Kidneys and Excretion
(with Notes on Nitrogen Excretion)

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An animal's body fluids are dynamic—continuously gaining and losing water, inorganic ions, and organic solutes. When a kangaroo rat in the desert is successfully maintaining water balance in moderate summer weather, about 15% of the water in its body fluids is lost and replaced each day. This rate of turnover is low for a mammal of its body size; the mice and rats of moist temperate habitats often turn over 35% or more of their water per day. Humans, being larger, have lower percentage turnover rates, but still when when people go about their ordinary daily lives, about 7% of their body water is lost and replaced every day. Inorganic ions and organic solutes in the body fluids also undergo incessant turnover.

The great dynamism of the body fluids means that their composition is continuously in danger of being shifted away from normal. As water, for example, leaves the body fluids and is replaced each day, any mismatch between the rates of loss and replacement can quickly cause the body fluids to become too dilute or too concentrated.

For the blood and other body fluids to be maintained at a normal composition, an animal requires organs that are capable of correcting any departures from normal that develop. The kidneys bear primary responsibility for this task in terrestrial animals such as kanagaroo rats. In aquatic animals such as fish and crayfish, the kidneys and gills are the organs primarily responsible.

Species of small mammals that have evolved in deserts are, among all animals, the extreme performers in their ability to concentrate their urine Excretion of dissolved wastes in as little water as possible is a key to maintaining water balance in water-poor environments. Around the world, each desert has its own assemblage of small mammals. Some of these unique animals—such as the kangaroo rats (main photo) of the American southwest—have been carefully studied. Others—such as the jerboas (inset) of Middle East deserts—have been little studied by physiologists. Shown here are Merriam's kangaroo rat (*Dipodomys merriami*) from the American southwest and the four-toed jerboa (*Allactaga tetradactyla*) from the northern Sahara in Egypt and Libya.

Kangaroo rats and other desert rodents that have been studied have a status in the physiology of kidney function not unlike the status of cheetahs in the physiology of running. The kidneys of these desert rodents represent the ultimate product of evolution in their ability to concentrate urine. Most types of animals cannot concentrate their urine at all: That is, most cannot produce urine with an osmotic pressure higher than the osmotic pressure of their blood plasma. Some insects can make urine that is 6–8 times higher in osmotic pressure than their blood plasma. Birds also can concentrate their urine, typically to an osmotic pressure that is 3 times their blood osmotic pressure, or lower. Many desert rodents, however, achieve far higher urine concentrations than any of these other groups of animals. Certain species of kangaroo rats can make urine that is 14 times higher in osmotic pressure than their blood plasma, and some of the desert hopping mice of Australia can make urine more than 20 times higher.[1]

The advantage of the high concentrating ability of the kidneys of desert rodents is made clear when we recognize the challenges these animals face in their severe environments. During the dynamic daily flux of water and solutes in and out of the body, the blood plasma of desert rodents tends often to be shifted toward concentrations higher than normal; dehydration in the desert, for example, raises the plasma concentration. When an animal's kidneys can produce urine with a higher osmotic pressure than the blood plasma, they can dilute the blood.[2] The kidneys of desert rodents, with their unique abilities to produce urine that is very hyperosmotic to the blood plasma, are exceptionally suited to lowering the osmotic pressure of the blood plasma when the blood has become too concentrated.

Considering animals in general, what are **kidneys**? We will see in this chapter that the kidneys of various types of animals are very diverse in morphology as well as in details of their physiology. All kidneys, however, have three features in common. First, they all consist of tubular elements that discharge directly or indirectly to the outside world. Second, they all produce and eliminate aqueous solutions derived from the blood plasma or other extracellular body fluids. Third, *their function is the regulation of the composition and volume of the blood plasma and other extracellular body fluids by means of controlled excretion of solutes and water.*

Urine, the product of the kidneys, is typically a complex solution containing multiple inorganic and organic solutes. All the constituents of the urine—including the water—are drawn from the blood plasma, and the urine concentration of each affects the blood concentration according to the principles we discussed in Chapter 27 (see page 733 and Figure 27.7). The urine often contains nitrogenous wastes, but the role of the urine is much more far-reaching than merely excreting waste nitrogen. The urine of a mammal, for example—although it contains urea (the nitrogenous end product)—also contains Na^+, Cl^-, K^+, PO_4^{3-}, SO_4^{2-}, creatinine, and numerous other components. The kidneys excrete each of these in greater or lesser amounts day by day, closely regulating the concentration of each in the blood plasma. The kidneys also excrete greater or lesser amounts of H^+ in the urine, thereby helping to maintain a steady blood pH. Moreover, the kidneys regulate the osmotic pressure of the blood by means of the controlled excretion of water relative to total solutes. It seems almost impossible—but is true—that the kidneys perform all these functions *simultaneously* by structuring the composition of a *single* fluid output: the urine.

Basic Mechanisms of Kidney Function

Urine formation can usually be conceptualized as occurring in two steps, although these "steps" may sometimes be partly contemporaneous. First, an aqueous solution, called **primary urine**, is introduced into the kidney tubules. Second, this solution is modified as it moves through the kidney tubules and other excretory passages, ultimately becoming the **definitive urine** that is eliminated.

Primary urine is introduced into kidney tubules by ultrafiltration or secretion

One widespread mechanism by which fluid is introduced into kidney tubules is *ultrafiltration*. This is the mechanism used in most vertebrates and in many invertebrates, such as molluscs and decapod crustaceans (e.g., crayfish and crabs). Ultrafiltration into a kidney tubule occurs when the hydrostatic pressure is higher outside the tubule than inside the tubule lumen[3] at a place where the tubule wall is structured in a specialized, minutely porous way that permits fluid to pass through the wall. Under these circumstances, the difference in hydrostatic pressure—provided the difference is great enough[4]—forces fluid to enter the tubule lumen through the wall by means of pressure-driven *bulk flow*, or *streaming*. This flow is termed **ultrafiltration**. The process is literally a form of *filtration* because solutes of large molecular size typically are unable to pass through the wall of the tubule. Thus the fluid introduced into the tubule lumen—which is termed a **filtrate** or **ultrafiltrate**—consists only of water and the subset of solutes that are able to stream through with the water. The blood plasma is the source of the water and solutes that stream through. Although there are exceptions in some groups of invertebrates, the *blood pressure produced by the heart* is typically the pressure that drives ultrafiltration, explaining why heart disease can interfere with urine formation.

To understand the formation of primary urine by ultrafiltration more fully, let's examine the process in the vertebrate kidney. Each kidney consists of many tubules, called **nephrons**, the walls of which consist of a single layer of epithelial cells (see Figure 2.6B). As diagrammed in **FIGURE 29.1A**, each nephron *begins blindly* with its walls thrown into a hemispherical, invaginated structure termed a **Bowman's capsule**, named after William Bowman (1816–1892), who first described it. Tucked inside each Bowman's capsule is an anastomosing cluster of blood capillaries, termed a **glomerulus** (**FIGURE 29.1B**), which is supplied with blood at relatively high pressure by branches of the renal artery. A Bowman's capsule and its glomerulus together constitute a

[1] Urine this concentrated is almost rich enough in salts for the dissolved salts to crystallize out of solution.

[2] This important point is explained in Chapter 27 (see Figure 27.7).

[3] The *lumen* of a hollow structure such as a kidney tubule is the open central cavity.

[4] We discuss this important topic shortly.

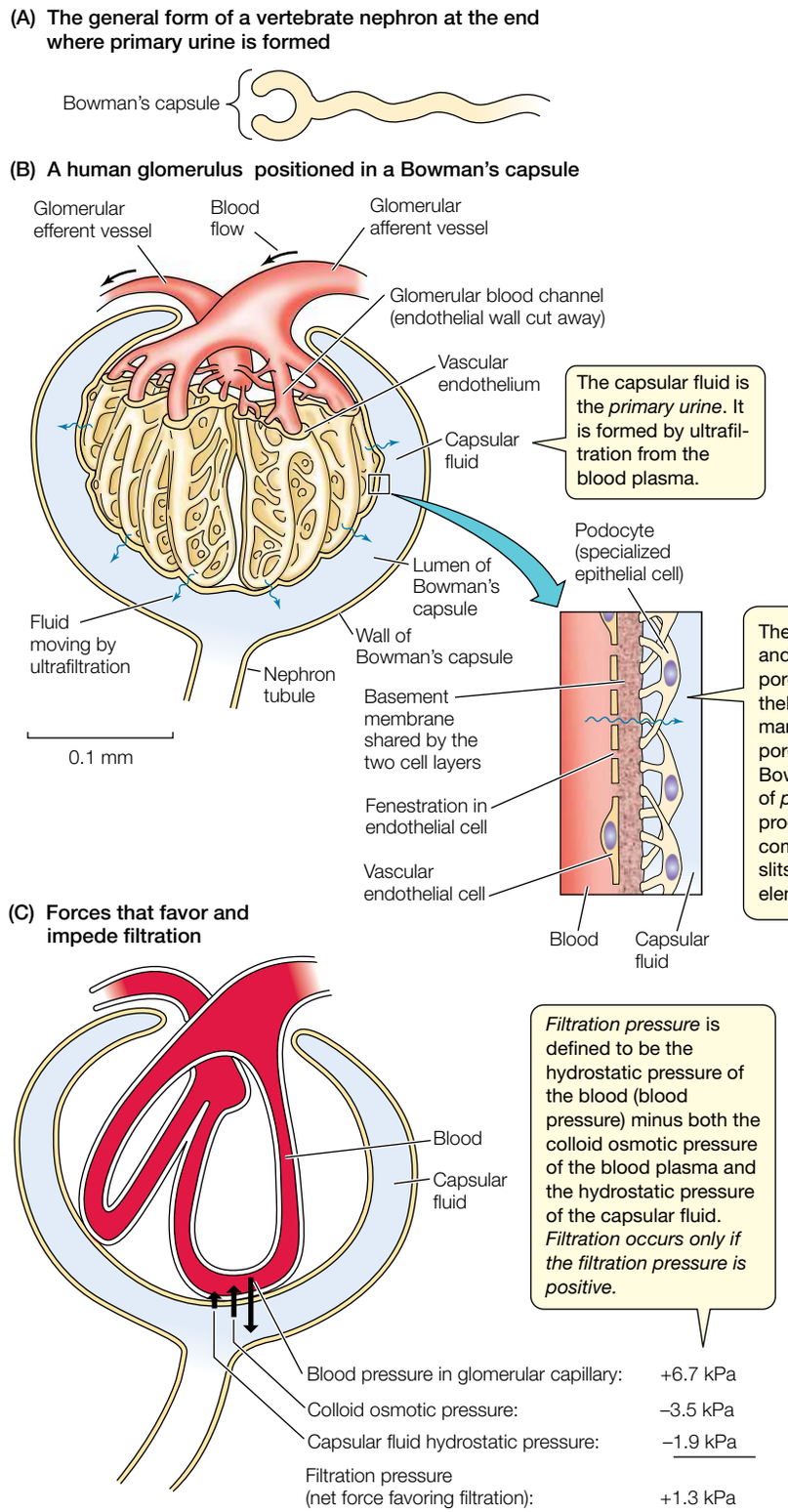


FIGURE 29.1 The structural and functional basis for formation of primary urine by ultrafiltration in the vertebrate kidney (A) The blind end of a vertebrate nephron, where ultrafiltration occurs. (B) A human renal corpuscle, consisting of glomerulus and Bowman's capsule. The vascular endothelium that forms the walls of the glomerular capillaries has been cut away at the top of the drawing, so that only the blood channels are shown there. The Bowman's capsule is drawn diagrammatically; the inner membrane of the capsule actually interdigitates with the sheets of vascular endothelium so that there is intimate juxtaposition of all blood capillaries and the capsular membrane. (C) The forces of hydrostatic pressure and colloid osmotic pressure that affect the rate of filtration: The relative lengths of the black arrows symbolize the relative magnitudes of these forces. (B after Elias et al. 1960.)

renal corpuscle.[5] The glomerular capillaries are intimately juxtaposed to the inner wall of the Bowman's capsule. Moreover, the wall of each capillary consists of a single layer of epithelial cells (the capillary endothelium), just as the wall of the Bowman's capsule consists of a single layer of cells. The lumen of the capillaries, therefore, is separated from the lumen of the Bowman's capsule by only two layers of cells and a nonliving, porous basement membrane between the cell layers (see inset in Figure 29.1B). These intervening structures have a specialized morphology and act as a filter. Fluid is driven through this filter from the blood plasma into the lumen of the Bowman's capsule by the hydrostatic pressure of the blood. The fluid that accumulates in the lumen of the Bowman's capsule is known as the **capsular fluid** and is the *primary urine*. Although a critical determinant of whether a solute will pass through is its molecular size, molecular charge and shape can also be significant; thus the filter has complex features, which include, but are not limited to, simple physical pores or slits.

