodium absorption by the proximal tubules of amphibians, reptiles, birds, and freshwater-adapted teleosts (Table 4.2), and indicate that transepithelial sodium transport occurs against an electrochemical gradient (Fig. 4.1) (Boulpaep 1972; Dantzler and Bentley 1981; Giebisch 1961; Nishimura et al. 1983b; Sackin and Boulpaep 1981a and b; Stoner 1977; Windhager et al. 1959). The fractional absorption of chloride is essentially the same as the fractional absorption of sodium along the proximal tubules of lampreys (Logan et al. 1980a), amphibians (Giebisch 1956), reptiles (Dantzler and Bentley 1978a), and birds (Laverty and Dantzler 1982) (Table 4.1). This also appears to be the case in the first portion of the proximal tubules of elasmobranchs (Stolte et al. 1977a) and teleosts (Nishimura and Imai 1982). Thus it appears that the transepithelial absorption of sodium chloride by the proximal tubules of those nonmammalian vertebrates studied involves active sodium and passive chloride transport.

In mammals, too, transepithelial sodium chloride absorption in the proximal tubule involves an active step for sodium and passive chloride movement. However, the coupling of the movements may be somewhat different from that in nonmammalian vertebrates (Weinstein and Windhager 1985). Among other points, the transepithelial potential difference is slightly negative in the early portion and slightly positive in the late portion of the mammalian proximal tubules (Table 4.2). The initial lumen-negative potential reflects the presence of organic solutes and their electrogenic cotransport. with sodium (vide infra; Chap. 6). The small positive potential in the later portions of the mammalian proximal tubule apparently reflects the fact that chloride absorption lags behind sodium and bicarbonate absorption. This potential appears to be a diffusion potential generated by the transepithelial differences in chloride and bicarbonate concentrations and the greater permeability of the epithelium for chloride than for the other solutes present (Weinstein and Windhager 1985). As noted above, however, chloride absorption does not lag behind sodium absorption in the proximal tubules of nonmammalian vertebrates. Instead, sodium and chloride appear to be absorbed together.

The transepithelial resistance of those proximal tubules of nonmammalian vertebrates in which it has been measured is low but not so low as that of mammalian proximal tubules (Table 4.2). These data indicate that mammalian proximal tubules are somewhat more leaky electrically than nonmammalian proximal tubules, a characteristic that also appears to be reflected in the generally lower transepithelial potentials in mammalian than in nonmammalian proximal tubules (Table 4.2).

A detailed analysis of ionic transport in amphibian proximal tubules involving intracellular recordings with ion-sensitive and conventional microelectrodes as well as the transepithelial recordings has supplied information on the basolateral membrane potential (Table 4.2), the relative conductances across the basolateral and apical membranes, the transepithelial ionic shunt pathways, and the intracellular ion activities (Boulpaep 1976; Guggino et al. 1982a, 1982b, 1983; Sackin and Boulpaep 1981a, 1981b; Spring and Kimura 1978). Studies with vesicles from brush-border membranes of *Necturus maculatus* have also supplied detailed information on some of the transport steps across that membrane (Seifter and Aronson 1984). The data suggest that during the transepithelial absorptive process sodium enters the cells across the luminal membrane down an electrochemical gradient by a saturable process that is limiting for transepithelial transport (Fig. 4.1). This sodium entry step apparently involves primarily sodium-hydrogen exchange (Fig. 4.1), but other processes, e.g., coupled entry with organic solutes (Chap. 6, Figs. 6.1 and 6.2), are also certainly involved. Sodium is then transported out of the cells across the basolateral membrane by a rheogenic active process, apparently involving Na-K-ATPase, that is not saturated over a range of sodium concentrations greater than those normally found in the cells (Fig. 4.1). Saturation of the sodium entry step across the luminal membrane apparently results from changes in the sodium permeability of the luminal membrane in response to increasing intracellular sodium concentrations. Chloride appears to enter the cells across the luminal membrane during the absorptive process by an electroneutral, carrier-mediated process that is driven by the activity gradient for sodium (Fig. 4.1). However, this process apparently does not involve a direct sodium-chloride cotransport step. Instead, it appears to involve an anion-exchange process, probably chloride-bicarbonate exchange, coupled to the sodium-hydrogen exchange (Fig. 4.1). Chloride absorption by a similar sodium-coupled process probably occurs in the proximal tubules of all nonmammalian vertebrates, as indicated by the less direct studies above, but probably does not account for at least a portion of the chloride transport by mammalian proximal tubules. In these amphibian tubules during the net absorptive process, chloride appears to exit cells across the peritubular membrane by an electroneutral mechanism, probably involving exchange for sodium and bicarbonate (Fig. 4.1). There is also a shunt pathway with a high chloride conductance between these amphibian tubule cells (Fig. 4.1).

Beyenbach and his colleagues have attempted to define the mechanism for net sodium chloride secretion in those proximal tubules in which it occurs. They have applied an electrophysiological analysis similar to that described above for amphibians to the isolated, perfused second segment of the proximal tubule of an elasmobranch (dogfish shark, *S. acanthias*) (Beyenbach and Fromter 1985)

Table 4.2 Electrical properties by tubule segment

Tubule segment and species	V_T mV	V_{BL} mV	R_T KΩcm	R_T Ωcm^2	References
PROXIMAL					
Fishes					
Elasmobranchii					
Dogfish shark, *Squalus acanthias*					
Unstimulated	1.2 ± 0.6 (16)	–61.3 ± 1.6 (16)	2.87 ± 0.23 (16)	36.8 ± 3.1 (16)	Beyenbach 1986; Beyenbach and Fromter 1985
Stimulated	– 1.6 ± 0.7 (7)	–48.0 ± 5.1 (17)		31.9 ± 3.3 (7)	
Teleostei					
Rainbow trout, *Salmo gairdneri* (freshwater)	– 5.0 ± 0.7 (15)				Nishimura et al. 1983a
Winter flounder, *Pseudopleuronectes americanus*	– 1.9 ± 0.2 (113)			25.6 ± 2.7 (28)	Beyenbach et al. 1986
Amphibia					
Anura					
Leopard frog, *Rana pipiens*	– 6.5 ± 1.6 (4)				Stoner 1977
Toad *Bufo marinus*	– 6.6 ± 1.7 (4)				Stoner 1977
Urodela					
Tiger salamander *Ambystoma tigrinum*	– 4.5 ± 0.2 (137)	–59.6 ± 2 (84)		52.1 ± 3.0 (81)	Sackin and Boulpaep 1981a
Mudpuppy, *Necturus maculosus*	–15.4 ± 0.6 (93)	–61.0 ± 3.7 (10)		69.9 ± 8.97 (14)	Boulpaep 1972
Reptilia					
Squamata					
Ophidia					
Garter snake, *Thamnophis* spp.	– 0.49 ± 0.15 (14)				Dantzler and Bentley 1981
Aves					
European starlings, *Sturnus vulgaris*					
Reptilian-type nephrons	– 2.0				G. Laverty personal communication
Mammalia					
Rabbit, *Oryctolagus cuniculus*					
Convoluted segment	– 2.0 to 0	–51 ± 1.63 (24)		7.0	Biagi et al. 1981; Schafer and Andreoli 1979
Straight segment	+ 2.0 to +3.1	–47.0 ± 0.97 (94)		8.2	Biagi et al. 1981; Schafer and Barfuss 1982; Schafer and Andreoli 1979

Table 4.2 Continued

Tubule segment and species	V_T mV	V_{BL} mV	R_T KΩcm	R_T Ωcm^2	References
EARLY DISTAL "DILUTING"					
Fishes					
Teleostei					
Rainbow trout, *Salmo gairdneri* (freshwater)	+17.8 ± 1.4 (53)				Nishimura et al. 1983a
Amphibia					
Anura					
Leopard frog, *Rana pipiens*	+13.5 ± 1.1 (35)				Stoner 1977
Toad, *Bufo marinus*	+ 4.6 ± 1.4 (4)				Stoner 1977
Urodela					
Tiger salamander, *Ambystoma tigrinum*	+13.6 ± 1.7 (14)	−54 ± 2			Sackin et al. 1981 Stoner 1977
Congo eel, *Amphiuma means*	+ 9.0 ± 0.5 (70)	−71.0 ± 0.5 (28)		10 – 30	Oberleithner et al. 1982; Stanton et al. 1982
Japanese newt, *Triturus* sp.	+13.7 ± 1.0 (35)	−50.6 ± 1.6		29 ± 9 (4)	Hoshi et al. 1981
Aves					
Japanese quail, *Coturnix coturnix*					
Reptilian-type nephrons	+ 4.5 ± 1.7 (7)				Nishimura et al. 1986
Mammalian-type nephrons, thick ascending limb	+ 9.1 ± 0.72 (35)				Nishimura et al. 1986
Mammalia					
Rabbit, *Oryctolagus cuniculus*					
Medullary thick ascending limb	+ 3 to + 7				Burg 1982
Cortical thick ascending limb	+ 3 to +10			25 – 34	Burg 1982
LATE DISTAL AND COLLECTING TUBULE					
Amphibia					
Urodela					
Tiger salamander, *Ambystoma tigrinum*	−41.2 ± 4.2 (25)				Stoner 1977
Congo eel, *Amphiuma means*					

Table 4.2 Continued

Tubule segment and species	V_T mV	V_{BL} mV	R_T KΩcm	R_T Ωcm^2	References
Late distal	− 4.7 ± 0.6 (66)	−83.5 ± 1.3 (66)			B. Cohen et al. 1984
Collecting tubule	− 8.0 ± 2.0	−70 ± 4			Stanton et al. 1982
Japanese newt, *Triturus* sp.	− 6.5 ± 2.2 (11)			764	Hoshi et al., 1981
Reptilia					
Squamata					
Ophidia					
Garter snake, *Thamnophis* sp..	−34.9 ± 2.1 (27)		23.4 ± 1.6 (27)	83.1	Beyenbach et al. 1980
Aves					
Japanese quail, *Coturnix coturnix*	− 3.2 ± 0.83 (7)				Nishimura et al. 1986
Mammalia					
Rabbit, *Oryctolagus cuniculus*	− 5 to −40				Gross et al. 1975; Shareghi and Stoner 1978
COLLECTING DUCT					
Amphibia					
Urodela					
Tiger salamander, *Ambystoma tigrinum*	− 8.9 ± 1.9 (17)		31 ± 5 (9)	626	Delaney and Stoner 1982
Japanese newt, *Triturus* sp.	−23 ± 5				Hoshi et al. 1981
Mammalia					
Rabbit, *Oryctolagus cuniculus*					
Cortical	−23	−96	15.2		Koeppen et al. 1983
Outer medullary	−30 to +10				Stokes et al. 1978
Papillary	~ 0	−15 to +24			Rocha and Kudo 1982 Terreros et al. 1981

Values are means, ranges, or means ± SE. They are taken directly or calculated from the references. Numbers in parentheses indicate numbers of determinations. V_T indicates transepithelial potential difference; sign indicates lumen relative to peritubular side. V_{BL} indicates potential difference across basolateral membrane; sign indicates inside of cell relative to outside of cell on basolateral side. R_T indicates transepithelial resistance.

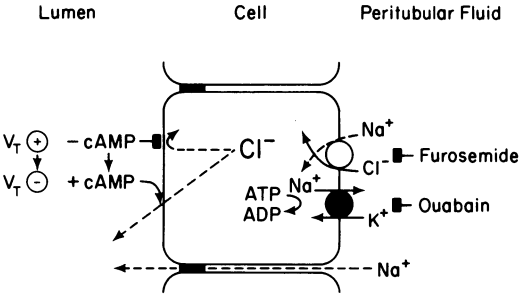


Fig. 4.2. Model for net sodium chloride secretion by proximal tubules based on studies of elasmobranch proximal tubules by Beyenbach (1986). Symbols have same meanings as in legend for Fig. 4.1. Note that transepithelial potential difference changes from lumen- positive to lumen-negative when the chloride conductance across the luminal membrane is increased by cAMP

and a less rigorous analysis, because the tubule cells are too small to hold intracellular electrodes for long, to the second segment of the proximal tubule of a marine teleost (winter flounder, *P. americanus*) (Beyenbach et al. 1986). This tubule segment from both species has a relatively low transepithelial resistance and, when secreting, a small, lumen-negative transepithelial potential (Table 4.2). The low transepithelial resistance appears to reflect a paracellular shunt pathway for sodium chloride that is more selective for sodium than chloride (Beyenbach 1986; Beyenbach and Fromter 1985; Beyenbach et al. 1986). Secretion of sodium and chloride by the elasmobranch tubule, and probably also the teleost tubule, is stimulated by cAMP and inhibited by furosemide and ouabain (Beyenbach and Fromter 1985).

The electrophysiological studies on elasmobranch tubules, involving cAMP stimulation and furosemide and ouabain inhibition, are consistent with a secondary active transport process for sodium chloride secretion, in which, as in the case of sodium chloride absorption, the primary energy is provided by the ouabain-sensitive Na-K-ATPase on the basolateral membrane (Fig. 4.2) (Beyenbach and Fromter 1985; Beyenbach 1986). The generation of a low intracellular sodium concentration by the primary active transport of sodium out of the cells at the basolateral membrane provides a substantial electrochemical gradient for the movement of sodium into the cells from the peritubular side (Fig. 4.2). Movement of chloride into cells from the peritubular side is coupled to energy provided by this sodium gradient by means of a furosemide-sensitive sodium-chloride co-transporter (Fig. 4.2). The sodium that has entered the cells in this co-transport process is again transported out across the basolateral membrane by the Na-K-ATPase-driven pump (Fig. 4.2). The chloride, however, moves passively from cells to the lumen through chloride channels in the luminal membrane, the conductivity of which is regulated by cAMP (Fig. 4.2). The transport of chloride into the tubule lumen appears to be electrically balanced by the paracellular movement of sodium from the peritubular side into the lumen (Fig. 4.2). In support of this model, stimulation of sodium chloride secretion (either spontaneously or with cAMP) in proximal tubules of *Squalus acanthias* decreases transepithelial resistance and resistance of the luminal membrane, depolarizes the potential across the basolateral membrane towards zero, and makes the transepithelial potential lumen-negative (Table 4.2; also Beyenbach and Fromter 1985).

4.2.2.3 Early Distal Tubules

As indicated above, significant sodium and chloride absorption occurs along the early portion of the distal tubules of mammals and of all those nonmammalian vertebrates studied, except freshwater lampreys. Such absorption also occurs to a significant extent along the comparable thick ascending limb of nephrons in mammals and of mammalian-type nephrons in birds. In all these cases, this absorption appears to be involved in dilution of the tubular fluid (vide infra; Chap. 7). Moreover, as described above (Chap. 2), the cells of these early distal and ascending loop segments have certain common structural features.

The most detailed studies of sodium and chloride transport by the early distal tubule and thick ascending limb segments have been made on amphibians (Bott 1962; Giebisch 1961; Hoshi et al. 1981, 1983; Oberleithner et al. 1982; Sackin et al. 1981; Stoner 1977, W. J. Sullivan 1968; Walker et al. 1937) and mammals (Greger 1985), although less detailed studies have been made on freshwater teleosts (Nishimura et al. 1983a) and birds (Miwa and Nishimura 1986; Nishimura et al. 1984, 1986). In vivo or in vitro microperfusions of early distal or thick ascending limb segments from each species studied reveal a lumen-positive transepithelial potential and a significant chloride absorptive flux (Tables 4.1 and 4.2), both of which are dependent on the presence of both sodium and chloride in the lumen and are eliminated by the addition of furosemide or bumetanide to the perfusate or ouabain to the peritubular bathing medium (Greger 1985; Hoshi et al. 1981, 1983; Miwa and Nishimura 1986; Nishimura et al. 1983a, 1984, 1986; Oberleithner et al. 1982; Stoner 1977). No such measurements have been made on the early distal tubules of reptiles, but, as noted earlier (Chap. 2), the cells of this segment do not necessarily have the structural characteristics described above for other species. A few preliminary measurements on the thin intermediate nephron segment from one reptile (garter snake, *Thamnophis* spp.) indicate that it has a significant lumen-positive transepithelial potential and, therefore, may absorb sodium chloride and dilute urine by the same process as the early distal tubule of other nonmammalian vertebrates (S. D. Yokota and W. H. Dantzler unpublished observations).

For the early distal tubules of amphibians (Congo eel, *A. means*), and, it is assumed, for comparable tubule segments from other nonmammalian vertebrates, intracellular recordings with ion-sensitive and conventional microelectrodes as well as transepithelial recordings indicate that the presence of furosemide in the lumen or the absence of chloride or sodium from the lumen leads to hyperpolarization of the basolateral membrane to an extent equivalent to the decrease in the transepithelial potential (Oberleithner et al. 1982). At the same time, there is a significant decrease in the intracellular chloride activity from a control value that is about three times that expected at electrochemical equilibrium (Oberleithner et al. 1982). Removal of potassium from the lumen also reduces the intracellular activities of sodium and chloride (Oberleithner et al. 1983b and 1983d). Greger and his colleagues have obtained similar data on the thick ascending limb of mammalian nephrons (Greger 1985). Taken together, these data support the general concept of chloride absorption by a secondary active, sodium-coupled mechanism, the energy for which is derived from the

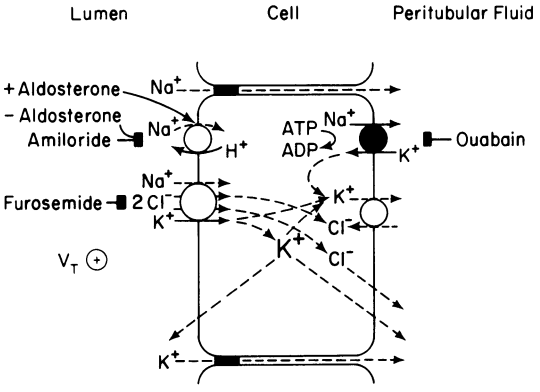


Fig. 4.3. Model for sodium, chloride, potassium, and hydrogen ion transport by the early distal tubules of nephrons of nonmammalian vertebrates and thick ascending limbs of loops of Henle of mammalian-type avian nephrons and mammalian nephrons. Symbols have same meanings as in legend for Fig. 4.1. Although the details are based primarily on studies on amphibians and mammals, the steps for sodium chloride absorption apparently hold for the early distal tubules of teleost nephrons, amphibian nephrons, and reptilian-type avian nephrons; the thick ascending limb of Henle's loop of mammalian-type avian nephrons and mammalian nephrons, and possibly the thin intermediate segment or early distal tubule of reptilian nephrons

ouabain-sensitive Na-K-ATPase at the basolateral membrane (as in the case of the proximal tubule sodium chloride secretion discussed above) (Fig. 4.3; also Greger 1985; Oberleithner et al. 1982). The primary active transport of sodium out of the cells across the basolateral membrane establishes a chemical gradient for coupled, electroneutral movement of sodium and chloride into cells across the luminal membrane by a furosemide-sensitive transporter, which also transports potassium (vide infra; Fig. 4.3; also Greger 1985; Oberleithner et al. 1982, 1983a, 1983d). The chloride probably leaves the cells across the basolateral membrane via both a chloride-conductive pathway and a coupled electroneutral pathway with potassium (Fig. 4.3; also Greger and Schlatter 1983; Oberleithner et al. 1982). From these data, it appears very likely that the same mechanism for sodium and chloride absorption and the consequent dilution of the tubular fluid exists in the early distal tubules of teleost nephrons, amphibian nephrons, and reptilian-type avian nephrons; the thick ascending limb of Henle's loop of mammalian-type avian nephrons and mammalian nephrons; and possibly the thin intermediate segment or early distal tubule of reptilian nephrons.

In addition to this primary process for sodium chloride absorption, there is a sodium-hydrogen exchange mechanism in the luminal membrane of the early distal tubule of amphibian nephrons (vide infra; hydrogen ion transport) and possibly in the thick ascending limb of mammalian nephrons (Fig. 4.3; also Good et al. 1984). In amphibians, at least, this exchanger apparently functions primarily for the regulation of hydrogen ion transport and secondarily for the regulation of potassium secretion (vide infra; potassium and hydrogen ion transport). However, during chronic exposure of amphibians to a high potassium environment, when the exchanger is stimulated (vide infra; potassium and hydrogen ion transport), the sodium-chloride-potassium co-transport system is depressed by some 30% so that the sodium-hydrogen exchange system may account for some 30% of the sodium entering the cells (Oberleithner et al. 1984). As in the

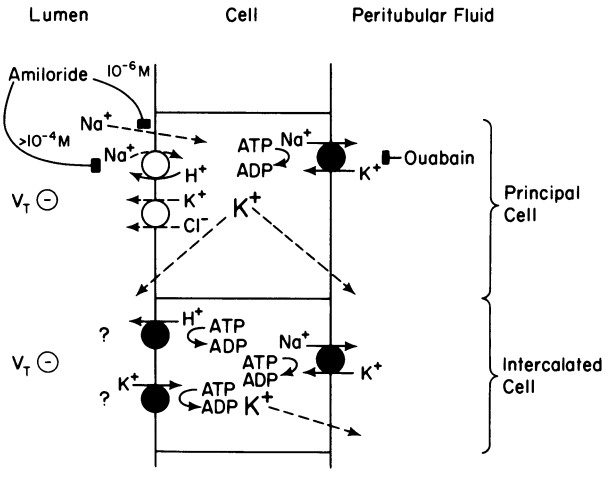


Fig. 4.4. Model for sodium, chloride, potassium, and hydrogen-ion transport by late distal tubules, collecting tubules, and collecting ducts. Symbols have same meanings as in legend for Fig. 4.1. The uncertainty about the processes in different cell types in nonmammalian vertebrates, particularly those processes in the intercalated cells is indicated by *question marks*

case of a sodium entry by the sodium-chloride-potassium co-transport system, the sodium is transported out of the cells across the basolateral membrane by the primary Na-K-ATPase system (Fig. 4.3). No information is available on the presence of such a sodium-hydrogen exchange system in this nephron segment in other nonmammalian vertebrates or on the quantitative role of this system in mammals.

4.2.2.4 Late Distal and Collecting Tubules

As indicated earlier, substantial sodium and chloride absorption does occur along the late distal tubules and collecting tubules of those nonmammalian vertebrates in which it has been measured (primarily amphibians and reptiles) and some absorption also occurs along these regions of the nephrons of mammals (Table 4.1). Direct studies of this absorptive process are available only for the late distal tubules of amphibians, reptiles, birds, and mammals and the collecting tubules of amphibians and mammals. Because of the confusion about the relationship of these structures in one species to those in another (vide supra; Chap. 2), the data from these studies are considered together as if they truly represented the analogous segment in all species (Table 4.2). This is undoubtedly an oversimplification, but much more information must be obtained on the relationship between structure and function in vertebrates other than the amphibians and mammals before a clearer picture will emerge. In each of these segments studied by in vivo or in vitro microperfusion, there is a significant transepithelial voltage (Table 4.2) (Fig. 4.4), and sodium absorption occurs against an electrochemical gradient (Stoner 1985). The transepithelial potential and the net sodium absorption are reduced or eliminated by amiloride (an agent that blocks sodium entry across the luminal membrane, in low concentrations by blocking entry through channels and in high concentrations by blocking sodium-hydrogen exchange) in the lumen or ouabain on the peritubular side (Fig. 4.4; also Beyenbach and Dantzler 1978; B. Cohen et al. 1984; Hoshi et al. 1981; Nishimura et al. 1983b; 1986). In late distal and collecting tubule segments of urodele

amphibians, there is a large potential difference across the basolateral membrane (Table 4.2; Fig. 4.4). This potential is a saturable function of the sodium concentration in the tubule lumen and is depolarized by the addition of amiloride to the lumen (Cohen et al. 1984). These data all suggest that there is an electrogenic sodium transport process at the peritubular membrane, apparently involving Na-K-ATPase, which plays a major role in generating the transepithelial potential difference and in absorbing sodium (Fig. 4.4; also Cohen et al. 1984).

In vitro microperfusion studies on the late distal tubules of garter snakes (*Thamnophis* spp., Beyenbach and Dantzler 1978; Beyenbach et al. 1980; Beyenbach 1984) suggest that there may be some intrinsic cellular regulation of sodium absorption in this region. The substantial lumen-negative transepithelial voltage (Table 4.2) and the calculated short-circuit current, apparently representing sodium absorption, although dependent on the presence of sodium in the lumen, decay rapidly when its concentration exceeds 30 mmol l^{-1}. The decays in voltage and short-circuit current appear to result from an increase in resistance to sodium transport through the active pathway. Because sodium that enters the cells across the luminal membrane at this time cannot be extruded rapidly enough, the cells swell. This response to an excessive sodium load may prevent the distal tubules from absorbing too much sodium when there is a need for additional sodium excretion. This adjustment of the transport system to operate effectively only at a low sodium concentration also permits further dilution of luminal fluid in which the sodium concentration is already low. Whether or not such a process operates in the terminal regions of distal tubules in other reptiles is unknown.

4.2.2.5 Collecting Ducts

Although substantial sodium absorption occurs along the collecting ducts of some fishes, amphibians, and reptiles (Table 4.1) and may even occur along the collecting ducts of birds, information bearing on the mechanism of transport exists only for urodele amphibians and mammals (Table 4.2). And, as noted above, the fraction of filtered sodium absorbed along mammalian collecting ducts is very low (Table 4.1). In vitro micropuncture measurements on the collecting ducts of a newt (*Triturus* sp.) (Hoshi et al. 1981) demonstrate a lumen-negative transepithelial potential (Table 4.2) that is abolished by the replacement of sodium in the incubation medium with choline. The solute absorption, presumably sodium absorption, that this voltage appears to reflect is substantially reduced by the addition of either amiloride or furosemide to the incubation medium. Segments of collecting ducts from a salamander (*A. tigrinum*), isolated and perfused in vitro also display a ouabain-sensitive, lumen-negative transepithelial voltage (Table 4.2), a substantial transepithelial specific resistance (Table 4.2), and a rate of sodium absorption (Table 4.1) equivalent to the measured short circuit current (Stoner 1977; Delaney and Stoner 1982). These data indicate that this segment actively absorbs sodium, at least in urodele amphibians (Stoner 1985). Although the process may be similar for the collecting ducts of other nonmammalian vertebrates, it has yet to be evaluated and, given the

structural heterogeneity among species (vide supra; Chap. 2), there may be specific differences. Moreover, in mammals, whereas sodium transport in the cortical collecting ducts appears to resemble that discussed above for the late distal tubules and collecting tubules (Table 4.2), such ouabain-sensitive transport may not exist in medullary collecting ducts (Stokes 1982; Stoner 1985). Much more work is required before the exact transport mechanisms in these structures are understood and the comparative relationships between structure and function among the vertebrate classes are determined.

4.2.3 Control of Transport

4.2.3.1 Hormonal Control

4.2.3.1.1 Antidiuretic Hormones

In view of the stimulatory effects of the antidiuretic neurohypophysial peptides on sodium transport by a number of nonrenal epithelia, e.g. the toad urinary bladder, it has long appeared likely that these hormones might stimulate sodium absorption by the renal tubules. Such stimulation of sodium and chloride absorption along the thick ascending limb of Henle's loop in mammalian nephrons or in mammalian-type nephrons in birds could enhance the function of the countercurrent multiplier system and hence improve concentrating ability (vide infra; Chap. 7). Indeed, arginine vasopressin stimulates adenylate cyclase activity and sodium chloride cotransport in the thick ascending limbs of some mammalian species (rat and mouse) but not of others (rabbit and human) (Hall and Varney 1980; Hebert et al. 1981a and b; Morel 1981; Sasaki and Imai 1980). The adaptive significance of this difference among species is not clear. Moreover, arginine vasotocin has no effect on the transepithelial voltage, presumably indicative of sodium chloride cotransport, in the thick ascending limbs of Henle's loops from the one avian species studied (Japanese quail, *Coturnix coturnix;* also Nishimura et al. 1984, 1986).

Arginine vasotocin also appears to have a variable effect on sodium absorption by renal tubules of other nonmammalian vertebrates. It apparently has no effect on tubular sodium absorption in teleost fishes (Nishimura 1985), but it does produce a natriuresis in lungfish (*P. aethiopicus*) out of proportion to the increase in glomerular filtration rate that it also produces (Sawyer 1970). However, even this natriuretic effect appears to reflect not a direct inhibition of sodium absorption but a failure of sodium absorption to keep pace with the increasing filtered load (Sawyer 1972). Clearance studies suggest that arginine vasotocin stimulates tubular sodium absorption in frogs (*R. esculenta*) (Jard and Morel 1963), water snakes (*Nerodia sipedon*) (Dantzler 1967), and chickens (Ames et al. 1971); inhibits tubular sodium absorption in freshwater turtles (*Chrysemys picta belli*) (Butler 1972); and has no effect on tubular sodium absorption in lizards (*V. gouldii*) (Bradshaw and Rice 1981).

The tubular sites of any of these hormonal effects are unknown. As noted above, there is no evidence of a direct effect of the hormone on the thick ascending limbs of mammalian-type nephrons in birds. It also has no effect on

the transepithelial voltage in isolated, perfused segments of late distal tubules from garter snakes (*Thamnophis* spp.) (Beyenbach 1984). If arginine vasotocin does have direct effects on the tubular transport of sodium, it may act at sites other than those studied. No data are yet available from nonmammalian vertebrates on receptors for arginine vasotocin in the nephrons.

4.2.3.1.2 Angiotensin

Whether or not angiotensin has a direct physiological effect on sodium transport by the renal tubules of mammalian or nonmammalian vertebrates is not completely clear (Burg 1986; Dantzler 1985; Jackson et al. 1985; Nishimura 1985). Some micropuncture and peritubular microperfusion studies do suggest that low, apparently physiological doses of angiotensin II can stimulate sodium absorption in mammalian proximal tubules, whereas higher doses inhibit it (Harris and Young 1977; Steven 1974). This concept of a direct, dose-related effect on the sodium transport system is also supported by preliminary measurements of hormone binding and sodium efflux in isolated mammalian proximal tubules (Freedlander and Goodfriend 1977). Among the nonmammalian vertebrates, pressor doses of angiotensin II can produce a natriuresis in some teleost fishes (e.g., American eel, *A. rostrata*), but this response, which may be pharmacological, appears to result primarily from an increase in GFR (Nishimura and Sawyer 1976; Nishimura 1985). Moreover, even high doses of angiotensin have little or no effect on sodium excretion by aglomerular teleosts (goosefish, *Lophius americanus;* toadfish, *Opsanus tau*), tending to confirm the absence of a direct effect on the tubules (Churchill et al. 1979; Nishimura 1985; Zucker and Nishimura 1981).

In contrast to the teleosts, infusions of angiotensin into the renal portal system of amphibians, reptiles, and birds suggest that the hormone may have a direct effect on sodium transport by renal tubules. In an anuran amphibian (Chilean toad, *Calyptocephallela cardiverbela*), infusions of low, nonpressor doses produce an antidiuresis and antinatriuresis without a change in GFR, whereas larger doses produce a diuresis and natriuresis despite an apparent reduction in GFR (Galli-Gallardo and Pang 1978; Nishimura 1985). Likewise, in a chelonian reptile (freshwater turtle, *Pseudemys scripta elegans*), infusion of a nonpressor dose through the renal portal system produces an antinatriuresis (Nishimura 1985), and, in chickens, infusion of a moderate dose produces a diuresis and natriuresis predominately on the ipsilateral side (Nishimura 1985; Stallone and Nishimura 1985). Together, these data from renal portal infusions in amphibians, reptiles, and birds suggest that, as in mammals, angiotensin II may act directly on the tubules at low levels to enhance absorption of sodium and at high levels to inhibit absorption of sodium. However, these studies on nonmammalian vertebrates are more indirect than those on mammals; there is no information on possible tubule sites or mechanisms of action.

4.2.3.1.3 Adrenocorticosteroids

Among mammals, the importance of aldosterone, the natural mineralocorticoid, in stimulating the absorption of sodium by the renal tubules is well documented,

a subject of continuing study, and frequently reviewed (Burg 1986; Hierholzer 1985). The primary site of aldosterone action in mammals appears to be the cortical collecting tubules, although there may be some effect in the late distal tubules, the medullary collecting ducts, and even the thick ascending limb of Henle's loop (Burg 1986; Hierholzer 1985). Although the detailed mechanism of action of the hormone is still not absolutely certain, substantial evidence indicates that it enhances Na-K-ATPase activity especially along the cortical collecting duct (Hierholzer 1985).

Among nonmammalian vertebrates, natural mineralocorticoids are not even clearly defined for many species and their effects on sodium transport by the renal tubules are often even less clear. In fact, as Nishimura (1985) points out, individual adrenocorticosteroids often have both glucocorticoid and mineralocorticoid effects in nonmammalian vertebrates. Adrenocorticoids have been identified in the blood of cyclostomes (Atlantic hagfish, *M. glutinosa;* marine lamprey, *P. marinus*) (Sandor et al. 1976), but their biological function is unknown. Similarly, aldosterone is present in the blood of a number of teleost species (Sandor et al. 1976). Although partial adenalectomy of freshwater European eels (*A. anguilla*) produces an increased fractional excretion of sodium (Chan et al. 1969), suggesting that aldosterone may stimulate sodium absorption, there is no direct evidence on this point. Aldosterone is present in amphibians and clearly stimulates sodium transport in nonrenal epithelia (e.g., urinary bladder of the toad, *Bufo marinus*), but an effect of it or any other corticosteroid on net sodium absorption by the renal tubules has not been demonstrated (Nishimura 1985). Aldosterone apparently does stimulate the sodium-hydrogen exchange process in the luminal membrane of amphibian early distal tubules during chronic exposure to a high potassium environment (vide infra; potassium and hydrogen ion transport) and thereby stimulates sodium absorption by this process. However, this may only compensate for decreased absorption by the sodium-chloride-potassium co-transport system and thus may not produce any increase in net sodium absorption.

The observed renal effects of adrenocorticoids in reptiles are so variable that a clear picture has yet to emerge. Indeed, the data suggest that the effects in some species are exactly opposite to those in other species. For example, clearance studies suggest that aldosterone, a naturally occurring adrenal steroid in reptiles, stimulates sodium absorption by renal tubules in sodium chloride-loaded snakes (*Nerodia cyclopion*) and turtles (*C. picta*), but has no effect on water-loaded or control animals (Brewer and Ensor 1980; Elizondo and LeBrie 1969). Although administration of aldosterone to desert iguanas (*Dipsosaurus dorsalis*) appears to have no effect on sodium excretion, hypophysectomy or dexmethasone administration in these animals and in a species of Australian agamid lizard (*Amphibolarus ornatus*) suggests that the adrenocorticosteroids may actually inhibit absorption by renal tubules (Bradshaw 1975; Bradshaw et al. 1972). More recent studies on a varanid lizard (*V. gouldii*), however, indicate that both plasma levels of aldosterone and fractional absorption of sodium decrease during administration of a salt load (Bradshaw and Rice 1981). The fractional absorption of sodium in *V. gouldii* is also reduced following adrenalectomy and restored to control level by the administration of aldosterone (Rice et al. 1982).

Together, these conflicting data suggest that in some reptiles aldosterone has the same stimulatory effect on sodium absorption as in mammals whereas in others it, or some other adrenocorticoid, has no effect or the opposite effect. However, no direct studies of tubular effects or possible sites of action have yet been made.

Finally, in birds, at least ducks, *Anas platyrhynchos,* adrenalectomy and administration of corticosteroids suggest that both aldosterone and corticosterone, two naturally occurring adrenal corticosteroids, have physiological roles in stimulating sodium absorption by renal tubules (Holmes and Phillips 1976). As in the case of the reptiles, however, no direct studies of tubular effects, sites of action, or mechanisms of action have been made.

4.2.3.1.4 Prolactin

The fragmentary data available suggest that prolactin may have an effect on sodium absorption by renal tubules in some nonmammalian vertebrates, but they are far from convincing. For example, the administration of the hormone to freshwater turtles (*C. picta*) (Brewer and Ensor 1980) and goldfish (Lahlou and Giordan 1970; Lahlou and Sawyer 1969) appears to stimulate sodium absorption, but it is possible that the prolactin preparations were contaminated with neurohypophysial peptides and that these may have played some role in the observed effects. Although specific binding sites for prolactin have been described along the proximal tubules of anuran amphibians (Gona 1982; Nishimura 1985; B. A. White and Nicoll 1979), no studies of the possible effects of the hormone on sodium transport have yet been made. No data are available on the possible effects of the hormone on sodium absorption by renal tubules of birds and there is no evidence of a direct effect on sodium absorption by the renal tubules of mammals (Burg 1981).

4.2.3.2 Neural Control

Adrenergic renal nerves apparently play some role in stimulating sodium absorption along the proximal tubules of mammalian nephrons (Gottschalk et al. 1985). However, whether only α-agonists or both α- and β-agonists are involved is not yet clear (Gottschalk et al. 1985). Although there may be some neural regulation of sodium absorption in Henle's loop (DiBona and Sawin 1982), it is not clear whether there is any neural regulation in the more distal portions of the nephron (Gottschalk et al. 1985). In addition, the physiological importance of this neural regulation in the overall regulation of sodium excretion is the subject of dispute and continuing study (for extensive review, see Gottschalk et al. 1985).

Although the studies on the neural regulation of sodium excretion in mammals are far from complete, they are certainly more extensive than those on nonmammalian vertebrates. In fact, only one study in amphibians has any direct bearing on this problem. In bullfrogs (*R. catesbeiana*), the systemic arterial infusion of phenoxybenzamine, an α-adrenergic blocker, initiates a bilateral natriuresis, whereas renal portal venous infusion of the same drug produces a natriuresis only from the ipsilateral kidney (Gallardo et al. 1980). Neural elements in close proximity to the renal tubules are also observed in these animals (Gallardo et al. 1980). Thus, it is possible that α-adrenergic nerves play

some role in stimulating sodium absorption in these animals. However, no additional, more direct information is available on amphibians or any other nonmammalian vertebrates.

4.3 Potassium

4.3.1 Direction, Magnitude, and Sites of Net Transport

Clearance studies indicate that either net absorption or net secretion of potassium by the renal tubules can occur in mammals (Giebisch 1975), representatives of all classes of nonmammalian tetrapods (Dantzler 1976a; Holmes et al. 1968; Shoemaker 1967; Skadhauge and Schmidt-Nielsen 1967a; Stolte et al. 1977b; Wiederholt et al. 1971), and freshwater teleosts (Hickman and Trump 1969). This also may be the case for marine elasmobranchs (Hickman and Trump 1969; Stolte et al. 1977a). However, net tubular absorption generally occurs in euryhaline teleosts adapted to seawater and net tubular secretion, in euryhaline teleosts adapted to freshwater (Hickman and Trump 1969; Schmidt-Nielsen and Renfro 1975). Only net tubular absorption has been observed in freshwater lampreys (Logan et al. 1980a) and only net tubular secretion in hagfishes (Alt et al. 1980; Stolte and Schmidt-Nielsen 1978).

As in the case of sodium excretion (vide supra), these measurements of net secretion and net absorption have been made only with the aqueous phase of the urine. In birds and uricotelic reptiles, some filtered or secreted potassium may be combined with urate precipitates in the tubular fluid (vide infra) so that true values for the magnitude and, in some cases, even the direction of net transport may be different from those generally reported (Dantzler 1978b).

Sites of net transport along the renal tubules and magnitude of such transport are more variable among nonmammalian vertebrates than the mammals. In all mammals studied, about 80% of the filtered potassium is absorbed along the proximal tubules whether or not overall net secretion by the tubules is occurring (Giebisch 1975). However, among those nonmammalian species in which micropuncture measurements have been made, potassium transport along the proximal tubules is much less consistent. About 25–35% of filtered potassium is absorbed along the proximal tubules of freshwater lampreys (river lamprey, *L. fluviatilis*) (Logan et al. 1980a), anuran amphibians (bullfrog, *R. catesbiana*) (Long and Giebisch 1979), and lizards (blue spiny lizard, *S. cyanogenys*) (Stolte et al. 1977b), but little or no net transepithelial transport occurs along the proximal tubules of urodele amphibians (mudpuppy, *N. maculosus,* and Congo eel, *A. means*) (Garland et al. 1975; Oken and Solomon 1963; Wiederholt et al. 1971); transepithelial transport can vary from net absorption to no net transport to possible net secretion along the proximal tubules of the superficial reptilian-type nephrons of birds (starlings, *S. vulgaris*) (Laverty and Dantzler 1982).

In mammalian and nonmammalian vertebrates, however, overall net secretion of potassium by the renal tubules, when it occurs, results from net secretion

along the distal portions of the nephrons (Dantzler 1976a; Giebisch 1975; Stolte et al. 1977a; Stolte et al. 1977b; Stoner 1977, 1985; Wiederholt et al. 1971). In mammals, the late distal tubule, or connecting tubule, and the cortical collecting duct are the principal sites of net potassium secretion, whereas the thick ascending limb of Henle's loop, the diluting segment, is a site of net potassium absorption (F. S. Wright and Giebisch 1985). In amphibians, however, net potassium secretion can occur along the early distal tubule, the diluting segment, as well as along the late distal tubule (Oberleithner et al. 1983a and b; Stoner 1977 and 1985). The specific distal nephron sites of net potassium secretion are not known for other nonmammalian vertebrates.

4.3.2 Mechanism of Transport

4.3.2.1 Proximal Tubules

Micropuncture and microperfusion studies of mammalian (rabbit and rat) and amphibian (bullfrog, *R. catesbeiana*) proximal tubules, involving transepithelial electrical potential measurements and intracellular measurements with ion-sensitive and conventional microelectrodes, indicate that potassium is transported into the cells against an electrochemical gradient at the luminal membrane (Fujimoto et al. 1980; F. S. Wright and Giebisch 1985). For amphibians, this appears to be the primary step in transepithelial potassium absorption (Fig. 4.5). This may also be the case for mammals, in which, however, there also appears to be a passive intercellular absorptive step driven by the positive luminal potential and by net fluid absorption (Fig. 4.5; also F. S. Wright and Giebisch 1985). The relative importance of the active and passive processes in transepithelial absorption is unknown. Nor is it known if a passive step is important for net absorption in amphibians. Nothing further is known about the absorptive mechanism in the proximal tubules of amphibians or other nonmammalian vertebrates.

4.3.2.2 Distal Tubules

Studies of the mechanism of potassium transport along the distal nephrons have been made only for mammals and amphibians. These studies on amphibians include micropuncture and microperfusion of tubules in intact and doubly

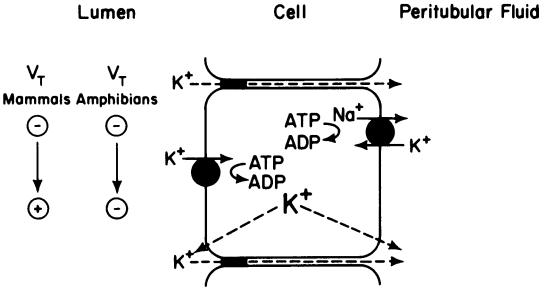


Fig. 4.5. Model for potassium absorption by the proximal tubules based on studies of mammals and amphibians. Symbols have same meanings as in Legend for Fig. 4.1. Note that transepithelial potential difference changes from lumen-negative in the early proximal tubule to lumen-positive in the late proximal tubules in mammals but remains lumen-negative in the proximal tubules of amphibians

perfused kidneys and microperfusion of isolated renal tubules. They involve transepithelial electrical potential measurements and intracellular measurements with ion-sensitive and conventional microelectrodes and kinetic analyses of tracer washout. Similar microelectrode studies have been made for portions of the mammalian distal nephrons. These studies indicate, as noted above, that net absorption or net secretion of potassium can occur in the early distal diluting segment of both anuran and urodele amphibians (Oberleithner et al. 1983c; Stoner 1977 and 1985) whereas only net absorption occurs along the thick ascending limb of Henle's loop in mammals (F. S. Wright and Giebisch 1985). Even in amphibians, net absorption generally occurs in this tubule segment during control conditions. This absorptive process in both the early distal segment of amphibian nephrons and the thick ascending limb of Henle's loop in mammalian nephrons apparently involves entry into the cells across the luminal membrane by the secondary active co-transport system with sodium and chloride discussed above (vide supra; Fig. 4.3; also Oberleithner et al. 1983c; F. S. Wright and Giebisch 1985). Potassium that enters the cells can diffuse back into the lumen and be recycled (Fig. 4.3), or, during net absorption, exit across the peritubular membrane either by a potassium conductive pathway or by the coupled electroneutral pathway with chloride noted above (vide supra; Fig. 4.3). Both pathways for potassium exit across the peritubular membrane may exist in single cells of mammalian thick ascending limbs. However, in the early distal tubules of amphibians (*A. means*) there appear to be two cell types, one with one pathway for potassium exit and the other with the other pathway (Guggino 1986). Even during the process of net absorption, active transport of potassium into the cells, involving ouabain-sensitive Na-K-ATPase, occurs across the peritubular membrane (Fig. 4.3).

Net secretion in this tubule region, and possibly in more distal nephron regions, in amphibians, and possibly in other nonmammalian vertebrates, is produced by adaptation to a high potassium environment or a high potassium intake and by alkalosis or administration of carbonic anhydrase inhibitors (Dantzler 1976a; Oberleithner et al. 1983c; Wiederholt et al. 1971). Net secretion in the early distal tubules of amphibians is influenced by peritubular potassium concentration (Oberleithner et al. 1983c; Wiederholt et al. 1971). Electrophysiological and kinetic studies indicate that in both anurans (*R. catesbeiana*) and urodeles (*A. means*) net secretion results from increased uptake across the peritubular membrane involving the ouabain-sensitive Na-K-ATPase, increased potassium conductance of the luminal membrane, and decreased potassium co-transport into the cells across the luminal membrane (Fig. 4.3; also Oberleithner et al. 1983c; L. P. Sullivan et al. 1977; Wiederholt et al. 1971; Wilkinson et al. 1983). Increased potassium conduction of the luminal membrane appears to be of primary importance in adaptation to a high potassium environment (Oberleithner et al. 1985).

Detailed information on the mechanism of potassium transport, particularly potassium secretion, in the more distal portions of amphibian distal nephrons, or late distal nephrons of any other nonmammalian vertebrates, is not yet available. However, as noted above, structural studies on *A. means* demonstrate that the collecting tubules, like the mammalian cortical collecting ducts, have both prin-

cipal and intercalated cells (Fig. 2.17), which may also be true for the late distal tubules and collecting ducts of reptiles and birds (vide supra; Chap. 2). In mammals, the principal cells are those responsible for net potassium secretion and are the ones most involved in the regulation of potassium excretion (F. S. Wright and Giebisch 1985). The structural studies on *A. means* indicate that the surface density of the basolateral membrane of the principal cells increases with exposure to high environmental potassium just as it does with a high potassium diet in mammals (Stanton et al. 1984a). As in mammals, these morphological changes may be related to high aldosterone levels (vide infra) and may reflect net potassium secretion (Fig. 4.4; also Stanton et al. 1984a).

4.3.3 Hormonal Control

4.3.3.1 Antidiuretic Hormones

There is no evidence that arginine vasopressin has any effect on potassium transport by mammalian renal tubules. Although arginine vasotocin appears to have some effect on potassium transport by renal tubules of nonmammalian vertebrates, its physiological importance is unclear. The administration of apparently physiological doses of the hormone appears to reduce the fraction of filtered potassium excreted by some reptiles (water snakes, *N. sipedon,* but not sand goannas, *V. gouldii*) (Bradshaw and Rice 1981; Dantzler 1967), but not by amphibians (Jard and Morel 1963) or birds (Ames et al. 1971; Bradley et al. 1971). If all filtered potassium is absorbed by reptilian renal tubules and that excreted is derived solely from tubular secretion, then a reduction in fractional excretion suggests that the hormone inhibits secretion. If all filtered potassium is not absorbed, then a reduction in fractional excretion could mean that the hormone stimulates absorption. However, it is not clear that the effective doses of the hormone are truly physiological; no direct studies of hormone action on the potassium transport process have yet been made.

4.3.3.2 Adrenocorticosteroid Hormones

In mammals, aldosterone and similar mineralocorticoids stimulate net potassium secretion apparently by acting primarily on the principal cells of the cortical collecting ducts to enhance transport into the cells across the peritubular membrane, to increase the potassium conductance of the luminal membrane, and, over time, to increase the area of the basolateral membrane, and with it the total Na-K-ATPase activity (F. S. Wright and Giebisch 1985). A considerable amount of information is also now available on the effects of adrenocorticosteroids on potassium transport by renal tubules of amphibians. The plasma level of aldosterone increases with chronic exposure to a high potassium environment at the same time that the potassium conductance of the luminal membrane of the early distal tubule is increased and net potassium secretion by this tubule segment is stimulated (vide supra; also Oberleithner et al. 1983c; Oberleithner et al. 1985). Direct studies involving intracellular as well as transepithelial

recordings with conventional as well as ion-sensitive microelectrodes now indicate that aldosterone (but not glucocorticoids) stimulates hydrogen ion secretion by these cells by enhancing sodium-hydrogen exchange at the luminal membrane (Fig. 4.3) (vide infra; hydrogen ion transport), thereby alkalinizing the interior of the cells, and, at the same time, stimulates potassium secretion (Oberleithner et al. 1987; Weigt et al. 1987). It appears that alkalinization of the cell interior can increase potassium conductance of the luminal membrane and that aldosterone enhances potassium secretion initially in the early distal tubules by stimulating sodium-hydrogen exchange (Oberleithner et al. 1985; Oberleithner et al. 1987). Chronic exposure to a high potassium environment has no effect on the structure of the early distal tubules, but, in urodeles (*A. means*) at least, it increases the surface density of the principal cells of the collecting tubules (vide supra; also Stanton et al. 1984a). There are no direct measurements of potassium transport by the cells of collecting tubules from *A. means*. However, the structural changes suggest, by analogy with those in mammalian cells, that net potassium secretion stimulated by aldosterone may occur here, and that the hormone action, in contrast to its initial effect in the early distal tubules, involves primarily an increase in the activity of the Na-K-ATPase at the basolateral membrane.

The effects of adrenocorticoids on potassium transport by the tubules of other nonmammalian vertebrates are far from clear. There is no evidence of any effect in fishes. Aldosterone and corticosterone apparently increase the fractional excretion of potassium in birds (Holmes 1972), but the mechanism involved in this effect is unknown. The effects of these hormones in reptiles are highly variable. They appear to reduce fractional potassium excretion in sodium-chloride-loaded water snakes (*N. cyclopion*) (LeBrie and Elizondo 1969), to increase fractional excretion in freshwater turtles (*C. picta* and *Pelomedusa subrufa*) (Brewer and Ensor 1980), and to stimulate net potassium secretion in lizards (*V. gouldii*) (Rice et al. 1982). The mechanisms involved in these variable responses are unknown.

4.3.3.3 Prolactin

The administration of this hormone to one species of freshwater turtle (*P. subrufa*) increases potassium excretion (Brewer and Ensor 1980), but whether this results from an increase in GFR, a decrease in tubular absorption, or an increase in tubular secretion is unknown. Nor is it clear that this response is even of physiological significance. No data on the effects of prolactin on potassium excretion are available for other nonmammalian vertebrates or for mammals.

4.4 Hydrogen Ion

4.4.1 Magnitude and Sites of Net Transport

Very little is known about hydrogen ion transport or even about the role of the kidneys in the regulation of acid-base balance in nonmammalian vertebrates. In

many, extrarenal structures, e.g., gills in teleost fishes, bladder in chelonian reptiles, are more important than the renal tubules for the elimination of hydrogen ions and have been subject to much more intensive study than the kidneys in this regard. The tubular transport of hydrogen ions may be related to the excretion of the end products of nitrogen metabolism (vide infra; Chap. 6) and to the function of these extrarenal routes for ion excretion or for the modification of ion excretion.

Ureteral urine of elasmobranchs, at least of little skate, *R. erinacea* and spiny dogfish, *S. acanthias,* is normally always acid with a fixed pH of about 5.8 (Smith 1939) and that of alligators (*Alligator mississippiensis*) is normally alkaline (Coulson and Hernandez 1964) regardless of dietary intake, whereas that of other reptiles, as well as that of amphibians, birds, and mammals normally can vary from alkaline to acid depending on dietary intake. Moreover, ureteral urine of mammals, birds, and amphibians can be greatly acidified (pH about 4.5 with blood pH about 7.4) during an acid load, whereas that of some reptiles (water snakes, *N. sipedon*) can only be acidified to a modest extent (pH 5.8 even with blood pH about 7.0) (Dantzler 1968, 1976a; Koeppen et al. 1985). These data suggest that the renal tubules of some reptiles may not be able to secrete hydrogen ions against a steep gradient and that significant acidification may occur in regions distal to the kidneys, e.g., bladder or cloaca. As noted above, hydrogen ion secretion by the turtle bladder has been well documented and is the subject of continuing study (Steinmetz 1967, 1974).

In elasmobranchs (e.g., *R. erinacea and S. acanthias*), micropuncture studies involving the injection of buffer dyes indicate that acidification occurs in the earliest portion of the proximal tubule and can go to completion at any point along the proximal tubule (Deetjen and Maren 1974; Kempton 1940). The rate of net acid secretion by the proximal tubules of *R. erinacea* amounts to about 40 μmol liter (of cells)$^{-1}$ min^{-1} (Deetjen and Maren 1974), a rate that compares favorably with that determined for intact, free-swimming *S. acanthias* (Hodler et al. 1955). Moreover, the acid secretory process is nearly inexhaustible (Swenson and Maren 1986).

In contrast, micropuncture studies on both anuran (*R. pipiens; R. esculenta*) and urodele (*N. maculosus*) amphibians indicate that the pH of the tubular fluid only falls below that of the blood along the distal tubules, beginning with the early distal diluting segment (Giebisch 1956; Montgomery and Pierce 1937; Oberleithner et al. 1984; O'Regan et al. 1982). These observations also indicate that the bicarbonate concentration does not change along the proximal tubules of amphibians. In birds, the micropuncture measurements on the proximal tubules of superficial reptilian-type nephrons indicating that sodium and chloride are absorbed at equivalent rates (vide supra; Laverty and Dantzler 1982) suggest that the bicarbonate concentration does not fall and that the tubular fluid is not acidified along this region of the avian nephron. Lack of acidification along the proximal tubules has recently been confirmed and the presence of acidification at the level of the superficial cortical collecting ducts has been demonstrated by direct micropuncture measurements of pH along these nephron segments (Laverty and Alberici 1987). These observations on amphibians and birds differ from those on mammals that show significant acidification along the proximal tubules

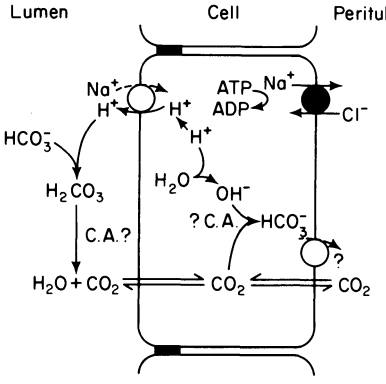


Fig. 4.6. Model for hydrogen ion secretion and bicarbonate absorption by the proximal tubules based on studies of mammals and amphibians. *C.A.* indicates carbonic anhydrase. Other symbols have same meanings as in legend for Fig. 4.1. *Question marks* indicate uncertainty about role of carbonic anhydrase in amphibians and about exit step for bicarbonate across peritubular membrane in both mammals and amphibians

(Koeppen et al. 1985). Although acidification does occur along the proximal tubules of elasmobranchs, the process involved appears to be quite different from that in mammals (vide infra).

4.4.2 Mechanism of Transport

4.4.2.1 Proximal Tubules

As in the case of sodium and chloride transport, detailed information bearing on the mechanism of hydrogen ion transport by proximal renal tubules of nonmammalian vertebrates is only available for urodele amphibians. These studies involve intracellular as well as transepithelial pH measurements, generally on doubly perfused kidneys of *N. maculosus* and *A. means* (Boron and Boulpaep 1983a, 1983b; O'Regan et al. 1982), and transport measurements with brush-border membrane vesicles from kidneys of *N. maculosus* (Seifter and Aronson 1984). The data indicate that the hydrogen ion concentration within the cells is maintained below electrochemical equilibrium apparently primarily by extrusion across the luminal membrane by the same coupled sodium-hydrogen exchange process discussed above (Figs. 4.1 and 4.6). The energy for this hydrogen extrusion is derived from the movement of sodium into the cells down its electrochemical gradient (Fig. 4.1). Extrusion of hydrogen ions across the basolateral membrane also may occur, although the mechanism of such extrusion is unknown (Boron and Boulpaep 1983a, b).

Although the fluid in the lumen of the proximal tubules is not acidified under control conditions, a reduction in luminal pH does occur when the lumen is perfused with high concentrations of bicarbonate (O'Regan et al. 1982). However, at steady-state no significant bicarbonate or pH gradient can be established across the epithelium (O'Regan et al. 1982). The rate of bicarbonate absorption in these experiments cannot be explained by passive movement (O'Regan et al. 1982). These observations suggest that, in view of hydrogen ion secretion across the luminal membrane, most bicarbonate absorption is driven by hydrogen ion secretion. Apparently, as in the mammalian kidney, such absorption involves conversion of bicarbonate to carbon dioxide and water in the tubule lumen and then reconversion to bicarbonate within the renal tubule cells (Fig. 4.6). Carbonic anhydrase is usually presumed to play a role in this process within renal tubule cells (Fig. 4.6). However, the presence of carbonic anhydrase

in amphibian proximal tubules is not well documented. Although histochemical evidence is conflicting, it suggests that, if the enzyme is present, there is much less than in the distal nephrons (vide infra; also Lonnerholm and Ridderstrale 1974; Ridderstrale 1976; Rosen 1972). In any case, under control, free-flow conditions, the lack of any significant transepithelial bicarbonate or pH gradient along the proximal tubules indicates that net bicarbonate absorption is proportional to net fluid absorption.

Although the exact mechanism of acidification along the proximal tubules of elasmobranchs is unknown, preliminary data indicate that it is very different from that in proximal tubules of mammals. First, these animals have no renal carbonic anhydrase (Maren 1967). Second, in the isolated perfused kidneys of *R. erinacea,* transepithelial voltage and pH measurements with microelectrodes in the presence and absence of inhibitors on the peritubular side suggest that both the coupled sodium-chloride-potassium co-transport system discussed above (Fig. 4.2) and a chloride-bicarbonate exchange system play a role in the acidification process (Silbernagl et al. 1986).

No details of cellular hydrogen ion or bicarbonate transport are known for the proximal renal tubules of other nonmammalian vertebrates, but the observation that the administration of carbonic anhydrase inhibitors to water snakes (*N. sipedon*) results in an alkaline urine, an increased excretion of sodium and potassium, and an unchanged excretion of chloride suggest that the mechanisms in these reptiles may be similar to those in amphibians and mammals (Dantzler 1968). However, one group of reptiles, the crocodilians, are clearly different (Coulson and Hernandez 1964, 1983; Lemieux et al. 1985). The kidneys of alligators (*Alligator mississippiensis*) normally produce an alkaline urine containing bicarbonate and ammonia, despite a high protein, acid-ash diet (Coulson and Hernandez 1964, 1983). Net tubular secretion of bicarbonate occurs and the blood pH is low (around 7.1- 7.2) compared to that of other nonmammalian vertebrates or mammals at co ıparable temperatures (Coulson and Hernandez 1983; Lemieux et al. 1985). In ıese animals, the administration of carbonic anhydrase inhibitors results in a mil ly acid urine and the conversion of net secretion to net absorption of bicarbona : (Coulson and Hernandez 1964; Lemieux et al. 1985). It appears that carb ıic anhydrase plays a role in bicarbonate production by renal tubule cells nd that hydrogen ions may be extruded from the cells into the blood as bicar ɔnate is secreted into the lumen (Lemieux et al. 1985). However, nothing more s really known about the tubule sites or mechanisms involved.

4.4.2.2 Distal Tubules

Specific information on the hydrogen ion secretory process in the distal tubules of nonmammalian vertebrates is available only for the early distal diluting segment of anuran amphibians (*R. esculenta* and *R. pipiens*). Studies on this process have involved transepithelial and intracellular recordings with conventional and ion-sensitive microelectrodes in isolated, doubly perfused frog kidneys (Oberleithner et al. 1984; Oberleithner et al. 1987; Weigt et al. 1987) and intracellular recordings on fused "giant cells" from frog diluting segments

(Oberleithner et al. 1987). These studies all indicate that hydrogen ion secretion involves an amiloride-sensitive secondary active sodium-hydrogen exchange process at the luminal membrane (Fig. 4.3). This process is similar to that described above for the proximal tubules. The energy for the hydrogen extrusion from the cells into the lumen is derived from the movement of sodium down its electrochemical gradient from the lumen into the cells. This electrochemical gradient for sodium is maintained, in turn, by the primary, energy-consuming sodium-potassium pump at the peritubular membrane.

Activity of the sodium-hydrogen exchanger appears to depend on the metabolic state of the animal (Oberleithner 1985). Adaptation to a high potassium environment stimulates sodium-hydrogen exchange, thereby raising the intracellular pH and lowering the luminal pH, whereas adaptation to a high sodium environment suppresses sodium-hydrogen exchange, thereby leaving hydrogen ions distributed at electrochemical equilibrium (Oberleithner 1985; Oberleithner et al. 1984). As noted above, aldosterone secretion is stimulated by adaptation to a high potassium environment and suppressed by adaptation to a high sodium environment, suggesting that this hormone may be responsible for the observed changes in hydrogen ion secretion (Oberleithner et al. 1983c). Direct evaluation of the effects of aldosterone indicate that it does, indeed, stimulate the sodium-hydrogen exchange and that such stimulation can be inhibited by spironolactone (Oberleithner et al. 1987; Weigt et al. 1987). In fact, aldosterone appears to be required for activation of the exchange process (Fig. 4.3; also Weigt et al. 1987). These data all support the concept that this hormone is responsible for the enhanced hydrogen ion secretion observed with adaptation to a high potassium environment.

In isolated, perfused late distal tubules of urodele amphibians (*A. means*) direct pH measurements indicate that there is significant electrically silent hydrogen ion secretion that is inhibited by acetazolamide and luminal amiloride (Stanton et al. 1984b). These data suggest that the enzyme carbonic anhydrase is important for the intracellular generation of hydrogen ions, an idea supported by histochemical evidence that significant amounts of carbonic anhydrase are found in amphibian distal nephrons (Lonnerholm and Ridderstrale 1974). The data also suggest that hydrogen ions are secreted into the lumen by a sodium-hydrogen exchange mechanism like that discussed above for the urodele proximal tubule and the anuran diluting segment (Fig. 4.4).

Virtually nothing is known about possible hydrogen ion secretory processes in the distal tubules of other nonmammalian vertebrates. Because a primary electrogenic hydrogen ion secretory pump, similar in nature to that in the cells of the turtle bladder, apparently exists in the luminal membranes of the intercalated cells of mammalian connecting tubules and cortical collecting ducts (Koeppen et al. 1985), it seems likely that such a hydrogen ion secretory system exists in the cells of the late distal nephrons or perhaps collecting tubules or initial collecting ducts of other nonmammalain vertebrates (Fig. 4.4). However, as pointed out above, the existence of such a system in the bladder of the freshwater turtle (*P. scripta*) also suggests that postrenal structures (bladder or cloaca) may be more important than the renal tubules in regulating acid excretion in many nonmammalian vertebrates.

4.5 Calcium

4.5.1 Direction, Magnitude, and Sites of Net Transport

Clearance studies indicate that overall net tubular secretion of calcium occurs in marine teleosts and euryhaline teleosts adapted to seawater (Hickman and Trump 1969). It has also been observed in one marine elasmobranch species (little skate, *R. erinacea*) (Stolte et al. 1977a) whereas overall net tubular absorption of calcium has been observed in the only other marine elasmobranch species studied (dogfish shark, *S. acanthias*) (Hickman and Trump 1969). Whether or not net secretion always occurs in skates and net absorption always occurs in dogfish sharks is unknown. However, it would seem advantageous for marine species to eliminate excess calcium. Micropuncture studies on the little skate indicate that the net secretion in this species occurs primarily along the second portion of the proximal tubule (Stolte et al. 1977a).

In all other nonmammalian vertebrates studied and in mammals, clearance studies indicate that overall net tubular absorption of filtered calcium occurs (Agus and Goldfarb 1985; Clark and Dantzler 1972, 1975; Dantzler 1976a; Hickman and Trump 1969; Laverty and Clark 1981). In most of these species, the fraction of filtered calcium excreted in the ureteral urine is about equal to the fraction of filtered sodium excreted. Thus, the excretion of calcium is quite low.

Micropuncture and microperfusion studies on mammals indicate that about 60% of the filtered calcium is absorbed along the proximal tubules, about 30% along the loops of Henle, and most of the remainder along the distal tubules (Agus and Goldfarb 1985). Similar studies on urodele amphibians (*N. maculosus*) indicate that all significant net absorption occurs along the distal tubules, not along the proximal tubules (Garland et al. 1975). A complete description of tubular transport sites is not available for other nonmammalian vertebrates. However, micropuncture studies in birds (starlings, *S. vulgaris*) (Laverty and Dantzler 1982) do show that, as in mammals, net absorption can occur along the proximal tubules of the superficial, reptilian-type nephrons. But in contrast to mammals, the rate of absorption of calcium along these avian proximal tubules exceeds the rate of absorption of sodium and water. In some of these birds, however, for reasons that are not yet clear, net secretion of calcium occurs along the proximal tubules of the superficial, reptilian-type nephrons (J. S. Roberts and W. H. Dantzler unpublished observations). Because almost all filtered calcium, even in these particular birds, is still absorbed before the final urine is reached (J. S. Roberts and W. H. Dantzler unpublished observations), substantial net absorption must take place in the distal portions of these nephrons and in the collecting ducts as well as in the mammalian-type nephrons. However, as noted above, no information is available about other possible absorptive sites for calcium in the renal tubules of birds or other nonmammalain vertebrates.

4.5.2 Mechanism of Transport

Very little is known about the mechanisms of tubular calcium transport in mammalian or nonmammalian vertebrates. The most detailed information available involves the net secretory process in teleosts. This process has long been known to respond directly to the concentration of calcium in the peritubular fluid, increasing with an increase in concentration and decreasing with a decrease in concentration (Hickman and Trump 1969). Transport of calcium across the peritubular surface into the cells of proximal renal tubules isolated from the kidneys of seawater-acclimated winter flounder (*P. americanus*) involves a fast and a slow component (Renfro 1978). These studies and others with isolated tubules and plasma membrane vesicles indicate that the slow component, which may be more important for net calcium secretion, is saturable, ATP-dependent, sodium-dependent (but apparently not directly linked to the sodium gradient) and partially inhibited by magnesium (Renfro 1978; Renfro et al. 1982).

The net absorption along the proximal tubules of mammals apparently involves mainly passive movement between the cells because the concentration in the tubule fluid is always greater than that in the ultrafiltrate of the plasma, but some active transcellular absorption may also occur (Agus and Goldfarb 1985). Net absorption along the proximal tubules of the superficial, reptilian-type nephrons of birds, when it occurs, probably does not involve only passive paracellular movement because the concentration in the tubule fluid is below that in the ultrafiltrate of plasma (Laverty and Dantzler 1982) and the transepithelial electrical potential difference appears to be too small (Table 4.2) to account for simple passive movement. However, this process has not been rigorously evaluated. Some active transcellular absorption of calcium must also occur along the distal nephrons of mammals and all those nonmammalian vertebrates that show overall net tubular absorption (Agus and Goldfarb 1985; Clark and Dantzler 1972, 1975; Dantzler 1976a; Hickman and Trump 1969; Laverty and Clark 1981). However, there is considerable disagreement about the presence of active or passive absorption along the thick ascending limbs of Henle's loops in mammals. Moreover, recent measurements with conventional and ion-sensitive microelectrodes on the diluting segments in nephrons of the isolated, perfused kidney of the frog (*Rana pipiens*) suggest that all absorption is passive via the paracellular shunt pathway and is driven by the lumen-positive transepithelial voltage (Dietl and Oberleithner 1987).

The mechanism involved in any active net transcellular absorptive process is not understood. The very low intracellular calcium activity (about 10^{-7} M) in amphibian and mammalian proximal tubules, and presumably in all tubule cells of mammalian and nonmammalian vertebrates (C. O. Lee et al. 1980; Murphy and Mandel 1982), indicates that calcium can enter the tubule cells from the lumen down an electrochemical gradient but must be transported out of the cells on the peritubular side against an electrochemical gradient. Transport of calcium out of the cells across the peritubular membrane can involve countertransport for sodium that enters the cells across this membrane down its electrochemical gradient (Taylor and Windhager 1979). However, it appears doubtful that this process could account for significant net transepithelial absorption. It appears

more likely that a primary active transport step for calcium out of the cells across the peritubular membrane provides the driving force for net transcellular transport. Recent data on mammals suggests that this may involve a magnesium-independent calcium ATPase (Tsukamoto et al. 1986), although a calcium, magnesium ATPase also may be involved (Doucet and Katz 1982).

4.5.3 Hormonal Control

4.5.3.1 Parathyroid Hormone

In mammals and in most birds studied (starling, *S. vulgaris;* Japanese quail, *C. coturnix;* domestic chicken, *G. gallus*), parathyroid hormone (PTH) appears to be required for normal absorption of calcium by the renal tubules because parathyroidectomy markedly reduces absorption and the administration of PTH to parathyroidectomized animals restores absorption to control levels (Agus and Goldfarb 1985; Clark and Wideman 1977; Clark and Sasayama 1981; Mok 1978). In mammals, at least in rabbits, most physiological stimulation of calcium absorption appears to occur in the distal convoluted tubules and collecting ducts (Agus and Goldfarb 1985), although pharmacological and possibly physiological stimulation can also occur in the thick ascending limb of Henle's loop (Bourdeau and Burg 1980).

Preliminary micropuncture studies on starlings indicate that PTH does not stimulate calcium absorption along the proximal tubules of the superficial reptilian-type nephrons (G. Laverty and W. H. Dantzler unpublished observations). Parathyroid hormone may, of course, stimulate calcium absorption along the proximal tubules of the deeper mammalian-type nephrons. However, in view of the lack of significant effect on the proximal tubules of mammals, any proximal effect seems unlikely. The stimulatory effect is more likely to occur in the more distal regions of the nephrons. In fact, because the response of the whole kidney in starlings is similar to that in mammals and because PTH can stimulate calcium absorption by the thick ascending limb of Henle's loops in mammals, the whole-kidney response in these birds may reflect the presence of thick ascending limbs of Henle's loops in the mammalian-type nephrons. In this regard, it is particularly noteworthy that PTH does not appear to stimulate calcium absorption in reptiles, where the nephrons lack loops of Henle. Neither parathyroidectomy nor the administration of PTH to intact or parathyroidectomized snakes (*Nerodia* spp.) or turtles (*Chrysemys* spp.) has any observable effect on absorption of calcium by the renal tubules (Clark and Dantzler 1972; Laverty and Clark 1981). However, it should also be noted that in two species of birds (herring gull, *Larus argentatus* and great black-backed gull, *L. marinus*), which, of course, do have mammalian-type nephrons with loops of Henle, neither parathyroidectomy nor the administration of PTH to intact or parathyroidectomized animals appears to affect net tubular absorption of calcium (Clark and Mok 1986). These differences among species of birds do not appear to be the result only of differences in age or sex and, thus, of differences in the reproductive state and in the requirement for calcium for egg shell formation (Clark and Mok 1986). They also

do not appear to be the result of differences in the maturity of the calcium transport system (Clark and Mok 1986). No information is available on the effects of PTH on the transport of calcium by the renal tubules of other nonmammalian vertebrates.

4.5.3.2 Calcitonin

Although calcitonin may stimulate calcium absorption by the thick ascending limb of mammalian nephrons, the effects are far from clear and their physiological meaning is uncertain (Agus and Goldfarb 1985). Among the nonmammalian vertebrates, the administration of calcitonin to intact or parathyroidectomized snakes (*Nerodia* spp.) has no effect on calcium transport by the renal tubules (Clark and Dantzler 1975). Also, there is no evidence to date of an effect of calcitonin on calcium transport by the renal tubules of any other nonmammalian vertebrates. Finally, although the corpuscles of Stannius have a role in regulating calcium metabolism in teleost fishes, no specific role in regulating calcium transport by the renal tubules has yet been demonstrated.