Current theories regarding the function of the filter place particular importance on the cellular wall of the Bowman's capsule, which is composed of specialized cells called **podocytes** (drawn highly diagrammatically in Figure 29.1B). The podocytes have processes, and the processes of neighboring podocytes interdigitate in geometrically intricate ways, creating countless narrow slits between the processes. The assembly of processes and slits is called the *slit diaphragm*, believed to be the most critical part of the filter.

Inorganic ions and small organic molecules such as glucose, urea, and amino acids move freely with filtered fluid as it passes from the blood plasma into the lumen of a Bowman's capsule. Thus the concentrations of these solutes are virtually the same in the capsular fluid—the primary urine—as in the blood plasma. In contrast, solutes with molecular weights of about 10,000 daltons or more—such as albumins and other plasma proteins—are essentially unable to pass through the structures that separate the blood plasma and the capsular fluid. The primary urine, therefore, closely resembles the blood plasma in its composition of inorganic ions and low-molecular-weight organic solutes, but differs from the plasma in being almost devoid of high-molecular-weight organic solutes such as proteins.

Because proteins remain more concentrated in the blood plasma than in the capsular fluid, the osmotic pressure of the blood plasma is higher than the osmotic pressure of the capsular fluid (see page 126). This difference in osmotic pressure is called the *colloid osmotic pressure* of the blood. Taking the colloid osmotic pressure into account, there are two processes that tend to cause water (H_2O) to move between the blood plasma and capsular fluid. The first is the *difference in osmotic pressure*, which tends to cause osmosis of water from the capsular fluid into the blood

[5] Another name for *renal corpuscle* is *Malpighian corpuscle*. Sometimes the entire renal corpuscle is called a *glomerulus*.

plasma. The second is the *difference in hydrostatic pressure*, which tends to cause bulk flow of water from the blood plasma into the capsular fluid. *Net filtration* of fluid into the capsular lumen will occur only if the difference in hydrostatic pressure is greater than the difference in osmotic pressure. In the renal corpuscles of the species of mammals that have been used as model systems for research, the blood pressure (hydrostatic pressure of the blood) is about 6.7 kPa, and the opposing hydrostatic pressure in the capsular fluid is about 1.9 kPa, meaning that the difference in hydrostatic pressure is about 4.8 kPa. The colloid osmotic pressure averages about 3.5 kPa. Thus the net force favoring filtration—termed the *filtration pressure*—is about 1.3 kPa, as shown in **FIGURE 29.1C**. The blood pressure in the glomerular capillaries is significantly higher than the blood pressure in most capillaries in mammals, helping to promote filtration and formation of primary urine. Part of the reason for the high capillary blood pressure is that the arterioles leading to the glomeruli are relatively large in diameter and thus offer a relatively low resistance as blood flows to the glomeruli.

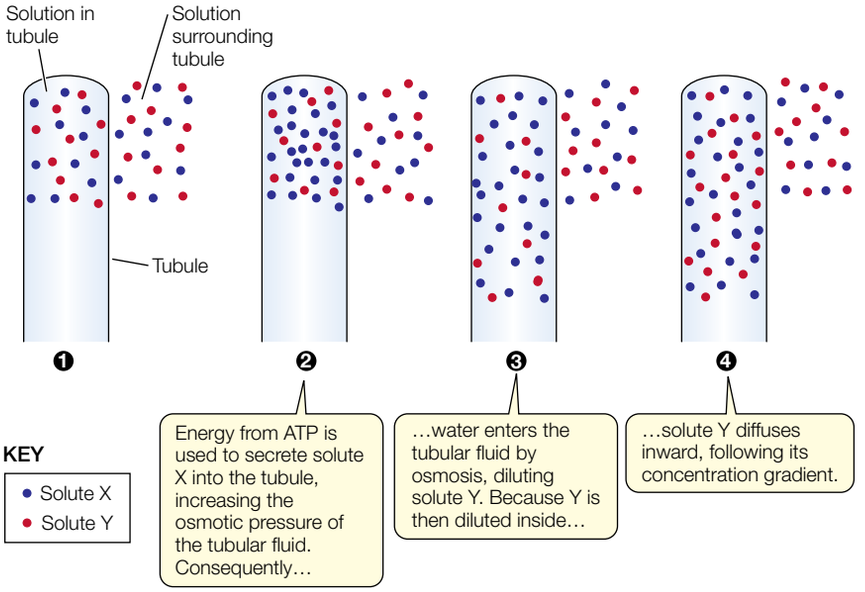


FIGURE 29.2 Formation of primary urine by active solute secretion
In this model system, there are two uncharged solutes. Although the renal tubule is completely surrounded by the outside solution, only a small sample of the outside solution is shown at the upper right of the tubule in each step. For simplicity, the outside solution is assumed to stay constant in volume and composition. Movement of water into the tubule is represented by an increase in the length of the tubule filled with solution.

The rate of primary-urine formation by all of an animal's kidney tubules taken together is called the **filtration rate**. In vertebrates, it is termed specifically the **glomerular filtration rate**, or **GFR**. Adult humans, for example, have a GFR of about 120 mL/min. At this rate, *the equivalent of all the plasma water in a person's body is filtered about every 30 minutes!* This fact points to an important property of vertebrates, namely that the GFR *greatly* exceeds the rate of excretion of definitive urine. Most of the filtered water is ultimately reabsorbed back into the blood, rather than being excreted. The sheer magnitude of the rate of filtration means, however, that the nephrons have very intimate access to the blood plasma to carry out their function of regulating plasma composition.

The rate of production of definitive urine by an individual vertebrate animal can, in principle, be controlled in part by regulating the GFR. This mode of controlling urine flow is employed to some degree by mammals. It is employed to a greater extent by other types of vertebrates. There are two principal ways to adjust the GFR. One is to vary the rate of filtration into all the nephrons of the kidneys collectively. The second is to increase or decrease the numbers of nephrons that are actually functioning as filtration units at any given time. The latter strategy is the norm in nonmammalian vertebrates. The rate of filtration into an individual nephron depends on the nephron's glomerular blood pressure, which is modulated by vasomotor changes in the diameter (and hence flow resistance) of the glomerular afferent vessel. Vasomotor changes of this sort are under the control of the autonomic nervous system and circulating hormones. Variation in the GFR is not the only way in which the rate of production of definitive urine can be controlled. Animals can also modulate the rate at which the nephrons reabsorb filtered fluid prior to excretion; this, in fact, is the preeminent process of urine volume control in mammals, as discussed later.

In addition to ultrafiltration, **active solute secretion** is the second mechanism by which water and solutes can be moved into kidney tubules to form the primary urine. This is the mechanism employed, for example, by insects and some marine fish.

To see how urine formation can be initiated by secretion, consider **FIGURE 29.2**, which presents the essentials of a secretory system in a conceptual, stepwise fashion. For simplicity, only two uncharged solutes are assumed to be present. Moreover, the fluid outside the tubule is assumed to be abundant, so that over short periods of time, movements of solutes and water into the tubule do not greatly modify its composition. At the start, which is labeled step ❶, the osmotic pressure and the concentrations of both solutes are equal on the inside and outside of the kidney tubule. In step ❷, an active-transport pump uses energy from ATP to secrete a quantity of solute X (symbolized with blue dots) into the lumen of the kidney tubule, increasing the inside concentration of X and also increasing the inside osmotic pressure. In step ❸, water moves inward by osmosis—following the osmotic gradient that was set up by secretion of solute X—and the volume of fluid in the tubule increases. Because of this increase in volume, the inside concentration of solute Y, initially the same as the outside concentration, is reduced so that it is now lower than the outside concentration. In step ❹, solute Y diffuses inward following its concentration gradient. Although simplified and artificial, this model system demonstrates that *active secretion of even just a single solute into a kidney tubule can lead to passive influx of water and other solutes*. Thus a complex solution of many solutes can be introduced into the lumen of

a kidney tubule from the body fluids bathing the tubule by a secretory mechanism. During the operation of a secretory system, the epithelium of the kidney tubule acts as something of a filter. The permeability of the epithelium to the various solutes that *might* passively diffuse into the tubular lumen determines which solutes *do*, in fact, enter.

Whether the process of primary-urine formation is ultrafiltration or secretion, energy is required. In ultrafiltration systems, energy is expended in maintaining a suitably high blood pressure to cause net filtration. In secretory systems, energy is expended by the active-transport pump responsible for solute secretion.

The predominant regulatory processes in kidney function: After primary urine forms, solutes and water are recovered from it for return to the blood, and some solutes are added from the blood

As the fluid introduced into a kidney tubule moves down the tubule and through other parts of an animal's excretory system, it is typically altered extensively in volume and composition before it is eliminated as definitive urine. Most of the water in the primary urine is usually reabsorbed and returned to the blood plasma. Solutes may be reabsorbed and returned to the blood plasma—lowering the amounts excreted—or they can be added from the blood.

These processes that occur *after* primary-urine formation are the *predominant regulatory processes* in kidney function. That is, they are the predominant processes by which the formation of urine ultimately regulates the composition and volume of the blood plasma and other body fluids. This last statement is of central importance. *Regulating the composition and volume of the blood plasma and other body fluids is the function of the kidneys*. Their function is not to regulate the urine composition and volume. Instead, the formation of urine is a means to an end. It is a means to the specific end of regulating the body fluids.

As urine flows through a kidney tubule, it is separated from blood capillaries or blood spaces by the epithelial wall of the tubule, a single layer of cells. This epithelium is typically differentiated into distinct regions along the length of the tubule. Within each of these regions, the epithelial cells express distinctive membrane proteins, such as ion channels, transporters, and aquaporins; and the cells may have a distinctive structure. These properties give each region of the tubule distinctive abilities to reabsorb water and solutes from the tubular fluid—returning them to the blood plasma—and to secrete solutes from the blood plasma into the tubular fluid. The processes carried out by each region of a kidney tubule, and the permeability properties of each region, are commonly under endocrine control and hormonally modulated in regulatory ways.

In mammals, the regulatory exchange of solutes and water between urine and blood plasma is complete (and the composition of the definitive urine is fixed) when the urine leaves the kidneys. This is not always the case, however. In many types of animals other than mammals, solutes and/or water are further exchanged between the urine and blood plasma in the urinary bladder, cloaca, or other postrenal ("after kidney") structures, before the urine is finally excreted from the body.

SUMMARY

Basic Mechanisms of Kidney Function

- Primary urine is formed by ultrafiltration or by active solute secretion.
- During ultrafiltration, fluid is driven by elevated hydrostatic pressure from the blood plasma into the kidney tubules through intervening epithelia and basement membranes that act as a filter. The filtrate, which is the primary urine, is almost identical to blood plasma in its composition, except that it lacks high-molecular-weight solutes such as plasma proteins.
- In cases in which primary urine is formed by active solute secretion, the process that initiates and drives primary-urine formation is the active transport of one or more solutes into the kidney tubules. Water then follows by osmosis, and other solutes enter by diffusion, following electrochemical gradients set up by the active solute transport and osmosis.
- As primary urine flows through the kidney tubules, it undergoes exchange with the blood plasma by active or passive transport of solutes and by osmosis of water across the epithelial walls of the tubules. These processes are the *predominant regulatory processes in the kidney tubules*: They determine the ways in which the production of urine ultimately alters the composition and volume of the blood plasma. The urine produced by the kidneys is sometimes (as in mammals) the definitive urine, but in many animals, further regulatory exchange between urine and blood plasma occurs by postrenal processing.

Urine Formation in Amphibians

The amphibians provide an excellent starting point for the study of vertebrate nephron function. Much is known about the amphibian nephron because of practical considerations that make the nephrons of amphibians relatively easy to study. Furthermore, the amphibian nephron can reasonably be considered a "generalized" vertebrate nephron. Our purpose in this section is not only to describe how amphibians form urine, but also to bring out many additional general principles of vertebrate kidney function by example.

Each nephron of an amphibian (**FIGURE 29.3A,B**) consists of (1) a Bowman's capsule; (2) a convoluted segment known as the **proximal convoluted tubule**; (3) a short, relatively straight segment of small diameter, the **intermediate segment**; (4) a second convoluted segment known as the **distal convoluted tubule**; and (5) a relatively straight segment, the **collecting tubule**.[6] The nephrons are microscopic in diameter but macroscopic in length; in an average-sized toad, for example, each might be 1 cm long. Hundreds or thousands of nephrons are found in each kidney, and the nephrons constitute much of the bulk of the kidney tissue. In each nephron, the structure and function of the nephron epithelium change along the length of the nephron, from one segment to the next of the major nephron segments we have described. Structure

[6] The names of the nephron segments are not standardized. For example, the *collecting tubule* is sometimes called the *initial collecting duct*.

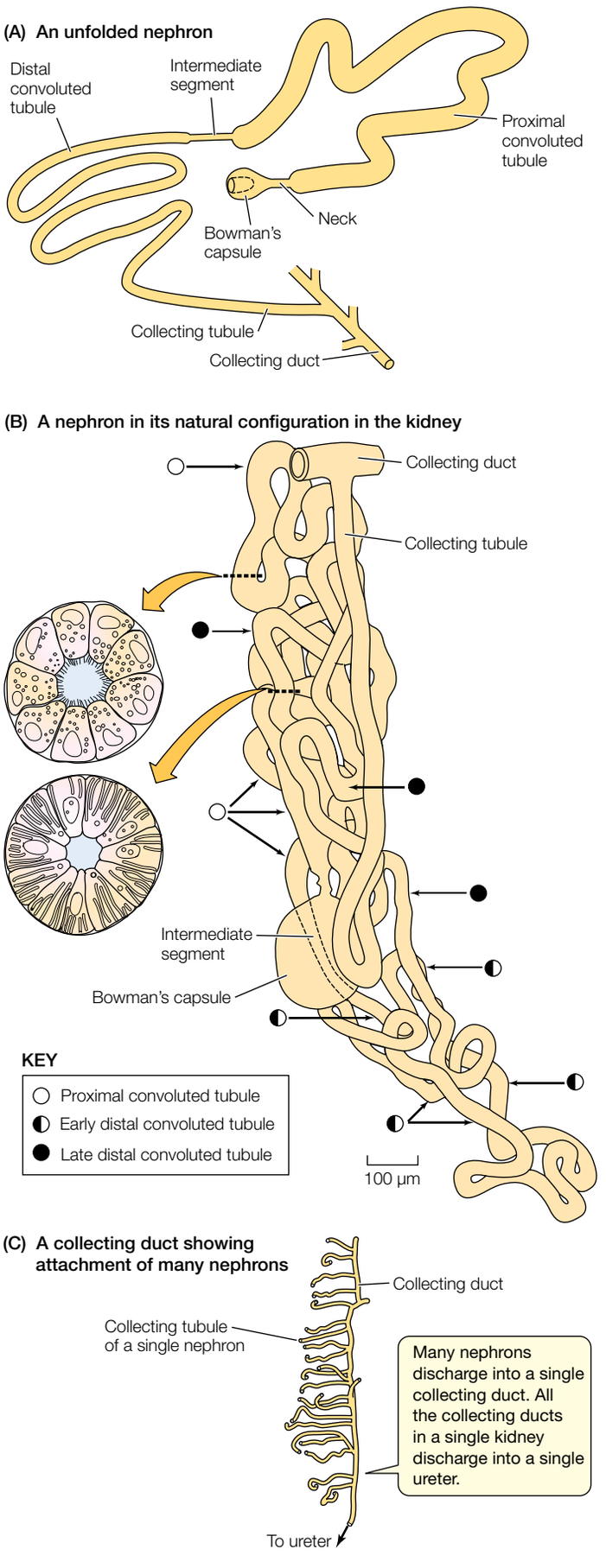


FIGURE 29.3 Amphibian nephrons and their connections to collecting ducts (A) An unfolded amphibian nephron. (B) A nephron of the toad *Bufo bufo*, shown realistically in its natural configuration. Symbols help to trace the nephron along the course of its intricate geometry. Two of the segments—part of the proximal convoluted tubule and part of the late distal convoluted tubule—are shown in cross section at greater magnification than the main drawing. (C) A single collecting duct, showing the connections of the collecting tubules of many nephrons. (B after Møbjerg et al. 1998; C after Huber 1932.)

and function also often change *within* each major nephron segment (e.g., along the length of the distal convoluted tubule). In each kidney, the collecting tubules of all the nephrons feed into **collecting ducts** (FIGURE 29.3C), and all the collecting ducts connect to a single **ureter**, which carries fluid from the kidney to the bladder.

The proximal convoluted tubule reabsorbs much of the filtrate—returning it to the blood plasma—without changing the osmotic pressure of the tubular fluid

In an amphibian's kidneys, the amount of water filtered each day typically far exceeds the amount that needs to be excreted. The same can be said of Na^+ and Cl^-, which are the principal solutes in the blood plasma and therefore also in the filtrate: Na^+ and Cl^- enter the Bowman's capsules as briskly as water does, yet amphibians often need ultimately to conserve Na^+ and Cl^- to the maximum possible extent (see page 744). A high rate of filtration ensures that the nephrons have intimate access to the blood plasma to perform regulatory functions, as noted earlier. However, a high filtration rate also necessitates reabsorption of much of the water and NaCl filtered.

The reabsorption begins in the proximal convoluted tubule. Na^+ is actively reabsorbed across the walls of the proximal tubule. Cl^- may also be reabsorbed actively in some species, but in general its reabsorption is passive, induced by the electrical gradient set up by active Na^+ reabsorption. Although the quantities of Na^+ and Cl^- reabsorbed in the proximal tubule are substantial, the osmotic pressure of the tubular fluid does not fall in the proximal tubule. Instead, the tubular fluid—which is isosmotic to the blood plasma when introduced into the Bowman's capsule by ultrafiltration[7]—remains isosmotic to the plasma as it flows through the proximal tubule. Its osmotic pressure remains unchanged because as NaCl is reabsorbed, a proportional reabsorption of water from the tubular fluid occurs simultaneously. The epithelial walls of the proximal tubule are freely permeable to water.[8] Water therefore moves out of the tubular fluid by osmosis rapidly enough that the active reabsorption of NaCl does not produce a lower osmotic pressure in the tubular fluid than in the blood; the water is said to undergo *near-isosmolar transport* driven by the NaCl reabsorption. In those species of amphibians that have been studied, 20%–40% of the filtered NaCl and water are reabsorbed in the proximal tubule. Even as these large *amounts* of NaCl and water are reabsorbed,

[7] The difference in osmotic pressure that actually exists between the filtrate and the blood plasma—the colloid osmotic pressure—is large enough to affect filtration, as discussed earlier (see Figure 29.1C). However, not only in amphibians but also in other vertebrates, the difference in osmotic pressure is less than 1% of the *absolute* osmotic pressure of either the filtrate or plasma. Thus for most purposes, the filtrate and the plasma can be considered isosmotic.

[8] This high permeability is presumably a consequence of abundant constitutive (i.e., chronically present) aquaporins in the cell membranes of the epithelial cells.

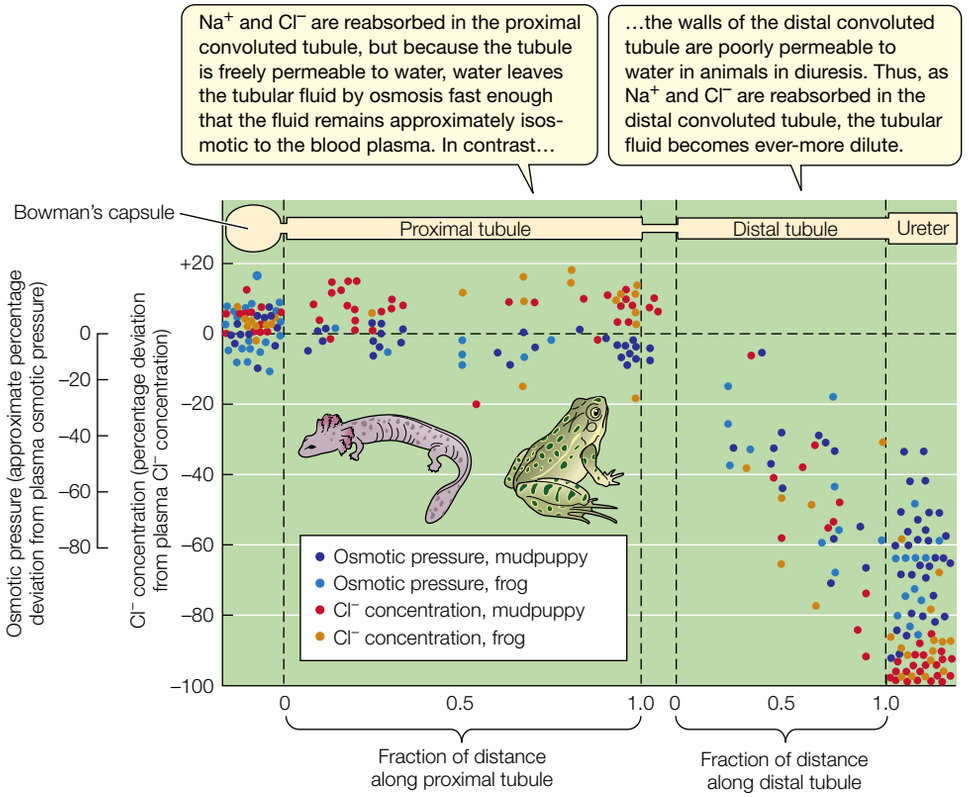


FIGURE 29.4 Urine formation in amphibians during diuresis (rapid urine flow) The osmotic pressure and Cl^- concentration of urine as it flows through the nephrons of two species of amphibians—the semiterrestrial leopard frog (*Rana pipiens*) and the aquatic mudpuppy (*Necturus maculosus*). Fluid was sampled for analysis by use of minute pipettes inserted into the nephrons (see Box 29.2). Concentrations are expressed as percentage deviations from plasma concentrations; for example, a value of –40 indicates that the concentration in the tubular fluid was below the plasma concentration by an amount equal to 40% of the plasma concentration. (After Walker et al. 1937.)

the *concentrations* of ions and water in the tubular fluid—as shown in **FIGURE 29.4**—remain unaltered because ions and water are removed in proportion to each other (**BOX 29.1**).

Another important process that takes place in the proximal tubule is the reabsorption of glucose. Glucose in the blood plasma is a valuable metabolite that—because of its small molecular size—cannot be withheld from the primary urine during ultrafiltration. However, in amphibians and other vertebrates, glucose is promptly reclaimed and returned to the blood. Glucose is reabsorbed into the cells of the proximal tubule (and then passed to the blood plasma) by *secondary* active transport driven by the *primary* active transport of Na^+—a mechanism similar to that diagrammed in Figure 5.12. Amino acids are also valuable organic molecules that are freely carried into the Bowman's capsules by ultrafiltration because of their small size. Their reabsorption begins in the proximal tubule. **BOX 29.2** discusses some of the methods used to study kidney function: methods that have played important roles in creating the knowledge discussed here and throughout the chapter.

The distal convoluted tubule can differentially reabsorb water and solutes, thereby regulating the ratio of water to solutes in the body fluids

Active reabsorption of NaCl from the tubular fluid continues in the distal convoluted tubule. In this way, the quantity of NaCl destined for excretion from the body—that is, removal from the body fluids—is gradually lowered toward the level that is appropriate for maintenance of internal NaCl balance.