4.6 Phosphate

4.6.1 Direction, Magnitude, and Sites of Net Transport

Clearance and micropuncture studies have revealed both net absorption and net secretion of phosphate by the renal tubules of birds, reptiles, and fishes (Clark et al. 1976; Clark and Dantzler 1972; Hickman and Trump 1969; Knox et al. 1973; Schneider et al. 1980; Laverty and Dantzler 1982; Levinsky and Davidson 1957). Among amphibians, net tubular absorption definitely occurs and net tubular secretion also may occur, but the data are not convincing (Walker and Hudson 1937c; Schneider et al. 1980). Among mammals, however, only net tubular absorption has been documented (Knox and Haramati 1985; Mizgala and Quamme 1985; Schneider et al. 1980). In fact, micropuncture experiments suggest that a tubular secretory process is not involved at all in the excretion of phosphate by the mammalian kidney (Greger et al. 1977).

Among fishes, hagfishes, lampreys, marine elasmobranchs, and stenohaline marine teleosts exhibit net tubular secretion (Hickman and Trump 1969; Stolte et al. 1977a; Cliff et al 1986). Net tubular absorption always appears to occur among the true stenohaline freshwater teleosts (Hickman and Trump 1969). In general, euryhaline teleosts exhibit net absorption when adapted to freshwater and net secretion when adapted to seawater (Hickman and Trump 1969), but the mechanism involved in the change in direction of transport is unknown. Moreover, net tubular secretion has been observed in euryhaline eels (*A. anguilla*) adapted to freshwater (Chester Jones et al. 1969).

Among reptiles, clearance studies normally reveal net tubular secretion requiring the presence of parathyroid hormone in water snakes (*Nerodia* spp.)

and freshwater turtles (*C. picta* and *C. picta elegans*) (vide infra; also Clark and Dantzler 1972; Laverty and Clark 1981). This also may be the case for alligators (*A. mississippiensis*) (Dantzler 1976a). Under control conditions in water snakes, the rate of excretion of phosphate can actually be 2.5 times the rate of filtration, a relative rate of net tubular secretion higher than that found in any other vertebrate species (Clark and Dantzler 1972).

Among birds, clearance studies normally reveal net tubular absorption, but this changes to net tubular secretion with phosphate loading or the administration of parathyroid hormone (Clark et al. 1976; Levinsky and Davidson 1957). Moreover, as among the reptiles, net tubular secretion always requires the presence of parathyroid hormone (vide infra).

Descriptions of the tubular sites of net transport are not truly complete for any vertebrates. Among the mammals, about 80% of the filtered phosphate is absorbed by the renal tubules, and micropuncture and microperfusion studies suggest that 90% (if not all) of this absorption occurs along the proximal tubules (Knox and Haramati 1985). The degree of net absorption, if any, along the distal convoluted tubules and collecting ducts is not yet resolved (Knox and Haramati 1985; Mizgala and Quamme 1985). Micropuncture measurements on a marine elasmobranch species (little skate, *R. erinacea*) indicate that the major site of secretion is the second segment of the proximal tubule (Stolte et al. 1977a). In vitro microperfusion of tubules from a marine teleost species (winter flounder, *P. americanus*) suggests that net secretion also occurs in the second segment of the proximal tubule in these animals (Cliff et al. 1986). Nothing further is known about the sites of transport in fishes. Although micropuncture and microperfusion studies indicate that net absorption of phosphate can occur along the proximal tubules of amphibians and reptiles, they do not clearly demonstrate the site of marked net secretion in reptiles (Walker and Hudson 1937c; Dantzler 1985). Micropuncture studies on starlings indicate that in intact control animals either net absorption or net secretion can be observed along the proximal tubules of the superficial reptilian-type nephrons (Laverty and Dantzler 1982). It is not yet clear whether this variation in direction of net transport is time-dependent or indicates simultaneous differences among the individual nephrons in the transport of phosphate. Nothing more is known about the site of transport in birds or in any other vertebrates.

4.6.2 Mechanism of Transport

The cellular mechanism involved in phosphate absorption by mammalian renal tubules has been and still is the subject of intensive investigation, whereas little research effort has been expended on the mechanism of phosphate transport by the renal tubules of nonmammalian vertebrates. This latter situation is particularly unfortunate because net secretion under the control of parathyroid hormone is found only among the nonmammalian vertebrates. The studies on the mammalian absorptive mechanism have been reviewed extensively elsewhere (see, for example, Mizgala and Quamme 1985) and are beyond the scope of this volume. Briefly, the net absorptive process by the mammalian

proximal tubule is a saturable one involving transport into cells across the luminal membrane against an electrochemical gradient by a sodium-dependent secondary active transport process. Phosphate then moves out of the cells across the peritubular membrane down an electrochemical gradient by a carrier-mediated process.

Although much less work has been done on the mechanism of phosphate transport in nonmammalian vertebrates, one recent study with isolated brush-border membrane vesicles from chick kidneys reveals a sodium-dependent transport step for phosphate similar to that found with brush-border membrane vesicles from mammalian kidneys (Renfro and Clark 1984). This transport step is assumed to be involved in the absorptive flux of phosphate. No similar membrane studies are available concerning the secretory flux of phosphate in nonmammalian vertebrates. However, Wideman and Braun (1981), taking advantage of the avian renal portal system to deliver phosphate-containing compounds to the renal tubules without having them first pass through the glomerular circulation, demonstrated that when net phosphate secretion occurs, the transepithelial peritubular-to-lumen flux of inorganic phosphate represents only a minor component of the net tubular secretion. The exact source of the phosphate secreted in these circumstances has not been identified.

4.6.3 Control by Parathyroid Hormone

In mammals, PTH apparently regulates phosphate excretion by inhibiting the phosphate absorptive process primarily in the proximal tubule, but the hormone apparently can act in any segment in which phosphate absorption occurs (Mizgala and Quamme 1985). In birds and reptiles, as pointed out above, net tubular secretion of phosphate appears to require the presence of parathyroid hormone. However, whether the hormone solely inhibits an absorptive flux, directly stimulates a secretory flux, or does both in these animals is not yet known. In starlings (*S. vulgaris*), net secretion is converted to net absorption by parathyroidectomy (Clark and Wideman 1977; Wideman et al. 1980). Net secretion can be restored by the administration of PTH with or without phosphate loading but not by phosphate loading alone (Clark and Wideman 1977; Wideman et al. 1980). Similarly, in both freshwater snakes (*Nerodia* spp.) and freshwater turtles (*C. picta* and *C. picta elegans*), net secretion of phosphate changes to net absorption following parathyroidectomy and is restored by the administration of PTH (Clark and Dantzler 1972; Laverty and Clark 1981). Moreover, the marked net tubular secretion in intact water snakes noted above can be greatly enhanced by the administration of PTH (Clark and Dantzler 1972).

As already noted, the presence of PTH-dependent net tubular secretion of phosphate in birds and reptiles offers an opportunity to examine both the site and underlying mechanism involved. With regard to the site of this effect, preliminary micropuncture measurements on starlings indicate that in birds with intact parathyroid glands the administration of exogenous PTH can induce net phosphate secretion by the proximal tubules of all superficial, reptilian-type nephrons (Laverty and Dantzler 1983a). Moreover, if the change from net secre-

tion to net absorption and vice versa in these superficial nephrons in the absence of exogenous PTH is time-dependent (vide supra), it may reflect fluctuations in the rate of secretion of endogenous PTH. If, instead, simultaneous differences in the direction of net transport occur among these nephrons (vide supra), it would raise intriguing questions about the distribution of endogenous hormone or the number and sensitivity of hormone receptors. Nothing is yet known about transport in other segments of the superficial reptilian-type nephrons or in any segment of the mammalian-type nephrons. The site or sites of hormonal effects on phosphate transport by the renal tubules of reptiles are not yet fully known. However, preliminary studies with isolated, perfused proximal renal tubules from garter snakes (*Thamnophis* spp.) indicate that PTH can partially inhibit net absorption in these segments but cannot induce net secretion (Dantzler 1985).

For both net secretion and net absorption to occur in a given nephron, both a unidirectional secretory flux and a unidirectional absorptive flux must exist. As already noted above, in birds and reptiles, PTH could inhibit the absorptive flux only, as in mammals, stimulate the secretory flux, or do both. Since the rate of PTH-stimulated net secretion of phosphate can be two or more times the rate of filtration of phosphate (Clark and Dantzler 1972; Laverty and Dantzler 1983a), inhibition of absorption alone by the hormone would mean that very large unidirectional secretory and absorptive fluxes must always exist. Although this appears energetically wasteful, a precedent for the presence of relatively large unidirectional transepithelial fluxes in the same tubule segment exists in regard to the transport of some organic cations by reptilian renal tubules (vide infra; Dantzler and Brokl 1986).

Some data do exist on the possible cellular mechanism of the PTH effect on phosphate transport in the avian kidney, but they shed little light on the regulation of the secretory process. Sodium-dependent phosphate transport is significantly stimulated in brush-border membrane vesicles from parathyroidectomized chicks and significantly inhibited in these vesicles from intact or parathyroidectomized chicks given exogenous PTH (Renfro and Clark 1984). These findings, which are similar to those for mammals (Mizgala and Quamme 1985), apparently reflect hormone action on the absorptive transport step. No direct studies of a possible secretory step at the basolateral membrane or of hormone action on such a step have yet been made. However, specific receptors for PTH have been demonstrated in plasma membranes from the superficial regions of the chicken kidney (Nissenson et al. 1981). PTH also stimulates adenylate cyclase in this superficial avian renal tissue, this stimulation being modulated by guanidyl nucleotides (Nissenson et al. 1981). More detailed studies with precise localization of the hormone action are required to explain the mechanism by which this hormone induces or enhances net tubular secretion of phosphate in birds and reptiles.

4.7 Magnesium

4.7.1 Direction, Magnitude, and Sites of Net Transport

Clearance studies reveal net absorption of magnesium by renal tubules of mammals and most nonmammalian tetrapods and net secretion by the renal tubules of all marine fishes. However, in some marine reptiles (e.g., sea snakes, *A. laevis*) either net secretion or net absorption by the renal tubules can occur (Benyajati et al. 1985). Net secretion of magnesium in these marine reptiles, as in marine teleosts, may play an important role in the elimination of magnesium ingested in seawater, particularly at low glomerular filtration rates.

Although net tubular secretion of magnesium occurs in all marine fishes examined (hagfishes, elasmobranchs, and teleosts), it is particularly striking among the pauciglomerular and aglomerular teleosts (Hickman and Trump 1969; Stolte et al. 1977 a). Even among glomerular marine teleosts, almost all excreted magnesium derives from tubular secretion (Renfro 1980). However, net secretion ceases or is markedly reduced with adaptation of euryhaline teleosts to freshwater (Hickman and Trump 1969; Schmidt-Nielsen and Renfro 1975). When need to excrete magnesium exceeds need to excrete sulfate (vide infra), a relatively large fraction of filtered chloride also appears in the urine (Hickman and Trump 1969). In vivo micropuncture experiments on elasmobranchs (little skate, *R. erinacea*) (Stolte et al. 1977 a) and in vitro tubule-perfusion experiments on marine teleosts (flounder, *P. americanus*) (Beyenbach 1982; Beyenbach et al. 1986; Cliff et al. 1986) suggest that the primary site of magnesium secretion is the second segment of the proximal tubule.

As noted above, clearance studies on sea snakes (*A. laevis*) reveal net secretion or net absorption of magnesium. The observation that net secretion of magnesium is most significant at low filtered loads, when almost all of the filtered magnesium should be absorbed, suggests that magnesium is absorbed at a proximal site by a transport system with a high capacity and then secreted at a more distal point (Benyajati et al. 1985). If magnesium were secreted at a relatively proximal location, most secreted magnesium should be absorbed distally and net secretion of magnesium should not be apparent with a low filtered load. However, these possible sites of magnesium transport need to be examined by more direct methods.

Although the amount of magnesium excreted by these sea snakes at low urine flow rates may exceed the filtered load by an order of magnitude, the estimated rate of tubular secretion, if it is assumed that all excreted magnesium is secreted, is much lower than that for marine teleosts (1 mmol kg^{-1} h^{-1} in sea snakes versus 17 mmol kg^{-1} h^{-1} in American eel, *A. rostrata*) (Benyajati et al. 1985; Schmidt-Nielsen and Renfro 1975). In addition, the highest magnesium concentration observed in the urine of these sea snakes is about 30 mmol l^{-1}, whereas that in the urine of some marine teleosts can be as high as 130 mmol l^{-1} (Benyajati et al. 1985; Foster 1975). Magnesium excretion has not yet been studied in other reptiles or in amphibians.

Although clearance studies on birds reveal only net tubular absorption of magnesium (Laverty and Dantzler 1982; Robinson and Portwood 1962), micropuncture measurements on starlings (*S. vulgaris*), made simultaneously with clearance measurements, indicate that both net absorption and net secretion can occur along the proximal tubules of the superficial, reptilian-type nephrons (Laverty and Dantzler 1982). The data suggest that net secretion occurs in the early portion of the tubules and net absorption in the late portion. If this is the case, then net absorption along the late proximal tubules or distal segments of these nephrons must more than compensate for the early proximal secretion. In addition, as in mammals (vide infra), absorption of magnesium in the loops of Henle of the mammalian-type nephrons may contribute significantly to the conservation of filtered magnesium by the whole kidney. However, no direct measurements of magnesium transport by these nephrons have yet been made.

In mammals, micropuncture and microperfusion studies indicate that only 20–30% of the filtered magnesium is absorbed along the proximal tubules, the bulk, about 65%, being absorbed along the loops of Henle, particularly along the thick ascending limb (Quamme and Dirks 1985). Very little additional absorption occurs along the distal tubules and collecting ducts, and about 5–10% of the magnesium filtered appears in the final urine (Quamme and Dirks 1985).

4.7.2 Mechanism of Transport

Little information is available, even for mammals, on the mechanism of magnesium absorption by renal tubules. What is available for mammals is reviewed in detail elsewhere (Quamme and Dirks 1985). Briefly, current data suggest that magnesium absorption in the thick ascending limb of Henle's loop, the major site of magnesium absorption, is voltage-dependent and secondary to active absorption of sodium chloride. Magnesium may enter the cells across the luminal membrane by a carrier-mediated mechanism down its electrical gradient. It would then have to be transported out of the cells across the peritubular membrane against an electrochemical gradient by a primary or secondary active transport process. A secondary active transport process may involve sodium-magnesium exchange (Quamme and Dirks 1985).

Even less information is available on the mechanism of magnesium transport by the renal tubules of nonmammalian vertebrates. However, recent kinetic studies with isolated, perfused tubules of the flounder (*P. americanus*) suggest that the transepithelial secretion of magnesium involves a high affinity, low capacity system (Cliff et al. 1986). As noted above, magnesium tends to inhibit the slow component of calcium uptake across the peritubular membrane of isolated, nonperfused flounder tubules (Renfro 1978), and it may be transported by a similar mechanism. However, nothing more is known about the cellular transport processes. No information is available on local or systemic control of magnesium transport in nonmammalian vertebrates, but PTH, arginine vasopressin, calcitonin, and glucagon all may enhance magnesium absorption by the thick ascending limb of Henle's loop in mammals (Greger 1985).

4.8 Sulfate

4.8.1 Direction, Magnitude, and Sites of Net Transport

Clearance studies reveal net absorption of filtered sulfate by the renal tubules of mammals, birds, and amphibians (Mudge et al. 1973). This is probably the case for all tetrapods. However, as in the case of magnesium (vide supra), net tubular secretion of sulfate occurs in all marine fishes (hagfishes, elasmobranchs, and teleosts) examined (Hickman and Trump 1969; Stolte et al. 1977a). Such net secretion is most obvious in the pauciglomerular or aglomerular marine teleosts (Hickman and Trump 1969), but even in the glomerular marine teleosts almost all the excreted sulfate derives from secretion by the renal tubules (Renfro 1980). Again, as in the case of magnesium, net tubular secretion of sulfate ceases or is markedly reduced with the adaptation of euryhaline teleosts to freshwater (Hickman and Trump 1969). Micropuncture in vivo of the renal tubules of elasmobranchs (little skate, *R. erinacea*) (Stolte et al. 1977a) and perfusion in vitro of renal tubules from marine teleosts (flounder, *P. americanus;* Beyenbach 1982; Beyenbach et al. 1986) suggest that the primary site of sulfate secretion is the second segment of the proximal tubule. In mammals, net absorption of sulfate apparently occurs primarily along the proximal tubules (Mudge et al. 1973; Ullrich et al. 1980), but no information is available about the site of net sulfate absorption in nonmammalian vertebrates.

4.8.2 Mechanism of Transport

In mammals, studies with intact proximal convoluted tubules perfused in vitro indicate that both saturable secretory and saturable absorptive sulfate fluxes exist in the same segment, but that the absorptive flux predominates (Brazy and Dennis 1981). Findings with intact tubules and with membrane vesicles indicate that the sulfate absorptive flux involves electroneutral, sodium-dependent transport into the cells across the luminal membrane against an electrochemical gradient (Lucke et al. 1979; Ullrich et al. 1980). Movement out of the cells down an electrochemical gradient across the peritubular membrane then involves a carrier-mediated anion exchange process (Brazy and Dennis 1981; Pritchard and Renfro 1983). This anion exchange process on the peritubular membrane may be involved in the secretory flux as well (Brazy and Dennis 1981; Pritchard and Renfro 1983).

All studies of the sulfate transport mechanism in nonmammalian vertebrates have involved the secretory process in marine teleosts. These studies with isolated, nonperfused tubules and membrane vesicles from two species of flounder (winter flounder, *P. americanus,* and southern flounder, *P. lethostigma*) have supplied substantial information about the transport process and revealed similarities and differences from the process in mammals (Renfro and Dickman 1980; Renfro and Pritchard 1982, 1983). The vesicle studies suggest that the transepithelial process involves uptake at the basolateral membrane driven by a pH

gradient (inside of the vesicles alkaline) followed by exit at the brush border via anion exchange (Renfro and Pritchard 1982, 1983). Both membrane steps are electroneutral. Sodium is not required by either carrier, and a sodium gradient alone does not stimulate sulfate transport with vesicles from either membrane. However, in intact, nonperfused tubules, maneuvers that decrease the sodium gradient across the membranes, e.g., incubation in sodium-free medium or in the presence of ouabain, reduce sulfate uptake into the cells across the peritubular membrane (Renfro and Pritchard 1982). It is possible that the sodium dependency of sulfate transport across the peritubular membrane reflects a decrease in sodium-hydrogen exchange at the luminal membrane and, therefore, a decrease in intracellular pH. This would decrease the driving force for sulfate uptake across the basolateral membrane (Renfro and Pritchard 1982). The pH-driven anion-exchange process across the basolateral membrane may be essentially the same as that found in mammals, but the absence of a sodium-dependent transport step into the cells across the luminal membrane may result in net secretion in marine teleosts instead of net absorption (Pritchard and Renfro 1983).

Chapter 5

Transport of Fluid by Renal Tubules

5.1 Introduction

A large fraction of the fluid filtered by glomerular nephrons does not appear in the urine and, therefore, must be absorbed by the renal tubules. In addition, as already noted (vide supra; Chap. 3), the rate of fluid excretion in some nonmammalian vertebrates is determined, in part, by secretion by the renal tubules. The extent to which these processes have been studied or are understood in mammalian and nonmammalian vertebrates varies greatly, but some general patterns can be ascertained.

5.2 Fluid Absorption

5.2.1 Magnitude and Sites of Net Absorption

Although a large fraction of the filtered fluid is absorbed by the renal tubules of all tetrapods, only in mammalian and avian kidneys — kidneys capable of producing a urine hyperosmotic to the plasma — can the tubules absorb nearly all the filtered fluid — more than 99% during dehydration (Jamison and Kriz 1982; Skadhauge 1973). Moreover, among those vertebrates studied, only the proximal renal tubules of mammals and birds are capable of absorbing over half, 60–65%, the filtered fluid (Table 5.1). About 15–45% of the filtered fluid is absorbed along the proximal renal tubules of amphibians and reptiles (Table 5.1). However, a substantial fraction of the filtered fluid, as much as 25–45%, can be absorbed along the late distal tubules and collecting ducts of these animals (Table 5.1). Significant fluid absorption, perhaps as much as 20% of that filtered (Jamison and Kriz 1982), can also occur along the collecting ducts of mammals and birds, but the amount absorbed along these structures in mammals and birds and in some amphibians and reptiles depends on the requirements for water conservation and the presence of antidiuretic hormone (vide infra; Chap. 7). In addition, in amphibians, reptiles, and birds, substantial filtered fluid can be absorbed, depending on the requirements of the animals, by structures distal to the kidneys: colon, cloaca, or bladder.

Among fishes, fluid absorption by the renal tubules is even more variable and less well understood than among the tetrapods. Approximately 40% of the filtered fluid is absorbed by the nephrons of the primitive river lamprey (*L.*

Table 5.1 Fluid transport by tubule segment

Tubule segment and species	J_V % Filt. load	J_V nl $min^{-1}mm^{-1}$	Osmolal TF/P	References
PROXIMAL				
Fishes				
Petromyzonta				
River lamprey, *Lampetra fluviatilis* (freshwater)	10	0.2	1.0	Moriarty et al. 1978; Logan et al. 1980a
Elasmobranchii				
Dogfish shark, *Squalus acanthias* (stimulated)		−27.6 ± 3.9 (21)		Beyenbach 1986; D.B. Sawyer and Beyenbach 1985
Teleostei				
Winter flounder, *Pseudopleuronectes americanus*		−36.6 ± 4.2 (53)		Beyenbach 1986; Beyenbach et al. 1986
Killifish, *Fundulus heteroclitus* (seawater)		−49.0 ± 8.7 (16)		Beyenbach 1986
Amphibia				
Anura				
Bullfrog, *Rana catesbeiana*	17	0.34 ± 0.07 (29)	0.99 ± 0.02 (23)	Irish and Dantzler 1976; Long 1973
Urodela				
Tiger salamander, *Ambystoma tigrinum*		0.26 ± 0.01 (14)		Sackin and Boulpaep 1981a
Mudpuppy, *Necturus maculosus*	29	0.39 ± 0.06 (47)	1.01 (22)	Boulpaep 1972; Garland et al. 1975
Reptilia				
Squamata				
Ophidia				
Garter snake, *Thamnophis* spp.	45	0.87 ± 0.04 (127)	1.00 ± 0.01 (12)	Dantzler and Bentley 1978a
Sauria				
Blue spiny lizard, *Sceloporus cyanogenys*	35	0.18	0.99 (8)	Stolte et al. 1977b
Aves				
European starling, *Sturnus vulgaris*				
Reptilian-type nephrons	60	0.21		Laverty and Dantzler 1982
Mammalia				
Rabbit, *Oryctolagus cuniculus*				

Table 5.1 Continued

Tubule segment and species	J_V % Filt. load	J_V nl $min^{-1}mm^{-1}$	Osmolal TF/P	References
Convoluted segment	65	0.9 – 1.0	1.0	Jacobson 1982
Straight segment	65	0.4 – 0.6	1.0	Schafer and Barfuss 1982
EARLY DISTAL "DILUTING"				
Fishes				
Petromyzonta				
River lamprey, *Lampetra fluviatilis* (freshwater)	1	0.07	0.87	Moriarty et al. 1978; Logan et al. 1980a
Amphibia				
Anura				
Leopard frog, *Rana pipiens*		0.07 ± 0.09 (6)		Stoner 1977
Bullfrog, *Rana catesbeiana*	1		0.75 ± 0.11 (11)	Long 1973
Urodela				
Mudpuppy, *Necturus maculosus*	0 – 1		0.48	Garland et al. 1975
Congo eel, *Amphiuma means*		0.07 ± 0.01 (12)		Oberleithner et al. 1982
Aves				
Japanese quail, *Coturnix coturnix*				
Mammalian-type nephrons, thick ascending limb		–0.01 ± 0.04 (9)		Nishimura et al. 1984; Miwa and Nishimura 1986
Mammalia				
Rabbit, *Oryctolagus cuniculus*				
Thin ascending limb	0	~0		Imai and Kokko 1974
Medullary thick ascending limb	0	~0		Rocha and Kokko 1973
Cortical thick ascending limb	0 – 1	0.13 ± 0.02		Burg and Green 1973
LATE DISTAL AND COLLECTING TUBULE				
Amphibia				
Urodela				
Tiger salamander *Ambystoma tigrinum*				
(control)		0.01 ± 0.02 (7)		Stoner 1977
(ADH)		0.02 ± 0.02 (7)		Stoner 1977
Mudpuppy, *Necturus maculosus*	2 – 25		~0.25	Garland et al. 1975

Table 5.1 Continued

Tubule segment and species	J_V % Filt. load	J_V nl $min^{-1}mm^{-1}$	Osmolal TF/P	References
Congo eel, *Amphiuma means*	25			Wiederholt et al. 1971
Reptilia				
Squamata				
Ophidia				
Garter snake, *Thamnophis* spp.				
(control)		0.07 ± 0.04 (14)		Beyenbach 1984
(ADH)		0.08 ± 0.03 (14)		Beyenbach 1984
Sauria				
Blue spiny lizard, *Sceloporus cyanogenys*	2.5	0.05	0.85	Stolte et al. 1977b
COLLECTING DUCT				
Fishes				
Petromyzonta				
River lamprey, *Lampetra fluviatilis* (freshwater)	28.4	0.43	0.18	Logan et al. 1980a; Moriarty et al. 1978
Amphibia				
Anura				
Bullfrog, *Rana catesbeiana*	46.9			Long 1973
Urodela				
Tiger salamander, *Ambystoma tigrinum*				
(control)		−0.09 ± 0.09 (4)		Stoner 1977
(ADH)		−0.02 ± 0.05 (4)		Stoner 1977
Reptilia				
Squamata				
Sauria				
Blue spiny lizard, *Sceloporus cyanogenys*	36.4			Stolte et al. 1977b
Mammalia				
Rabbit *Oryctolagus cuniculus*				
Cortical				
No osmotic gradient		0.3		Grantham and Burg 1966
200 mOsm Osmotic Gradient				
−ADH		0.9		Grantham and Burg 1966
+ADH		3.0		Grantham and Burg 1966

Values are means, ranges, or means ± SE. They are taken directly or calculated from the references. Numbers in parentheses indicate number of determinations. J_V indicates net transepithelial movement of fluid. Minus sign in front of values indicates secretion rather than absorption. Osmolal TF/P indicates ratio of osmolality in fluid collected from tubule (TF) to osmolality in plasma (P).

fluviatilis) in freshwater. However, only 10% is absorbed along the proximal tubules, the remaining 30% being absorbed along the collecting ducts (Table 5.1). An average of 40% of the filtered fluid also is absorbed by the nephrons of freshwater teleosts, but this can vary from 25% to 75%; the sites of absorption along the renal tubules are unknown (Hickman and Trump 1969). It is difficult to determine the magnitude of net fluid absorption for marine elasmobranchs and teleosts and euryhaline teleosts adapted to seawater because of the net fluid secretion that also takes place (vide infra). However, the data suggest that the fraction of filtered water absorbed by the renal tubules can range from 9% to 93% (average, about 70–85%) in marine elasmobranchs and can exceed 90% in marine teleosts and euryhaline teleosts adpated to seawater (Hickman and Trump 1969). The major sites of absorption along the renal tubules in these marine fishes are unknown.

When the river lamprey is adapted to seawater to mimic its marine phase, almost 90% of the filtered water is absorbed by the renal tubules (Logan et al. 1980 c). As in the animals adapted to freshwater, most of this absorption appears to occur along the distal tubules and collecting ducts (Logan et al. 1980 c). Finally, in the marine cyclostomes, e.g., the Atlantic hagfish (*M. glutinosa*), which conform to their environment, there is no net fluid absorption along the primitive archinephric ducts (Stolte and Schmidt-Nielsen 1978).

5.2.2 Mechanism and Control of Fluid Absorption

The process by which filtered fluid is absorbed by the proximal renal tubules of mammalian and nonmammalian vertebrates is not well understood. However, micropuncture or microperfusion experiments on lampreys (Logan et al. 1980 a), amphibians (Windhager et al. 1959), reptiles (Dantzler and Bentley 1978 a; Stolte et al. 1977 b), birds (Laverty and Dantzler 1982), and mammals (Walker et al. 1941; Gottschalk and Mylle 1959) indicate that during transepithelial volume absorption the fluid in the tubule lumen remains isosmotic with the plasma. Thus, transepithelial fluid absorption appears to be an isosmotic process. Studies on urodele amphibians (*N. maculosus*) also demonstrate that fluid absorption, although apparently isosmotic, is secondary to and apparently dependent upon sodium absorption (Windhager et al. 1959). How this coupling of solute and water movement actually occurs is not yet completely understood for either mammalian or nonmammalian vertebrates; it remains one of the most important unsolved problems in epithelial transport.

The possible mechanisms for mammalian tubules, which have been reviewed in detail elsewhere (e.g., Schafer 1984), are beyond the scope of the present discussion. However, a number of studies on nonmammalian vertebrates, especially on urodele amphibians, have shed some light on this process for nonmammalian vertebrates and, possibly, for mammals. Analysis of the transport process in the proximal tubules of mudpuppies (*N. maculosus*) suggests that net absorption may be slightly hyperosmotic but that this may be experimentally indistinguishable from isosmotic absorption (Sackin and Boulpaep 1975). This suggestion has also been made with regard to the apparent isosmotic absorption by

mammalian proximal tubules (Schafer 1984). Furthermore, a quantitative structural evaluation of the proximal tubules of two urodele amphibian species (tiger salamander, *A. tigrinum,* and mudpuppy, *N. maculosus*) demonstrates that the basal portion of the basolateral membrane of the former has a highly elaborate organization and much greater amplification than that of the latter (Maunsbach and Boulpaep 1984). This elaboration of the basal membrane may form an additional compartment that plays some role in the absorptive process in this amphibian species (Maunsbach and Boulpaep 1984). In addition, micropuncture studies on *N. maculosus* suggest that organic substrates such as lactate, alanine, glutamate, lysine, butyrate, and glucose in the tubule lumen and all of them except glucose in the peritubular blood are important for fluid absorption (Forster et al. 1980). However, it is not clear which of these are most important or whether they act by enhancing luminal permeability for sodium, regulating metabolism, reducing passive paracellular backleak for sodium, or by some other process (Forster et al. 1980).

Among reptiles, in vivo micropuncture studies of lizard (*S. cyanogenys*) proximal tubules (Stolte et al. 1977 b) and in vitro microperfusion studies of proximal tubules of garter snakes (*Thamnophis* spp.) (Dantzler and Bentley 1978 a) indicate that sodium and water can be absorbed at osmotically equivalent rates. However, they do not prove that fluid absorption always must be dependent on sodium absorption. In fact, substitutions for sodium or chloride or both in the solutions used for bathing and perfusing the snake tubules in vitro suggest that neither one of these ions may be essential for normal fluid absorption (Dantzler and Bentley 1978 a). When sodium in the perfusate is replaced with choline, net fluid absorption almost ceases (Fig. 5.1). However, when sodium in the bathing medium is also replaced with choline, so that both solutions are identical, net fluid absorption returns to the control rate (Fig. 5.1). The results are the same when sodium is replaced by tetramethylammonium, when sodium and the equivalent amount of chloride are replaced with sucrose, and when chloride alone is replaced with methyl sulfate (Fig. 5.1). However, net fluid absorption does not change from the control rate when lithium replaces sodium in the perfusate alone or in both the perfusate and bathing medium simultaneously (Fig. 5.1). Fluid absorption at control rates, regardless of the composition of the perfusate and bathing medium, is isosmotic within the limits of the measurements and can be at least partly inhibited by cold and cyanide (Dantzler and Bentley 1978 a). Thus, it appears that isosmotic fluid absorption can proceed at control rates when lithium replaces sodium or when some other substance replaces sodium or chloride or both in the perfusate and bathing medium simultaneously.

Even with sodium present, however, net fluid absorption by these snake tubules cannot be inhibited by ouabain or other cardiac glycosides or by the removal of potassium from the bathing medium (Dantzler and Bentley 1978 a, b); it is also not dependent on the nature of the buffer (bicarbonate, phosphate, or Tris) used (Dantzler and Bentley 1978 a). With sodium present, net fluid absorption is reduced about 18–25% by the removal of colloid from the peritubular fluid (Dantzler and Bentley 1978 a).

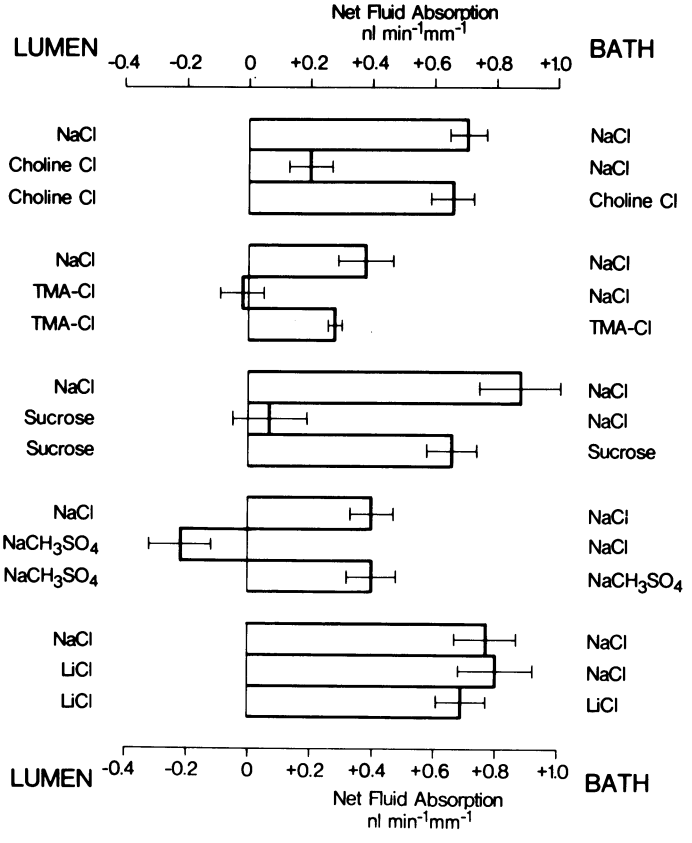


Fig. 5.1. Net fluid movement in isolated perfused snake proximal renal tubules. Composition of solution in lumen and bath in terms of sodium and chloride and of substitutes for them is shown at sides of figure for each experiment. Each *bar* represents mean net fluid movement with lumen and bath composition shown. *Horizontal lines* at end of each bar represent SE. From 6 to 13 tubules were used in each experiment. (Dantzler 1978a)

Although these observations on snake proximal tubules suggest that isosmotic fluid absorption can occur in the absence of both sodium and chloride, they do not provide any information on the mechanism involved. However, recent quantitative structural studies on these isolated, perfused tubules (Dantzler et al. 1986) show that within a few minutes after substitution of choline for sodium in both the perfusate and bathing medium significant morphological changes take place (fig. 5.2). Cells double in size and intercellular spaces nearly quintuple. At the same time, the areas of the lateral and apical cell membranes approximately double, but their surface densities remain essentially constant. Therefore, the larger cells in the absence of sodium have proportionately larger surface areas but the volume-to-surface area ratio remains constant. The rapid increase in membrane area most likely involves incorporation of additional membrane from other areas, possibly from intracellular membrane vesi-

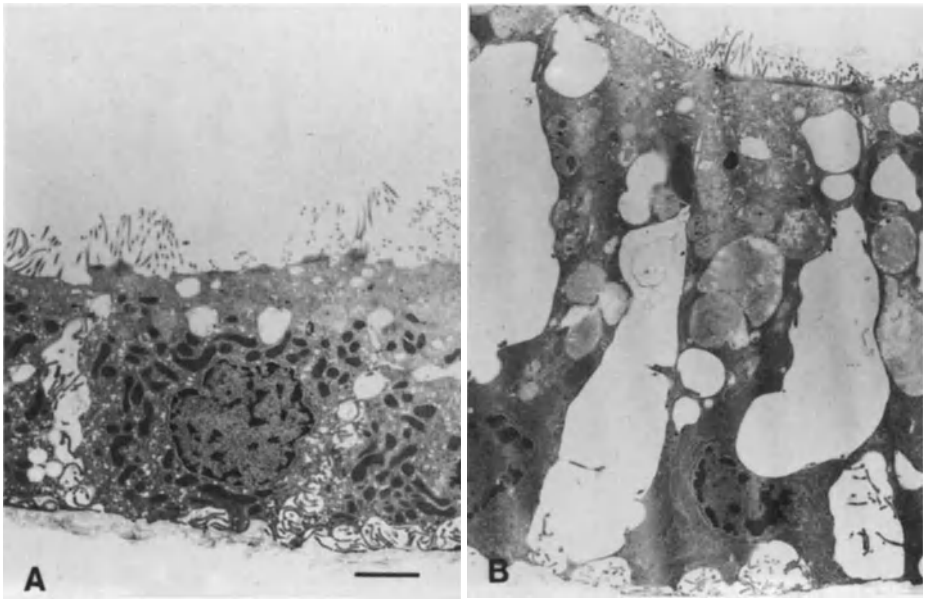


Fig. 5.2 A, B. Electron micrographs of cross section of isolated perfused proximal renal tubules of garter snake (*Thamnophis* sp.). A Proximal tubule perfused and bathed with medium containing sodium. B Proximal tubule perfused and bathed with medium in which sodium has been replaced with choline. Both tubules are shown at same magnification. Note increase in cell height and widening of intercellular spaces in B, the tubule perfused and bathed with medium in which choline replaced sodium. Bar = 2.0 μm. (Dantzler et al. 1986)

cles, but the exact source is unknown. In any case, these changes are correlated with the maintenance of a control level of net fluid absorption and may permit a small, previously unimportant driving force, e.g., the osmotic pressure generated by colloid in the peritubular fluid, to produce a control level of net fluid absorption.

In general, the extent to which hydrostatic and colloid osmotic pressures in the peritubular capillaries or sinuses contribute to fluid absorption by the proximal tubules of nonmammalian or even mammalian vertebrates is unknown. However, these pressures, if they influence net fluid absorption significantly, could be particularly important in nonmammalian vertebrates because the renal venous portal system contributes to a variable extent to the peritubular blood supply. Alterations in the portal contribution could alter fluid absorption and, in this way, factors that regulate portal flow could also influence net fluid absorption by the proximal tubules. Net fluid absorption in the distal portions of the nephrons, although variable and regulated in many species by neurohypophysial peptides, clearly is driven by transepithelial osmotic gradients established primarily by sodium and chloride absorption (vide infra).

5.3 Fluid Secretion

As discussed above (Chap. 3), net secretion of fluid by the proximal tubules, which has now been demonstrated for a number of primarily marine vertebrates, is particularly important for marine aglomerular and even some glomerular fishes (Table 5.1). Although in vivo studies originally suggested that such fluid secretion was dependent on magnesium and, to a lesser extent, sulfate and calcium secretion (Babikir and Rankin 1978; Berglund and Forster 1958; Bieter 1935; Hickman 1968; Hickman and Trump 1969; Renfro 1980), recent studies with isolated, perfused proximal tubules from marine glomerular teleosts (winter flounder, *P. americanus*), euryhaline teleosts adapted to seawater (killifish, *F. heteroclitus*), and marine elasmobranchs (dogfish shark, *S. acanthias*) have demonstrated that the dominant ions in the secreted fluid are sodium and chloride and that the fluid secretion depends upon their secretion (Beyenbach 1982, 1986; Beyenbach et al. 1986; Sawyer and Beyenbach 1985). The probable mechanism involved in the secretion of sodium chloride by these tubule segments has been discussed above (Chap. 4). The secretion of magnesium and sulfate, which also occurs in this tubule segment, as discussed above (Chap. 4), enhances fluid secretion, but the essential driving force remains the secretion of sodium and chloride (Beyenbach 1982; Cliff et al. 1986). There are no known humoral or neural controls of fluid secretion.

Chapter 6

Transport of Organic Substances by Renal Tubules

6.1 Introduction

The excretion of numerous organic substances is regulated by renal tubular transport. For a few of these substances, enough information is now available on the transport processes in nonmammalian species that they can be discussed, compared, and contrasted. To a limited extent, comparisons and contrasts with transport processes in mammals are possible.

6.2 Glucose

6.2.1 Direction, Magnitude, and Sites of Net Transport

Glucose is freely filtered and almost completely absorbed by the glomerular nephrons of all those vertebrates studied (Table 6.1; also Dantzler 1981; von Baeyer and Deetjen 1985). A low rate of net secretion is observed in aglomerular teleosts (e.g. *Lophius americanus,* Malvin et al. 1965). However, even aglomerular nephrons appear to have retained an absorptive process for glucose because the administration of phlorizin, an inhibitor of glucose absorption in glomerular nephrons, to these aglomerular teleosts produces an increase in the urine-to-plasma glucose concentration ratio (Malvin et al. 1965).

The proximal tubule has been identified as the primary site of net glucose absorption in amphibians by micropuncture (Walker and Hudson 1937 a), in reptiles by perfusion of isolated renal tubules (Barfuss and Dantzler 1976), and in mammals by micropuncture (Walker et al. 1941) and by perfusion of isolated renal tubules (Tune and Burg 1971). Although some absorption may be possible in nephron segments distal to the proximal tubule, this does not appear to occur under normal circumstances (von Baeyer and Deetjen 1985). It is generally assumed that the proximal tubule is the primary site of glucose absorption in all other vertebrates.

In garter snakes (*Thamnophis* spp.) the maximum rate of net tubular absorption is twice as great in the distal portion of the proximal tubule as in the proximal portion (Barfuss and Dantzler 1976), a pattern that is almost exactly the opposite of that in mammals (Tune and Burg 1971). However, no studies of intranephron heterogeneity have been made in other nonmammalian vertebrates to determine if the pattern of glucose transport is similar to that in either reptiles or mammals.

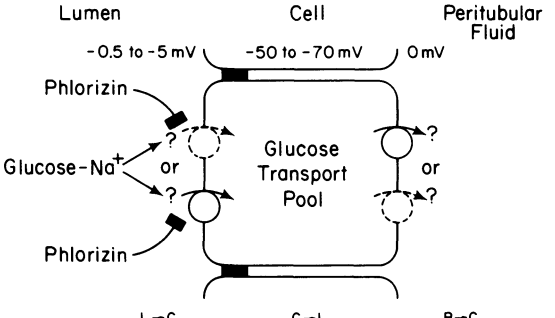
Table 6.1 Normal direction of net tubular transport of some organic substances observed with whole-kidney clearance studies

Substance	Fishes		Amphibia		Reptilia		Aves		Mammalia	
	Net absorption	Net secretion	Net absorption	Net secretion	Net absorption	Net secretion	Net absorption	Net secretion	Net absorption	Net secretion
Glucose	+	+	+		+		+		+	
Bicarbonate			+		+	+	+		+	
Amino acids	+	+	+		+	+	+		+	
Urea	+		+	+	+	+	+	+?	+	
Ammonia		+		+		+		+		+
Organic acids and anions (except amino acids, urate and lactate)		+	+	+		+		+		+
Urate				+		+		+	+	+
Lactate					+				+	
Organic bases and cations					+	+	+	+	+	+

6.2.2 Mechanism of Transport

6.2.2.1 Net Absorption

The transepithelial transport process is saturable and sodium-dependent in all mammalian and nonmammalian species studied (Barfuss and Dantzler 1976; Coulson and Hernandez 1964; Khuri et al. 1966; Sperber 1960; Vogel and Kroger 1966; Vogel et al. 1965; Vogel and Stoeckert 1966; von Baeyer and Deetjen 1985). Studies of the transport step across the luminal membrane into the cells of the proximal tubule have been made in teleost fishes (flounder, *P. americanus,* and mullet, *Mugil cephalus*) with brush-border membrane vesicles (Eveloff et al. 1979; Lee and Pritchard 1983 a); in amphibians (newt, *Triturus pyrrhogaster*) with intracellular and transepithelial potential measurements on perfused tubules (Maruyama and Hoshi 1972); in reptiles (garter snakes, *Thamnophis* spp.) with brush-border membrane vesicles (Benyajati and Dantzler 1986 d) and with isolated, perfused tubules (Barfuss and Dantzler 1976); and in mammals with brush-border membrane vesicles (Kinne et al. 1975) and isolated, perfused tubules (Tune and Burg 1971). All these studies indicate that glucose crosses the luminal membrane and enters the cells via a saturable, phlorizin-sensitive, electrogenic co-transport step with sodium (Fig. 6.1). The vesicle data from all species and the data from isolated, perfused proximal tubules of mammals indicate that glucose enters the cells against a concentration gradient and thus is a



	$P_L^{L\to C}$ cm sec^{-1}	$P_L^{C\to L}$ cm sec^{-1}	$P_P^{B\to C}$ cm sec^{-1}
Control	10.0×10^{-5}	0.65×10^{-5}	0.46×10^{-5}
0 Na^+ in Lumen	5.98×10^{-5}		
Phlorizin in Lumen	0.86×10^{-5}	0.65×10^{-5}	0.46×10^{-5}

Fig. 6.1. Model for net tubular absorption of glucose based on studies on amphibians, reptiles, and fish. *Solid circles with solid arrows* indicate either primary or secondary active transport against electrochemical gradient. *Broken circles with broken arrows* indicate mediated transport down electrochemical gradient. *Question marks* indicate tentative suggestions. *Lines with bar at end* indicate inhibition. Apparent permeabilities of luminal membrane in lumen-to-cell ($P_L^{L\to C}$) and cell-to-lumen ($P_L^{C\to L}$), directions and of peritubular membrane in bath-to-cell ($P_P^{B\to C}$) direction are shown for various conditions for snake renal tubules

secondary active transport process coupled to the movement of sodium down its electrochemical gradient. However, it is not clear that glucose movement into the cells, although coupled to sodium, is against a chemical gradient in all nonmammalian species. Indeed, the studies with isolated, perfused snake proximal tubules indicate that glucose enters the cells down a concentration gradient (Barfuss and Dantzler 1976). Whether this difference between the isolated, perfused tubules and the brush-border membrane vesicles from the same species reflects a purely technical difference between the types of studies or whether it reflects a difference between what can be demonstrated in an isolated membrane and what actually occurs in an intact asymmetrical epithelium containing cytosol and two membranes in series is not yet known. It may be, since the intracellular glucose concentration in the isolated, perfused snake tubules was measured only with sufficient glucose in the lumen to saturate the transport system, that normal transport against a concentration gradient when the luminal concentration is low was obscured (Barfuss and Dantzler 1976). In any case, it is not possible at this time to decide whether the mediated entry step for glucose across the luminal membrane is always a form of secondary active transport or whether it may be a form of facilitated diffusion in some nonmammalian species (Fig. 6.1).

The transport step for glucose out of the cells across the peritubular membrane appears to occur down a concentration gradient in most species (Eveloff et al. 1979; Lee and Pritchard 1983 a; Tune and Burg 1971). Although this movement could occur by simple passive diffusion, studies with isolated, nonperfused proximal tubules of flounders (Kleinzeller and McAvoy 1973) and isolated, perfused proximal tubules of rabbits (Tune and Burg 1971) suggest that this step should be mediated in some fashion (Fig. 6.1). Moreover, glucose transport out of the cells at the peritubular side of isolated, perfused snake tubules appears to be against a concentration gradient (Barfuss and Dantzler 1976). When glucose entry across the luminal membrane of these tubules is blocked with phlorizin, this gradient increases because the concentration of glucose within the cells decreases. When these tubules spontaneously stop absorbing glucose, this gradient disappears because the concentration within the cells increases to equal that in the bathing medium. In addition, the apparent passive permeability of the peritubular membrane of these snake tubules to glucose is too low to permit glucose absorption across this membrane at the observed rate by simple passive diffusion (Barfuss and Dantzler 1976). Therefore, glucose transport out of the cells at the peritubular side, although it may differ in different species, probably involves at least a carrier-mediated step and may involve a primary or secondary active transport step in some nonmammalian species (Fig. 6.1).

6.2.2.2 Net Secretion

The small net secretory flux observed in aglomerular teleosts could be explained by some form of passive process because the concentration in the urine is always far below that in the plasma even when absorption is blocked by phlorizin (Malvin et al. 1965). However, the steps in transport across the tubule epithelium have yet to be examined directly.

6.3 Bicarbonate

6.3.1 Direction, Magnitude, and Sites of Net Transport

Bicarbonate transport by mammalian nephrons, which has been studied extensively, is still the subject of intensive investigation. However, very little information is available on the transport of bicarbonate by the renal tubules of nonmammalian vertebrates or on the role of such transport in acid-base balance. As noted above (Chap. 4), in many nonmammalian vertebrates, extrarenal structures, e.g., gills in teleost fishes, bladder in chelonian reptiles, which have been intensively studied, are more important than the renal tubules in the removal of acid or alkali. For example, it is now well documented that the turtle bladder can secrete bicarbonate as well as hydrogen ions (Husted et al. 1979; Leslie et al. 1973). The tubular transport of bicarbonate and its regulation may be related to the excretion of the end products of nitrogen metabolism and to the function of the extrarenal routes for ion excretion.

Most of the major aspects of bicarbonate transport have been covered in the discussion of hydrogen ion transport (Chap. 4), for there have been only a few studies in which bicarbonate excretion or the possible tubular transport of bicarbonate has actually been measured in nonmammalian vertebrates. As already noted (Chap. 4), mammals, birds, and amphibians are capable of producing a ureteral urine substantially more acid (ca. pH 4.5) than the initial filtered plasma (blood pH ca. 7.4) during an acid load, indicating indirectly that under these circumstances the filtered bicarbonate can be essentially completely absorbed by the renal tubules. Elasmobranch renal tubules also normally absorb essentially all the filtered bicarbonate (Hodler et al. 1955).

Although some reptiles (e.g., water snakes, *N. sipedon*) acidify ureteral urine to a lesser extent than mammals, birds, and amphibians during an acid load, the decrease in pH still indicates a substantial absorption of the filtered bicarbonate (Dantzler 1968, 1976 a). Moreover, as noted previously (Chap. 4), the alkaline urine resulting from the administration of carbonic anhydrase inhibitors to these water snakes supports the concept that bicarbonate absorption occurs and that it involves a process similar to that observed in mammals and amphibians (Dantzler 1968). Among crocodilian reptiles, however, as noted previously (Chap. 4), clearance studies have revealed net tubular secretion of bicarbonate that can be converted to net tubular absorption by the administration of carbonic anhydrase inhibitors (Lemieux et al. 1985).

The tubule sites of net bicarbonate transport, although studied extensively in mammals, are very poorly evaluated in nonmammalian vertebrates. In mammals, as much as 90% of the filtered bicarbonate is absorbed along the proximal tubules (Koeppen et al. 1985). Additional absorption can occur along the distal tubules and cortical and medullary collecting ducts, but net secretion of bicarbonate can also occur along the cortical collecting ducts and, possibly, along the terminal portion of the distal tubules (initial collecting duct) (Koeppen et al. 1985; Levine and Jacobson 1986). In elasmobranchs, all filtered bicarbonate appears to be absorbed along the proximal tubules because the pH

of the tubular fluid does not decrease beyond this point and essentially no filtered bicarbonate appears in the final urine (Deetjen and Maren 1974; Hodler et al. 1955). In both anuran (*R. pipiens*) and urodele (*N. maculosus*) amphibians, however, micropuncture studies indicate that the pH of the tubular fluid does not fall below that of the plasma along the proximal tubules and that net bicarbonate absorption is proportional to net fluid absorption (Giebisch 1956; Montgomery and Pierce 1937; O'Regan et al. 1982). Similarly, micropuncture measurements on avian (*S. vulgaris*) proximal tubules indicating that sodium and chloride are absorbed at equivalent rates and that the pH of the tubule fluid does not fall below that of the peritubular plasma (Laverty and Dantzler 1982; Laverty and Alberici 1987) suggest that the bicarbonate concentration does not change and that bicarbonate and fluid absorption are proportional.

Additional bicarbonate absorption must occur in the distal portions of the nephrons in amphibians and birds to account for the low pH observed in the final urine during an acid load. The micropuncture experiments revealing a substantial fall in pH along the distal portions of amphibian nephrons and avian cortical collecting ducts suggest that this is the case (Giebisch 1956; Montgomery and Pierce 1937; Laverty and Alberici 1987). More recently, studies involving direct measurements of bicarbonate transport with isolated, perfused renal tubules from urodele amphibians (*A. maculatum* and *A. tigrinum*) indicate that net bicarbonate absorption occurs to the greatest extent along the late distal tubules (Yucha and Stoner 1986). There is, of course, some net absorption along the proximal tubule. Although the study of Yucha and Stoner (1986) reveals no net bicarbonate absorption along the early distal tubules in urodeles, the electrical potential and bicarbonate concentration are still compatible with absorptive bicarbonate transport in this region. Moreover, net absorption of bicarbonate, as determined indirectly by hydrogen ion secretion, apparently can occur in the early distal tubules of anuran amphibians (*R. pipiens* and *R. esculenta;* vide supra; Chap. 4), but the relative importance of absorption in this region compared with more distal regions in these anurans is unknown. Some net bicarbonate absorption generally occurs along the initial collecting ducts of urodeles, but this is highly variable and net secretion may even occur along these segments in some animals (Yucha and Stoner 1986).

Nothing is known about the site of net bicarbonate secretion in alligators. However, in view of the magnitude of the transport, which leads to net overall secretion for the entire kidney, the proximal tubule must be involved.

6.3.2 Mechanism of Transport

Most of the information available concerning the mechanism of bicarbonate absorption in the proximal tubules of nonmammalian vertebrates is derived from studies on urodele amphibians, which have been reviewed in connection with the discussion of hydrogen ion transport in Chapter 4. Briefly, these studies indicate that in the proximal tubules of amphibians, as in mammals, most bicarbonate absorption is driven by hydrogen ion secretion. Apparently, such absorption involves conversion of bicarbonate to carbon dioxide and water in the tubule

lumen and then reconversion to bicarbonate within the tubule cells (Fig. 4.6). As also noted previously, carbonic anhydrase is presumed to play a role in this process within the tubule cells (Fig. 4.6), but its presence in the proximal tubule cells of amphibians is not clearly documented (Chap. 4). As noted above (Chap. 4), no carbonic anhydrase is present in the kidneys of elasmobranchs; the titration studies of Deetjen and Marin (1974) on the proximal tubules of *R. erinacea* suggest that bicarbonate is absorbed directly without conversion to carbon dioxide and water. This absorptive process may involve the chloride-bicarbonate exchanger suggested by the studies of Silbernagl et al. (1986; also Chap. 4). Although no direct information is available about the process of bicarbonate absorption in other nonmammalian vertebrates, the observation that the administration of carbonic anhydrase inhibitors to water snakes results in an alkaline urine, an increased excretion of sodium and potassium, and an unchanged excretion of chloride suggests that the mechanism for bicarbonate absorption in these reptiles is the same as in mammals and amphibians (Dantzler 1968, 1976 a).

Few direct studies have been made on the mechanism of bicarbonate absorption in the distal nephrons of nonmammalian vertebrates. However, in the early distal tubules of amphibians, the absorptive process is probably driven by the sodium-hydrogen exchanger (vide supra; Chap. 4; hydrogen ion transport) (Fig. 4.3). Some bicarbonate absorption, apparently driven by a sodium-hydrogen exchanger, can occur in the thick-ascending limb of mammalian nephrons (Good et al. 1984), but, as noted above, the process must be quantitatively more important in amphibians than in mammals. Less complete studies on the late distal tubules of urodeles showing sodium and carbonic anhydrase dependence of bicarbonate absorption (Yucha and Stoner 1986) and involving luminal pH measurements (Stanton et al. 1984 b) suggest that the absorptive process in this tubule region is also driven by a sodium-hydrogen exchanger and involves carbonic anhydrase (vide supra; Chap. 4; hydrogen ion transport). Perhaps a similar process occurs in the distal nephrons of other nonmammalian vertebrates.

Although little direct information is available about the mechanism of bicarbonate secretion for any vertebrate renal tubules, the clearance studies on alligators, which normally reveal marked net tubular secretion, are highly suggestive. As already noted, the administration of carbonic anhydrase inhibitors to these animals results in a mildly acid urine and the conversion of net tubular secretion of bicarbonate to net absorption (Coulson and Hernandez 1964; Lemieux et al. 1985). These observations suggest that carbonic anhydrase plays a role in the production of bicarbonate within the tubule cells and that hydrogen ion is secreted from the cells into the blood, accounting for the persistently low plasma pH. Bicarbonate is then secreted from the cells into the tubule lumen — in general, the reverse of the process shown in Fig. 4.6 (Lemieux et al. 1985). However, this process has not been demonstrated directly, and absolutely nothing is known about the detailed mechanism for hydrogen ion or bicarbonate movement across the cell membranes. Chloride is required for bicarbonate secretion by mammalian cortical collecting ducts, suggesting that the secretory bicarbonate movement across the luminal membrane involves chloride-bicarbonate exchange (Koeppen et al. 1985), but no such information is available for the secretory process in nonmammalian vertebrates.

Parathyroid hormone appears to suppress and thyroid hormone appears to enhance bicarbonate absorption by mammalian proximal tubules, although neither appears to be of major importance under normal circumstances (Batlle and Kurtzman 1985). Aldosterone may even have a direct effect on bicarbonate secretion by mammalian cortical collecting tubules, but this is not yet certain (Levine and Jacobson 1986). There are no available data on possible hormonal effects on bicarbonate transport by the tubules of nonmammalian vertebrates.

6.4 Amino Acids

6.4.1 Direction, Magnitude, and Sites of Net Transport

Filtered amino acids are almost completely absorbed by the renal tubules of mammals and most nonmammalian vertebrates. However, clearance studies reveal that net tubular secretion of amino acids also can occur in some species of fishes and reptiles (Table 6.1). For example, the sulfonated amino acid, taurine, which plays an important role in cellular osmoregulation in marine fishes, can undergo net secretion by the renal tubules of euryhaline marine teleosts (winter flounder, *P. americanus*) and marine elasmobranchs (dogfish, *S. acanthias;* little skate, *R. erinacea*) (Schrock et al. 1982). Net secretion of taurine by the renal tubules of marine and terrestrial snakes (olive sea snake, *A. laevis;* garter snake, *T. sirtalis*) also can occur (Benyajati and Dantzler 1986 a, c). Clearance studies reveal only net tubular secretion of taurine in flounders but either net tubular secretion or net tubular absorption in the dogfish, skates, and snakes (Benyajati and Dantzler 1986 a, c; Schrock et al. 1982). Moreover, either net tubular secretion or net tubular absorption of other endogenous β-amino acids (β-alanine and β-aminoisobutyric acid) and an endogenous analog of the β-amino acids (L-cysteic acid) can occur in sea snakes and garter snakes (Benyajati and Dantzler 1986 a, c). The variable direction of net tubular transport of taurine in some fishes may function to control the plasma levels which are rapidly altered by osmotic stress and high protein meals. This is especially likely because taurine is not readily metabolized in these animals. However, the function of tubular secretion of taurine and other β-amino acids in snakes is not at all clear. It also should be noted that taurine is one of the few amino acids for which a significant fractional excretion, some 6–40% of the filtered load, occurs in mammals (Silbernagl 1985), but the physiological significance of this high excretion rate is not known.

In the snakes studied, the endogenous amino acids other than those discussed above are largely absorbed by the renal tubules (Benyajati and Dantzler 1986 a, c). However, whereas in mammals and birds such absorption usually amounts to more than 99% of the filtered load, in these reptiles it is often substantially less (Benyajati and Dantzler 1986 a, c). For example, in both garter snakes and sea snakes, only about 80% of the filtered histidine is absorbed. The absorption of filtered serine also is often this low, and in garter snakes adapted to cold the

absorption of phenylalanine and glutamic acid also falls to about 60% of the filtered load. In contrast, in the only other reptile species studied, the alligator (*A. mississippiensis*), absorption of all filtered amino acids appears to be more than 99% complete (Hernandez and Coulson 1967). Because ureteral urine was collected from the snakes and cloacal urine was collected from the alligators, it appears possible that the apparent difference in tubular absorption results from the difference in the site of collection and that amino acid absorption or, possibly even secretion, can occur in the cloaca as well as the renal tubules (Benyajati and Dantzler 1986 a). This possibility is supported by preliminary data on sea snakes in which ureteral and cloaca urine samples were collected from the same animals (Benyajati and Dantzler 1986 a), but a more direct and detailed evaluation is required before the idea can be accepted or discarded.

The sites of amino acid transport along the nephrons of nonmammalian vertebrates are only poorly understood. Much more information is available concerning the transport sites and pathways along the nephrons of mammals. However, the mammalian sites and pathways which are multiple and complex, are beyond the scope of this volume (for recent detailed discussion, see Silbernagl 1988). Nevertheless, it can clearly be stated that the primary site of amino acid absorption in the mammalian nephron is the proximal tubule (Silbernagl 1988). Among the nonmammalian vertebrates, studies with isolated tubules from flounders certainly indicate that secretion of taurine occurs along the proximal tubule (King et al. 1982), and it appears likely that the absorption of other filtered amino acids also occurs along this tubule segment. In snakes, net tubular secretion and absorption of taurine probably both occur along the proximal tubule, but analysis of the net tubular transport rates as a function of the filtered load suggests that net secretion occurs distal to the primary site of net absorption (Benyajati and Dantzler 1986 c). Again, it appears likely that absorption of other amino acids occurs along the proximal tubule in reptiles, but this has not been examined directly and no information is available about transport sites for amino acids beyond the proximal tubule. One micropuncture study on urodele amphibians (*N. maculosus*), for which only net absorption has been reported, suggests that amino acid absorption occurs beyond the end of the proximal tubule (Oken and Weise 1978). For example, almost all filtered aspartic acid, but only 30% of the filtered glutamic acid, is absorbed by the end of the proximal tubule in these animals. This pattern of fractional absorption is similar to that observed in the ureteral urine of snakes (Benyajati and Dantzler 1986 a), but whether additional absorption of amino acids occurs in the more distal portions of nephrons of *N. maculosus* in the cloaca, or not at all, has not been determined. As already noted, in birds, clearance studies indicate that more than 99% of the total filtered amino acids are absorbed by the renal tubules (Boorman and Falconer 1972; Sykes 1971). The studies of Boorman (Boorman and Falconer 1972) suggest that there are at least two separate absorptive pathways in birds — one for basic amino acids and one for neutral and acidic amino acids — but no information is available on the sites of transport or the specificity for individual amino acids.

6.4.2 Mechanism of Transport

6.4.2.1 Net Tubular Absorption

In mammals, the process of amino acid absorption has been studied with cortical brush-border-membrane vesicles and with transepithelial potential measurements in tubules perfused in vitro and in vivo (Silbernagl 1988). The detailed studies are beyond the scope of this discussion. However, they all indicate that most amino acids are absorbed by a process involving electrogenic co-transport with sodium across the luminal membrane. As in other secondary active transport processes discussed previously, the primary driving force for amino acid entry into the cells is the electrochemical gradient for sodium.

Among the nonmammalian vertebrates, the only studies on the possible mechanisms for amino acid absorption involve urodele amphibians, i. e., the newt, *T. pyrrhogaster* (Hoshi et al. 1976), and teleost fishes, i. e., mullet, *M. cephalus* (Lee and Pritchard 1983b). The studies on newts utilized measurements of the potential difference across the whole epithelial wall and the basolateral membrane of the proximal tubule during perfusion of the lumen with various amino acids in the presence and absence of sodium. These studies also involved the uptake of amino acids by isolated, nonperfused renal tissue in the presence and absence of sodium. The results indicate that neutral amino acids, e. g., alanine, in the lumen depolarize the peritubular membrane potential only in the presence of sodium; dibasic amino acids, e. g., lysine, depolarize it in the presence or absence of sodium; and acidic amino acids, e. g., aspartic acid, do not depolarize it at all. However, the uptake of acidic amino acids by the tubules, apparently from the luminal side, requires sodium, and some uptake of neutral amino acids and substantial uptake of dibasic amino acids can occur in the absence of sodium. When these kinetic data are considered in light of the electrophysiologic data, they suggest that neutral amino acids cross the luminal membrane either in an electrogenic co-transport step with excess sodium or in a non-electrogenic transport step without sodium (Fig. 6.2); dibasic amino acids cross the luminal membrane in a sodium-independent transport step that is electrogenic because of the positive charge of these acids at physiologic pH (Fig. 6.2); and acidic amino acids apparently cross the luminal membrane in an electroneutral co-transport step with sodium (Fig. 6.2).

The studies on mullet were performed on brush-border membrane vesicles only (Lee and Pritchard 1983 b). The results, like those from the studies with mammalian vesicles and from the studies with newt tubules, support the concept that neutral amino acids, e.g., leucine, enter the cells across the luminal membrane via a secondary active transport process in which the transport of the amino acid is coupled to the electrochemical gradient for sodium (Fig. 6.2). They also support the concept that the transport of the dibasic amino acids, e.g., lysine, into the cells across the luminal membrane is independent of the sodium gradient (Fig. 6.2). In addition, they indicate that the transport of these basic amino acids may involve coupling to a proton gradient from the lumen into the cells and that this co-transport system is enhanced by the electrical gradient from

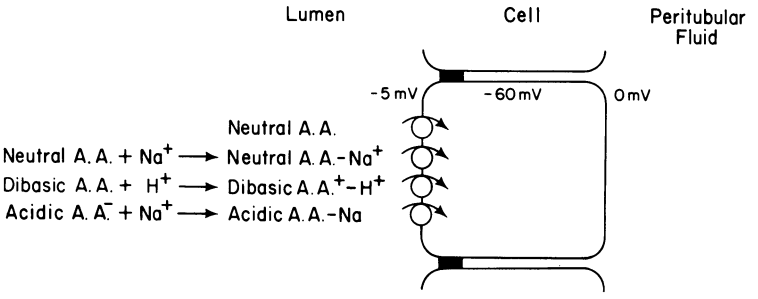


Fig. 6.2. Model for amino acid entry across luminal membrane based on studies on amphibians and fish. Symbols have same meaning as in legend for Fig. 6.1. Absence of positive charge on sodium indicates electroneutral entry step

the lumen to the cell interior (Fig., 6.2). Therefore, as suggested for the newt tubule, this entry step for the basic amino acids is probably electrogenic (Fig. 6.2).

6.4.2.2 Net Tubular Secretion

Net tubular secretion of taurine has been studied with kidney slices from elasmobranchs (dogfish, *S. acanthias;* Schrock et al. 1982), isolated perfused and nonperfused proximal tubules from teleosts (flounder, *P. americanus;* killifish, *F. heteroclitus*) (King et al. 1982; Wolff et al. 1986), and renal brush-border membrane vesicles from teleosts (*P. americanus;* King et al. 1985). The primary energy-requiring step in the transepithelial secretory process appears to involve transport into the cells against a concentration gradient at the peritubular side (Fig. 6.3). This transport step across the basolateral membrane is apparently shared by other β-amino acids, e.g., β-alanine, but not by α-amino acids, e.g., α-aminoisobutyric acid, or other unrelated acids, e.g., p-aminohippurate. It is absolutely dependent on the presence of external sodium and is stimulated by the presence of external chloride. The kinetic studies on killifish suggest that, in the presence of external chloride, the stoichiometric relationship between sodium and taurine is 2:1 and that, in the absence of external chloride, it is 1:1. Wolff et al. (1986) suggest that chloride affects the binding of sodium to the carrier for taurine and that this transmembrane transport step may involve the co-transport of sodium, chloride, and taurine (Fig. 6.3).

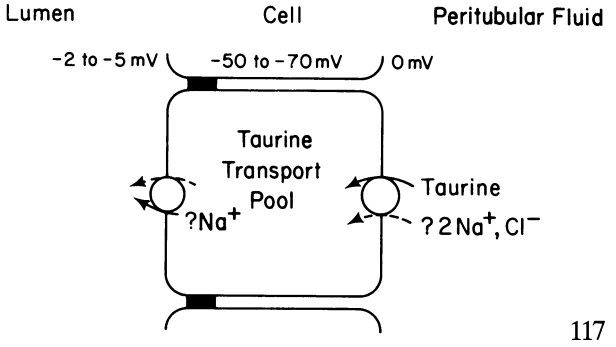


Fig. 6.3. Model for net taurine secretion based on studies with teleosts. *Broken arrows* always indicate movement down electrochemical gradient. Other symbols have same meaning as in legend for Fig. 6.1

Taurine that has been transported into the cells against a concentration gradient at the peritubular side moves down a concentration gradient from the cells to the lumen during the net secretory process (King et al. 1982; Wolff et al. 1986). However, the mechanism involved is less well understood than the transport step at the peritubular membrane. It probably cannot involve simple passive diffusion unless the permeability of the luminal membrane to taurine is much greater than would be expected; a carrier-mediated process appears more likely (King and Goldstein 1985). A sodium-dependent, electrogenic carrier-mediated transport step for taurine that is shared by other β-amino acids has been found in brush-boder membrane vesicles from flounder proximal tubules (King et al. 1985). King et al. (1985) suggest that, under appropriate conditions in vivo, this transport process may facilitate taurine movement out of the cells into the lumen during the net secretory process (Fig. 6.3). However, it has yet to be demonstrated directly that the electrochemical gradients in vivo are appropriate to account for taurine and sodium movement into the lumen by this process.

6.5 Urea

6.5.1 Direction, Magnitude, and Sites of Net Transport

Urea is freely filtered and, in mammals and most other vertebrates, a variable amount is then absorbed passively by the renal tubules (Table 6.1) (Dantzler 1976a; Mudge et al. 1973). Filtration and net passive tubular absorption determine urea excretion even in most of those vertebrates in which it is the major excretory end-product of nitrogen metabolism, i.e., all mammals; chelonian reptiles from mesic, semi-aquatic, and aquatic habitats; adult urodele amphibians; and many anuran amphibians (Table 6.2; also Dantzler 1976 a; Forster 1970; Mudge et al. 1973; Walker and Hudson 1937 b). In mammals, some 50% of the filtered urea is passively absorbed along the proximal tubule. An additional fraction is passively absorbed from the medullary collecting ducts, the amount depending on the presence or absence of antidiuretic hormone. The urea absorbed from the collecting ducts is trapped to a large extent in the medulla, playing a role in the concentrating process (vide infra). Some of it also passively enters the thin ascending and descending loops of Henle and is recycled. The details of these movements of urea in the mammalian kidney and their relationship to the concentrating mechanism are beyond the scope of this volume, but are well reviewed by Jamison and Kriz (1982). The site or sites of passive absorption of urea in the renal tubules of those nonmammalian vertebrates in which such absorption of filtered urea predominates are not yet clearly defined. However, in view of the large fraction absorbed, the proximal tubule must play an important role.