A major function of the distal convoluted tubule in many amphibians—a function sometimes shared by the collecting ducts and urinary bladder—is control of the excretion of *pure* water, often termed **osmotically free water**. By controlling the excretion of free water, the distal tubule helps to control both the amount of water in the body fluids and the concentrations of solutes in body fluids, including concentrations in the blood plasma. Recall from Chapter 28

BOX 29.1 Quantity versus Concentration

When analyzing kidney function, it is important to maintain a clear distinction between measures of *quantity* (or *mass*) and measures of *concentration*. The importance of this distinction is illustrated nicely by the events in the proximal tubule of amphibians. As shown in Figure 29.4, the *concentrations* of Na^+, Cl^-, and water in the tubular fluid remain, on average, unchanged. Yet the *quantities* of these substances exiting the proximal tubule are much lower than those entering.

Measures of quantity and concentration are each informative, although in different ways. Quantity is an absolute measure, whereas concentration is a relative measure (quantity of solute relative to quantity of water). As a general principle, measures of *quantity* provide the most direct insight into questions of salt and water *balance*. For instance, to determine whether an animal is in Na^+ balance, you would measure the quantity of Na^+ gained per day and the quantity lost per day (including the quantity lost in urine) and compare them. Although urine *concentrations* are not directly useful for balance calculations, *concentrations* provide the most direct insight into the effects of urine production on blood-plasma composition. For instance—as explained in Figure 27.7—if you wanted to know whether the kidneys are lowering the Na^+ concentration of the blood plasma, you would examine the urine Na^+ concentration relative to the plasma Na^+ concentration. Urine production is lowering the plasma Na^+ concentration if the urine Na^+ concentration is greater than the plasma Na^+ concentration (meaning that Na^+ U/P > 1.0).

BOX 29.2 Methods of Study of Kidney Function: Micropuncture and Clearance

Some of the methods used to study kidney function, although technically difficult, are intuitively easy to understand. A technique of this sort that has revolutionized renal physiology is **micropuncture**. Fine micropipettes are inserted into individual nephrons at identified points, permitting samples of tubular fluid to be withdrawn for analysis of composition. Such samples from amphibian nephrons reveal, for example, that the glucose concentration falls virtually to zero by the end of the proximal convoluted tubule. This is how we know that the proximal tubule is the site of glucose reabsorption.

A method that is not so intuitively simple to understand—but important in both physiological research and medical practice—is the study of **renal clearance**. Clearance studies are used to measure the glomerular filtration rate and can be used to quantify the reabsorption or secretion of solutes in the renal tubules. **Box Extension 29.2** explains the principles and uses of renal clearance studies.

(see page 776) that the water in urine may be considered to consist of two parts: (1) water that is *required* to accompany excreted solutes and (2) additional water that may be excreted but is not required for solute excretion. The second component may be considered to represent an excretion of pure, or "free," water—a regulated removal of water from the body fluids—precisely because it is not required for solute excretion.

In amphibians, the amount of water that is *required* to be excreted with solutes is determined by the fact that the maximum possible osmotic U/P ratio is 1.0: The urine osmotic pressure cannot exceed the plasma osmotic pressure.[9] This means that *at least* enough water must be excreted with solutes in the urine to create a solution that is isosmotic to the blood plasma. If the urine osmotic pressure of an amphibian in fact equals the animal's plasma osmotic pressure, the urine contains *only* water that is *required* for solute excretion. That is, the urine contains no water of the second kind: no pure, osmotically free water. However, if the urine osmotic pressure of an amphibian is less than the animal's plasma osmotic pressure (osmotic U/P < 1), the urine carries an "extra" quantity of water, an amount not strictly required by solute excretion. This extra quantity represents an excretion of pure water—removal of water from the body fluids. This excretion of pure water can be varied: The more dilute the urine, the more free water it contains. Thus *an animal can control its excretion of water independently of its excretion of solutes by varying the osmotic pressure of its urine*.

The extent of pure-water excretion is controlled in the distal convoluted tubule by varying the degree to which osmotic water reabsorption keeps pace with solute reabsorption there. The extent of water reabsorption is controlled by modulating the permeability of the walls of the tubule to water. This control of permeability is exercised at least partly by **antidiuretic hormone** (**ADH**) secreted

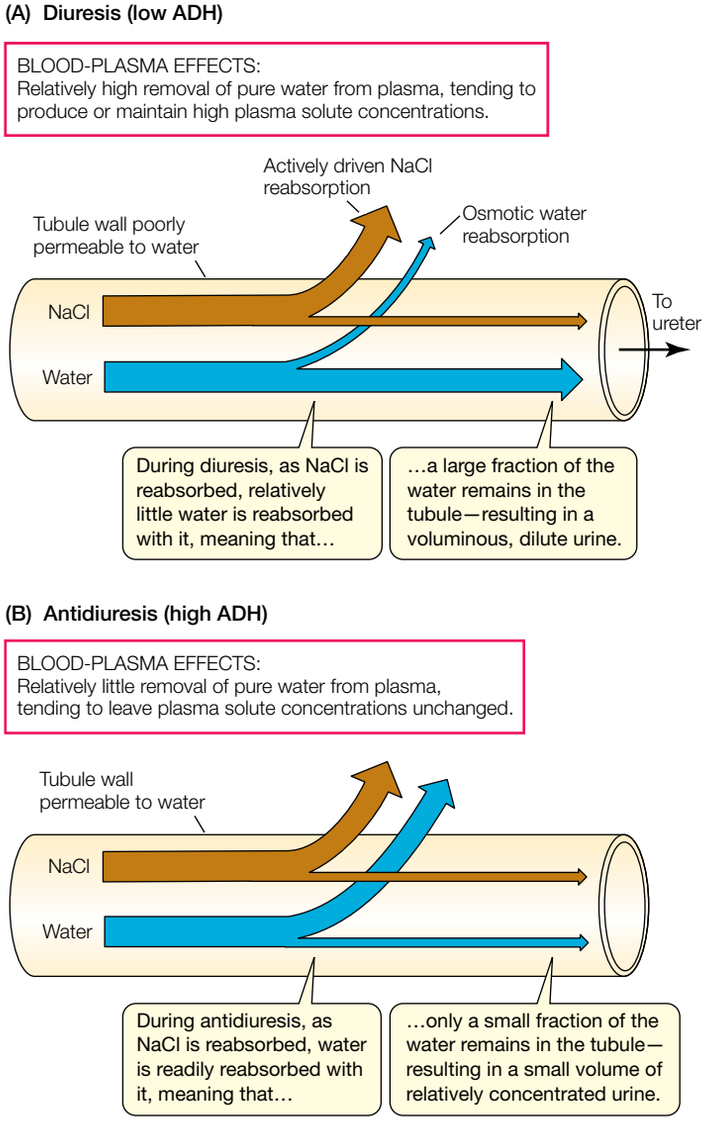


FIGURE 29.5 Major solute and water fluxes in the late distal convoluted tubule during diuresis and antidiuresis The pathways followed by NaCl and water are symbolized by the relative sizes of the arrows. (A) In diuresis, low permeability of the tubule walls to water impedes osmotic water reabsorption; thus a relatively large fraction of the water remains in the tubule, and the ratio of solute to water (the osmotic pressure) in the tubular fluid is dramatically reduced. (B) In antidiuresis, the tubule walls are more permeable to water; thus a larger fraction of the water is reabsorbed, and the ratio of solute to water in the tubular fluid is affected relatively little. This conceptual diagram is simplified in two ways: First, it assumes that NaCl and water reabsorption occur in the same parts of the tubule, and second, it ignores solutes other than NaCl (e.g., urea).

by the neurohypophysis (posterior pituitary gland).[10] As we now explain these points, refer to **FIGURE 29.5** for a visual summary.

When ADH levels are low (see Figure 29.5A), the wall of the distal convoluted tubule has a low permeability to water. Consequently, NaCl reabsorption and water reabsorption from the tubular fluid are significantly uncoupled. The active reabsorption of NaCl tends to dilute the tubular fluid and thus create an osmotic gradient that favors water reabsorption by outward osmosis. However, the low permeability of the tubule wall to water impedes osmosis. This limitation of water

[9] Recall from Chapter 27 that the osmotic U/P ratio is the ratio of urine osmotic pressure to plasma osmotic pressure.

[10] The antidiuretic hormone of amphibians, birds, and nonavian reptiles is *arginine vasotocin*; see Table 16.2 on page 438.

reabsorption has three important and complementary consequences. First, relatively little water is returned to the body fluids. Second, NaCl reabsorption makes the tubular fluid more dilute than the blood plasma, both in osmotic pressure and in ion concentrations. This dilution is progressive: As fluid flows through the distal tubule, the fluid becomes ever-more dilute as ever-more ions are reabsorbed from it (see Figure 29.4). The active reabsorption of solutes from the urine across tubule walls that are poorly permeable to water is, in its fundamentals, the *universal mechanism by which animals make urine hyposmotic to the blood plasma*, and here we see that mechanism in action. The third principal consequence of low permeability to water in the amphibian distal convoluted tubule is that a high proportion of the water that enters the distal tubule passes through to be excreted in the urine. Considering the second and third consequences together, one can see that in the presence of low levels of ADH, the urine is *dilute* and *voluminous*. It carries away far more water than is necessary just to excrete solutes, and thus has a high content of pure, osmotically free water. The most important points to understand are the effects on the body fluids, because the function of producing urine is to regulate the volume and composition of the blood plasma and other body fluids. Under the circumstances we are discussing, the urine extracts relatively large amounts of pure water from the body fluids, tending to reduce the volume of the body fluids and raise the concentrations of solutes in them (see Figure 27.7).

When ADH levels are high (see Figure 29.5B), the presence of ADH induces the wall of the late distal convoluted tubule (the half or so of the distal tubule closest to the collecting tubule) to become relatively permeable to water, and the distal tubule then functions more like the proximal tubule. Osmotic water reabsorption is promoted. Thus, as NaCl is reabsorbed, more water is reabsorbed than when ADH levels are low. Focusing on immediate consequences, there are again three. First, a relatively large amount of water is returned to the body fluids. Second, the tubular fluid stays more nearly isosmotic to the blood plasma than when ADH levels are low. Third, a smaller proportion of the water that enters the distal tubule passes through to be excreted. In the presence of high levels of ADH, therefore, the urine is relatively *concentrated* and *scanty*.[11] It contains relatively small amounts of water extracted from the body fluids and carries away little or no pure, osmotically free water. As before, the most important points to understand are the effects on the body fluids, because the function of producing urine is to regulate the volume and composition of the body fluids. Under the circumstances we are now discussing, the urine extracts little or no pure water from the body fluids, tending to leave the concentrations of solutes in the body fluids unaltered (see Figure 27.7).

You can see now why ADH has the name it does. Recall from Chapter 28 that **diuresis** is production of abundant urine. High levels of ADH promote the opposite: **antidiuresis**.

ADH is believed to control the water permeability of the amphibian distal convoluted tubule by controlling the insertion and retrieval of aquaporin proteins (see page 800) in cell membranes in parts of the tubular epithelium. When the level of ADH is high, aquaporins are inserted into the cell membranes, and—with the water channels therefore in place in the cell membranes—water can pass through the epithelium relatively readily by osmosis. When the level of ADH is low, aquaporins are retrieved from the cell membranes (i.e., returned to intracellular locations where they are nonfunctional), and osmosis through the epithelium is impeded. More will be said of aquaporin function later in this chapter.

Active H^+ secretion into the tubular fluid is an additional function that is known to occur in the distal convoluted tubule. The amount of H^+ added is adjusted to maintain a normal pH in the body fluids.

ADH exerts an elaborate pattern of control over nephron function

In amphibians—and also in birds, lizards, and other reptiles—ADH not only increases the permeability of parts of the distal convoluted tubule to water, but also decreases the glomerular filtration rate. Specifically, ADH reduces the GFR in these vertebrate groups by reducing the numbers of actively filtering nephrons, an effect mediated by inducing vasoconstriction in glomerular afferent blood vessels. The decrease in GFR tends to reduce urine flow and promote water retention in the body, thereby complementing the increase in water reabsorption induced by ADH in the distal tubules.

ADH has also been shown in some frogs and toads to increase the rate of active NaCl reabsorption from the renal tubules. This effect, like the others mentioned, also tends to reduce urine volume and promote water retention because it enhances solute-driven water reabsorption and decreases the solute load of the urine.

Clearly, ADH mediates a multifaceted *pattern* of control over nephron function. If an amphibian experiences excess water influx—as can occur during hours of immersion in freshwater—secretion of ADH is reduced. Then the GFR is relatively high, distal-tubule reabsorption of water is relatively low, and a voluminous, dilute urine results. If dehydration sets in, ADH is secreted from the neurohypophysis, apparently under the control of osmoreceptors (which detect an increase in body-fluid osmolarity) and of pressure or stretch receptors (which signal a decrease in blood volume). The ADH induces a reduction in GFR, an increase in distal-tubule water reabsorption, and an increase in NaCl reabsorption, thereby promoting water retention and production of a scanty, concentrated urine. The renal responses to ADH are not as well developed in some amphibian species from consistently moist or wet habitats as they are in species that are more terrestrial and thus more likely to experience dehydration (see pages 770–771).

The bladder functions in urine formation in amphibians

In many species of amphibians, the bladder not only stores urine but also plays a substantial role in adjusting the volume and composition of the urine. In these species, the function of the bladder can be described very much in the way we have described that of the distal convoluted tubules. The bladder wall is poorly permeable to water when ADH levels are low but becomes quite permeable to water when ADH levels are high; the participation of aquaporins in these changes of permeability in the amphibian bladder has been directly demonstrated. NaCl is actively reabsorbed across the bladder wall, and this reabsorption is stimulated by ADH.

The amphibian excretory system has mechanisms to promote excretion of urea

Urea is the principal compound used to excrete waste nitrogen in most adult amphibians. The nephrons, bladder, and other excretory passages of adults seem generally to be poorly permeable to urea.

[11] Recall that in amphibians, the maximum urine concentration is isosmotic to the plasma.

Thus urea introduced into the tubular fluid tends to be retained in it and removed from the body by excretion; moreover, as water is reabsorbed from the tubular fluid, urea in the fluid tends to be concentrated. Filtration is one process by which urea enters the nephrons, and in many amphibians it is probably the sole process. However, in at least some ranid frogs (e.g., bullfrogs), urea is also actively secreted into the tubular fluid across the nephron walls.

SUMMARY

Urine Formation in Amphibians

- A primary function of the proximal convoluted tubule of the amphibian nephron is the return of both water and solutes to the body fluids by the isosmotic reduction of urine volume. NaCl is actively reabsorbed from the tubular fluid. Because the epithelial wall of the proximal tubule is permeable to water, water exits the tubular fluid by osmosis, keeping the tubular fluid isosmotic to the blood plasma.
- Glucose and amino acids are actively reabsorbed from the tubular fluid in the proximal tubule, returning them to the body fluids.
- The distal convoluted tubule *differentially* returns water and solutes to the body fluids; in the process it helps regulate plasma composition and determines the volume and osmotic concentration of the definitive urine produced by the kidney. An important mechanism by which control of distal-tubule function is exercised is that the epithelial wall of the distal convoluted tubule can have high or low permeability to water, depending on blood levels of antidiuretic hormone (ADH) secreted by the neurohypophysis (posterior pituitary).
- When ADH levels are low, the distal-tubule epithelium is poorly permeable to water. Active reabsorption of NaCl returns NaCl to the body fluids and dilutes the tubular fluid. However, relatively little water is returned to the body fluids because water cannot readily move out of the tubular fluid by osmosis. The volume of the tubular fluid remains high, and both the osmotic pressure and the NaCl concentration of the fluid become progressively lower as the tubular fluid flows through the tubule.
- When ADH levels are high, aquaporins are believed to be inserted into cell membranes in the distal-tubule epithelium, causing the water permeability of the epithelium to become high. As active reabsorption of NaCl takes place, osmosis carries water out of the tubular fluid. Thus relatively high amounts of water are returned to the body fluids. The volume of the tubular fluid is reduced, and the fluid remains similar to the blood plasma in its osmotic pressure and NaCl concentration.

Urine Formation in Mammals

The nephrons of amphibians, as noted earlier, may reasonably be considered to represent the generalized vertebrate condition. The nephrons of lizards, snakes, turtles, and crocodilians resemble them. Mammalian nephrons differ, however. Compared with an amphibian nephron, each nephron of a mammal has an added, long segment of tubule, positioned between the proximal and distal convoluted tubules. This added segment is arranged in the shape of a hairpin loop and—having first been described by Jacob Henle in the 1860s—is called the **loop of Henle** (pronounced *Hen-lee*). An additional "innovative" feature of the mammalian kidney is that the loops of Henle of the various nephrons in a kidney, along with the collecting ducts, are arranged in parallel arrays, giving the kidney a pronounced macroscopic structure not seen in the kidneys of amphibians or reptiles. The loops of Henle and their parallel arrangement provide the anatomical basis for the production of urine that is more osmotically concentrated than blood plasma: *hyperosmotic urine*. Amphibians, lizards, snakes, turtles, and crocodilians—lacking these anatomical attributes—cannot produce hyperosmotic urine.

We saw in Chapter 28 that the ability of mammals to concentrate their urine is one of their most dramatic and important adaptations for life on land. Now, as we study the mammalian kidney, we will examine the mechanism by which their urine is concentrated. Mechanisms for producing urine that is hyperosmotic to the blood plasma might seem simple to evolve. The history of life offers a very different verdict, however. In the entire animal kingdom, only three major groups have mastered the task: mammals, birds, and insects. In each case, the ability to concentrate the urine has opened up new habitats and ways of life—such as by aiding certain small mammals, like the kangaroo rats described at the start of this chapter, to survive as seed eaters in deserts. Thus, as we examine the mammalian mechanism of concentrating urine, we focus on a physiological attribute of enormous ecological and evolutionary significance.

The nephrons, singly and collectively, give the mammalian kidney a distinctive structure

The loop of Henle in a mammalian nephron consists of two long and parallel tubes, termed *limbs*, connected by a hairpin bend: The **descending limb** leads from the proximal convoluted tubule to the bend, and the **ascending limb** runs from the bend to the distal convoluted tubule (**FIGURE 29.6B**). The descending limb begins with a segment of relatively large diameter termed a **thick segment**, and the ascending limb terminates with a thick segment. Interposed between these thick segments, at various positions and for various lengths, is a segment of very small diameter, the **thin segment**. The epithelium of the thin segment differs cytologically from that of the intermediate segment discussed earlier and occurs only in mammals and birds. The loop of Henle varies considerably in length among species of mammals and among the nephrons within the kidneys of any one species.

As can be seen in Figure 29.6B, the Bowman's capsules and convoluted tubules of the nephrons in each kidney of a mammal are aggregated toward the outer surface of the kidney, whereas the loops of Henle and collecting ducts project inward, toward the **renal pelvis**, a tubular structure that represents the expanded inner end of the ureter that drains the kidney (**FIGURE 29.6A**). Because of this highly ordered arrangement of the renal tubules, histologically distinct layers are evident in the gross structure of the kidney tissue. In sagittal section, the tissue of each kidney consists of an outer layer, the **cortex**, which surrounds an inner body of tissue, the **medulla** (see Figure 29.6A). The cortex (see Figure 29.6B) consists of Bowman's capsules, convoluted tubules, the beginnings of collecting ducts, and associated vasculature. The medulla consists of loops of Henle and collecting ducts, as well as their associated vasculature. Within the medulla, the loops of Henle and collecting ducts run in parallel to one another.

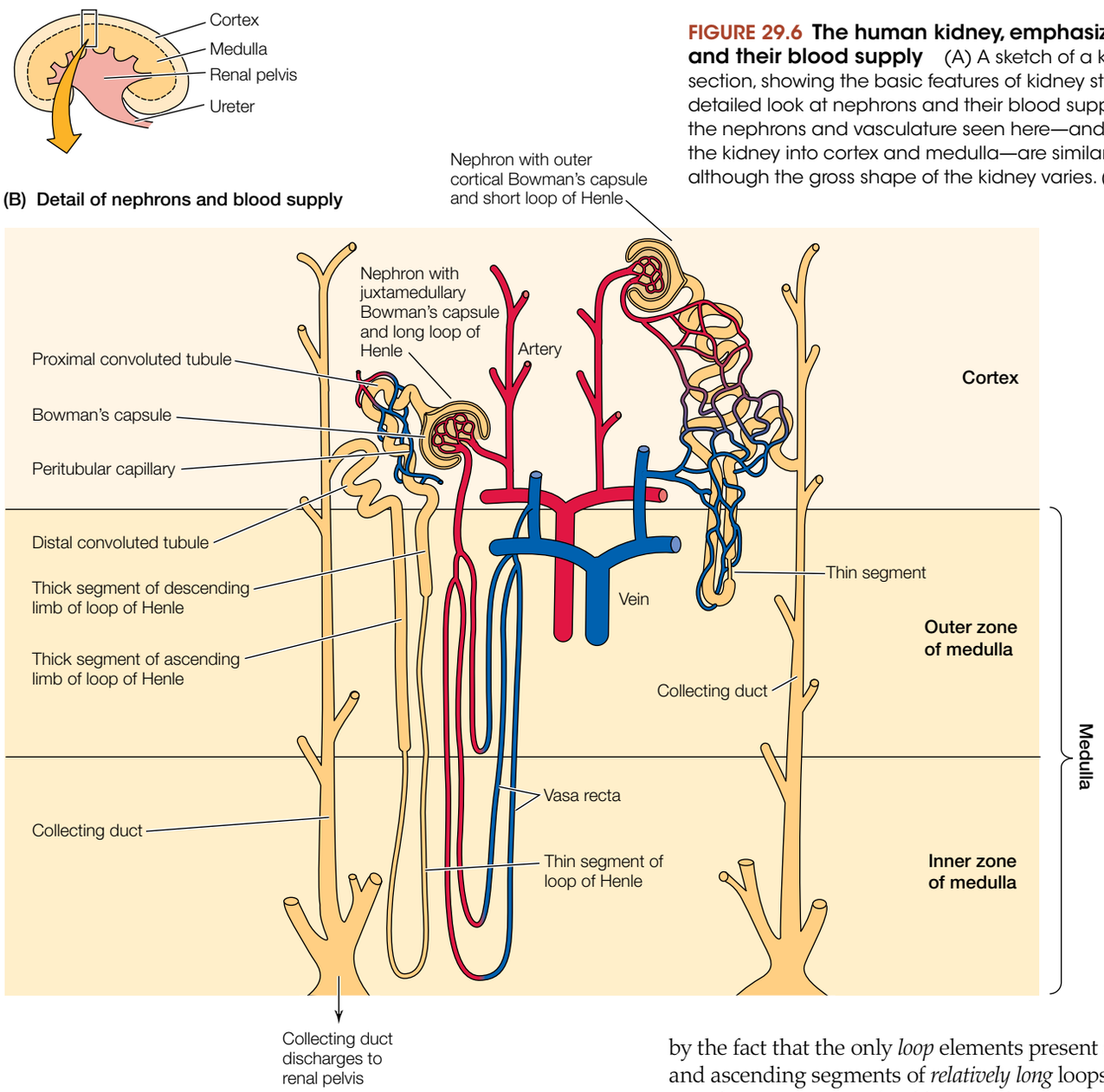


FIGURE 29.6 The human kidney, emphasizing the nephrons and their blood supply (A) A sketch of a kidney in sagittal section, showing the basic features of kidney structure. (B) A more detailed look at nephrons and their blood supply. The structures of the nephrons and vasculature seen here—and the subdivision of the kidney into cortex and medulla—are similar in all mammals, although the gross shape of the kidney varies. (B after Smith 1951.)