Although only net passive absorption of filtered urea occurs in many urotelic anuran amphibians, net tubular secretion has been demonstrated by clearance and micropuncture studies in a number of species of primarily aquatic anuran

Table 6.2 Approximate percent of total urinary nitrogen as urates, urea and ammonia

	Percent of total urinary nitrogen as:			References
	Urates	Urea	Ammonia	
Mammalia	1 – 2	80 – 90	2 – 8	Dantzler 1970
Aves				
Chicken, *Gallus gallus*	55 – 72	2 – 11	11 – 21	McNabb and McNabb 1975
Moist terrestrial				
Duck *Anas platyrhynchos*	54	1.5	29	Stewart et al. 1969
Reptilia				
Testudinea				
Wholly aquatic	5	20 – 25	20 – 25	Moyle 1949
Semi-aquatic	5	40 – 60	6 – 15	Baze and Horne 1970
Wholly terrestrial				
Mesic environment	7	30	6	Moyle 1949
Xeric environment	50 – 60	10 – 20	5	Baze and Horne 1970
Desert tortoise, *Gopherus agassizii*	20 – 25	15 – 50	3 – 8	Dantzler and Schmidt-Nielsen 1966
Freshwater turtle, *Pseudemys scripta*	1 – 24	45 – 95	4 – 44	Dantzler and Schmidt-Nielsen 1966
Crocodilia	70	0 – 5	25	Khalil and Haggag 1958
Squamata				
Sauria	90	0 – 8	Insignificant to highly significant	Khalil 1951; Dessauer 1952; Perschmann 1956; Minnich 1972
Ophidia	98	0 – 2	Insignificant to highly significant	Khalil 1948a 1948b; Minnich 1972
Rhynchocephalia *Sphenodon punctatum*	65 – 80	10 – 28	3 – 4	Hill and Dawbin 1969
Amphibia				
Anura				
Mesic-xeric terrestrial				
African tree frog, *Chiromantis xerampelina*	60 – 75	20 – 35	1 – 8	Loveridge 1970
South American tree frog, *Phyllamedusa sauvegei*	80 – 90	3 – 11	5	Shoemaker and McClanaham 1975
Mexican tree frog, *Pachymedusa dacnicolor*	2 – 7	90	5	Shoemaker and McClanahan 1975
Mesic terrestrial semi-aquatic				
South American tree frog, *Hyla pulchella*	0	94	6	Shoemaker and McClanahan 1975
Bullfrog, *Rana catesbeiana*	0	84	12	Munro 1953
Aquatic environment (freshwater)				
South African clawed toad, *Xenopus laevis*		20 – 28	72 – 80	Munro 1953; McBean and Goldstein 1967

amphibians of the genus *Rana* (*R. catesbeiana, R. pipiens,* and *R. clamitans;* Long 1973; Mudge et al. 1973). In doubly perfused kidneys of the bullfrog (*R. catesbeiana*) there is a substantial unidirectional passive absorptive flux for urea as well as a unidirectional secretory flux, but the net flux is always secretory (Love and Lifson 1958). Indeed, when net tubular secretion in these animals is abolished by the administration of dinitrophenol (DNP) (vide infra), passive net absorption is revealed (Forster 1970). In micropuncture studies on *R. catesbeiana,* Long (1973) observed that net secretion of urea occurs in both the proximal and distal tubules but not in the collecting ducts. Apparently, no net transtubular movement of urea occurs beyond the distal tubules (Long 1973).

Clearance studies have also revealed net tubular secretion of urea in some lizard species (*Lacerta viridans* and *S. cyanogenys*), in the only living representative of the rhychocephalian reptiles (*Sphenodon punctatum*), and in ducks (*Anas platyrhynchos*) (Dantzler 1976 a; Stewart et al. 1969). However, such net secretion is rarely observed except during extreme diuresis and, in ducks, may result from synthesis by the renal tubules (Dantzler 1976 a; Stewart et al. 1969). Therefore, even in these animals, filtration and net passive absorption appear to play the primary roles in determining the excretion of urea.

Net tubular absorption of urea that may have an active component has been demonstrated by clearance and micropuncture studies in marine elasmobranchs (Forster 1970). In these animals, high concentrations of urea in the body fluids help to maintain osmotic equilibrium with the surrounding seawater, and such absorption may be of physiological significance in regulating these levels. Micropuncture studies on dogfish (*S. acanthias*) suggest that the site of such absorption is between the end of the proximal tubule and the beginning of the collecting duct, apparently in some portion of the distal tubule that is inaccessible to micropuncture (Schmidt-Nielsen 1972).

6.5.2 Mechanism of Transport

6.5.2.1 Net Tubular Absorption

As already noted, net tubular absorption of urea appears to be a passive process in mammals and most other vertebrates, for the concentration in the urine exceeds that of plasma. However, the concentration of urea in the urine and in the early collecting duct segments of elasmobranchs is below the concentration in the plasma (Schmidt-Nielsen 1972). Because this concentration difference between the collecting ducts and the plasma cannot be explained by the addition of water to the tubule lumen, it suggests that urea is being removed from the lumen by some form of primary or secondary active transport. However, this transport process is not saturable or blocked by the usual metabolic inhibitors (Forster 1970). On the other hand, it does appear to be shared by amide containing compounds, e.g., acetamide, but not thiourea, to be sodium-dependent, and to be suppressed by inhibitors of sodium transport and phloretin (Roch-Ramel and Peters 1981; Schmidt-Nielsen 1972). Net urea absorption by elasmobranch tubules could involve some sort of primary active step across the

luminal membrane, although this appears unlikely. It could also involve a secondary active step in which urea movement is coupled directly to the movement of sodium down its electrochemical gradient, as in the case of glucose or amino acid absorption, although this has yet to be demonstrated directly. However, the complex anatomical arrangement of the elasmobranch nephrons (vide supra; Chap. 2, Fig. 2.3) led Boylan (1972) to suggest that the apparent uphill movement of urea could be passive and indirectly coupled to the transport of sodium. He proposed that the proximal tubule segments in some manner create an environment of low urea concentration around the terminal segment by absorbing sodium and water and that urea moves passively out of the terminal segment of the nephron into the surrounding space. More recently, P.A. Friedman and S.C. Hebert (personal communication) have suggested that, if the appropriate permeabilities for urea and water exist in the encapsulated nephron segments, the countercurrent arrangement could concentrate urea within the tubule lumen and permit its passive movement out of the terminal segment down its concentration gradient (P.A. Friedman personal communication). However, as noted by P.A. Friedman (personal communication), this model is insufficient to explain a reduction in the concentration of urea in the tubule lumen below the concentration in the plasma. In any case, whether urea absorption along the elasmobranch nephrons involves a primary or directly coupled secondary active process, a purely passive process, or a combination of these has not yet been determined.

6.5.2.2 Net Tubular Secretion

Studies of the secretory process have been performed only on freshwater frogs, primarily bullfrogs, *R. catesbeiana*. Secretory transport saturates in vivo, suggesting that it involves some form of mediated transport (Forster 1970). The urea concentration in renal tissue obtained from animals that are secreting urea in vivo is greater than that in the plasma, suggesting that a primary or secondary active transport step for urea into the cells exists at the peritubular membrane (Fig. 6.4; also Long 1973; Schmidt-Nielsen and Shrauger 1963). The possibility of a mediated transport step at the peritubular side of the cells is supported by the observations that net secretion is inhibited by the urea analog, thiourea, but not by methylurea or acetamide, by p-aminohippurate (PAH), and by probenecid, apparently in a competitive fashion (Forster 1970; Schmidt-Nielsen and Shrauger

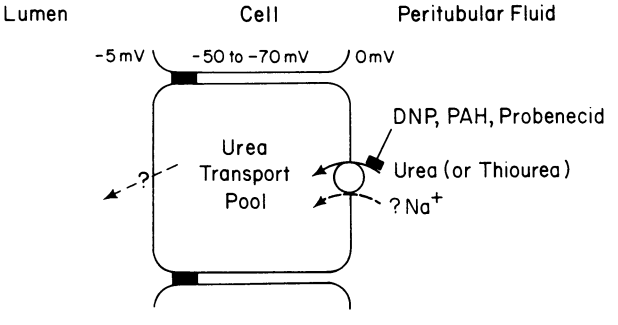


Fig. 6.4. Model for net tubular secretion of urea based on studies on amphibians. Symbols have same meaning as in legend for Fig. 6.1

1963). DNP also inhibits both net tubular secretion and tissue accumulation of urea and thiourea in vivo, further supporting the concept of an active transport step for these substances at the peritubular membrane (Fig. 6.4; also Forster 1970; Schmidt-Nielsen and Shrauger 1963). Studies by Vogel and Kurten (1967) with isolated, doubly perfused frog kidneys indicate that net urea secretion is dependent upon sodium absorption, suggesting that the transport step at the peritubular membrane is coupled in some fashion, direct or indirect, to sodium transport (Fig. 6.4). A linear correlation between the rate of net urea secretion and the urine flow rate in these frogs has been cited as indicating that urea diffuses passively from the tubule cells to the lumen during the net secretory process (Fig. 6.4), but this need not be the case; furthermore, no direct measurements of the transport across the luminal membrane have been made.

Lastly, it is necessary to note that in vitro observations are not in agreement with the tentative model shown in Fig. 6.4. No transport into the cells against a concentration gradient occurs in kidney slices from *R. catesbeiana* or in teased proximal tubules, although avid PAH transport into the cells is observed under the same circumstances (Irish 1975; O'Dell and Schmidt-Nielsen 1961). Moreover, no net active secretion of urea is observed with isolated, perfused frog proximal tubules in which net PAH secretion and net fluid absorption occur at rates comparable to those in vivo (Irish 1975). Some factor or factors necessary for urea transport may be missing from the in vitro preparations. However, Irish (1975) has also suggested that active transport occurs only in the distal tubule and, because of the convolutions of the frog nephron, recirculation may account for apparent active transport in the proximal tubule.

6.6. Ammonia

6.6.1 Magnitude and Sites of Net Secretion

Very little ammonia is normally present in systemic blood of most vertebrate species; that excreted in urine is produced by the renal tubule cells and secreted into the tubule lumen. In mammals, ammonia is produced within the proximal tubule cells (Good and Knepper 1985). Nothing is known about the exact site of production in the tubules of other vertebrates, but the proximal tubule is almost certainly involved.

The primary function of renal ammonia production and excretion in most vertebrates is to help maintain acid-base balance by enhancing the removal of excess acid, or, more correctly, by leading to renal production of an equivalent quantity of bicarbonate. Therefore, in most species the magnitude of ammonia production and secretion by the renal tubules is dependent on acid-base status. Production and secretion increase with an acid load. This is certainly true for mammals, although it varies among species, the capacity for enhanced production apparently being much greater in species such as rats and dogs that generally excrete an acid urine than in rabbits that generally excrete an alkaline urine

(Halperin et al. 1985; King and Goldstein 1985). An increase in ammonia production and excretion with an acid load is also true of those avian species (domestic fowl) and amphibian species (*R. catesbeiana* and *Xenopus laevis*) studied (Craan et al. 1982; Long and Skadhauge 1983; Stetson 1978; Yoshimura et al. 1961).

Although acid-base regulation in fishes occurs primarily via the gills, King and Goldstein (1985) note that there is accumulating evidence of a role for the renal excretion of ammonia in acid-base balance even in these animals, especially when they are exposed to an acid environent. Apparently, at low environmental pH, acid-base regulation via the gills is impaired and the animals actually tend to gain acid across the gills (McDonald and Wood 1981). This leaves the kidney as the only route for acid removal. There is now evidence for increased renal production and excretion of ammonia with an acid load in freshwater teleosts (rainbow trout, *S. gairdneri;* channel catfish, *Ictalurus punctatus;* goldfish, *Carassius auratus;* Cameron and Kormanik 1982; King and Goldstein 1983 b; McDonald and Wood 1981) and in marine elasmobranchs (dogfish shark, *S. acanthias;* King and Goldstein 1983 a).

Among reptiles, a clear ammonigenic response to an acid load has not been demonstrated, although there is a suggestion that this might occur in freshwater snakes (*Nerodia* spp., Dantzler 1976 a). On the other hand, few studies of the effects of an acid load on renal ammonia production and excretion have been made in reptiles. Of particular interest, ammonia is a major excretory end product of nitrogen metabolism in crocodilians (alligators, *A. mississippiensis;* Coulson and Hernandez 1964) and in aquatic and semi-aquatic chelonian reptiles (e.g., freshwater turtle, *Pseudemys scripta;* Table 6.2, also Dantzler 1978 b). In alligators, urates account for about 75% of the excreted nitrogen and ammonia for about 25% (Dantzler 1978 b). The quantity of ammonia excreted by hydrated alligators on a standard meat died (about 2.4 mEq kg^{-1} day^{-1}) is greater than that recorded for any other vertebrate species (Coulson and Hernandez 1970; Kind and Goldstein 1985). In semi-aquatic and aquatic turtles, the excreted nitrogen is often about equally distributed between urea and ammonia, although urea excretion tends to predominate (Table 6.2). In any case, the primary function of ammonia excretion in these reptiles is the elimination of nitrogen. Among amphibians, ammonia excretion via the kidney, although it increases with an acid load, is the primary means of nitrogen elimination in the aquatic clawed toad (*Xenopus laevis;* Table 6.2; also Balinski and Baldwin 1961).

6.6.2 Process of Production and Secretion

In mammals, renal ammonigenesis occurs in the proximal tubules by a process for which the steps and the control have been reviewed in great detail elsewhere (Goldstein 1976; Silbernagl and Scheller 1986; Tannen 1978). Briefly, glutamine is taken up by the renal tubule cells and then enters the mitochondria. Here it is deaminated by phosphate-dependent glutaminase I to form glutamate and NH_4^+. Glutamic acid is then deaminated via glutamate dehydrogenase to form α-ketoglutarate and NH_4^+. As noted by King and Goldstein (1985), the control

of the increased renal production of ammonia in mammals during acidosis has been attributed to almost every step in the production pathway.

Among birds, renal ammonia production has been investigated only for domestic chickens (Craan et al. 1982, 1983). The appropriate enzymes for the production of ammonia from glutamine or alanine are present in homogenates of the whole avian kidney and in tubules separated from the superficial regions of the kidney, apparently the reptilian-type nephrons. In the kidneys of acidotic chickens, as in those of mammals, the activities of glutaminase I, glutamate dehydrogenase, and alanine aminotransferase increase (Craan et al. 1982). Moreover, ammonia production is increased in superficial tubules from acidotic chickens compared with those from control chickens when the tubules are incubated in vitro with glutamine or alanine (Craan et al. 1982). Because only the uptake of glutamine, however, not alanine, is enhanced in tubules from acidotic animals, the former appears to be the preferred substrate for the increased ammonia production (Craan et al. 1983). Unfortunately, the exact tubule segment, or for that matter, the exact tubule population involved in ammonia production is not yet known.

Studies on the enzymes involved in ammonia production have also been performed on alligators (Lemieux et al. 1984). Glutaminase I is present in kidney mitochondria at suitable activities for ammonia production, but it is absent from the liver. However, glutamine synthetase is found only in the liver, suggesting that this organ may be a source of glutamine for ammonia production by the kidney. Glutamate dehydrogenase and alanine aminotransferase activities are also high in the kidney and low in liver and muscle. Moreover, isolated renal tubule fragments can produce ammonia from glutamine and alanine in vitro (Lemieux et al. 1984). Unfortunately, as noted above, there is no direct information available on the tubule segment involved in ammonigenesis.

It should also be noted that during periods of dehydration in alligators, the renal production of ammonia as a major end product of nitrogen metabolism is reduced and even more nitrogen is removed as uric acid (King and Goldstein, 1985). It is possible that with low urine flow accumulation of ammonia in the renal tissue could drive the reversible deamination reactions in the direction of amino acid formation (King and Goldstein 1985), but this process has not been examined directly. It also seems likely that a decrease in renal blood flow during dehydration would decrease the delivery of amino acids to the renal tissue, thereby reducing the amount of substrate for ammonigenesis (King and Goldstein 1985).

Among amphibians, studies with renal tissue from *X. laevis* and *R. temporaria* indicate that glutamine and alanine, among the amino acids, are most readily deaminated in vitro (Balinski and Baldwin 1962). Moreover, acidosis leads to a 30% increase in renal glutaminase I activity in *X. laevis* (Stetson 1978). The data suggest that, in *X. laevis* at least, glutamine is an important source for the increased ammonia excretion observed during acidosis and that regulation of renal ammonigenesis may be similar to that in mammals. The very high concentrations of ammonia in the urine of *X. laevis* compared to the concentrations in the blood also suggest that, although ammonia is the major excretory end product of nitrogen metabolism in these animals, it is produced almost entirely

by the kidney (King and Goldstein 1985). As in the case of the alligators, renal ammonia production as a major excretory end-product of nitrogen metabolism is reduced during dehydration when more nitrogen is excreted as urea (King and Goldstein 1985). The mechanisms involved in this reduction in ammonia production may be the same as those suggested above for alligators (King and Goldstein 1985), but they have yet to be examined directly.

King and Goldstein (1983 a; 1983 b) have explored some aspects of ammonigenesis in a species of freshwater teleost (goldfish, *C. auratus*) and a species of marine elasmobranch (dogfish shark, *S. acanthias*). As noted above, acidosis or an acid environment increases renal ammonia production in both species. In vitro the dogfish kidney has the capacity to synthesize ammonia from a number of amino acids, but the greatest production apparently comes from glutamine. The activities of glutaminase I and glutamine synthetase, localized to the renal mitochondria, suggest that the production of ammonia by renal tissue in these animals may be regulated by the activities of these enzymes working antagonistically in a cycle of substrates between glutamine and glutamate plus NH_4^+ (King and Goldstein 1983 a; King and Goldstein 1985). In vitro studies also indicate that the goldfish kidney has the capacity to synthesize ammonia from a number of amino acids. However, in addition to glutamine, aspartate and alanine appear to be important precursors of ammonia production. In fact, measurements of enzyme activities and of ammonia production both suggest that aspartate has the greatest potential of any substrate analyzed as a precursor of renal ammonia synthesis, at least in vitro (King and Goldstein 1983 b). King and Goldstein (1985) also note that ammonia production from aspartate in these teleost kidneys occurs via transdeamination rather than via the purine nucleotide cycle as in mammalian kidneys.

In general, in mammals, the transport of ammonia from the tubule cells, where it is produced, into the tubule lumen (net secretion) is thought to involve nonionic diffusion of free ammonia (NH_3) and trapping in the lumen as ammonium ion (NH_4^+). Such diffusion trapping is enhanced by the acidity of the fluid in the tubule lumen relative to that in the cells. Such a process for net ammonia secretion could also be important in those nonmammalian vertebrates that produce a urine of relatively low pH. However, even in mammals this process of nonionic diffusion and trapping in the lumen cannot account for all ammonia transport by the renal tubules (Good and Knepper 1985). For example, along the mammalian proximal tubule, where ammonia is produced, a portion of the ammonia must move from the cells to the lumen in the form of NH_4^+ (Good and Knepper 1985). In other segments of the mammalian nephron, e.g., the thick ascending limb of Henle's loop, ammonia may be absorbed as NH_4^+ (Good and Knepper 1985).

The transport of ammonia as NH_4^+ appears to be a very likely possibility for net secretion by the alligator nephrons which, as noted above, always produce an alkaline urine containing large amounts of bicarbonate. This transport may involve the substitution of ammonium ions for hydrogen ions on a sodium-hydrogen exchanger (vide supra; Chap. 4; Fig. 4.1) at the brush-border of proximal tubule cells. Such substitution has been demonstrated in vitro with brush-border membrane vesicles from mammalian proximal tubules (Good and

Knepper 1985). It is also possible, since alligators maintain a rather low blood pH (about 7.1), that the initial filtrate has a pH below that of the cells and that some ammonia may enter the lumen by nonionic diffusion in the first part of the proximal tubule (Lemieux et al. 1985). Neither secretory system would explain the lack of diffusion from the lumen back into the cells further along the nephrons as the tubule fluid becomes highly alkaline. If ammonia secretion involves a carrier-mediated process, the tubule cell membranes may simply have a much lower passive permeability for ammonia than those of other species. The details of this intriguing problem of large amounts of ammonia secretion in an alkaline urine have yet to be examined directly. Moreover, nothing further is known about the mechanism involved in ammonia secretion in those other nonmammalian vertebrates that produce an acid urine and in which renal ammonia production increases with acidosis.

6.7 Organic Acids and Anions (Except Amino Acids, Urate, and Lactate)

6.7.1 Direction and Sites of Net Transport

A diverse group of organic acids that exist as anions at physiological pH, such as PAH, iodopyracet (Diodrast), and phenolsulfonphthalein (PSP, phenol red), undergo net secretion by a common process in the renal tubules of all vertebrates studied except a species of urodele amphibian, *N. maculosus,* and a species of hagfish, *M. glutinosa* (Table 6.1; also Dantzler 1985; Weiner 1973). Both net secretion and net absorption of PAH and iodopyracet by the proximal tubules of *N. maculosus* have been described, but net absorption is generally observed (Tanner 1967; Tanner and Kinter 1966). Moreover, no net tubular transport of phenol red is observed in these animals (Tanner et al. 1979). The renal tubules of *M. glutinosa* also do not transport phenol red or, it is generally assumed, any other organic anion (Fange and Krog 1963; Rall and Burger 1967).

The site of net secretion has been determined in flounders (*P. americanus*), frogs (*R. catesbeiana*), snakes (*Thamnophis* spp.), and rabbits (*Oryctolagus cuniculus*) by perfusion of isolated renal tubules (Burg and Weller 1969; Dantzler 1974 a; Irish and Dantzler 1976; Woodhall et al. 1978). In flounders, net secretion against a concentration gradient from bath to lumen apparently occurs in all segments of the proximal tubule (Burg and Weller 1969). In frogs, however, it is limited to the proximal and intermediate segments of the proximal tubule (Irish and Dantzler 1976). In snakes, such net secretion occurs only in the distal portion of the proximal tubule (Dantzler 1974 a). In rabbits, and probably in other mammals, it occurs primarily in the S_2 segment of the proximal tubule, the central segment that includes approximately the second half of the convoluted portion and the first half of the straight portion (Woodhall et al. 1978).

6.7.2 Mechanism of Transport

6.7.2.1 Net Absorption

As pointed out above, net tubular absorption does not occur in most species and the passive backflux that does occur is small and apparently crosses the cells (vide infra). However, in proximal tubules of *N. maculosus*, net absorption of PAH and iodopyracet does occur against a transepithelial concentration gradient (Tanner 1967). Arterial injections of fatty acids, such as octanoate, inhibit net absorption (Tanner 1967), but no other information is available about the transport process.

6.7.2.2. Net Secretion

Saturation of the organic acid secretory system has been shown by clearance techniques in many species (Weiner 1973) and by perfusion of isolated proximal tubules from flounders, frogs, snakes, and rabbits (Burg and Weller 1969; Dantzler 1974 a; Irish and Dantzler 1976; Shimomura et al. 1981). Saturation of the transepithelial transport system occurs at about the same bath concentration with isolated tubules from flounders, frogs, and snakes, but at about five times that concentration with isolated tubules from rabbits. In the case of the frogs, this in vitro concentration corresponds closely to the plasma concentration at which the secretory system saturates in vivo (Irish and Dantzler 1976; Schmidt-Nielsen and Forster 1954). The apparaent K_m for net PAH secretion, determined from saturation studies with isolated tubules, is about the same for frog tubules and snake tubules but about 15–20 times higher for rabbit tubules (Table 6.3; also Dantzler 1976 b; Irish and Dantzler 1976; Shimomura et al. 1981). The V_{max} for net PAH secretion is about 10–15 times greater in rabbit tubules than in frog and snake tubules (Table 6.3; also Dantzler 1974 a; Irish and Dantzler 1976; Shimomura et al. 1981). Thus, the affinity of the transport system is much higher but the capacity is much lower in frog and snake tubules than in rabbit tubules.

Most recent studies of tubular transport have been concerned with the transport steps at the peritubular and luminal membranes. The studies on nonmam-

Table 6.3 PAH transport by isolated, perfused proximal tubules

Species	Tubule segment	K_m μM	V_{max} fmol min^{-1} mm^{-1}	References
Bullfrog, *Rana catesbeiana*	Proximal	15	659	Irish and Dantzler 1976
Garter snake, *Thamnophis* spp.	Distal-proximal	10	325	Dantzler 1974a, 1976b
Rabbit, *Oryctolagus cuniculus*	Proximal, S_2	195	7430	Shimomura et al. 1981

K_m indicates substrate (PAH) concentration at which rate of transport is one-half the maximum rate. V_{max} indicates the maximum rate of transport for PAH.

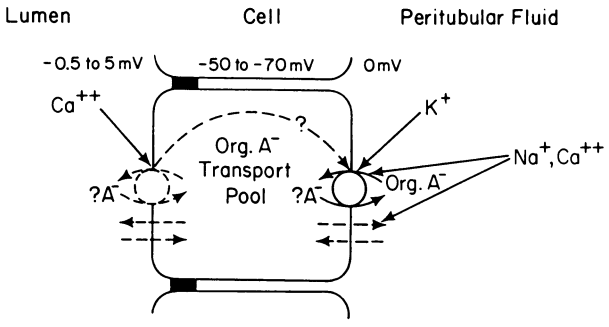


Fig. 6.5. Model for net tubular secretion of organic anions (*Org. A*$^-$) based on studies on fish, amphibians, reptiles, and birds. *A*$^-$ indicates anion of unspecified nature. *Arrows* from K^+, Na^+, and Ca^{++} indicate sites of effects of these inorganic cations. *Broken arrow with question mark* leading from transport step at luminal membrane to transport step at peritubular membrane indicates possible feedback coupling between the transport steps. Other symbols have the same meaning as in legend for Fig. 6.1

malian vertebrates have been particularly revealing, but very similar, if somewhat less complete, data have been obtained on mammals. Substances such as phenol red and PAH are taken up, apparently across the peritubular membrane, by cells of teased proximal tubules or by slices from fish, amphibian, reptilian, avian, and mammalian kidneys (Dantzler 1969, 1974 a; Hoshi and Hayashi 1970; Irish and Dantzler 1976; Weiner 1973, 1985). Furthermore, during the net secretion of iodopyracet or PAH against a transepithelial concentration gradient by isolated, perfused proximal tubules of flounder, frog, snake, and rabbit, the concentration of the acid in tubule cells is greater than that in either bath or lumen (Burg and Weller 1969, Dantzler 1974 a; Irish and Dantzler 1976; Tune et al. 1969). Because there is no evidence of significant binding or trapping of the organic acids within the cells, because the inside of the cells is negative compared to the bath, and because the organic acids apparently are transported as anions, these data are compatible with transport into the cells against an electrochemical gradient at the peritubular membrane (Fig. 6.5; also Dantzler 1974 a; Irish and Dantzler 1976; Tune et al. 1969). These organic anions can then move down an electrochemical gradient from the cells to the lumen (Fig. 6.5). An apparent permeability of the luminal membrane to PAH some five to ten times the apparent permeability of the peritubular membrane is also compatible with this general model for net transepithelial secretion (Dantzler 1974 a, b; Irish and Dantzler 1976; Tune et al. 1969).

In the isolated, perfused renal tubules of frogs and snakes, the transepithelial permeability to PAH determined directly from PAH efflux from the lumen is almost identical to that calculated from the independently measured permeabilities of the luminal and peritubular membranes, suggesting that the small transepithelial efflux crosses the cells (Dantzler 1976 b). Indeed, the measured transepithelial efflux is almost identical to that predicted from the membrane permeabilities in both frog and snake tubules (Dantzler 1976 b; Irish and Dantzler 1976). Essentially the same relationship between the efflux and the less directly determined membrane permeabilities is observed with isolated,

perfused rabbit tubules (Tune et al. 1969). These data showing only a low transepithelial permeability and a small lumen-to-bath efflux that apparently crosses the cells are also consistent with the observation that net organic acid secretion is little influenced by perfusion rate in renal tubules of flounder, frog, snake, and rabbit (Burg and Weller 1969; Dantzler 1974 a; Irish and Dantzler 1976; Tune et al. 1969).

The relationship of inorganic cations — particularly potassium, sodium, and calcium — to the transport step for organic anions at the peritubular membrane, long a subject of study, is still poorly understood. Removal of potassium from the medium bathing teased flounder renal tubules, snake, chicken, and rabbit kidney slices, and isolated, perfused snake renal tubules apparently inhibits the active transport step into the cells at the peritubular membrane without affecting the passive permeability (Fig. 6.5; also Dantzler 1969, 1974 b; Weiner 1973, 1985). The mechanism involved in this requirement for potassium is completely unknown.

Sodium is also required for the net transepithelial secretion of organic anions (Fig. 6.5), but, as in the case of the potassium requirement, the mechanism involved is not yet completely understood. Studies with doubly perfused frog kidneys suggest that both sodium absorption and the intracellular sodium concentration may be important for net PAH secretion (Vogel and Kroger 1966; Vogel et al. 1965; Vogel and Stoeckert 1966). A kinetic analysis of phenol red uptake by teased renal tubules of goldfish (*C. auratus*) and of PAH uptake by isolated kidneys of the newt (*T. pyrrhogaster*) suggest that sodium may influence the relationship of the organic acid with the carrier at the peritubular membrane (Hoshi and Hayashi 1970; Kikutu et al. 1979; Kikutu and Hoshi 1979). Moreover, in newts, measurements of PAH uptake by renal tissue and of the potential across the peritubular membrane of the renal cells suggest for this species, at least, that organic anions may enter the cells in a co-transport step with sodium driven by the sodium electrochemical potential (Kikutu et al. 1979; Kikutu and Hoshi 1979).

However, in other species in which transepithelial transport and membrane transport events have been studied more directly, the relationship of sodium to the transport process is far from clear. In isolated, perfused snake proximal tubules, net PAH secretion is reversibly depressed by the removal of sodium from the bathing medium (Dantzler and Bentley 1976). This depression appears to involve both inhibition of the concentrative transport step into the cells across the peritubular membrane and an increase in the passive permeability of that membrane (Fig. 6.5). These effects are not simply the result of an increase in the concentration of calcium in the cytosol secondary to the removal of the extracellular sodium (Dantzler and Brokl 1984 a). In reptiles, as in mammals, the requirement for sodium does not appear to involve a direct co-transport step with the organic anion. However, the inhibitory effect of 5-acetamido-4'-isothiocyano-2, 2'-disulfonic stilbene (SITS) on the transport step for PAH into the cells across the peritubular membrane of isolated, perfused snake tubules (Dantzler and Bentley 1980) suggests that this step may involve countertransport for other anions whose transport might, in turn, be dependent on or coupled to sodium transport (Fig. 6.5). This concept is also suggested for mammals by

recent work with basolateral membrane vesicles of the rat (Kasher et al. 1983). These studies indicate that a sodium gradient (outside to inside) only stimulates concentrative uptake for radioactively labeled PAH when there is an opposing gradient (inside to outside) for unlabeled PAH. Thus, it appears most likely that the sodium requirement for transport at the peritubular membrane is linked in some fashion to an anion countertransport system (Fig. 6.5).

With regard to the possible calcium requirement for organic anion secretion, studies on the effects of a low calcium concentration in the medium, of lanthanum, and of verapamil on PAH transport by isolated, perfused snake tubules suggest that the entry of calcium into the cells, but probably not the cytosolic concentration of calcium, is important for the transport step at the peritubular membrane and for the maintenance of the normal passive permeability of that membrane (Fig. 6.5; also Dantzler and Brokl 1984 a, b). Lastly, the inhibitory effect of the mercaptide-forming reagent p-chloromercuribenzoate (PCMB) on the PAH transport step across the peritubular membrane in these snake tubules suggests that sulfhydryl groups may be involved (Dantzler and Bentley 1983).

As pointed out above, during the net tubular secretion of organic anions, such as PAH, the movement from the cells to the lumen is apparently down an electrochemical gradient (Fig. 6.5). Although this movement could occur by simple diffusion, the initial studies with isolated, perfused proximal tubules of frog, snake, and rabbit that revealed an apparent PAH permeability of the luminal membrane much higher than that of the peritubular membrane (vide supra) suggested that the transport was mediated in some fashion (Fig. 6.5; also Dantzler 1974 a, b; Irish and Dantzler 1976; Tune et al. 1969). This concept is strongly supported by studies with brush-border vesicles from flounder, snake, rabbit, and rat tubules (Benyajati and Dantzler 1986 b; Blomstedt and Aronson 1980; Eveloff et al. 1979; Kinsella et al. 1979) and by additional studies with isolated, perfused snake proximal tubules (Dantzler and Bentley 1979, 1980, 1981). The movement of radioactively labeled PAH across the luminal membrane, which it apparently crosses as an anion, is inhibited and the apparent permeability of this membrane to PAH is reduced by the presence of unlabeled PAH, phenol red, probenecid, and SITS (Benyajati and Dantzler 1986 b; Eveloff et al. 1979; Dantzler and Bentley 1979, 1980). This mediated transport step for PAH, and presumably for other organic anions that share this system, is not influenced by the presence or absence of sodium or potassium in the lumen of intact snake tubules (Dantzler 1974 b; Dantzler and Bentley 1976). However, very preliminary studies with brush-border membrane vesicles from these same snake tubules suggest that PAH movement across this membrane may be stimulated by a sodium gradient (Benyajati and Dantzler 1986 d).

This luminal transport step for PAH in the intact snake tubules is not dependent on the presence of chloride in the lumen, but may be dependent on the presence of an equivalent quantity of some anion to which the luminal membrane is highly permeable, and may involve anion exchange (Fig. 6.5; also Dantzler and Bentley 1981). That this step involves an anion exchanger is further supported by data on mammalian brush-border vesicles (Kinsella et al. 1979). The transport step across the luminal membrane, at least in intact snake tubules, also may be dependent on the entry of calcium into the cells (Fig. 6.5; also

Dantzler and Brokl 1984 a, b) and may involve sulfhydryl groups (Dantzler and Bentley 1983). Finally, with isolated, perfused snake proximal renal tubules, inhibition, by any means, of organic anion movement from the cells to the lumen during net anion secretion appears secondarily to reduce the transport of the organic anion into the cells at the peritubular membrane (Dantzler and Bentley 1979, 1980, 1981; Dantzler and Brokl 1984 a, b). These data suggest that there may be some feedback coupling between the transport systems on the luminal and peritubular membranes (Fig. 6.5).

6.8 Urate[1]

6.8.1 Direction, Magnitude and Sites of Net Transport

Urates form the major excretory end products of nitrogen metabolism in birds, in most reptiles, except chelonians from mesic, semi-aquatic, and aquatic habitats; and in some amphibians (South American tree frogs of the genus *Phyllomedusa* and African tree frogs of the genus *Chiromantis*) (Table 6.2; Dantzler 1978 b). Urate is always a very minor end product of total nitrogen metabolism in mammals (Table 6.2), but in those mammals lacking uricase to convert urate to allantoin — humans, the great apes, and dalmation coach hounds — it is the major excretory end-product of purine metabolism (Weiner 1985). Urate appears to be freely filtered in mammals, reptiles, and amphibians and probably in birds, although a small amount of binding to plasma proteins may occur in the last (Dantzler 1978 b; Weiner 1985). Clearance measurements on mammals generally reveal that some 96–99% of the filtered urate is absorbed by the renal tubules (Weiner 1985). However, net tubular secretion is observed normally in pigs and frequently in rabbits (Weiner 1985). Thus, either net tubular absorption or net tubular secretion can be observed in mammals, depending on the species studied (Table 6.1). In all those birds, reptiles, and uricotelic amphibians studied, clearance measurements reveal only net secretion of urate by the renal tubules (Table 6.1; also Dantzler 1978 b). This is true even for those chelonian reptiles in which urate is not the primary excretory end-product of nitrogen metabolism (Dantzler 1978 b). However, in chickens, infusions of the diuretics ethacrynic acid and furosemide via the renal portal system can increase net urate secretion, suggesting that the drugs may be inhibiting an absorptive flux (Shideman et al. 1981). Moreover, urate synthesized by the renal tubule cells contributes about 3% of that excreted in fasted chickens, about 20% in normally fed chickens, and up to 50% in chickens infused systemically with hypoxanthine (Chin and Quebbemann 1978). It contributes about 17% of the urate excreted in

[1] The term "urate" in this volume refers to all forms that contain the urate anion (uric acid, uric acid dihydrate, and monobasic urate salts).

normally fed alligators (Lemieux et al. 1985). No information is available on the synthesis of urate by the tubules of other species.

The site or sites of net urate transport in the renal tubules have been studied in mammals by micropuncture and by perfusion of isolated renal tubules, in reptiles (garter snakes, *Thamnophis* spp.) by perfusion of isolated renal tubules, and in birds (starlings, *Sturnus vulgaris*) by micropuncture. In mammals, in which both absorption and secretion have been found in the proximal tubules, it is now well accepted that urate excretion is the sum of filtration, tubular secretion, and tubular absorption (Weiner 1985). In pigs, in which clearance studies reveal net secretion, the secretory process appears to involve the proximal convoluted tubule and the proximal straight tubule, but the proximal convoluted tubule appears to predominate (Roch-Ramel et al. 1980; Schali and Roch-Ramel 1981). In rabbits, the primary secretory flux, which can result in overall net secretion, appears to occur in the S_2 segment of the proximal tubule (Weiner 1985). In snakes, net secretion from bath to lumen against a concentration gradient occurs throughout the proximal tubule but not in the distal tubule (Dantzler 1973, 1976 b). There is no evidence for net absorption in these animals, but a passive unidirectional absorptive flux can also occur throughout the proximal tubule (Dantzler 1973). In the starlings, net secretion occurs along the proximal tubules of the superficial reptilian-type nephrons, but it is not clear whether any additional secretion can occur in more distal segments of these nephrons (Laverty and Dantzler 1983 b). There is no micropuncture evidence of net absorption in these birds (Laverty and Dantzler 1983 b), and no data are yet available on urate transport by the mammalian-type nephrons.