To get oriented to fluid-flow patterns in the mammalian kidney, let's now trace the path of fluid through a nephron, focusing on the nephron to the left in Figure 29.6B. After filtration into the Bowman's capsule, fluid moves first through the proximal convoluted tubule and then descends into the medulla in the loop of Henle. After rounding the bend of the loop, the fluid returns to the cortex, passes through the distal convoluted tubule, and leaves the nephron to enter a collecting duct. The fluid then again passes through the medulla, this time in the collecting duct. After the fluid is discharged from the collecting duct into the renal pelvis, it flows into the ureter and to the bladder to be excreted. A convention worthy of note is that when fluid flows from the cortex toward the medulla, it is said to move *deeper* into the kidney.

We have already mentioned that the various nephrons in the kidney of a species may have loops of Henle of different lengths. Nephrons differing in this regard are positioned differently within the kidney, a fact that contributes to gross kidney structure. As can be seen at the left side of Figure 29.6B, there is a region deep in the medulla—termed the **inner zone** of the medulla—that is defined by the fact that the only *loop* elements present are *thin* descending and ascending segments of *relatively long* loops of Henle. The surrounding, more superficial layer of the medulla is the **outer zone**. Loops of Henle that project into the inner zone are termed **long loops**. Loops that turn back within the outer zone of the medulla or within the cortex are called **short loops**. The thin segments of long and short loops of Henle differ cytologically. Bowman's capsules may be positioned near the outer cortical surface, at mid-depth in the cortex, or within the cortical tissue next to the medulla; the last location is termed the *juxtamedullary* ("near the medulla") position. As depicted in Figure 29.6B, nephrons with short loops tend to have their Bowman's capsules positioned toward the outer cortex, whereas those having long loops tend to have midcortical or juxtamedullary capsules. Laboratory rats have about 30,000 nephrons (of all types combined) in each kidney. Domestic dogs have about 400,000, and humans have 0.4–1.2 million.

A final morphological feature of importance is that the thick ascending segment of each nephron, near its outer (upper) end, passes immediately next to the Bowman's capsule of the very same nephron.[12] At the point where this occurs, the wall of the thick

[12] Figure 29.6B is drawn to emphasize other features and does not show this.

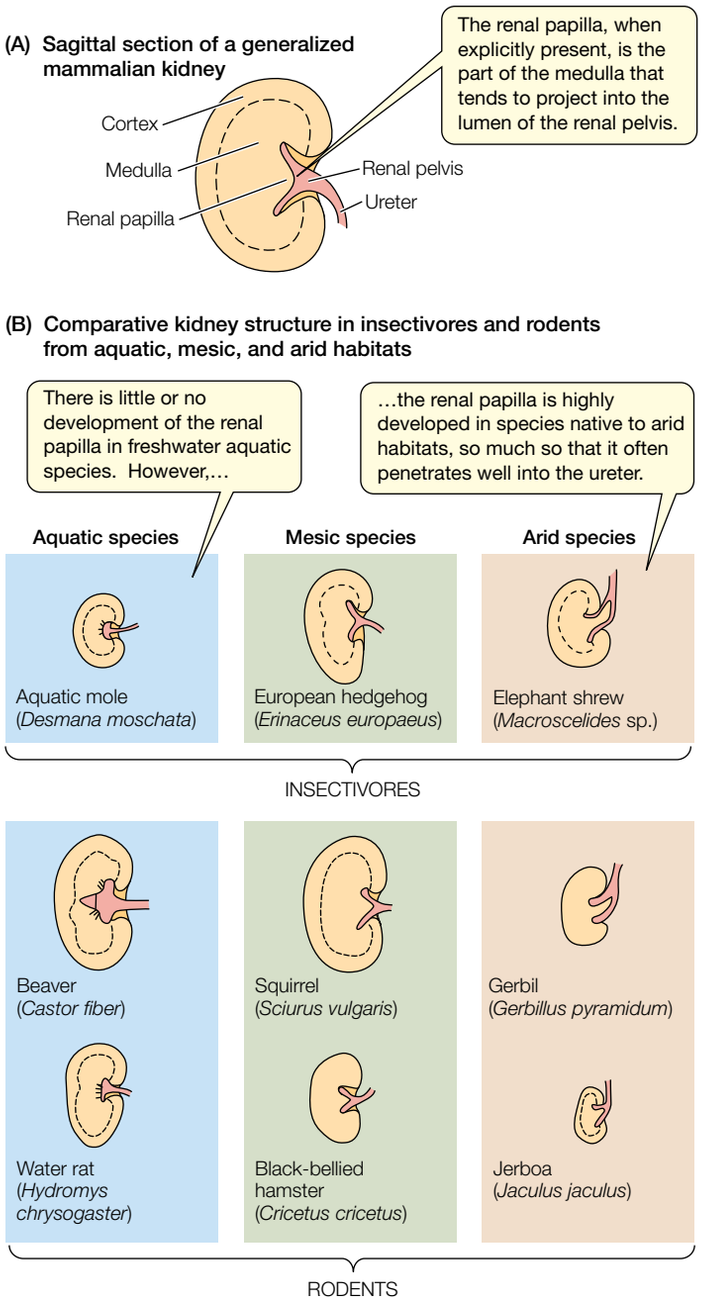


FIGURE 29.7 Evolutionary development of the renal papilla in mammals native to different habitats (A) Sagittal section of a generalized mammalian kidney, showing the location of the renal papilla. (B) Kidney structures of insectivores (e.g., shrews and moles) and rodents (e.g., rats and squirrels) from aquatic, mesic, and arid habitats. (After Sperber 1944.)

ascending segment is modified, forming a set of specialized cells, the **macula densa**. The macula densa and other associated cells form a structure called the **juxtaglomerular apparatus**.[13] Specialized vascular endothelial cells in this apparatus are responsible for secreting the key hormone renin (pronounced *ree-nin*), which controls secretion of another hormone, aldosterone, which is a major controller of renal ion excretion (see pages 450–451 and Figure 16.17).

[13] Be certain not to confuse the juxtamedullary capsules and the juxtaglomerular apparatus.

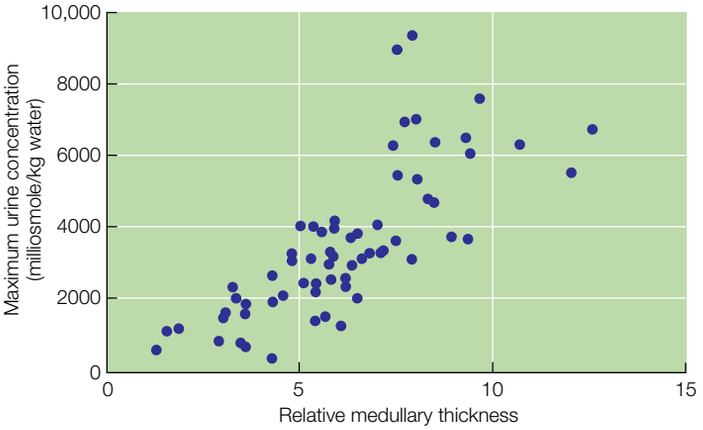


FIGURE 29.8 Maximum urine concentration correlates with the relative thickness of the medulla The relation is plotted for 68 species of mammals, each point representing a different species. The relative medullary thickness is a dimensionless number. To calculate it, an index of kidney size is first calculated by taking the cube root of the product of the three principal linear dimensions (length, width, and thickness) of the kidney. Medullary thickness is then expressed as a ratio of the index of kidney size to obtain relative medullary thickness. (After Beuchat 1990.)

Comparative anatomy points to a role for the loops of Henle in concentrating the urine

Even before the physiology of the loops of Henle began to be understood, morphological evidence strongly suggested that the loops are intimately involved in the production of urine that is hyperosmotic to the blood plasma. This evidence helped to center attention on the physiology of the loops.

One type of comparative morphological evidence comes from studies of certain species of mammals—characteristic of freshwater environments—that lack long loops of Henle and have only short loops, so that they have no inner medulla. Hippos, mountain beavers (*Aplodontia*), and muskrats are examples. Such species are noted for having only meager abilities to concentrate their urine. Long loops are essential for achieving high urinary concentrations; in mammals that achieve high concentrations, at least 15%–20% of the nephrons have long loops of Henle.

Another type of comparative morphological evidence comes from studies of the **renal papilla** (**FIGURE 29.7A**). Not all mammals have a grossly apparent renal papilla. Commonly, however, the renal medulla has a roughly pyramidal shape and forms a projection into the lumen of the renal pelvis. This projection, the renal papilla, is composed in major part of long loops of Henle. Thus the prominence of the renal papilla provides an indication of the number and length of long loops in a mammal's kidney. In 1944, Ivar Sperber (1914–2006) reported seminal observations on the papilla in about 140 species of mammals from diverse habitats. He found that the papilla was uniformly poorly developed in species inhabiting freshwater habitats. The papilla was more evident in species from mesic (moderately moist) habitats and was most developed in species from arid habitats (**FIGURE 29.7B**). Insofar as habitat may be taken as an indicator of demand for urinary concentration, Sperber's results indicated that there is a greater evolutionary development of the long loops of Henle in species that produce relatively concentrated urine.

Inspired by Sperber's work, comparative studies have since been conducted on **medullary thickness**. The thickness of the medulla provides a measure of the lengths of the longest loops of Henle. A problem that needs to be addressed in such comparative studies is

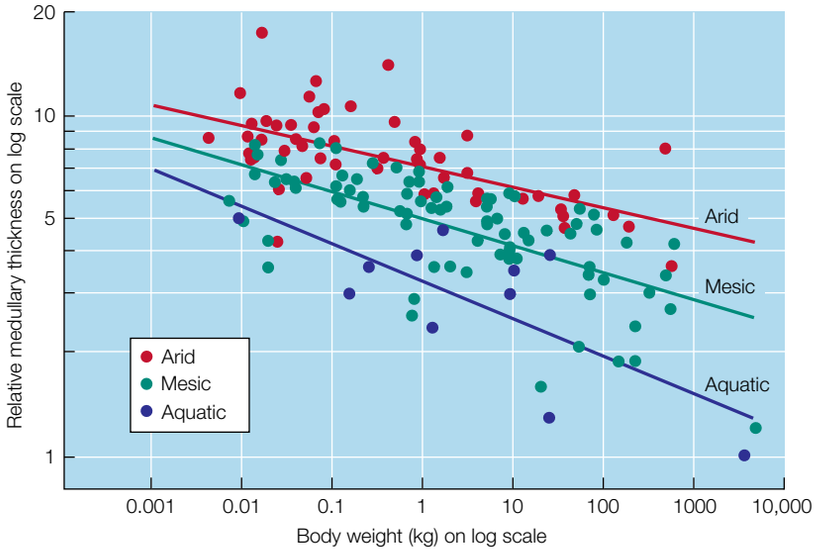


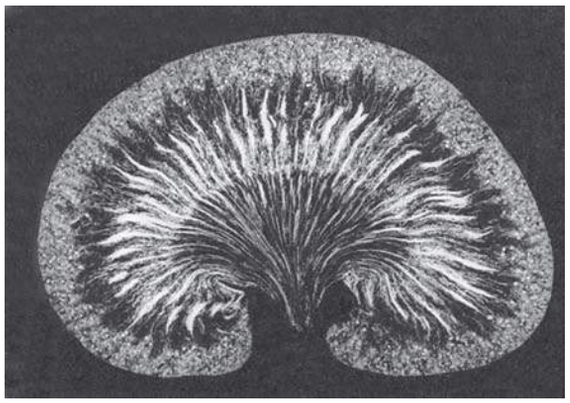
FIGURE 29.9 The relation between relative medullary thickness and body size depends on whether mammals are native to arid, mesic, or aquatic habitats Each point represents a different mammal species. The three lines are fitted statistically through the points for the arid, mesic, and freshwater aquatic species. The straight lines on this log–log plot indicate that the relations are allometric (see Appendix F). To interpret this plot, keep in mind that logarithmic scales tend to cause visual compression of data. The differences in relative medullary thickness among the arid, mesic, and aquatic mammals of a particular body size are substantial; for example, the medullary thickness of a representative 1-kg arid species is more than twice that of a 1-kg aquatic species. See the legend of Figure 29.8 for a description of how relative medullary thickness is calculated. (After Beuchat 1996.)

that medullary thickness depends on the body size of mammals; humans, for example, have a very thick medulla compared with all species of mice, merely because humans are more than 1000 times larger than mice. To remove the effects of absolute kidney size, medullary thickness is expressed as a ratio of kidney size. This ratio is called **relative medullary thickness**. A high relative medullary thickness means that the longest loops of Henle are long relative to the overall dimensions of the kidney. By now, data are available on many species, and as **FIGURE 29.8** shows, urinary concentrating ability is strongly correlated with relative medullary thickness: Species with high relative medullary thickness tend to be able to produce especially concentrated urine.