6.8.2 Mechanism of Transport

6.8.2.1 Net Absorption

The only evaluations of the net absorptive process have been made on mammals in which urate is not a major excretory end-product of nitrogen metabolism. Studies have been made on the mechanism involved in this process with brush-border-membrane vesicles from kidneys of dog, rat, and rabbit. The detailed mammalian studies, which are beyond the scope of the current discussion, have been reviewed recently by Kahn and Weinman (1985). Briefly, however, the studies with renal brush-border vesicles of dog and rat indicate that urate transport, presumably the initial step in the saturable net transepithelial absorptive process, involves an anion exchange system with an affinity, not only for urate, but also for PAH, hydroxyl ions, and bicarbonate (Blomstedt and Aronson 1980; Kahn and Aronson 1983; Kahn et al. 1983; Kahn and Weinman 1985). Recently, Abramson and her co-workers (Abramson et al. 1986; Pordy et al. 1986) have provided evidence that uricase associated with the rat renal brush border membranes may function as a carrier for urate. This may be very important for those mammals in which uricase is present. On the other hand, for rabbits, in which net tubular secretion frequently predominates, studies with renal brush border vesicles suggest that urate transport is predominantly nonmediated

(Boumendil-Podevin et al. 1979; Kippen et al. 1979; Kahn and Weinman 1985). Similarly, preliminary studies with renal brush-border vesicles from snakes, in which there is no evidence for net urate absorption, suggest that urate movement across this membrane is largely nonmediated (Benyajati and Dantzler 1986 d).

6.8.2.2 Net Secretion

Net secretion of urate by the renal tubules, as already noted, is the dominant process in urate excretion in nonmammalian vertebrates and is especially important in the uricotelic species. Although a few studies with avian and reptilian kidney slices have contributed to the understanding of the net secretory process, studies with isolated perfused renal tubules of snakes (*Thamnophis* spp.) have provided most of the detailed information (Dantzler 1978 b; 1985). A few studies with isolated, perfused rabbit tubules have suggested that the net tubular secretion that occurs in these mammals involves a process very similar to that in snakes (Chonko 1980). As in the case of the transport of other organic anions discussed above, these studies on urate transport indicate that net secretion involves transport into the cells against an electrochemical gradient at the peritubular side followed by movement from the cells into the lumen down an electrochemical gradient (Fig. 6.6; also Chonko 1980; Dantzler 1973, 1978 b). The effect of SITS on the transport step into the cells at the peritubular membrane in isolated, perfused snake renal tubules suggests that this step involves anion exchange (Fig. 6.6; also Mukherjee and Dantzler 1985). Although this basic model for urate secretion appears very similar to that for other organic anions described above, there are some significant differences. First, in the snake tubules in which the apparent passive permeabilities of the membranes to urate have been measured, that of the peritubular membrane is much greater than that of the luminal membrane (Fig. 6.6; also Dantzler 1976 b). These observations, which are the opposite of those for PAH, suggest an inefficient system if urate transported into the cells across the peritubular membrane is to move readily into the lumen and not back into peritubular bathing medium. Second, there is no convincing evidence that the movement of urate from the cells to the

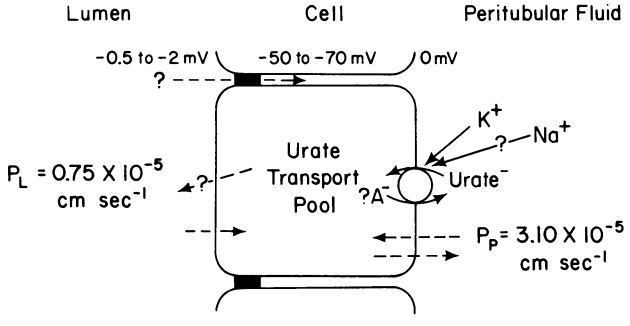


Fig. 6.6. Model for net tubular secretion of urate based on studies with reptiles and birds. Apparent permeabilities for luminal (P_L) and peritubular (P_P) membranes for control conditions in snake tubules are shown. Other symbols have the same meaning as in legend for Fig. 6.1

lumen during the net secretory process is mediated in any fashion. Neither unlabeled urate, probenecid, nor SITS has any effect on the movement of radioactively labeled urate from the cells to the lumen or on the apparent permeability of the luminal membrane in isolated, perfused snake proximal renal tubules (Dantzler and Bentley 1979; Mukherjee and Dantzler 1985). Moreover, preliminary studies of urate transport with brush-border membrane vesicles from these snake tubules, as noted above, provide no evidence of concentrative transport, sodium dependence, or a significant effect of probenecid (Benyajati and Dantzler 1986 b). Similarly, as pointed out above, studies with rabbit brush-border vesicles, although variable, suggest that urate movement across this membrane is largely nonmediated (Boumendil-Podevin et al. 1979; Kippen et al. 1979). These studies, in contrast to those on the transport of PAH and other organic anions, are all suggestive of urate movement across the luminal membrane by simple passive diffusion (Fig. 6.6), although they do not prove it.

Third, in isolated, perfused snake renal tubules, the transport step into the cells across the peritubular membrane appears to be dependent, in part, on movement of glomerular filtrate, or an equivalent artificial perfusate, through the lumen (Dantzler 1973). Fourth, net urate secretion by isolated, perfused snake and rabbit tubules varies directly with the perfusion rate, suggesting significant back-diffusion at low perfusion rates (Chonko 1980; Dantzler 1973). A flux from lumen to bath that appears to be passive and that varies with the perfusion rate has been demonstrated with isolated, perfused snake tubules (Dantzler 1973). Moreover, the transepithelial permeability determined from this lumen-to-bath flux is four times that calculated from the independently measured luminal and peritubular membrane permeabilities, suggesting that much of this backflux must occur between cells (Fig. 6.6; also Dantzler 1976 b). It appears possible that, in the reptilian kidney, the relatively high apparent passive permeability of the peritubular membrane, the low apparent permeability of the luminal membrane and the lack of evidence for mediated transport, the apparent dependence of the peritubular transport step on luminal perfusion, and the apparent large passive back-leak between the cells all function to reduce the accumulation of urate in the cells or lumens of nephrons that are not filtering.

Fifth, the kinetic data for net urate secretion in isolated, perfused snake renal tubules differ from those for net PAH secretion. The net secretory system for urate saturates at a much higher bath concentration than that for the net PAH secretory system (Dantzler 1973, 1974 a). The apparent K_m for net urate secretion in these snake tubules (about 150μM), determined from the saturation data, is approximately 15 times that obtained for PAH under similar circumstances (Table 6.3; see Dantzler 1982 b). However, the V_{max} for net urate secretion (about 150 fmol min^{-1} mm^{-1}) in these tubules is only about one-half that for net PAH secretion (Table 6.3; also Dantzler 1973; 1974 a). Even though the K_m for urate transport is substantially higher than the K_m for PAH transport, it is still well below the normal plasma urate level (400–500 μM) in these animals. This observation suggests that the urate secretory mechanism in snakes is normally saturated and that changes in plasma urate levels do not greatly alter the net urate secretion. Instead, the rate of flow through the lumen and, thus, the back-diffusion described above may be particularly important in determining the net

secretion and the final net excretion. In contrast, the K_m for urate secretion in rabbit tubules (about 238 μM) is well above the normal plasma urate level (about 35μM), suggesting that the plasma urate level may play an important role in determining the net urate secretion in these mammals (Grantham 1982). In addition, there appears to be a protein in normal rabbit serum that inhibits urate secretion, possibly by allosteric modification of the transporter on the basolateral membrane (Tanner et al. 1983).

Sixth, the inorganic cation requirements for net secretion of urate are different from those for net secretion of other organic anions. Net urate secretion by isolated, perfused snake tubules, in contrast to net PAH secretion, is completely unaffected when all the sodium in the bathing medium is replaced by choline (Randle and Dantzler 1973). Therefore, the transport of urate into the cells at the peritubular membrane has neither a direct nor indirect dependence on sodium in this species. There are no direct data implicating sodium in urate secretion by rabbit tubules although the transport system is sensitive to ouabain (Chonko 1980). However, this sensitivity may relate to a requirement for potassium (vide infra). Studies of urate uptake by chicken kidney slices do suggest that sodium may be important for the transport step at the peritubular membrane in avian kidneys (Dantzler 1969, 1978 b). This variable role for sodium in the transport step at the peritubular membrane is indicated by the arrow with the question mark in Fig. 6.6. Also, in contrast to PAH transport, urate transport by isolated, perfused snake tubules does not appear to be sensitive to calcium entry into the cells (Dantzler and Brokl 1984 c).

As in the case of the transport of other organic anions, however, potassium is essential for urate transport in all nonmammalian vertebrate species examined (Dantzler 1978 b). In view of the sensitivity of urate secretion by rabbit tubules to ouabain, potassium may be necessary in these animals also. In any case, removal of potassium from the bathing medium suppresses urate uptake by kidney slices from snake, lizard, and chicken and reversibly inhibits net urate secretion in isolated, perfused snake renal tubules (Dantzler 1969, 1978 b; Randle and Dantzler 1973). At the time of maximum suppression of net urate secretion in these isolated, perfused tubules, the urate concentration in cell water is lower than that in the peritubular bathing medium but greater than that in the lumen (Randle and Dantzler 1973). These findings suggest that the transport step against an electrochemical gradient across the peritubular membrane is completely inhibited in the absence of potassium and that urate moves from the bath to the lumen by a purely passive process. However, as in the case of the transport of other organic anions, the mechanism involved in the requirement of urate transport at the peritubular membrane for potassium is unknown.

Seventh, there is additional evidence that urate is secreted by a pathway separate from that for other organic anions in the renal tubules of snakes and possibly birds. As already noted, studies with isolated, perfused snake proximal tubules indicate that urate is secreted uniformly along the entire length of the proximal tubule whereas PAH is secreted only along the distal portion of the proximal tubule (Dantzler 1973, 1974 a). Moreover, net urate secretion by snake renal tubules is not inhibited by high concentrations of PAH in vivo or in vitro, and net PAH secretion is not inhibited by high concentrations of urate in vitro (Dantzler

1978 b). In contrast, in rabbit proximal tubules, urate and PAH are secreted primarily by the same S_2 segment and apparently can share the same transport system on the peritubular membrane (Grantham 1982). In gouty chickens, net urate secretion is impaired, apparently from impairment of the transport step into the cells at the peritubular membrane, but net PAH secretion is only impaired when the plasma concentration is near the level at which transport is maximum (Austic and Cole 1972; Zmuda and Quebbemann 1975). Other in vivo data on normal chickens indicate that even levels of PAH sufficient to completely saturate the secretory system only partially inhibit net urate secretion and that, at this time, urate secretion can be further inhibited by adenine without influencing PAH secretion (Cacini and Quebbemann 1978). These data tend to support the suggestion, originally made by Weiner (Weiner and Tinker 1972) that there are two pathways for organic anion secretion in birds, both of which transport PAH but only one of which transports urate (Cacini and Quebbemann 1978; Zmuda and Quebbemann 1975).

The relationship of inorganic cations to urate excretion may have consequences for other tubular transport systems. Sodium and potassium and, sometimes, calcium, magnesium, or even ammonium may be found with urate precipitates in the ureteral urine of many birds and uricotelic reptiles and amphibians (Dantzler 1978 b, 1985). The predominant cation may be determined by the diet and ionic requirements of each species. The chemical structure of the urate precipitates and the manner in which the cations are combined with them have yet to be clearly defined (Dantzler 1978 b). The structural combination of urate and organic cations, however, may bear an important relationship to the solubility of the urates (Dantzler 1978 b). Regardless of the nature of this chemical combination, the inorganic cations held in urate precipitates are excreted without contributing to the osmotic pressure of the urine. Therefore, the limited ability of the avian kidney and the lack of any ability of the reptilian kidney to concentrate the solutes in the urine (vide infra) may not be true indications of the ability of these kidneys to excrete inorganic cations.

Some data suggest that when large amounts of sodium are combined with urate precipitates, the fraction of filtered water absorbed by the renal tubules may exceed the fraction of filtered sodium absorbed (Dantzler 1980). If this is true, then isosmotic fluid absorption without sodium of the type discussed above may be required (see Chap. 5). The combination of sodium with urate precipitates also may function in the distal reptilian nephrons to keep the concentration of free sodium low enough to permit continued absorption and maximum dilution by the sodium absorptive mechanism discussed above (see Chap. 4). In addition, the complexing of calcium with urate precipitates in the tubular fluid of some crocodiles (*Crocodylus porosus*) adapted to seawater (G. Grigg personal communication) may facilitate its excretion in these animals. A portion of these inorganic cations combined with urate precipitates in the ureteral urine may be reclaimed — depending on the requirements of the animals — by absorption in the colon, cloaca, or bladder where less complex urate precipitates may be formed and urate salts may be converted to uric acid (Dantzler 1978 b, 1980).

6.9 Lactate

Lactate, an endogenous monocarboxylic organic acid, which may be important in the energy metabolism of renal tubules, is freely filtered and then absorbed by the nephrons of those vertebrates in which its renal handling has been studied (Table 6.1) (Dantzler 1985; Kinne and Kinne-Saffron 1985). Among nonmammalian vertebrates, this has involved only reptiles. However, studies with isolated proximal tubules from garter snakes (*Thamnophis* spp.) have been particularly revealing with regard to the transport processes by which lactate might be conserved and utilized by the renal tubules (Brand and Stansbury 1980 a, b, 1981). Net transepithelial absorption occurs throughout the length of these snake proximal tubules via an energy-requiring, sodium-dependent process (Brand and Stansbury 1980 a, b). It appears likely that the primary absorptive transport step in these tubules, as demonstrated for mammalian nephrons with rat brush-border membranes (Barac-Nieto et al. 1980, 1982), involves an electrogenic, sodium-coupled secondary active transport process at the luminal membrane. Of particular interest, however, is the observation that lactate is transported by perfused snake tubules from the lumen to the bathing medium without being metabolized, whereas lactate that is transported into the cells of nonperfused snake tubules from the peritubular side is metabolized to a significant extent (Brand and Stansbury 1980 a, b). The transport step at the peritubular membrane in mammals and the relationship of such transport to the metabolism of the cells are not well understood (Kinne and Kinne-Saffron 1985). However, these data on snake tubules suggest, although they do no demonstrate conclusively, that absorbed and metabolized lactate are taken up by the tubule cells at opposite membranes and that the pools of absorbed and metabolized lactate are separate. They support the idea first put forward by Cohen (1964) that renal absorptive transport conserves substrate for the entire organism, whereas peritubular accumulation provides nutrients for the renal tubule cells (Brand and Stansbury 1980 a).

6.10 Organic Cations

6.10.1 Direction and Sites of Net Transport

Much less is known about transport of organic cations by renal tubules than about the transport of organic anions. In part, this stems from the fact that many organic cations transported by renal tubule cells are toxic in measureable systemic concentrations so that their transport is difficult to study in vivo. However, many toxic, or potentially toxic, compounds in the environment are organic cations that, if ingested in any manner, must be eliminated by the kidneys. In addition, the systemic concentrations of a number of essential organic cations, e.g., choline, are regulated, at least in part, by the kidneys.

Therefore, understanding of the renal transport of these compounds is more important than the limited number of studies in this area would suggest.

The problem of toxicity has led to the study of organic cation transport in vivo in nonmammalian vertebrates, primarily chickens, in which infusion of even highly toxic compounds through the renal portal system permits the examination of transport by the renal tubules without a significant systemic concentration (Rennick 1981 a, b). In these animals, net tubular secretion of a number of endogenous organic cations, e.g., N^1-methylnicotinamine and catecholamines, and exogenous organic cations, e.g., tetraethylammonium and morphine, occurs (Table 6.1; also Rennick 1981 b). Other endogenous organic cations, most notably choline, undergo net tubular absorption at normal plasma levels and net tubular secretion at elevated plasma levels (Table 6.1; also Rennick 1981 b). Studies involving direct infusions through the renal arteries as well as standard clearance techniques for nontoxic substances suggest that the same patterns hold for mammals (Table 6.1; also Rennick 1981 b, Weiner 1985).

Work with isolated, perfused renal tubules of snakes (*Thamnophis* spp.) indicate that net transport, secretion or absorption, of organic cations can occur throughout the proximal tubule (Dantzler and Brokl 1986; Dantzler unpublished observations; Hawk and Dantzler 1984). Similarly, stop-flow studies on dogs, micropuncture studies on rats, and perfusions of isolated rabbit tubules indicate that secretion and absorption in mammals are primarily functions of the proximal tubules (Weiner 1985). It appears most likely that the proximal tubule is the site of net transport of organic cations in birds as well as in reptiles and mammals.

6.10.2. Mechanism of Transport

In vivo studies in chickens involving portal infusions have shown that the net secretory transport of organic cations, such as tetraethylammonium (TEA) and N^1-methylnicotinamide (NMN), saturates and that it can be inhibited by other organic cations, e.g., mepiperphenidol, quinine, and cyanine 863, but not by organic anions, e.g., probenecid (Rennick 1981 b). Among the nonmammalian vertebrates, more detailed studies of the cellular transport process have been made only with isolated, perfused snake renal tubules (Dantzler and Brokl 1986; Hawk and Dantzler 1984). In studies on TEA transport with these tubules, net secretion resulting from the difference between a small, saturable unidirectional flux from lumen to bath and a sixfold larger, saturable unidirectional flux from bath to lumen is observed (Fig. 6.7) (Hawk and Dantzler 1984). Transport into the cells across the peritubular membrane during the bath-to-lumen flux and transport into the cells across the luminal membrane during the lumen-to-bath flux are both against an electrochemical gradient (Fig. 6.7). TEA then moves down an electrochemical gradient across the opposite membrane during each unidirectional flux (Fig. 6.7). Movement from the cells to the lumen, at least, although down an electrochemical gradient, is probably mediated in some fashion (Fig. 6.7). The transporters into the cells at both membranes have high affinities for TEA, but the affinity of the transporter on the peritubular

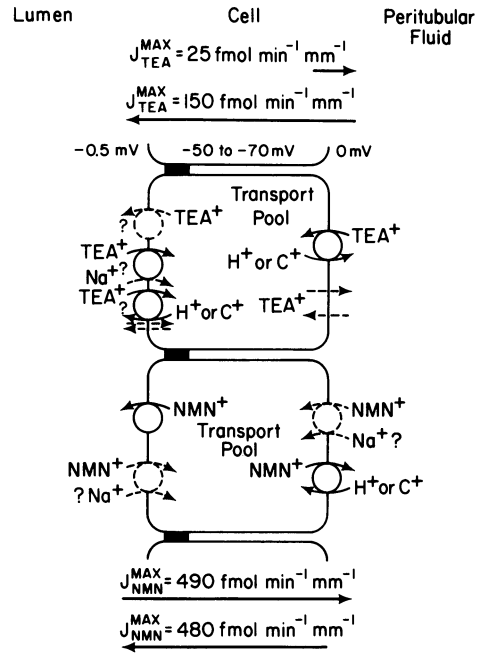


Fig. 6.7. Models for net tubular transport of tetraethylammonium (TEA^+) and N^1-methylnicotinamide (NMN^+) based on studies with snake proximal renal tubules and indicating differences in directions of net movement and in mechanisms of transport. C^+ indicates unspecified organic cation. Other symbols have same meaning as in legend for Fig. 6.1. Maximum unidirectional transepithelial fluxes are given and illustrated by the *length of the arrows for* TEA^+ *and* NMN^+ *at the top* and the *bottom of the figure,* respectively

membrane is only one-third that of the transporter on the luminal membrane. However, the capacity of the transporter at the peritubular membrane for TEA is apparently about six times that of the transporter at the luminal membrane, resulting in net secretion. The transporter on the luminal membrane is at least partly dependent on the presence of sodium in extracellular fluid whereas the transporter on the peritubular membrane is not (Fig. 6.7). In fact, it is possible that transport into the cells across the luminal membrane involves both a sodium-dependent and a sodium-independent step (Fig. 6.7). Preliminary data also suggest that the transport steps into the cells at both membranes involve countertransport for other organic cations, or, perhaps, for protons (Fig. 6.7; also Dantzler and Brokl 1987 a).

This process of TEA transport into the cells against an electrochemical gradient at the peritubular membrane, producing net secretion, observed in snake tubules is similar to the process observed in isolated, perfused rabbit tubules (Schali et al. 1983). No saturable lumen-to-bath flux was observed with the rabbit tubules, but the possibility was not examined in detail (Schali et al. 1983); thus such a flux may well exist. The pattern observed with intact perfused tubules from both snakes and rabbits is quite different from that suggested by studies with brush-border and basolateral membrane vesicles from rabbits and dogs (Kinsella et al. 1979; Rennick 1981 b; Wright 1985; S.H. Wright personal communication). These vesicle studies suggest that for both NMN and TEA an uphill transport step, involving countertransport, and probably driven by a proton gradient, exists only at the luminal membrane (Holohan and Ross 1980, 1981; Kinsella et al. 1979; Wright 1985). The data suggest that entry of these organic cations into the cells across the peritubular membrane, although

mediated and involving cation exchange, is down an electrochemical gradient (Holohan and Ross 1980; Kinsella et al. 1979). At present, it is not possible to reconcile the differences between the studies with intact mammalian renal tubules and mammalian brush-border and basolateral membranes.

However, studies of NMN transport with isolated, perfused snake proximal renal tubules reveal a transport process distinct from that for TEA which resembles, in part, the process suggested by studies with mammalian brush-border and basolateral membrane vesicles (Dantzler and Brokl 1986). Although NMN competes with TEA for transport in mammalian renal tubules (Rennick 1981 b), it inhibits TEA transport by isolated, perfused snake tubules only at extremely high concentrations (Hawk and Dantzler 1984). As in the case of TEA transport, the unidirectional lumen-to-bath and bath-to-lumen fluxes for NMN both saturate. In contrast to TEA transport, however, both fluxes are quite large, more than three times the bath-to-lumen flux of TEA, and the lumen-to-bath flux tends to exceed the bath-to-lumen flux at all concentrations of NMN (Fig. 6.7). Indeed, net flux measurements indicate that, in contrast to TEA, a small net absorptive flux always occurs. Thus, in contrast to TEA transport for which two relatively small unidirectional fluxes differ significantly producing a substantial net secretory flux (Hawk and Dantzler 1984), two relatively large unidirectional fluxes for NMN differ only slightly producing a small net absorptive flux (Fig. 6.7; also Dantzler and Brokl 1986). Also, in contrast to TEA transport, during each unidirectional NMN flux, NMN enters the renal cells down an electrochemical gradient at each membrane by mediated, sodium-dependent transport (Fig. 6.7). It is then transported out of the cells against an electrochemical gradient at the opposite membrane (Fig. 6.7). In these tubules, the transport of NMN across either membrane is not inhibited even by extremely high concentrations of TEA or choline, indicating that the presence of a quaternary ammonium group alone is not sufficient for transport by the NMN transport system. In fact, inhibitor studies with analogs of NMN and with mepiperphenidol suggest that a ring configuration containing quaternary ammonium is essential and that the transporter into the cells down an electrochemical gradient at the luminal membrane, which appears to be the dominant one, has a greater specificity for the NMN structure than the transporter into the cells at the peritubular membrane (Dantzler and Brokl 1986). Current data suggest that the transporter out of the cells at the peritubular membrane, which appears to be the dominant one, involves countertransport, perhaps for protons (Fig. 6.7; also Dantzler and Brokl 1986). As indicated above, the model for the movement of NMN from the peritubular side of the cells to the lumen by entering the cells down an electrochemical gradient across the peritubular membrane, and leaving the cells across the luminal membrane against an electrochemical gradient, is similar to the model for NMN and TEA secretion in mammalian tubules derived from the studies with membrane vesicles (Holohan and Ross 1980, 1981; Kinsella et al. 1979; Wright 1985). However, in these snake tubules, a similar transport process in the opposite direction dominates (Fig. 6.7).

Chapter 7

Diluting and Concentrating Processes

7.1 Introduction

The production of a dilute urine with an osmolality below that of the plasma enables an animal to excrete excess water. The greater the ability to dilute the urine, the greater is the ability to eliminate excess water rapidly. The ability to change the urine from one with an osmolality below that of the plasma to one isosmotic with the plasma enables an animal to conserve water. Of course, the ability to produce a urine significantly hyperosmotic to plasma — an ability found only in mammals and birds — enables an animal to conserve even more water while still eliminating excess inorganic and organic ions and nitrogenous waste. The ability to conserve water by these means is also related to the excretory end-products of nitrogen metabolism. This chapter covers the differences among vertebrate classes and species in ability to dilute and concentrate the urine, the process involved in diluting the urine, the process involved in concentrating the urine, and the regulation of these processes.

7.2 Range of Urine Osmolality

The kidneys of many species of fishes, amphibians, and reptiles, although incapable of producing a urine substantially hyperosmotic to the plasma, are capable of producing a urine with an osmolality ranging from about one-tenth that of the plasma to isosmotic with the plasma (Table 7.1). Those kidneys capable of producing very dilute urine are generally found in animals with a major need to excrete excess water, e.g., stenohaline freshwater fishes, some euryhaline fishes adapted to freshwater, and freshwater amphibians and reptiles (Table 7.1). However, the kidneys of some species appear to be capable of producing only urine hypoosmotic to the plasma regardless of the specific need to conserve or excrete water (Table 7.1). For a number of these species, this production of a hypoosmotic urine under all circumstances appears to be a truly functional limitation, e.g., the desert tortoise, *Gopherus agassizii;* the blue spiny lizard, *S. cyanogenys;* the South African clawed toad, *Xenopus laevis;* the stenohaline marine fishes; and probably, the stenohaline freshwater fishes (Dantzler and Schmidt-Nielsen 1966; Forster 1953; McBean and Goldstein 1970; Stolte et al. 1977 a). For other species, e.g., the bullfrog, *R. catesbeiana,* the maximum urine osmolality may simply not yet have been determined.

Table 7.1 Examples of range of osmolal urine-to-plasma ratios (U/P)

Species	Osmolal U/P (approximate maximum range)	Environment and mode of existence	References
Fishes			
Myxinoidea			
Atlantic hagfish, *Myxine glutinosa*	1.0	Marine	Stolte and Schmidt-Nielsen 1978
Petromyzonta			
River lamprey, *Lampetra fluviatilis*	0.1	Freshwater	Logan et al. 1980a
Elasmobranchii			
Little skate, *Raja erinacea*	0.96 0.80	Marine (placed in 100% seawater) (placed in 75% seawater)	Stolte et al. 1977a
Teleostei			
Goldfish, *Carassius auratus*	0.14	Freshwater	Hickman and Trump 1969
American eel, *Anguilla rostrata*	0.15 0.60	Euryhaline (adapted to freshwater) (adapted to seawater)	Schmidt-Nielsen and Renfro 1975
Southern flounder, *Paralicthys lethostigma*	0.96	Euryhaline (adapted to seawater)	Hickman and Trump 1969
Toadfish (aglomerular), *Opsanus tau*	0.85 0.90	Eurhyaline (adapted to freshwater) (adapted to seawater)	Lahlou et al. 1969
Goosefish (aglomerular), *Lophius americanus*	0.84	Marine	Forster 1953
Longhorn sculpin, *Myoxocephalus octodecimspinosus*	0.85 – 0.94	Marine	Forster 1953
Amphibia			
Anura			
Bullfrog, *Rana catesbeiana*	0.1 – 0.3	Freshwater semi-aquatic	Long 1973; Schmidt-Nielsen and Forster 1954
South African clawed toad, *Xenopus laevis*	0.14 0.65	Freshwater, aquatic (placed in freshwater) (placed in hyperosmotic saline)	McBean and Goldstein 1970
Urodelia			
Mudpuppy, *Necturus maculosus*	0.2 – 0.9	Freshwater, aquatic (TF/P osmolar ratio in distal tubule, before and following administration of arginine vasotocin).	Garland et al. 1975
Reptilia			
Testudinea			
Desert tortoise, *Gopherus agassizii*	0.3 – 0.7	Arid, terrestrial	Dantzler and Schmidt-Nielsen 1966

Table 7.1 Continued

Species	Osmolal U/P (approximate maximum range)	Environment and mode of existence	References
Freshwater turtle, *Pseudemys scripta*	0.3 – 1.0	Freshwater, semi-aquatic	Dantzler and Schmidt-Nielsen 1966
Crocodilia			
Crocodile, *Crocodylus acutus*	0.55 – 0.95	Freshwater and marine, semi-aquatic	Schmidt-Nielsen and Skadhauge 1967
Squamata			
Ophidia			
Bull snake, *Pituophis melanoleucus*	0.5 – 1.0	Arid, terrestrial	Komadina and Solomon 1970
Freshwater snake, *Nerodia spidedon*	0.1 – 1.0	Freshwater, semi-aquatic	Dantzler 1967
Olive sea snake, *Aipysurus laevis*	0.8 – 1.2	Marine, aquatic	Yokota et al. 1985b
Sauria			
Horned lizard, *Phrynosoma cornutum*	0.8 – 1.0	Arid, terrestrial	Roberts and Schmidt-Nielsen 1966
Blue spiny lizard, *Sceloporus cyanogenys*	0.3 – 0.7	Arid, terrestrial	Stolte et al. 1977b
Sand goanna, *Varanus gouldii*	0.4 – 1.0	Arid, terrestrial	Bradshaw and Rice 1981
Aves			
Chicken, *Gallus gallus*	0.1 – 2.0	Moist, terrestrial, gallinaceous	Ames et al. 1971; Dantzler 1966; Skadhauge and Schmidt-Nielsen 1967a
Gambel's quail, *Callipepla gambelii*	0.5 – 2.5	Arid, terrestrial gallinaceous	Braun and Dantzler 1972, 1975
Mammalia			
White rat, *(Rattus norvegicus alb.)*	0.2 – 8.9	Moist, terrestrial	Dantzler 1970

Values chosen appear to reflect measurements made on ureteral urine.

The kidneys of some other species appear to produce a urine nearly isosmotic with the plasma or with very little variation around isosmoticity (Table 7.1). This appears reasonable for stenohaline marine fishes; xerophilic lizards, e.g., horned lizard *Phrynosoma cornutum;* and marine snakes, e.g., olive sea snake, *A. laevis,* that rarely or never obtain excess water to excrete, but it is also the case for the euryhaline aglomerular toadfish, *Opsanus tau,* which is found in freshwater (Table 7.1; see also Lahlou et al. 1969).

Although it is certainly true that urates can be excreted with very little water (vide infra) whereas the excretion of urea or ammonia in significant amounts requires a large urine volume or a significantly concentrated urine, these differences in the ability to produce hypoosmotic urine or isosmotic urine or to regulate the urine osmolality cannot be related simply to differences in the primary

excretory end-products of nitrogen metabolism for there are species that excrete primarily urates, primarily urea, or primarily ammonia in each group (Table 6.2). However, in many species of amphibians and reptiles and even in some species of fishes, additional regulation of the urine osmolality almost certainly takes place distal to the kidney — in the baldder, cloaca, or colon (Dantzler 1970; Lahlou et al. 1969).

Even though the kidneys of fishes, amphibians, and reptiles are incapable of producing a urine substantially hyperosmotic to the plasma, as suggested by the arrangement and structure of their nephrons (see Chap. 2), some still appear capable of producing a slightly hyperosmotic urine. The production of a urine slightly hyperosmotic to plasma, i.e., urine:plasma osmolar ratio of about 1.2–.1.3, has been reported for a species of euryhaline teleost (*F. kansae;* Fleming and Stanley 1965; Stanley and Fleming 1964), marine turtle (*Chelonia mydas;* Prange and Greenwald 1979), xerophilic lizard (*Amphibolurus maculosus;* Braysher 1976), and marine snake (*A. laevis;* Yokota et al. 1985 b). In some cases, this may involve modification of the ureteral urine in the bladder or cloaca because ureteral urine was not collected directly. However, such is not the case for the sea snakes in which ureteral urine was collected directly and found to be slightly hyperosmotic to the plasma at low urine flows (Table 7.1) (Yokota et al. 1985 b). It appears most likely, although by no means proven, that this hyperosmolality results from tubular secretion of solutes, i.e., sodium, potassium, magnesium, or ammonia, into a small tubular fluid volume (Yokota et al. 1985 b). Similarly, the sodium chloride secretion that has been demonstrated in killifish proximal tubules (vide supra; Chap. 4) may account for the hyperosmotic urine observed when these animals are abruptly transferred from freshwater to seawater (Stanley and Fleming 1964; Beyenbach 1986). Although the secretion of ions by the renal tubules may be important for any of these animals in terms of regulating the plasma levels of these substances (vide supra; Chap. 4 and 6), the production of a urine slightly hyperosmotic to the plasma can be of little adaptive significance in the conservation of water for marine reptiles or teleosts because the osmolality of their plasma is so far below that of seawater.

One possible exception to the foregoing discussion is the marine catfish (*Cnidoglanis macrocephalus*). A few measurements suggest that bladder urine in these animals can be not only hyperosmotic to the plasma but also hyperosmotic to the surrounding seawater (Kowarsky 1973). If the production of urine hyperosmotic to the seawater by the animals is confirmed and if it is shown to emanate from the renal tubules, it may result from hyperosmotic sodium chloride secretion by the apparent chloride cells in the collecting tubules now identified by ultrastructural analysis (vide supra; Chap. 4; also Hentschel and Elger 1987).

Both avian and mammalian kidneys are capable of producing a urine hypoosmotic to the blood (Table 7.1). Among the birds, it appears that this diluting ability may be slightly better developed in those species that have most ready access to water (Table 7.1), but this possibility has not been examined systematically.

As noted in Chapter 2, the structural arrangement of the nephrons and blood supply in the medullary cones of the avian kidney suggests, by analogy with the

Table 7.2 Maximum osmolal urine-to-plasma ratio (U/P) and relative medullary thickness (RMT) for a number of birds

Species	Osmolal U/P	RMT[f]
Domestic fowl *(Gallus gallus)*	2.1[a]	
Bobwhite quail *(Colinus virginianus)*	1.6[a]	
California quail *(Callipepla californicus)*	1.7[a]	
Gambel's quail *(Callipepla gambelii)*[b]	2.5[a]	
House finch *(Carpodacus mexicanus)*	2.3[a]	3.59
Zebra finch *(Poephila guttata)*[b]	2.8[c]	4.71
Sengal dove *(Strepotopelia senegalensis)*	1.7[c]	
Kookaburra *(Dacelo gigas)*	2.7[c]	
Emu *(Dromaius novae-hollandiae)*[b]	1.4[c]	
Crested pigeon *(Ocyphaps lophotes)*[b]	1.8[c]	
Galah *(Cacatua roseicapilla)*[b]	2.6[c]	
Budgerigar *(Melopsittacus undulatus)*[b]	3.0[d]	5.06
Savannah sparrow *Passerculus sandwichensis beldingi)*	5.8[e]	3.24

[a] Reviewed in Dantzler (1970)
[b] Desert birds
[c] Skadhauge (1974)
[d] Krag and Skadhauge (1972)
[e] Poulson and Bartholomew (1962)
[f] Johnson (1974). Relative medullary thickness equals ten times the mean length of the medullary cones divided by the cube root of the kidney volume

mammalian kidney, that these kidneys would be capable of producing a urine hyperosmotic to the plasma. Indeed, this was known to occur long before anyone understood the meaning of these structural relationships (Tables 7.1 and 7.2; also Dantzler 1987). However, as shown in Tables 7.1., 7.2, and 7.3, the concentrating ability of birds is quite limited compared with that of mammals. Only one avian species studied to date, the salt marsh savannah sparrow (*Passerculus sandwichensis beldingi*), which can thrive on a high salt intake in the absence of a salt gland, can produce a urine osmolality more than two to three times as great as that of plasma (maximum U/P osmolar ratio: 5.8; Table 7.2; also Poulson and Bartholomew 1962). A slight gradation in the apparent maximum concentrating ability does exist among three closely related species of quail — bobwhite, *Colinus virginianus;* California quail, *Callipepla californicus;* and *C. gambelii* (Table 7.2) — the maximum urine concentration increasing with the aridity of the habitat (Dantzler 1970). However, even the xerophilic Gambel's quail is incapable of producing a urine much more concentrated than that of the mesophilic domestic chicken (Table 7.2). And other desert-dwelling birds are even less able to concentrate their urine (Table 7.2). It should be noted, however, that a direct comparison of the maximum U/P osmolar ratios of birds and mammals can be somewhat misleading because the plasma osmolality of birds tends to increase more than that of mammals with dehydration (Braun 1985). The reason for this greater lability of the plasma osmolality in birds than in mammals is unknown.