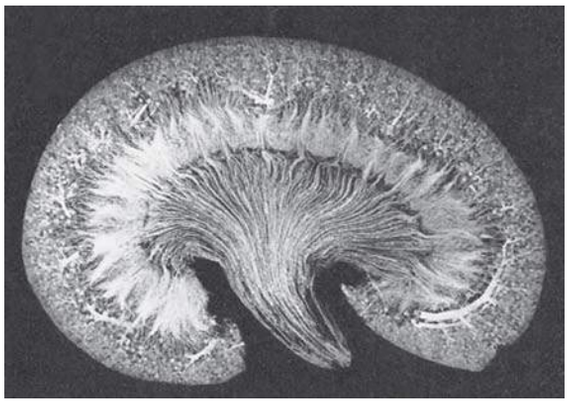
The latest incarnation of Sperber's work is shown in **FIGURE 29.9**, where relative medullary thickness is plotted as a function of body weight (size) for mammals from three types of habitats. This modern analysis reveals that the relative thickness of the medulla tends to decrease allometrically with body size (just as concentrating ability tends to decrease with body size, as seen in Figure 28.20). Habitat, however, is a significant factor. At any given body size, mammals from arid habitats tend to have the thickest medullas and longest loops of Henle, whereas those from freshwater aquatic habitats have the thinnest and shortest, and those from intermediate mesic habitats are in between.

A dramatic morphological comparison of the kidneys of three species of rodents of roughly similar body size is seen in **FIGURE 29.10**. Two of the species, the Mongolian gerbil and sand rat, evolved

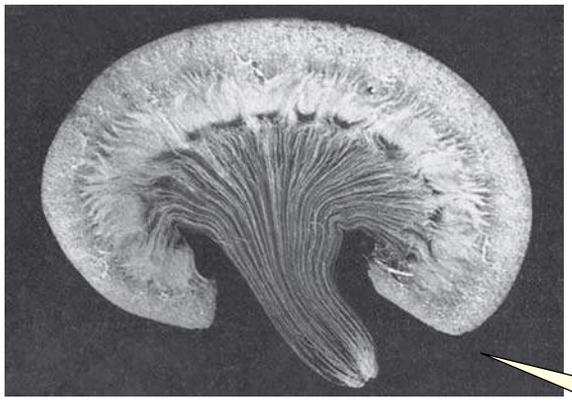
(A) Laboratory rat (*Rattus norvegicus*)



(B) Mongolian gerbil (*Meriones shawii*)



(C) Sand rat (*Psammomys obesus*)



(D) A long-looped nephron in a sand rat kidney

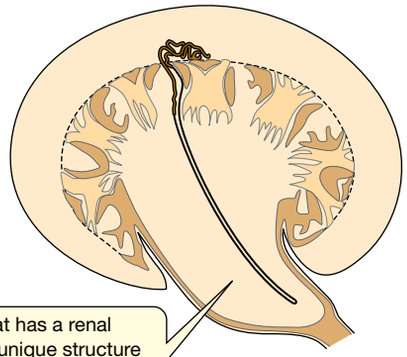


FIGURE 29.10 Kidney structure visualized by injection of the microvasculature (A–C) Midsagittal sections of the kidneys of three species of rodents of similar adult body size, in which the microscopic blood vessels of the kidneys have been injected with rubber for visualization. (D) A drawing of a sand rat kidney showing a nephron with a long loop of Henle. (Photographs in A–C courtesy of Lise Bankir [see Bankir and de Rouffignac 1985]; D after Kaissling et al. 1975.)

in deserts. Both have far more-prominent renal papillae (singular *papilla*) and thicker medullas than the laboratory rat (see Figure 29.10A–C). Moreover, the sand rat has a thicker, longer papilla than the gerbil. Detailed studies of the sand rat reveal that its renal medulla is particularly elaborately organized; in comparison with most mammals, an especially high proportion of the long loops of Henle in the sand rat extend far into the papilla (see Figure 29.10D), rather than turning back only a fraction of the way toward the tip. The sand rat, when living in its natural habitat, experiences far higher dietary salt loads than the gerbil because it subsists largely on succulent plants of very high salt content (see page 734), whereas the gerbil is a seed eater. The sand rat can produce a slightly more concentrated urine than the gerbil (6300 milliosmolar [m*Osm*] versus 5000 m*Osm*, respectively). What is more striking, however, is that the sand rat produces far greater *volumes* of highly concentrated urine than the gerbil. The longer, thicker papilla in the sand rat kidney correlates with the species' ability to produce an abundance of concentrated urine.

Countercurrent multiplication is the key to producing concentrated urine

When renal physiologists finally figured out how mammals make urine hyperosmotic to their blood plasma, they were guided to the loops of Henle by Sperber's studies of comparative kidney morphology. We will soon return to the loops, but first we need to distinguish *urea* and *nonurea solutes* and discuss the immediate concentrating process for the latter. The nonurea solutes are simply the solutes other than urea. They consist mostly of inorganic ions such as Na^+, K^+, Cl^-, and SO_4^{2-}. An important operational parameter is the osmotically effective concentration of all the nonurea solutes taken together, termed the **total concentration of nonurea solutes**.

THE IMMEDIATE CONCENTRATING PROCESS FOR NONUREA SOLUTES The immediate concentrating process for the nonurea solutes is the removal of water from the urine as it flows through the collecting ducts to leave the kidney. Recall that on its way out of the kidney, urine is discharged from the nephrons into the collecting ducts, and then flows down the collecting ducts—passing first through the renal cortex and then the medulla—prior to being discharged into the renal pelvis and ureter (see Figure 29.6B). At the point where urine enters the collecting ducts, the total concentration of nonurea solutes in the urine is *lower* than that in the blood plasma. However, when a mammal is in a state of antidiuresis, as the urine passes in the collecting ducts through deeper and deeper layers of the medulla, its total concentration of nonurea solutes is progressively elevated: The urine ultimately reaches a concentration of nonurea solutes far above the plasma concentration. The immediate mechanism that concentrates the nonurea solutes during this process is movement of water out of the urinary fluid by osmosis. Nonurea solutes are largely trapped within the collecting ducts because the collecting-duct walls are poorly permeable to such solutes. Thus, as water passes by osmosis out of the urine, the nonurea solutes in the urine become more concentrated. Why does water undergo osmosis out of the urine? The fluids that *surround* the collecting ducts in the medulla, known as the **medullary interstitial fluids**, have a high NaCl concentration. In fact, their NaCl concentration rises steadily with increasing depth in the medulla, so that in the deepest parts of the medulla the osmotic pressure attributable to NaCl is *far* above plasma osmotic pressure. During antidiuresis, the cells of the collecting-duct walls are freely permeable to water. As urine inside the collecting ducts flows deeper into the medulla and encounters ever-more-concentrated medullary interstitial fluids just on the other side of the collecting-duct epithelium, water progressively moves by osmosis out of the urine into the medullary interstitial fluids.

An important attribute of these processes is that *a high NaCl concentration on the outside of the collecting ducts serves to concentrate not only NaCl, but also many other nonurea solutes, on the inside*. This happens because the solutes involved cannot readily cross the walls of the collecting ducts, yet the cells in the duct walls (the duct epithelium) are freely permeable to water. Because of this difference between permeability to solutes and to water, when high interstitial NaCl concentrations are encountered deep in the medulla, the primary process of equilibration between the urine and the medullary interstitial fluid is osmosis. As this osmosis occurs, nonurea solutes in the urine are concentrated *indiscriminately* until their total osmotically effective concentration matches the total osmotically effective concentration of NaCl in the medullary interstitial fluids.

A SINGLE EFFECT BASED ON ACTIVE NaCl TRANSPORT Now we must consider how the gradient of NaCl concentration in the medullary interstitial fluids is created. The loops of Henle are responsible. The first step in understanding how the loops of Henle produce the NaCl gradient is to study a phenomenon, termed the *single effect*, that is well documented in the outer zone of the medulla, where the thick segments of the ascending limbs of the loops of Henle occur.

The cells in the walls of the ascending thick segment of a loop of Henle actively transport NaCl from the tubular fluid inside the loop into the adjacent medullary interstitial fluid. The *consequences* of this NaCl transport, illustrated in **FIGURE 29.11**, depend on the permeability characteristics of the ascending limb and the adjacent descending limb of the loop of Henle. The walls of the ascending limb are essentially impermeable to water. Thus the active transport of NaCl out of the tubular fluid inside the ascending limb creates a difference in osmotic pressure between that fluid and the adjacent interstitial fluid, in addition to decreasing the NaCl concentration of the fluid inside the ascending limb and increasing the NaCl concentration of the interstitial fluid. The permeability characteristics of the descending limb appear to vary from species to species. Nonetheless, by passive processes of one sort or another, the fluid inside the descending limb readily approaches equilibrium or near-equilibrium with the interstitial fluid in terms of osmotic pressure and ion concentrations.

In a few words, the active transport of NaCl out of the ascending limb lowers the NaCl concentration and osmotic pressure of the ascending-limb fluid and raises the NaCl concentration and osmotic pressure of both the adjacent interstitial fluid and adjacent descending-limb fluid. These differences between the ascending-limb fluid and the *adjacent* interstitial and descending-limb fluid represent the **single effect** of the active-transport mechanism.

COUNTERCURRENT MULTIPLICATION The major hurdle in understanding how mammals produce concentrated urine was crossed in the 1940s and 1950s when Werner Kuhn (1899–1968), Heinrich Wirz (1914–1993), and several other investigators demonstrated that the concept of **countercurrent multiplication** ap-

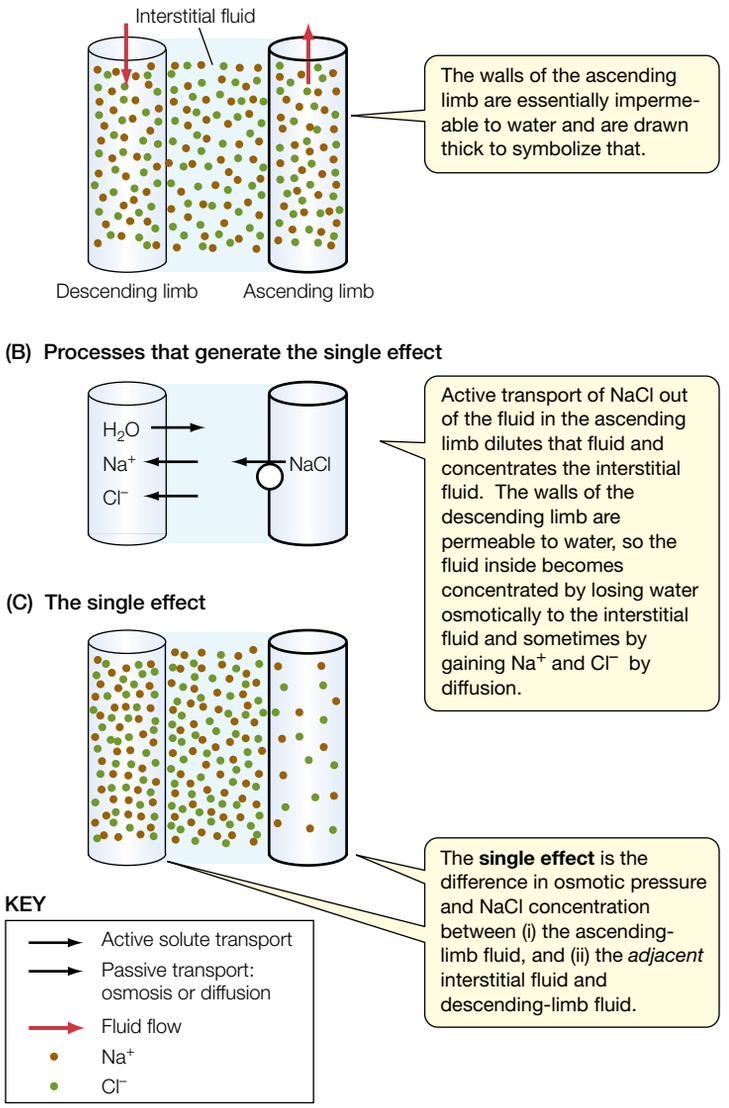


FIGURE 29.11 Generation of the single effect in the loop of Henle Shown here are the ascending limb of the loop of Henle and adjacent descending limb in the outer zone of the medulla, where the thick segments of the ascending limbs occur. As a thought exercise, the diagrams show how the single effect can be generated from scratch. (A) In the initial condition, all the fluids are identical in their osmotic pressures and ion concentrations. (B) The processes that generate the single effect. (C) The single effect that is produced. The osmotic pressure and the concentrations of ions in the ascending-limb fluid are lowered from their original levels, whereas the osmotic pressure and the concentrations of ions in the interstitial and descending-limb fluids are raised.

plies to the loops of Henle. In the classic model of countercurrent multiplication generated by their work, it was assumed that all parts of each ascending limb actively transport NaCl in the manner just described. Here we develop that classic model. Later we discuss complexities introduced by more recent research.