The nature of the concentrating process and some possible structural and other differences between birds and mammals are discussed below. However, the fact that birds excrete uric acid as the primary excretory end product of nitrogen

metabolism, rather than urea, makes it possible for them to conserve water without producing as concentrated a urine as mammals. In addition, in birds, significant modification of the ionic composition of the ureteral urine can take place by the transport of ions in structures distal to the kidney, primarily the coprodeum and colon (Skadhauge 1981). In this case, the production of a ureteral urine only modestly more concentrated than the plasma would reduce the tendency for water to move from the plasma into the colon. Moreover, uric acid as the major end product of nitrogen metabolism in the ureteral urine will not diffuse back into the plasma across the intestinal wall. It is not readily converted to ammonia and can be stored in the intestine during the postrenal modification period.

The observation that the low solubility of uric acid permits the excretion of nitrogenous waste with very little water is an old one, but it deserves further discussion in relation to the osmolality of the urine. Homer W. Smith (1953) noted that if the nitrogen from the metabolism of 1 g of protein is excreted as urea in a solution isosmotic with the plasma, it requires 20 ml of water. However, as Skadhauge (1981) noted, reptiles and birds can excrete this much nitrogen as uric acid in 1 ml of urine without significantly concentrating the urine. Urea must remain in solution whereas uric acid almost certainly does not. Much of the uric acid found in the ureteral urine of uricotelic vertebrates is in the form of precipitates. This can be as much as 90% of that excreted at the highest concentrations in domestic fowl (McNabb and Poulson 1970). These precipitates do not, of course, contribute to the osmotic pressure of the urine. In addition, as noted above (Chap. 6), in many uricotelic vertebrates, significant amounts of inorganic cations and ammonium may be included with these precipitates (Dantzler 1978 b). The actual amounts may be as high as 80–90% of the cations excreted and may vary with the diet, including the ionic intake, and with hydration (Dantzler 1978 b; Skadhauge 1981). These cations also do not contribute to the osmotic pressure of the urine. However, the degree to which such ion trapping occurs and the extent to which the molar ratios of these cations to urate exceed those expected for simple salts of urate are still controversial, primarily because of the techincal difficulty of determining the extent to which ions actually accompany urate precipitates in ureteral urine (Dantzler 1978 b; Skadhauge 1981). Clearly, this problem is worthy of further study.

However, even the amount of urate in the liquid phase of the ureteral urine in many uricotelic vertebrates exceeds the solubilities for uric acid (0.384 mM), sodium urate (6.76 mM), and potassium urate (12.06 mM) (Dantzler 1978 b). Therefore, much of the urate in the liquid phase must be in a colloidal state. This can amount to three-fourths of the urate in the liquid phase of the ureteral urine in birds (McNabb and Poulson 1970). The property of urates to form lyophobic colloids has been known for many years (Schade and Boden 1913; Bechhold and Ziegler 1914; Young and Musgrave 1932; Porter 1963). However, the concentrations of urates in the liquid phase of the urine of uricotelic vertebrates often exceed the stability limits of lyophobic colloids in aqueous solutions (Dantzler 1978 b). These findings suggest that the lyophobic urate colloids are converted to a lyophilic state by absorption to lyophilic macromolecules (Porter 1963). Such lyophilic colloids can exist in the liquid phase of the urine at concentrations

above the stability limits for lyophobic colloids. Mucoid materials that may serve as appropriate lyophilic macromolecules have been identified by histochemical techniques in the kidneys of birds (Longley et al. 1963; McNabb et al. 1973); similar materials may exist in the urine of reptiles (Dantzler 1978 b). The apparent formation of lyophilic colloids permits much more urate to remain in the liquid phase of the urine than would otherwise be the case. This may be important in protecting the urinary system by reducing the amount of urate precipitation. In any case, neither lyophobic nor lyophilic colloids contribute significantly to the osmotic pressure of the ureteral urine. Although, as noted above, the ability to excrete urate as the major end-product of nitrogen metabolism may not correlate exactly with the ability of kidneys to vary the urine osmolality, it does permit the conservation of water and certainly reduces the need of terrestrial species to produce a concentrated urine.

7.3 Process and Sites of Dilution

Formation of a urine hypoosmotic to the plasma requires absorption of solute (primarily sodium and chloride) in excess of water somewhere along the nephrons. As pointed out in Chapter 4, sodium and chloride absorption in excess of water appears to occur by a similar mechanism in the early distal tubules of teleost nephrons, amphibian nephrons, reptilian-type avian nephrons, the thick ascending limb of Henle's loop of mammalian-type avian nephrons and of mammalian nephrons, and possibly the thin intermediate segment or early distal tubule of reptilian nephrons. In vivo micropuncture studies document this early distal site for dilution in freshwater lampreys (*L. fluviatilis;* Logan et al. 1980 a), aquatic freshwater urodeles (*N. maculosus;* Garland et al. 1975), and xerophilic lizards (*S. cyanogenys;* Stolte et al. 1977 b), and indicate that dilution continues throughout the length of the distal tubule and collecting ducts. Also, as noted above (Chap. 4), extremely low water permeability and significant solute absorption have been demonstrated by in vivo or in vitro microperfusion of early distal segments of amphibian nephrons (Stoner 1977, 1985; Oberleithner et al. 1983 a), freshwater teleost nephrons (Nishimura et al. 1983 a), and reptilian-type avian nephrons (Miwa and Nishimura 1985; Nishimura et al. 1983 b), and of the thick ascending limbs of mammalian-type avian nephrons (Nishimura et a. 1983b) and of mammalian nephrons (Burg and Green 1973; Rocha and Kokko 1973). Preliminary in vitro microperfusion studies suggest that this also may be the case for the thin intermediate segment of reptilian nephrons (*Thamnophis* spp.; S.D. Yokota and W.H. Dantzler unpublished observations). Also, as discussed in Chapter 4, the late portion of these reptilian distal tubules may be specialized to permit additional dilution of tubular fluid in which the sodium concentration is already low (Beyenbach and Dantzler 1978; Beyenbach et al. 1980; Beyenbach 1984).

As just noted above, dilution of the tubular fluid occurs in some nonmammalian vertebrates despite an apparent need to conserve water (Table 7.1). In

marine elasmobranchs, which remain isosmotic with their environment by retention of urea and trimethylamine oxide (TMAO), some dilution occurs by absorption of filtered sodium and chloride in excess of water which becomes more marked when the animals are maintained in 75% seawater (Table 7.1; also Stolte et al. 1977 a). Micropuncture studies indicate that in elasmobranchs, in contrast to those nonmammalian vertebrates in which formation of a hypoosmotic urine is physiologically significant, the primary site of dilution is the collecting duct, although some dilution does occur in the distal tubule (Stolte et al. 1977 a). This dilution, which is not compensated by fluid absorption at more distal sites, appears to serve no adaptive function. In the blue spiny lizard (*S. cyanogenys*), dilution of the tubular fluid also occurs throughout the distal tubules and collecting ducts regardless of the state of hydration (Stolte et al. 1977 b). However, in these animals, further modification of the ureteral urine may occur in the cloaca.

The slight dilution of the urine observed in stenohaline marine teleosts, e.g., in the longhorn sculpin, *Myxocephalus octodecimspinosus* (Table 7.1), presents an even more curious situation than the above cases. The dilution apparently results from net absorption of filtered sodium and chloride in excess of water because secreted magnesium and sulfate and some filtered chloride account for most of the osmotic activity in the urine (Hickman and Trump 1969). Not only is this dilution of no obvious adaptive advantage, but, because these animals lack distal tubules, it must occur in the proximal tubules. However, no direct studies of the site or mechanism have been made.

The process involved in the changes in dilution by the renal tubules of euryhaline teleosts adapted to different salinities has not been studied in detail. When these animals are adapted to seawater, secreted magnesium and sulfate along with filtered sodium and chloride (and possibly some secreted sodium and chloride; see Chap. 4) are the principal osmotically active substances in the urine (Hickman and Trump 1969; Schmidt-Nielsen and Renfro 1975). However, substantial net tubular absorption of filtered sodium and chloride apparently still occurs during adaptation to seawater (Schmidt-Nielsen and Renfro 1975). With adaptation to freshwater, the tubular secretion of magnesium and sulfate ceases and the epithelium of the distal tubules apparently becomes impermeable to water (Hickman and Trump 1969). However, the distal absorption of sodium and chloride continues, producing a tubular fluid of much lower osmolality than that formed during adaptation to seawater (Table 7.1; Hickman and Trump 1969; Schmidt-Nielsen and Renfro 1975).

7.4 Process of Concentration

The general features involved in the production of a concentrated urine are now well understood, at least for mammals and apparently for birds. The loops of Henle in mammals function as countercurrent multipliers to produce a gradient of increasing interstitial osmolality from the corticomedullary junction to the tip

of the papilla. In the presence of antidiuretic hormone, arginine vasopressin in the case of mammals, the permeability of the collecting ducts to water increases and the fluid in the collecting ducts equilibrates with the hyperosmotic medullary interstitium to produce a urine significantly hyperosmotic to the plasma. Antidiuretic hormone also increases the permeability of the medullary collecting ducts to urea and during this antidiuresis, the solute forming the medullary and papillary interstitial gradient consists of about 60% sodium chloride and about 40% urea. During a water diuresis, in the absence of antidiuretic hormone, urea contributes less than 10% of the medullary and papillary interstitial osmolality.

Although the general features of this process are clear and much is now known about the mechanism involved in the active transport of solute in the thick ascending limb of Henle's loop, which functions both to dilute the tubular fluid and to help form the medullary osmotic gradient (see Chap. 4), the details of the process — particularly the transport properties of the thin limbs, the role of urea, and the function of the blood flow in the vasa recta — which are still not well understood, remain the subject of intensive study. These problems and the various models of the mammalian concentrating mechanism are beyond the scope of this volume. They have been described in detail and critically discussed by Jamison and Kriz (1982) in their monograph and by a number of other authors in the symposium edited by de Rouffignac and Jamison (1987). Roy and Jamison (1985) have also critically reviewed the current models of the mammalian countercurrent mechanism, and Bankir and de Rouffignac (1985) have reviewed specific aspects of the comparative anatomy of the mammalian kidney that relate to this process. Only a few comparative aspects of the process in mammals and those details that are known about the process in birds are discussed in the following sections.

Because, other things being equal, the effectiveness of a countercurrent multiplier increases with the length of the loop, a relationship between the length of the papilla relative to the kidney size and the maximum concentrating ability might be expected in mammals. Indeed, this was one of the first aspects of this process examined (Schmidt-Nielsen and O'Dell 1961) and a general correlation was observed (Table 7.3). However, it is obvious from Table 7.3 that this relationship does not hold for all species. For example, the chinchilla has a relatively long papilla, but cannot concentrate urine as well as a laboratory rat with a much shorter papilla. Similarly, the gundi (*Ctenodactylus vali*), another xerophilic species, can only concentrate urine to about 1400 mosmol/kg H_2O, a value similar to that of humans (Table 7.3), despite a papilla about the length of that of the gerbil (de Rouffignac et al. 1981; Bankir and de Rouffignac 1985). And two other xerophilic species, an Australian hopping mouse (*Notomys cervinus*) and the sand rat (*Psammomys obesus*), do not concentrate their urine nearly as much as would be expected from the length of their papillae (Table 7.3). These observations all indicate that a long papilla with a comparable length of some loops of Henle is insufficient to assure the production of a highly concentrated urine. Clearly, other factors must be involved.

The number of long-looped and short-looped nephrons is also highly variable and does not necessarily correlate with the concentrating ability of the mammalian kidney (Bankir and de Rouffignac 1985). Many xerophilic rodents capable

Table 7.3 Maximum osmolality and osmolal urine-to-plasma ratio (U/P), and relative medullary thickness (RMT) for a number of mammals

Species	Urine osmolality[a] (mosmol/kg H_2O)	Osmolal[a] U/P	RMT[a]
Human	1430	4.2	3.0
Chinchilla *(Chinchilla laniger)*[b]	2000	6.7	9.4
Pack rat *(Neotoma albigula)*[b]	2700	7.0	6.6
Camel *(Camelus dromedarius)*[b]	2800	8.0	
White rat *(Rattus norvegicus)*	2900	8.9	5.8
Cat *(Felis domestica)*	3250	9.9	4.8
Ground squirrel *(Citellus leucurus)*[b]	3900	9.5	
Hopping mouse *(Notomys cervinus)*[b]	4920	14.2	12.0
Kangaroo rat *(Dipodomys merriami)*[b]	5500	14.0	8.5
Gerbil *(Gerbillus gerbillus)*[b]	5500	14.0	
Sand rat *(Psammomys obesus)*[b]	6340	17.0	10.7
Jerboa *(Jaculus jaculus)*[b]	6500	16.0	9.3
Pocket mouse *(Perognathus penicillatus)*[b]	7600	23.5	16.8
Hopping mouse *(Notomys alexis)*[b]	9370	24.6	12.2
Hopping mouse *(Leggadina hermansburgensis)*[b]	8970	26.8	

[a] Reviewed in Dantzler (1970) except for *C. laniger* and *P. penicillatus*. Values for *C. laniger* are from Gutman and Beyth (1970). Values for *P. penicillatus* are from E.J. Braun, University of Arizona, November 1980, personal communication.

[b] Mammals from arid regions.

Relative medullary thickness (RMT) equals ten times the longest axis of the medulla divided by the cube root of the product of the renal dimensions of length, width, and breadth (Sperber 1944).

of producing a highly concentrated urine have fewer nephrons than mesophilic species of comparable body and kidney mass that are not capable of producing a highly concentrated urine, but at least one xerophilic species, *P. obesus,* has a large kidney mass and a very large number of nephrons for its body size (Bankir and de Rouffignac 1985). However, because it eats succulent plants with a high salt and water content, in contrast to many desert species that eat dry seeds, it must excrete a large volume of concentrated urine with a high salt content. The kidneys of *P. obesus* are more effective in concentrating sodium and chloride and less effective in concentrating urea than those of some other desert species. This trait may also be reflected in the high permeability of the thin descending limb of Henle to sodium chloride in this species (Jamison et al. 1979). Although this species may have unique requirements because of its diet and is certainly not a typical desert rodent, it is clear that the number of nephrons in a kidney does not correlate in a simple fashion with concentrating ability.

In a similar manner, the elaboration of pelvic fornices, the size and complexity of the vascular bundles of the inner stripe of the outer medulla, the incorporation of the thin descending limb of Henle in the vascular bundles, and the thickness of the inner stripe all may be correlated with concentrating ability in some species but not in others (Bankir and de Rouffignac 1985). However, Bankir and de Rouffignac (1985), from their anatomical and functional analysis, suggest that the complex vascular-tubular relations of the inner stripe of the outer medulla may be particularly important in generating or, especially, in maintaining the osmotic gradient and in enhancing the concentrating ability. They propose, on the basis of these relations, that the addition of urea to the thin

limbs of short-looped nephrons and the addition of sodium chloride to the thin limbs of long-looped nephrons are particularly important in enhancing the concentrating ability of the mammalian kidney.

As noted above, structural similarities between the avian medullary cones and the mammalian medulla (Chap. 2) and functional similarities between the thick ascending limbs of mammalian-type avian nephrons and mammalian nephrons (Chap. 4) suggest that the avian kidney produces a concentrated urine by a mechanism similar to that of the mammalian kidney. This possibility is further supported by observations of an osmotic gradient along the avian medullary cones analogous to that in the mammalian medulla (Emery et al. 1972; Skadhauge and Schmidt-Nielsen 1967 b). Skadhauge and Schmidt-Nielsen (1967 b) froze the kidneys of dehydrated, salt-loaded, and hydrated male domestic fowl and turkeys. They then dissected the medullary cones from the frozen tissue, extracted them in water following boiling, and determined the osmolality and urea, sodium, chloride, and potassium concentrations. A small osmotic gradient along the medullary cones was observed. The tips of the medullary cones of dehydrated and salt-loaded birds were some 30–50 mosmol/kg H_2O hyperosmotic to the cortex. This gradient of increasing osmolality along the medullary cones disappeared with hydration. Emery et al. (1972) used a microcryoscopic method to determine the osmolality of structures in the medullary cones of budgerigars (*Melopsittacus undulatus*) and savannah sparrows (*Passerculus sandwichensis rostratus*), species capable of producing a substantially more concentrated urine than domestic fowl (Table 7.2). The maximum osmolality of the collecting ducts was about 1200 to 1300 mosmol/kg H_2O in dehydrated and vasopressin-injected budgerigars and 1300 to 1500 mosmol/kg H_2O in dehydrated or salt-loaded savannah sparrows. This value was attained midway along the medullary cones and was about 3–3.5 times the osmolality of the collecting ducts in the cortex. However, the osmolality of the medullary structures studied — loops of Henle, vasa recta, and collecting ducts — remained relatively constant from the middle of the medullary cone to the tip. In budgerigars, which could be studied in more detail, the osmolality of the loops of Henle was slightly higher and the osmolality of the vasa recta was slightly lower than the osmolality of the collecting ducts along the length of the medullary cones.

Of particular interest, the analysis of Skadhauge and Schmidt-Nielsen (1967 b) revealed that, in striking contrast to the mammalian kidney, there is virtually no urea involved in the osmotic gradient in the avian kidney; sodium, chloride, and, to a very slight extent, potassium account for the osmolality. The absence of urea may account for the plateau in the osmotic gradient in the avian medullary cones observed by Emery et al. (1972). It also may be reflected in the anatomical structure of the ascending limbs of Henle's loops in the avian kidney, which, as noted in Chapter 2, have no thin segment. Instead, they consist entirely of a thick segment that apparently functions like the thick ascending limb of the mammalian loop of Henle to absorb sodium and chloride (Chap. 4). Although these observations all support the concept that the loops of Henle of the avian mammalian-type nephrons function as countercurrent multipliers to produce the osmotic gradient and to permit the animals to concentrate their urine, they also suggest that the countercurrent multiplication depends only on the transport of sodium chloride.

The details of the structural organization of the avian medullary cones and the relationship of this organization or of the length and number of the loops of Henle to the concentrating ability are less well studied than the comparable structure-function relationships in mammals. Early attempts to correlate the length of the loops of Henle with concentrating ability showed no obvious relationship (Poulson 1965). However, Poulson (1965) appeared to find a correlation between the number of medullary cones per cross-sectional area of the kidney, which, he argues, reflects the number of loops of Henle per cross-sectional area, and the concentrating ability of two races of savannah sparrow (*P. sandwichensis brooksi* and *P. sandwichensis beldingi*) and a house finch (*Carpodacus mexicanus*). This result appeared reasonable, but Poulson's method of counting the number of medullary cones per cross-sectional area of kidney was questioned because it did not take into consideration the fact that the medullary cones are not straight and that a given cone may appear more than once in a given section. Thus, the method may have over-estimated the number of cones per section. A more detailed analysis of the relative medullary thickness for 26 avian species by Johnson (1974) revealed a tendency for those animals from arid habitats to have a greater relative medullary thickness than those animals from mesic habitats. However, the correlation was not strong and when the relative medullary thickness is compared with maximum concentrating ability for those species for which both are known (Table 7.2), the correlation is even less striking. Thus there appears to be no simple relationship between the relative medullary mass and the concentrating ability.

The tubular elements of the avian medullary cone are arranged in an orderly pattern that somewhat resembles that of the outer medulla of many mammals (Braun 1985; Johnson and Mugaas 1970). The collecting ducts are arranged in a ring about two-thirds of the way from the center to the outer margins of the cone. The descending limbs of Henle's loops are situated inside this ring of collecting ducts and the thick ascending limbs are situated outside the ring. However, whereas the vasa recta of mammals tend to be straight and unbranched in the outer medulla, those of birds tend to form an anastomotic network for the full length of the medullary cones (Braun 1985). Although, as noted above, the relationship of the thin limbs of the short-looped nephrons to the vasa recta in the mammalian outer medulla may permit the recycling of urea, this is clearly not the case in birds in which urea is virtually absent from the medullary cones. The functional significance of the arrangement of the tubular structures in the avian medullary cones has yet to be determined.

7.5 Regulation of Urine Osmolality

As pointed out above, the kidneys of many species of fishes, amphibians, and reptiles are capable of producing a urine that varies from distinctly hypoosmostic to the plasma during hydration or adaptation to a moist environment or freshwater to isosmotic with the plasma during dehydration or adaptation to a dry

environment or seawater (Table 7.1). The production of an isosmotic urine by the kidneys of these amphibians and reptiles apparently results from the equilibration of tubular fluid that has been diluted in one portion of the nephrons (apparently, the early distal tubule or, possibly, the thin intermediate segment) with the interstitium surrounding a more distal portion of those same nephrons or the collecting ducts. It is generally assumed that this equilibration is facilitated by an increase in permeability of the distal portions of the nephrons or the collecting ducts to water when the production of a urine of higher osmolality is required. As described above, the increased osmolality of the urine of euryhaline teleosts adapted to seawater apparently results largely from the tubular secretion of magnesium and sulfate (and possibly sodium and chloride), but some increase in the permeability of the nephrons to water also may occur.

If an increase in the permeability of the distal tubules or collecting ducts to water occurs, it is generally considered to be produced by arginine vasotocin (AVT), which appears to be the physiological antidiuretic hormone in all nonmammalian tetrapods, and perhaps in some fishes (vide supra; Chap. 3). That a change in permeability does occur and that it is produced by AVT is indirectly supported by clearance studies on reptiles and amphibians. The administration of apparently physiological quantities of AVT to some reptiles and amphibians during a water diuresis produces a prolonged increase in urine osmolality and decrease in relative free-water clearance (Butler 1972; Bradshaw and Rice 1981; Dantzler 1967; Jard and Morel 1963). As discussed above (Chap. 3), these doses of AVT also produce a reduction in the whole-kidney GFR, apparently, via a reduction in the number of filtering nephrons. However, the decrease in relative free-water clearance is generally prolonged beyond the decrease in GFR, suggesting that there is a true change in the permeability of the tubules to water (Butler 1972; Dantzler 1967; Jard and Morel 1963). In addition, in one reptile species (*V. gouldii*), a decrease in relative free-water clearance during dehydration is clearly correlated with an increase in plasma osmolality and AVT level (Bradshaw and Rice 1981).

The only direct observation indicating an effect of AVT on the water permeability of the tubular epithelium of nonmammalian vertebrates has been made in one species of urodele amphibian (*N. maculosus*). In these animals micropuncture collections at the same distal tubule site before and after the administration of apparently physiological doses of AVT indicate that the tubular fluid-to-plasma osmolar ratio can increase from 0.2 to 0.9 (Table 7.1; also Garland et al. 1975). This striking change, which only occurs in this tubule segment, appears to indicate a direct effect of the hormone on the epithelium. However, the volume flow rate along the nephrons is markedly reduced following the administration of the hormone and its effect on the equilibration of the lumen and plasma osmolalities has not been evaluated.

In contrast to these observations on *N. maculosus,* in those lizards that always produce a dilute urine, such as *S. cyanogenys* (Table 7.1), micropuncture studies actually show that dilution continues throughout the distal tubules and collecting ducts even following the administration of very large, possibly pharmacological, amounts of AVT (Stolte et al. 1977 b). AVT also has no effect on the water permeability of the distal tubules or collecting ducts of another species

of urodele amphibian (*A. tigrinum;* Stoner 1977), the distal tubules of garter snakes (*Thamnophis* spp.; Beyenbach 1984), or the distal tubules of the freshwater-adapted rainbow trout (*S. gairdneri;* Nishimura et al. 1983 a) isolated and perfused in vitro. In some of these species, of course, AVT may increase the permeability to water of more distal regions of the nephrons than those examined or of the cloaca. However, in none of these species has the effect of the volume flow rate through the distal nephrons or collecting ducts on the equilibration of the tubule fluid with the surrounding interstitium been evaluated.

As indicated, an effect of AVT on the permeability to water of the distal tubules and collecting ducts has not been directly documented in most of these species and clearly does not occur in some species. However, as discussed in Chapter 3, AVT plays an important role in regulating the number of filtering nephrons during dehydration or adaptation to a marine environment. A reduction in the number of glomeruli filtering is practical for fishes, amphibians, and reptiles, in which the nephrons do not function in concert to produce a urine hyperosmotic to the plasma. It conserves water at the expense of not excreting some ions and nitrogenous waste. Of additional importance, it reduces the volume flow through the collecting ducts into which the nephrons drain. In euryhaline teleost fishes, amphibians, and reptiles, this could promote the production of a urine isosmotic to the plasma if a smaller volume of fluid in the collecting ducts has more time to equilibrate with the surrounding interstitium. As noted above, in many of these species, the number of nephrons filtering appears to be altered more readily than the permeability of the collecting ducts by AVT. In other species, however, the prolonged depression of the relative free-water clearance suggests that there may be a change in the permeability of the distal nephrons to water and that this may be influenced more readily by AVT than the number of filtering nephrons. More detailed information is required on the effect of AVT on the tubular permeability to water and on the relative importance of an increase in tubular permeability to water and a decrease in the number of filtering nephrons in the total antidiuretic effect of AVT in amphibians and reptiles.

The kidneys of birds and mammals, as discussed above, are capable of producing a urine that varies from markedly hypoosmotic to the blood to hyperosmotic to the blood, depending on the degree of hydration (Tables 7.1, 7.2, and 7.3). The effect of antidiuretic hormone (arginine vasopressin) on the permeability of mammalian collecting ducts to water is well documented and will not be discussed further here. In birds, the increase in urine osmolality, decrease in urine volume flow rate, and decrease in free-water clearance observed with dehydration can be mimicked by the administration of single injections of AVT (Ames et al. 1971) and by an infusion of AVT that reproduces the plasma levels of the hormone measured during dehydration (Stallone and Braun 1985). This antidiuretic effect is generally assumed, as in mammals, to result from an increase in the permeability of the collecting ducts to water, permitting the fluid in them to equilibrate readily with the surrounding hyperosmotic interstitium of the medullary cones (Ames et al. 1971; Skadhauge 1964). However, an effect of AVT on the permeability of the avian collecting ducts to water has not yet been measured directly. Moreover, because all the reptilian-type nephrons contribute

to the fluid flowing through the collecting ducts (Chap. 2; Fig. 2.6), a decrease in the number of these filtering in response to AVT will reduce volume flow through the collecting ducts, should enhance equilibration with the surrounding interstitium, and may contribute significantly to the production of a urine hyperosmotic to the plasma (Braun and Dantzler 1974). However, this possible effect of volume flow through the collecting ducts also has yet to be examined directly.

Although neither the effects of AVT on the permeability of the collecting ducts to water nor the effects of volume flow rate on equilibration with the interstitium have been examined directly, indirect evidence suggests that there is a change in permeability to water and that the permeability to water is more sensitive to AVT than the number of filtering nephrons. Stallone and Braun (1985) infused AVT into unanesthetized domestic fowl receiving a constant hypoosmotic saline infusion to mimic the plasma levels observed during dehydration and measured the whole-kidney GFR and the free-water clearance. They found that both the whole-kidney GFR and the free-water clearance decreased with increasing plasma levels of AVT. However, the initial decrease in the free-water clearance occurred at a lower plasma level of AVT than the initial decrease in the GFR. In fact, about 90% of the maximum decrease in the free-water clearance occurred before there was any significant decrease in the GFR. At plasma concentrations of AVT equivalent to those found with 24 hours or more of dehydration, there was a 30 % decrease in the GFR but little additional change in the free-water clearance.

Gerstberger et al. (1985) examined the effects of perfusions of the third ventricle of unanesthetized, salt-water-adapted ducks on plasma immunoreactive AVT levels, whole-kidney GFR, and free-water clearance. These animals, like the domestic fowl examined by Stallone and Braun (1985), were receiving a hypoosmotic saline infusion during the experiments. Although both the plasma AVT levels and the renal function in these salt-water-adapted animals with functioning salt glands are certainly closely related to the function of these glands and may not be quantitatively applicable to birds without salt glands, some of the observations are relevant to the sensitivity of the glomeruli and tubules to AVT. Perfusion of the third ventricle with a solution hyperosmotic to native cerebrospinal fluid resulted in an increase in the plasma level of AVT and a decrease in the whole-kidney GFR and free-water clearance. However, the whole-kidney GFR returned to the control level while the perfusion continued and the plasma AVT level remained elevated, but the free-water clearance remained depressed. Perfusion of the third ventricle with a solution isosmotic to the native cerebrospinal fluid had no effect on plasma AVT or renal function. On the other hand, perfusion of the third ventricle with a solution hypoosmotic to the native cerebrospinal fluid resulted in a decrease in the plasma level of AVT and an increase in the whole-kidney GFR and free-water clearance. In this case, the GFR tended to return to the control level while perfusion continued and the plasma AVT level was depressed, but the free-water clearance remained elevated. Single intravenous injections of small, apparently physiological doses of AVT in these animals depressed both the whole-kidney GFR and the free-water clearance (Gerstberger et al. 1985). However, as in some of the studies with reptiles and

amphibians (vide supra), the whole-kidney GFR returned to the control level before the free-water clearance.

The data on domestic fowl and ducks all suggest that AVT produces an increase in the permeability of the collecting ducts to water and that this permeability is more readily altered by AVT than the whole-kidney GFR, and thus, presumably the number of functioning nephrons and the volume flow through the collecting ducts. At plasma concentrations of AVT equivalent to those found with 24 hours of dehydration, significant decreases in the number of filtering reptilian-type nephrons apparently occur, conserving water by reducing the amount of filtrate and slightly enhancing the concentrating ability by reducing flow through the collecting ducts. It must be remembered, however, that changes in the tubular permeability to water have not yet been measured directly in birds. Moreover, this apparent pattern of greater tubular than glomerular sensitivity to AVT may not hold for all avian species, not even for all gallinaceous species. Finally, it should be noted that in some avian species, e.g., starling (*S. vulgaris*), an increase in the number of filtering mammalian-type nephrons during the administration of a salt load (vide supra; Chap. 3) when the plasma AVT levels are presumably increased may also enhance the concentrating ability by permitting more nephrons to produce a greater osmotic gradient along the medullary cones.

Chapter 8

Integrative Summary of Renal Function

8.1 Introduction

Understanding of renal glomerular and tubular functions discussed in the preceding chapters is far from complete, even for mammals. It is much less complete for the nonmammalian vertebrates that are the primary subjects of this volume. Nevertheless, it is possible to make some integrative generalizations about renal function in each major group of vertebrates and some general comparisons and contrasts of renal function among groups of vertebrates. However, these are just generalizations. They do not include all the functional details discussed earlier. Moreover, they may be modified in detail for individual species within a given vertebrate group.

8.2 Integrative Summary Within Each of the Major Vertebrate Groups

8.2.1 Fishes

8.2.1.1 Cyclostomes

Myxini (*hagfishes*). These primitive marine animals are in osmotic equilibrium with their seawater environment, the osmolality of their body fluids being determined primarily by the concentrations of sodium and chloride. Their kidneys have relatively few large glomeruli which empty via a short neck segment into a primitive archinephric duct (ureter). Both the whole-kidney and single-nephron glomerular filtration rates are high, possibly because of the large area available for filtration. As the filtrate passes along the archinephric duct, glucose is absorbed and urea, potassium, phosphate, magnesium, and sulfate are secreted, but essentially no filtered sodium or water is absorbed. The final urine is isosmotic with the body fluids and sea-water. Thus, the kidneys function to help regulate the body content of some organic compounds, divalent ions, and potassium and, possibly, the total body fluid volume. They do not, however, play any role in regulating the osmolality or sodium concentrations of the body fluids.

Petromyzones (*lampreys*). These primitive euryhaline animals, unlike the hagfishes, do not conform to their environment. Instead, they maintain their body fluids hyperosmotic to a freshwater environment and hypoosmotic to a

seawater environment. Their kidneys have nephrons with a gross structure similar to those of more advanced vertebrates. Each nephron has a relatively large glomerulus, short neck segment, proximal tubule, intermediate segment, distal tubule, and collecting duct. Animals adapted to freshwater have moderately high whole-kidney and single-nephron glomerular filtration rates. Filtration equilibrium is not reached along the glomerular capillaries; the single nephron filtration rate is particularly sensitive to changes in hydrostatic pressure but not to changes in plasma flow rate. Only about 40% of the filtered water is absorbed, most of it in the collecting ducts. However, over 90% of the filtered sodium, chloride, and even potassium is absorbed, again primarily along the distal tubules and collecting ducts. This absorption of ions, far in excess of the absorption of water, results in a final urine only one-tenth the osmolality of the plasma, thereby permitting the animals to eliminate excess water and helping them to maintain the osmolality of their body fluids well above that of the surrounding freshwater.

Much less information is available about lampreys adapted to seawater than about those adapted to freshwater. However, seawater-adapted lampreys have whole-kidney glomerular filtration rates far below those of freshwater-adapted animals, apparently resulting from a decrease in the filtration rate of each nephron, not from a decrease in the number of nephrons filtering. Under these circumstances, filtration equilibrium is achieved along the glomerular capillaries and the single nephron glomerular filtration rate is particularly sensitive to changes in the plasma flow rate along the glomerular capillaries. In contrast to the freshwater-adapted lampreys, 90% of the filtered water is absorbed by the renal tubules. As in the freshwater-adapted animals, however, this absorption of filtered water in seawater-adapted animals occurs primarily in the distal tubules and collecting ducts. With the decrease in filtration rate and the absorption of most of the filtered water the kidneys of seawater-adapted lampreys help to retain water and maintain the osmolality of the body fluids below that of the surrounding seawater. However, the factors regulating these changes in renal function between freshwater and seawater are unknown.

8.2.1.2 Elasmobranchs

Marine elasmobranchs, like the hagfishes, conform to the osmolality of their environment, but, unlike the hagfishes, much of the osmolality of the extracellular fluid is determined not by the concentrations of sodium and chloride but by the concentrations of urea and TMAO. Therefore, these animals that ingest seawater must excrete absorbed divalent ions, e.g., magnesium and sulfate and monovalent ions, e.g., sodium and chloride and retain urea and TMAO.

Although the elasmobranch nephrons contain all the standard components found in advanced vertebrates, the glomeruli are quite large and the proximal and distal tubules are long with a highly complex arrangement. This arrangement may play a role in urea absorption and retention, possibly by permitting some form of passive but apparently concentrative transport that is coupled indirectly to the transport of sodium.

Whole-kidney glomerular filtration rates in marine elasmobranchs are somewhat higher than those of marine teleosts and a large fraction of the filtrate can be absorbed by the renal tubules. However, the apparent fraction of filtrate absorbed can vary over an extremely large range. Moreover, it must be kept in mind that substantial fluid secretion by the renal tubules can occur, perhaps at a rate equaling the rate of glomerular filtration. And, although fluid secretion may be most important when glomerular filtration is low, providing a medium for the excretion of ions such as magnesium and sulfate, the exact integration and control of the processes of fluid filtration and fluid secretion are not understood.

Because the renal tissues of marine elasmobranchs have proved useful for physiological studies of transport in vitro, much is known about a number of specific transport processes but little about the overall integration of these processes. Sodium chloride is secreted in the second segment of the proximal tubule by a secondary active transport process which also provides the driving force for the secretion of water. However, sodium chloride is apparently absorbed in this segment as well as in other tubule segments. For both the absorptive and secretory processes, the primary energy is provided by the Na-K-ATPase on the basolateral membrane. Although the secretory process may be controlled in part by regulation of chloride channels in the luminal membrane by cAMP, the actual way in which the secretory and absorptive processes are integrated is not understood. In general, what appears to be two thirds of the filtered sodium chloride is absorbed by the renal tubules, although this may not be a true value because of the sodium chloride secretion. This absorption produces some variable dilution of the tubular fluid, apparently along the collecting duct, but the control and physiological significance of this dilution in these marine animals is unclear. Much of the sodium chloride absorbed by the renal tubules may be secreted by the rectal gland, a structure specialized to secrete these ions and to rid the animals of excess ingested sodium chloride. However, whether renal absorption of filtered and secreted sodium chloride is regulated in any way relative to the secretion by the rectal gland is unknown.

Magnesium, sulfate, calcium, and phosphate, although filtered, are also secreted in the second segment of the proximal tubule. Such net tubular secretion (and ultimate excretion) is important for the removal of these divalent ions ingested in seawater. In fact, this excretion of divalent ions is generally considered to be quantitatively more important physiologically than any renal excretion of other ions and water. Finally, the urine of marine elasmobranchs is always acid with an unchanging pH of about 5.8. The total acidification takes place in the early proximal tubule by a process involving direct bicarbonate absorption as well as hydrogen ion secretion. Since the pH of the final urine does not change, this acidification may be less significant for the regulation of acid excretion than for the maintenance of the solubility of the divalent ions secreted in the second part of the proximal tubule.

8.2.1.3 Marine Teleosts

The stenohaline marine teleosts, which maintain the osmolality of their body fluids well below that of the surrounding seawater, ingest seawater and must

excrete absorbed divalent ions, e.g., magnesium and sulfate, and monovalent ions, e.g., sodium and chloride. Their kidneys have relatively few small or no glomeruli and the nephrons lack distal tubules. The filtration rate for glomerular species is low and the urine flow rate for all species is low. Most of the filtered water is absorbed, apparently secondarily to absorption of sodium chloride. Magnesium, sulfate, calcium, and phosphate, for all of which the kidney is the primary excretory route, undergo net secretion apparently along the second segment of the proximal tubule. Net secretion of fluid, primarily dependent on the secretion of sodium chloride by the same mechanism as in the elasmobranchs, also occurs along the second segment of the proximal tubule. This fluid secretion determines the actual urine flow rate in aglomerular and, probably, glomerular species. The final urine is close to isosmotic with plasma, but the concentrations of sodium and chloride are usually low, and the urine may be slightly hypoosmotic. The excess sodium chloride absorbed by the nephrons and the gastrointestinal tract is excreted via the gills.

8.2.1.4 Freshwater Teleosts

The true stenohaline freshwater teleosts, which maintain the osmolality of their body fluids well above that of the surrounding freshwater, must excrete excess water and retain ions. The kidneys of most species have larger and more numerous glomeruli than those of the stenohaline marine teleosts, and the nephrons contain well-developed distal tubules. Whole-kidney glomerular filtration rates are much higher than those of marine teleosts, and the urine is copious and dilute. There is no net tubular secretion of divalent ions. Most of the filtered sodium chloride is absorbed; this absorption along the distal tubules, which are always essentially impermeable to water, can produce a urine with an osmolality one-tenth that of the plasma. The primary diluting process takes place in the early distal tubule where secondary active sodium-coupled chloride absorption involving a sodium-two-chloride-potassium transporter occurs. The few aglomerular freshwater teleosts apparently produce urine by net tubular secretion of ions (most likely sodium and chloride) and water in one nephron segment and the absorption of the ions without accompanying water in a more distal segment or bladder.

8.2.1.5 Euryhaline Teleosts

The euryhaline teleosts, which can maintain osmolality of body fluids below that of seawater when adapted to seawater and above that of freshwater when adapted to freshwater, must excrete excess ions and retain water in the former case and must excrete excess water and retain ions in the latter. Like the stenohaline freshwater teleosts, these animals have kidneys with numerous moderately large glomeruli and nephrons with distal tubules. In sea-water-adapted animals, the number of glomeruli filtering is low and, thus, the whole-kidney glomerular filtration rate is low. The low number of filtering glomeruli may result from enhanced α-adrenergic nerve activity, enhanced release of arginine vasotocin, enhanced release of angiotensin II, suppressed release of prolactin, or some combination of these factors. With adaptation to seawater, net

secretion of magnesium, sulfate, calcium, and phosphate occurs primarily in the second segment of the proximal tubule. Net secretion of sodium chloride and water by the same mechanism as in elasmobranchs and stenohaline marine teleosts also occurs in the second segment of the proximal tubule of euryhaline teleosts adapted to seawater. As in marine teleosts, this net fluid secretion may determine the actual rate of urine flow. Much of the filtered and secreted sodium chloride is apparently absorbed, but the control of the balance between secretion and absorption, as in other marine fishes, is unknown. This absorption can result in some dilution of the tubular fluid. However, this dilution is slight because the nephrons are quite permeable to water. The mechanism controlling this permeability is unknown. The final urine in these seawater-adapted animals tends to be close to isosmotic with the plasma and contains significant concentrations of divalent ions as well as sodium and chloride.

In freshwater-adapted animals in which the number of glomeruli filtering is high, whole-kidney glomerular filtration rate is consequently high. The large number of filtering glomeruli may result from depressed α-adrenergic nerve activity, suppressed release of arginine vasotocin, suppressed release of angiotensin II, enhanced release of prolactin, or some combination of these factors. Net secretion of divalent ions ceases. Net secretion of sodium chloride and water in the second segment of the proximal tubule may continue, but it apparently has little influence on the final rate of urine flow as long as the glomerular filtration rate is high. The epithelium of the distal tubules becomes impermeable to water, but absorption of sodium chloride continues and a dilute urine is produced, thus enabling the animals to excrete excess water. Dilution in the early distal tubule apparently involves the same secondary active, sodium-coupled chloride transport as in the stenohaline freshwater teleosts. The mechanism regulating the permeability to water of the epithelium of the distal tubules is unknown.

As these renal changes occur with adaptation to different aqueous environments, simultaneous appropriate changes in ion and water movements across the gills and bladder occur. In fact, the adaptation of all the fishes to their particular environment involves the coordinated regulation of ion and water transport through the kidneys, gills, integument, bladder, gastrointestinal tract, and specialized ion-transporting structures, such as the elasmobranch rectal gland.

8.2.2 Amphibians

Amphibians live, and indeed thrive in habitats ranging from completely aqueous — usually freshwater, but occasionally seawater — to arid terrestrial. Their kidneys contain nephrons with large glomeruli and all the standard segments. However, the nephrons do not contain loops of Henle and are not arranged in a manner to permit them to produce a urine hyperosmotic to the plasma.

For the wholly aquatic species, the number of glomeruli filtering and, consequently, the whole-kidney glomerular filtration rate decrease with adaptation to seawater and increase with adaptation to freshwater. For semi-aquatic

and terrestrial species, they decrease with dehydration and increase with hydration. The number of glomeruli filtering is controlled primarily by arginine vasotocin and secondarily by α-adrenergic nerves. The number filtering decreases with increased levels of arginine vasotocin and α-adrenergic activity and increases with decreased levels of the hormone and neural activity. The rates of filtration by individual glomeruli may also be controlled by a distal tubule-glomerular feedback mechanism in each nephron. The single nephron glomerular filtration rates of well-hydrated animals or animals in freshwater are, like those of the hagfishes, quite high for the observed net filtration pressure. This may reflect the large area available for filtration and may be important for the excretion of excess water.

Although the structure and arrangement of amphibian nephrons do not permit them to produce a urine hyperosmotic to the plasma, they are capable of producing a urine varying from one-tenth the osmolality of the plasma during hydration, when excretion of excess water is required, to nearly isosmotic with the plasma during dehydration, when retention of water is required. Luminal fluid hypoosmotic to the plasma is always generated in the early portion of the distal tubule where sodium chloride is absorbed without water. This absorption process involves the same secondary active, sodium-coupled chloride transport as in the early distal tubules of freshwater teleosts and euryhaline teleosts adapted to freshwater. During dehydration, arginine vasotocin apparently acts at a tubule site distal to this diluting area to increase the permeability of the epithelium to water and permit the fluid in the tubules to equilibrate with the surrounding interstitium, thus forming a final urine isosmotic with the plasma. The decrease in the number of glomeruli filtering during dehydration, in addition to conserving water by reducing the quantity of initial filtrate, also reduces the volume flow rate through the collecting ducts and this may contribute to the equilibration process.

Although the bulk of the filtered sodium is absorbed by the renal tubules of amphibians, some 10–15% is not, the remainder apparently being absorbed in the bladder or cloaca depending on the requirements of the animals. Of the sodium absorbed by the renal tubules, some one-half to two-thirds is absorbed in the distal tubules and collecting ducts. Sodium absorption, at least in the diluting segment and probably in other regions of the distal tubule, is stimulated by aldosterone, which may also play an important role in regulating sodium absorption in the bladder or cloaca. Sodium absorption, most likely in the distal portions of the nephrons, also may be stimulated by arginine vasotocin and angiotensin. Exactly how these endocrine controls are coordinated in maintenance of sodium balance in amphibians is unknown.

Much of the filtered potassium is absorbed, but net secretion can occur throughout the distal tubule, including the diluting segment, and possibly along the collecting duct. This net secretion is apparently under the control of aldosterone.

Amphibians, like birds and mammals, can significantly acidify their tubular fluid, but all such acidification occurs in the distal tubules. This acidification process, to a large extent, may invole a sodium-hydrogen countertransport mechanism for hydrogen ion secretion by the tubules, controlled in part by aldo-

sterone. In addition, acidification, in contrast to that in mammals, may involve the absorption of a large fraction of the filtered bicarbonate in the distal portion of the nephron.

For most amphibians, urea is the major excretory end product of nitrogen metabolism and may even be secreted by a primary or secondary active transport process in the renal tubules of some anuran species. However, for some xerophilic anuran amphibians urates are the major excretory end products and undergo net secretion by the renal tubules. Urates have low solubilities and can be excreted with very little water. Moreover, urate precipitates also may contain large quantities of inorganic cations that can therefore be excreted without contributing to the osmolality of the urine. Finally, as in the case of fishes, maintenance of fluid, electrolyte, and acid-base balance in amphibians adapted to various environments requires the coordinated regulation of renal function with ion and water movements across the skin and the colon, cloaca, or bladder.

8.2.3 Reptiles

Habitats of reptiles, like those of amphibians, range from completely aqueous, both freshwater and seawater, to arid terrestrial. Their nephrons contain glomeruli of modest size and the standard segments. As in amphibians, these nephrons lack loops of Henle, although the ciliated intermediate segment may vary greatly in length among species, and they are not arranged in a manner to permit them to produce a urine hyperosmotic to the plasma.

In general, as in amphibians, the number of glomeruli filtering and therefore whole-kidney glomerular filtration rate in reptiles tend to increase with hydration and decrease with dehydration. However, the changes are not always as rapid or as striking as in amphibians, possibly simply because of differences in the changes in hydration under which the measurements were made. The number of glomeruli filtering is apparently controlled primarily by arginine vasotocin but possibly also by prolactin, the number filtering decreasing with stimulated release of arginine vasotocin and suppressed release of prolactin and increasing with suppressed release of arginine vasotocin and stimulated release of prolactin.

Although the structure and arrangement of the nephrons in the reptilian kidney do not permit production of urine hyperosmotic to plasma, nephrons in many species are capable of producing a urine varying from one-tenth the osmolality of plasma to isosmotic with it, depending on the degree of hydration of the animal. In some species, however, the nephrons produce a ureteral urine that is always isosmotic with the plasma. Apparently, when necessary in these species, additional regulation of the urine osmolality takes place distal to the kidney — in the colon, cloaca, or bladder.

In those species capable of producing a urine of low osmolality, the initial dilution apparently occurs through the absorption of sodium chloride without water in the early distal tubule or, possibly, in the thin intermediate segment between the proximal and distal tubules. In some species, this diluting process may continue throughout the distal tubules and collecting ducts. The site of the

initial dilution and the mechanism of sodium chloride transport involved in that dilution are not as clearly defined in reptiles as in fishes, amphibians, birds, and mammals. In those species capable of varying the urine osmolality, the urine diluted in one portion of a nephron apparently can equilibrate to varying degrees with the interstitium surrounding a more distal portion of that nephron or the collecting ducts. The extent of the equilibration may be determined in part by the permeability of the distal tubule or the collecting ducts and in part by the volume flow through these structures. Permeability is controlled by arginine vasotocin and volume flow primarily by the number of filtering nephrons. The latter is also controlled primarily by arginine vasotocin. Therefore, arginine vasotocin, either directly through its action on epithelial permeability or indirectly through its regulation of the number of filtering nephrons and thus the volume flow rate through the collecting ducts, regulates the urine osmolality.

In reptiles, as in amphibians, although most of the filtered sodium is absorbed by the renal tubules, some 5–10% is not. The remainder apparently is absorbed in structures distal to the kidneys — colon, cloaca, or bladder — depending on the requirements of the animal. Again, as in the case of the amphibians, only about one-third to one-half the sodium absorbed by the nephrons is absorbed in the proximal tubule. The rest is absorbed in the more distal segments. Although there may be some regulation of distal absorption by aldosterone and possibly even by arginine vasotocin, hormonal control of sodium absorption in reptiles is not at all clear. Little is known about sodium transport processes in reptilian renal tubules but there appears to be an intrinsic cellular regulation of late distal sodium absorption in some species. This regulation appears poised to prevent additional absorption from relatively high luminal concentrations, thus permitting excretion of excess sodium when required, and enhancement of continued absorption from very low concentrations, thereby permitting further dilution of an already dilute urine and excretion of a water load.

Either net secretion or net absorption of potassium by the tubules of intact reptiles can be observed. Net absorption always occurs along the proximal tubules and overall net secretion, when it occurs, results from net secretion along the distal tubules. However, the transport mechanisms and the manner in which they are controlled in reptiles are not at all understood. Reptiles may not be able to acidify tubular fluid as much as mammals, birds, and amphibians, but further acidification apparently can occur in the bladder or cloaca.

In many species, net tubular secretion of phosphate, which is often quite high in the diet, can occur. This process is dependent on, and under the control of parathyroid hormone.

Urates are the major excretory end-products of nitrogen metabolism in all reptiles except some testudines from aquatic or mesic terrestrial habitats. In these latter species, urea is the major excretory end-product of nitrogen metabolism, but urates are still important. Although urate is filtered, its excretion depends primarily on net secretion by the proximal renal tubules. As noted above for the uricotelic amphibians, the low solubility of urate permits it to be excreted with very little water. Also, inorganic cations contained in urate precipitates can be excreted without contributing to the osmolality of the urine.

However, many of these cations may be absorbed in regions distal to the kidney — cloaca or bladder — where hydrogen ions may be secreted and urate complexes may be converted to uric acid. Indeed, maintenance of fluid, electrolyte, and acid-base balance in reptiles, as in fishes and amphibians, requires the coordinated regulation of renal function and the movements of ions and water across the integument, across the colon, cloaca, or bladder, and in some species, through structures specialized for the hyperosmotic excretion of ions, e.g., salt glands.

8.2.4 Birds

Habitats of birds, like those of amphibians and reptiles, range from aqueous — both freshwater and seawater — to arid terrestrial, although flight for many birds determines the intensity of exposure to a specific habitat. Avian kidneys contain a majority of nephrons resembling those of reptiles and most other nonmammalian vertebrates, i.e., reptilian-type nephrons, and a minority resembling those of mammals, i.e., mammalian-type nephrons. The parallel arrangement of the loops of Henle of the mammalian-type nephrons, the vasa recta arising from the efferent glomerular arterioles of these nephrons, and the collecting ducts draining all nephrons apparently permits birds, like mammals, to produce a urine hyperosmotic to the plasma. Birds also can produce a urine hypoosmotic to the plasma, apparently by absorbing sodium chloride without accompanying water along the thick ascending limbs of Henle's loops of the mammalian-type nephrons and the early distal tubules of the reptilian-type nephrons. This absorptive process apparently involves the same secondary active, sodium-coupled chloride transport as in the early distal tubules of teleost and amphibian nephrons and the thick ascending limbs of mammalian nephrons. Moreover, in birds the ascending limb of the mammalian-type nephrons consists entirely of a thick segment so that not only dilution but also concentration of the urine depend on sodium chloride absorption by this segment. Urea plays no role in the production of a urine hyperosmotic to the plasma.

The osmolality of the avian urine varies from about one-tenth that of the plasma during maximum hydration to about two to three times that of the plasma during maximum tolerable dehydration. This variation in urine osmolality with hydration is apparently controlled by arginine vasotocin, which regulates the permeability of the collecting ducts to water and the number of filtering nephrons. Both a change in the permeability of the collecting ducts to water and a change in the number of filtering nephrons and, hence, the volume flow through the collecting ducts, can influence the equilibration of fluid in the collecting ducts with the surrounding interstitium and thus the final osmolality of the urine. It appears, however, that the permeability of the collecting ducts to water is more sensitive to arginine vasotocin than the number of filtering nephrons.

In birds, as in mammals, the bulk of the filtered sodium and chloride is absorbed along the proximal tubule. The absorption of sodium by the distal tubules may be stimulated by arginine vasotocin, aldosterone, and cortico-

sterone, and inhibited by angiotensin II, but the direct effects of these hormones on the transport process and the integration of their effects in the adaptation of birds to their environment have yet to be evaluated. Although net potassium secretion may occur along the proximal tubules of some superficial reptilian-type nephrons, major excretion of potassium appears to result primarily from net secretion along the distal nephrons. This secretion may be stimulated by aldosterone and corticosterone but, again, this control process has not been studied in detail.

Birds, like both amphibians and mammals, can produce a highly acidified ureteral urine in response to an acid load. However, like amphibians but unlike mammals, little acidification appears to occur along the proximal tubules. Apparently, both significant bicarbonate absorption and significant hydrogen ion secretion can occur in the distal portions of the nephrons when acid excretion is required. Neither the exact tubule sites nor the mechanisms of bicarbonate and hydrogen ion transport have yet been studied.

Either net tubular absorption or net tubular secretion of phosphate occurs in birds, apparently depending on existing requirements. Both processes occur along the proximal tubules of the superficial reptilian-type nephrons and net secretion requires the presence of parathyroid hormone.

Urates are the major excretory end-products of nitrogen metabolism in all birds. Although urate is filtered, its excretion, as in reptiles, depends primarily on net secretion by the renal tubules. This certainly occurs along the proximal tubules of the superficial reptilian-type nephrons, but whether other tubule sites are involved is unknown. As in the case of the uricotelic amphibians and reptiles, the combination of inorganic cations with urate precipitates permits the excretion of a larger quantity of these than could be accommodated independently by the maximum urine osmolality. Finally, some birds of marine or arid terrestrial habitats have specialized extrarenal structures, i.e., salt glands, for the hyperosmotic excretion of ions. The maintenance of fluid, electrolyte, and acid-base balance in birds in the process of adaptation to their environment, as in other nonmammalian vertebrates, requires the coordinated regulation of renal functions and the transport of ions and water by these salt glands and across the colon and cloaca.

8.2.5 Mammals

Mammals, like amphibians, reptiles, and birds, have habitats ranging from completely aqueous to arid terrestrial. Nephrons of mammalian kidneys contain rather large glomeruli with highly complex multi-branched capillary tufts, proximal convoluted and straight tubules, loops of Henle with thin and thick limbs, distal convoluted tubules, connecting tubules, and collecting ducts. The loops of Henle, collecting ducts, and vasa recta are arranged in parallel, permitting production of a urine hyperosmotic to the plasma. Mammals also can produce a urine hypoosmotic to the plasma by absorbing sodium chloride without accompanying water primarily along the thick ascending limbs of Henle's loops. This absorptive process apparently involves the same secondary active, sodium-coupled chloride transport as in the early distal tubules of teleost

nephrons, amphibian nephrons, reptilian-type avian nephrons and in the thick ascending limb of mammalian-type avian nephrons. Sodium chloride absorption in the thick ascending limbs of Henle's loops in mammals is important not only for diluting the urine but also, as in birds, for the generation of the osmotic gradient necessary for the production of a concentrated urine. However, in contrast to the avian kidney, the concentrating process also depends on the presence of urea for the formation of this osmotic gradient.

The osmolality of the mammalian urine varies from about two-tenths that of the plasma during maximum hydration to as much as 25 times that of the plasma in some desert species that have no access to free water. This variation in urine osmolality with hydration is apparently controlled by arginine vasopressin, which regulates the permeability of the collecting ducts to water. In contrast to nonmammalian species, the number of filtering glomeruli does not normally vary; therefore, alterations in volume flow rate along the collecting ducts as a result of changes in glomerular filtration rate do not normally play a role in the concentrating or diluting processes.

The mammalian nephrons, like those of birds but unlike those of other nonmammalian vertebrates, are capable of absorbing almost all the filtered sodium and chloride. Mammals, unlike most nonmammalian vertebrates, do not have any extrarenal mechanisms for significant regulation of sodium chloride excretion. In mammals, as in birds but not as in other vertebrates, most of the filtered sodium and chloride is absorbed along the proximal tubules. Although the absorptive process for sodium chloride in the proximal tubules of mammals is quantitatively different from that in the proximal tubules of most nonmammalian vertebrates, some aspects of sodium absorption, i.e., transport across the luminal membrane coupled to organic solutes or by sodium-hydrogen exchange and transport across the basolateral membrane involving Na-K-ATPase, are the same in mammals and amphibians. Distal sodium absorption, which is of critical importance in mammals in determining the final sodium excretion, is clearly stimulated by aldosterone, primarily in the cortical-collecting tubules. This is in marked contrast to the limited or unclear effect of adrenocorticosteroids in most nonmammalian vertebrates. Arginine vasopressin may also stimulate sodium absorption in the thick ascending limb of some mammalian species.

As in most nonmammalian vertebrates, either net tubular absorption or net tubular secretion of potassium can occur in mammals. Net absorption always occurs along the proximal tubules and overall net secretion, when it occurs, results from net secretion along the distal nephrons, primarily the late distal tubule or connecting tubule and the cortical-collecting duct. This net secretion appears to be under the control of aldosterone.

Mammals, like amphibians and birds, can produce a highly acidified ureteral urine in response to an acid load. However, in contrast to amphibians and birds, significant net secretion of acid occurs along the proximal tubules where most of the filtered bicarbonate is absorbed. This process apparently involves the sodium-hydrogen exchanger at the luminal membrane. Additional acidification, apparently involving a primary electrogenic hydrogen-ion pump at the luminal membranes of the intercalated cells, can occur in the connecting tubules and cortical-collecting ducts. Although the remainder of the filtered bicarbonate can

be absorbed along the distal tubules and cortical and medullary collecting ducts, net bicarbonate secretion also can occur along the cortical collecting ducts when necessary.

Urea is the major excretory end-product of nitrogen metabolism in all mammals. Its excretion involves filtration and variable degrees of net tubular absorption. As noted above, urea contributes to the medullary osmotic gradient involved in the mammalian concentrating process. Such a concentrating process is required for the excretion of urea with little water. Finally, it must be stressed that there are no extrarenal routes contributing to the regulation of ion and fluid excretion in mammals adapted to different environments.

8.3 Summary Comparisons and Contrasts of Renal Function Among Groups of Vertebrates

8.3.1 Glomerular Filtration Rate — Stability Versus Lability

The importance of the ultrafiltration of the arterial plasma as the primary step in urine formation in almost all vertebrates has been discussed in Chapter 3. The differences in normal filtration rates among species and classes are directly dependent physically on the differences in pressures, plasma flows, surface areas, and hydraulic conductivities, but they also may relate to the balance between the conflicting requirements to eliminate water and metabolic wastes and to conserve water, salts, metabolites, and energy placed on any animal group by its habitat and mode of existence. Of particular interest in comparing the vertebrate groups, however, is the short-term lability or stability of the glomerular filtration rate with environmental changes, particularly changes in hydration.

In general, the whole-kidney glomerular filtration rate of mammals is remarkably stable during rapid moderate changes in hydration. The stability during dehydration probably reflects the ability of the renal tubules of most mammals to absorb almost all filtered water and to produce a urine far more concentrated than plasma. There is no need to reduce filtration to conserve water in response to moderate dehydration that might readily be experienced by these animals. However, a few species from arid habitats do show a decrease in whole-kidney GFR with what appears to be a degree of dehydration that they could routinely experience. Whether this degree of dehydration, although "physiological" in the sense that it might be regularly experienced, is simply too great to be compensated by tubular absorption is not completely clear, but a number of these species do not have an exceptional ability to concentrate their urine. Of course, all mammals will show a reduction in whole-kidney GFR with severe dehydration. Moreover, when changes in whole-kidney GFR do occur, whether physiological or not, they generally reflect changes in the filtration rates of individual nephrons with all nephrons filtering rather than in the number of nephrons filtering. Only in extreme circumstances — probably pathological dehydration — does the number of nephrons filtering decrease.

In contrast to mammals, most nonmammalian vertebrates have highly labile whole-kidney glomerular filtration rates. These tend to increase with hydration or adaptation to freshwater and to decrease with dehydration or adaptation to seawater. Modest changes in hydration usually can produce highly significant changes in whole-kidney GFR. These changes in whole-kidney GFR are most striking and occur most readily in amphibians and reptiles, but significant changes occur even in those fishes that adapt easily to seawater or freshwater, and in birds. The changes, particularly in fishes, amphibians, and reptiles, appear to reflect an inability to alter to as large an extent as mammals the amount of filtered water absorbed by the renal tubules. In some species, there is apparently no ability to control directly the fraction of filtered water absorbed by the tubules. Decreases in GFR with dehydration certainly reflect the inability to produce a urine hyperosmotic to plasma. Such changes in whole-kidney GFR with hydration may occur at the expense of regulating the excretion of certain ions and metabolic wastes, but this depends on the degree to which tubular and extrarenal transport processes can compensate for changes in filtration rate.

The changes in the whole-kidney GFR of nonmammalian vertebrates, also in contrast to mammals, reflect primarily changes in the number of nephrons filtering, although changes in the single nephron glomerular filtration rates also can occur. Such alterations in the number of nephrons filtering is practical for most nonmammalian vertebrales because the nephrons do not function in concert to produce a urine hyperosmotic to plasma. Also, almost all nonmammalian vertebrates showing glomerular intermittence have renal venous portal systems that supply oxygen and nutrients to the tubules in the absence of a post-glomerular arterial blood supply. Those few fishes that apparently show glomerular intermittence without a renal venous portal system may have some form of collateral circulation from post-glomerular arterioles. The one nonmammalian species that definitely does not show glomerular intermittence, the primitive lamprey, does not have a renal venous portal system. Changes in the number of filtering nephrons, often under the control of arginine vasotocin, alter the volume flow through the collecting ducts. Whether or not the permeability of these collecting ducts to water is increased by arginine vasotocin, the changes in volume flow rate may help to determine the degree to which tubular fluid diluted in an earlier portion of the tubules, usually the early distal tubules, equilibrates with the interstitium surrounding the collecting ducts.

Birds, with their reptilian-type and mammalian-type nephrons, lie somewhere between the mammals and the other nonmammalian vertebrates. Although capable of producing a urine with an osmolality varying from about one-tenth to two to three times the osmolality of plasma, birds still show changes in whole-kidney GFR with hydration. These changes, controlled mainly by arginine vasotocin, apparently involve primarily changes in the number of filtering reptilian-type nephrons, which do not function in concert and have a renal venous portal blood supply. As in other nonmammalian vertebrates, such changes alter the volume flow through the collecting ducts in the medullary cones and could influence equilibration with the surrounding interstitium and thereby the dilution and concentration of the urine. However, it appears that tubular permeability to water is more sensitive to arginine vasotocin and degree

of dehydration than the number of functioning nephrons. Thus, during changes in hydration, the whole-kidney GFR of birds appears to be less stable than that of mammals but less labile than that of other nonmammalian vertebrates.

8.3.2 Diluting and Concentrating Process

Representatives of all tetrapod vertebrate classes, as well as many fishes, are capable of producing a ureteral urine of lower osmolality than their plasma. Some fishes — primarily the stenohaline marine teleosts, as well as a number of reptiles — are not capable of producing a dilute urine. Those animals capable of producing the most dilute urine are those that are subject to the greatest water load. Many of those that cannot produce a dilute ureteral urine are never exposed to a water load, but others, particularly some reptiles that produce a urine of fixed osmolality, are exposed to varying amounts of water in their environment. Some of these animals may be capable of diluting the urine in regions distal to the kidney.

All animals capable of producing a dilute ureteral urine do so by absorbing filtered solute, primarily sodium and chloride, without accompanying water somewhere along the nephrons. The site of such absorption and the mechanism involved appear to be essentially the same in all vertebrates, i.e., apparently the early distal segment of teleost, amphibian, and reptilian-type avian nephrons and the thick ascending limb of Henle's loop of mammalian-type avian and mammalian nephrons. Although the site has not been determined for reptilian nephrons, it probably involves the early distal tubule or, possibly, the thin intermediate segment. The mechanism in all cases in which the site is known apparently involves secondary active, sodium-coupled chloride absorption, the energy for which is derived from primary active sodium transport out of the cells at the peritubular membrane via Na-K-ATPase. The coupled, electrically neutral transport step into the cells across the luminal membrane apparently involves two chlorides, one sodium, and one potassium.

Changing from a maximally dilute urine to an isosmotic urine conserves large amounts of water, just as changing from an isosmotic urine to a maximally dilute urine permits the excretion of large amounts of water. In animals that can produce a dilute urine, dilution always occurs along the tubules as described above. The degree to which final ureteral urine approaches isosmoticity then depends on the degree to which the diluted tubule fluid equilibrates with the surrounding interstitium in portions of the tubule distal to the diluting area. The degree of equilibration is apparently controlled by antidiuretic hormone — arginine vasotocin in nonmammalian vertebrates; arginine or lysine vasopressin in mammals — which determines the permeability of the distal nephrons (apparently the collecting ducts) to water. However, the effect of antidiuretic hormone on the permeability of the nephrons to water, although strongly suggested by indirect studies in many vertebrates, has only been demonstrated with certainty in mammals. In some animals, the hormone clearly acts on structures distal to the kidney, e.g., the urinary bladder of anuran amphibians, to produce the same effect and to alter the osmolality of the urine before it is excreted. As noted

above, changes in glomerular filtration, usually the number of filtering nephrons, in nonmammalian vertebrates, also controlled by arginine vasotocin, affect the volume flow rate through the collecting ducts. How important changes in volume flow rate are in the equilibration process relative to changes in epithelial water permeability is unknown. Therefore, how important the effect of antidiuretic hormone on the number of filtering nephrons is in the equilibration process relative to its effect on epithelial water permeability is also unknown. These relative effects await direct evaluation in nonmammalian vertebrates.

The production of a urine hyperosmotic to the plasma can conserve additional water, although not as much as changing from a maximally dilute urine to one isosmotic with plasma. In mammals the excretion of urea, the major excretory end product of nitrogen metabolism, with very little water requires the production of a concentrated urine. As noted above, urea also plays a role in the generation of the medullary osmotic gradient required by the mammalian concentrating process. In birds, uric acid, the major excretory end product of nitrogen metabolism, has a very low aqueous solubility. Therefore, its excretion with very little water does not require the production of urine with a high osmotality. Nevertheless, in birds a process remarkably similar to that in mammals has evolved for the production of a moderately concentrated urine. This process does not involve urea in birds. However, in both birds and mammals, as noted earlier, the primary driving force for the production of the medullary osmotic gradient is the coupled sodium-chloride absorption in the thick ascending limb of Henle's loops that is also responsible for diluting the urine. Again, in birds, in contrast to mammals, the volume flow rate through the collecting ducts may play some role in equilibration of the tubule fluid with the surrounding medullary interstitium.

Although the concentrating ability of birds appears modest compared with that of mammals, this may be misleading. Direct comparisons of the maximum U/P osmolar ratios of birds and mammals are not justified without considering the fact that the plasma osmolality of birds tends to increase more than that of mammals with dehydration. In addition, inorganic cations included with urate precipitates in avian ureteral urine are excreted without contributing to the osmotic pressure of the urine. Finally, a ureteral urine only moderately more concentrated than the plasma may permit efficient osmotically linked water absorption in the colon.

8.3.3 Sites and Mechanisms of Tubular Transport

Although data on sites and mechanisms of tubular transport among the vertebrates are still fragmentary, detailed information being available only for mammals and a few nonmammalian species, some general patterns of importance are emerging. As discussed above, the site and mechanism for dilution of the tubule fluid appear to be the same in all vertebrates — mammals and nonmammals — in which dilution occurs. In contrast to this uniformity among the vertebrates, the quantitative importance of the tubule sites for the absorption of filtered sodium and chloride varies. First, the absorption of filtered sodium and chloride along the renal tubules approaches completion only in

mammals and birds. Additional absorption in many nonmammalian vertebrates may occur in regions distal to the kidney — cloaca, colon, or bladder. To a significant extent this is true even for birds. Second, the proximal tubule is the major nephron site for the absorption of filtered sodium and chloride only in mammals and birds. The distal tubules and collecting ducts assume greater quantitative importance than the proximal tubules in most other nonmammalian vertebrates in which this process has been studied. There is as yet no evidence of an ultrastructural or subcellular biochemical basis for these quantitative differences.

A somewhat similar pattern in the quantitative importance of distal versus proximal transport sites exists for acid secretion and bicarbonate absorption. In mammals, significant acidification of the tubule fluid and the bulk of the bicarbonate absorption occurs along the proximal tubules. This process involves a sodium-hydrogen countertransport system at the luminal membrane and the action of carbonic anhydrase. In contrast, in amphibians, birds, and probably those reptiles that can produce an acidic ureteral urine, no acidification occurs along the proximal tubules. Although some bicarbonate absorption must occur in this portion of the tubules, it apparently is proportional to the rate of fluid absorption. Studies on amphibians suggest that, as in mammals, the process involves sodium-hydrogen exchange and may involve the action of carbonic anhydrase. However, the amount of carbonic anhydrase in the proximal tubule cells appears small and may reflect the limited quantitative importance of hydrogen ion secretion and bicarbonate absorption in this tubule region. Instead, the major acidification and the bulk of the bicarbonate absorption in amphibians, birds, and some reptiles appear to occur in the distal portions of the nephrons. Of course, further acidification and a quantitatively small fraction of bicarbonate absorption occur in the distal region of mammalian nephrons.

Studies on amphibians suggest that the distal acidification and bicarbonate absorption may occur in the early distal tubules, the diluting region, or the late distal tubules, the exact site perhaps differing in anurans and urodeles. The data indicate that the acidification and bicarbonate absorption processes involve a sodium-hydrogen countertransport system and the action of carbonic anhydrase. Histochemical data on amphibians suggest that the largest concentration of carbonic anhydrase occurs in the distal, rather than the proximal nephrons, again perhaps reflecting the quantitative importance of acid secretion and bicarbonate absorption in the distal nephrons.

In chelonian reptiles important acidification occurs even further distally, in the bladder, and apparently involves a primary electrogenic hydrogen-ion secretory system. An identical electrogenic hydrogen-ion secretory system appears to exist in mammalian connecting tubules and cortical collecting ducts, but significant bicarbonate absorption is not involved.

Of course, as noted above, data on nonmammalian vertebrates are still fragmentary and distinct exceptions to these patterns occur in some species. For example, maximum acidification and complete absorption of filtered bicarbonate occur along the proximal tubules of elasmobranchs, but there is no involvement of carbonic anhydrase. In alligators, net tubular secretion of bicarbonate, apparently involving the action of carbonic anhydrase, always occurs.

A few distinctive patterns of tubular transport — the net secretion of magnesium and sulfate by marine teleost proximal renal tubules; the net secretion of sodium chloride and water by teleost and elasmobranch proximal tubules; the net secretion or net absorption of taurine by proximal tubules of marine teleosts, marine elasmobranchs, and reptiles; and the net secretion of potassium under some circumstances by the distal nephrons of apparently all vertebrates — are being exploited as model systems to determine the mechanisms and controls involved. Studies of the net absorption of urea by the nephrons of some anuran amphibians have not yet revealed how the apparent active transport of this small polar molecule can occur, but this distinctive process deserves and probably will attract further study.

As is apparent from these summaries of major patterns as well as from the detailed coverage in the preceding chapters, a great deal remains to be learned about renal function among the nonmammalian vertebrates. Future studies may fill these gaps, revealing the adaptive patterns for individual groups and the basic mechanisms common to all groups.

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