The hairpin shape of a loop of Henle sets up two fluid streams that are oppositely directed (countercurrent), intimately juxtaposed, and connected. These properties are all requirements for a countercurrent multiplier system to operate. Such a system also requires a metabolic energy investment within the system (**BOX 29.3**). The energy investment in the loop of Henle is provided by the active NaCl transport we have already discussed, which—at an ATP cost—creates a difference in osmotic pressure and ion concentration between adjacent parts of the oppositely directed fluid streams—the single effect (see Figure 29.11C).

The countercurrent multiplier system multiplies the single effect. To be more specific, the single effect amounts to a difference of roughly 200 m*Osm* oriented *from side to side* in the loop of Henle. The countercurrent multiplier system multiplies this difference into a much larger difference in concentration *from end to end* in the loop (**FIGURE 29.12A**). An end-to-end difference of 600 m*Osm*

BOX 29.3 Countercurrent Multipliers versus Countercurrent Exchangers

When oppositely directed fluid streams are closely juxtaposed and commodities are actively or passively exchanged between them, the effect of the countercurrent arrangement is to preserve or magnify differences in the levels of those commodities from *end to end* along the axis of fluid flow. The countercurrent arrangement has this effect because it *impedes end-to-end flux* of commodities that are actively or passively exchanged between the fluid streams.

Two functional types of countercurrent systems are recognized: *active* and *passive*. The active systems are **countercurrent multipliers**, exemplified by the loops of Henle. The passive systems are called **countercurrent exchangers** (or *countercurrent diffusion exchangers*) and are exemplified by the heat exchangers in the appendages of mammals (see Figure 10.35B).

In an active system, metabolic energy is used *within the countercurrent system itself* to induce flux of commodities into or out of the fluid streams; within the loop of Henle, for example, ATP energy is used to transport NaCl out of the ascending limb. In a passive system, fluxes of commodities into or out of the fluid streams occur without expenditure of metabolic energy *in the countercurrent system itself*. In the heat exchanger in Figure 10.35B, for example, heat does not move out of one blood vessel and into another because of any metabolic energy expenditure *within* the countercurrent system; instead, heat follows temperature gradients that exist because energy expenditure *elsewhere* in the body has caused the body core to be warmer than the environment.

Active countercurrent systems produce—they *create*—differences in levels of commodities from end to end along their axis of flow. Note, for instance, that if energy expenditure (ion pumping) in the loops of Henle were turned off, the gradient of osmotic pressure and NaCl concentration from the outer to the inner end of the loops would disappear. Passive systems, by contrast, do not *create* end-to-end differences; instead they *preserve* or *accentuate* end-to-end differences that already exist for other reasons.

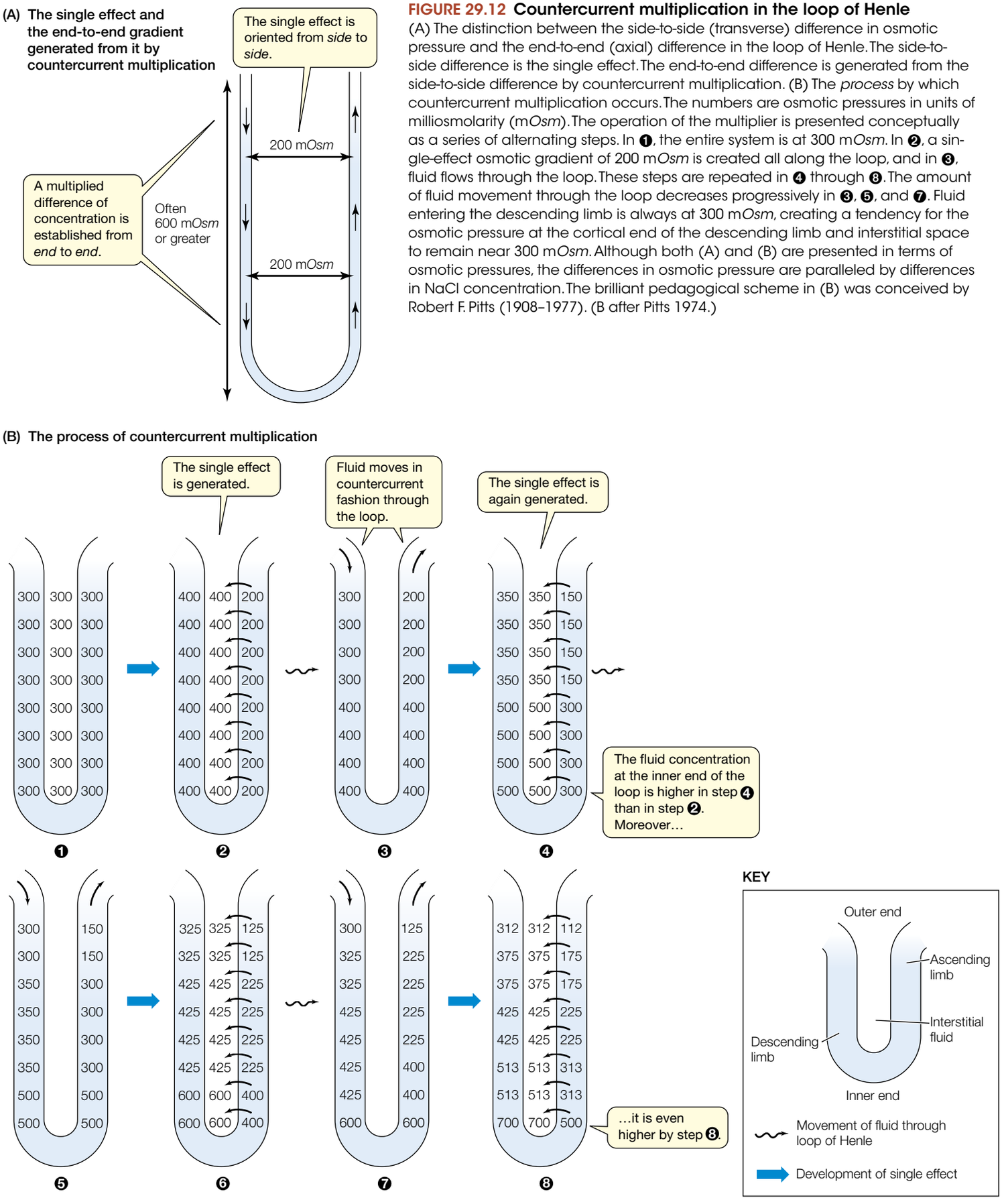


FIGURE 29.12 Countercurrent multiplication in the loop of Henle
(A) The distinction between the side-to-side (transverse) difference in osmotic pressure and the end-to-end (axial) difference in the loop of Henle. The side-to-side difference is the single effect. The end-to-end difference is generated from the side-to-side difference by countercurrent multiplication. (B) The *process* by which countercurrent multiplication occurs. The numbers are osmotic pressures in units of milliosmolarity (m*Osm*). The operation of the multiplier is presented conceptually as a series of alternating steps. In ❶, the entire system is at 300 m*Osm*. In ❷, a single-effect osmotic gradient of 200 m*Osm* is created all along the loop, and in ❸, fluid flows through the loop. These steps are repeated in ❹ through ❽. The amount of fluid movement through the loop decreases progressively in ❸, ❺, and ❼. Fluid entering the descending limb is always at 300 m*Osm*, creating a tendency for the osmotic pressure at the cortical end of the descending limb and interstitial space to remain near 300 m*Osm*. Although both (A) and (B) are presented in terms of osmotic pressures, the differences in osmotic pressure are paralleled by differences in NaCl concentration. The brilliant pedagogical scheme in (B) was conceived by Robert F. Pitts (1908–1977). (B after Pitts 1974.)

would not be unusual. Many mammals can create an end-to-end difference that is much greater.

The mechanism of countercurrent multiplication is diagrammed in **FIGURE 29.12B**. Although osmotic pressures are shown in the figure and the following discussion is phrased in those terms, it will be important to remember that differences in osmotic pressure in the loop of Henle are paralleled by differences in NaCl concentration. In step ❶ of Figure 29.12B, the entire loop of Henle and the interstitial space are filled with fluid of the same osmotic pressure as that exiting the proximal convoluted tubule—approximately isosmotic to the blood plasma (300 m*Osm*). In step ❷, active transport establishes a single-effect osmotic gradient of 200 m*Osm* all along the loop. In step ❸, fluid moves through the loop in countercurrent fashion. Fluid that was concentrated in the descending limb during step ❷ is thus brought around into the ascending limb and now lies opposite to the descending limb, so that both limbs and the interstitial space are filled with concentrated fluid at the inner end of the loop. Now when, in step ❹, the single-effect osmotic gradient is again established, the interstitial fluid is elevated to 500 m*Osm* at the inner end, rather than the 400 m*Osm* developed in step ❷, and the fluid in the descending limb also reaches this higher osmotic concentration of 500 m*Osm*. Steps ❺ and ❻, and steps ❼ and ❽, repeat this process and should be studied in order to see how the countercurrent multiplier works. *Fluid concentrated in the descending limb moves around into the ascending limb, setting the stage for the single effect to produce an ever-increasing osmotic concentration in the interstitial fluid and descending limb at the inner end of the loop.* Meanwhile, the steady influx of 300-m*Osm* fluid into the beginning of the descending limb, and the dilution of the ascending-limb fluid as it flows from deep in the medulla to the top of the ascending limb, combine to keep the osmotic pressure of the interstitial fluid at the outer (cortical) end of the loop near 300 m*Osm*. Thus the difference in osmotic pressure between the two ends of the loop becomes greater and greater, so much so that it greatly exceeds the single effect (see Figure 29.12A).

As noted previously, during the early years when the countercurrent multiplication concept was initially applied to understanding mammalian kidney function, the single effect was postulated to be created along the entire length of a loop of Henle by active NaCl transport out of the ascending limb. However, by 1970, research had established that the thin segment of the ascending limb deep in the medulla is unlikely to be carrying out such active transport. That discovery started a saga that continues unended today. Active transport of NaCl out of the tubular fluid occurs in the thick segment of the ascending limb, and a consensus exists that the single effect is created according to the classic model (see Figure 29.11) in the outer region of a loop of Henle where the thick ascending segment occurs. However, the single effect is now assumed to be created by some other mechanism in the inner region of a loop of Henle where the ascending limb is thin.

All this said, it is important to return to the big picture: Countercurrent multiplication of a single effect along much or all of the length of a loop of Henle creates a large gradient of osmotic pressure from one end of the loop to the other. In the medulla, there are thousands of loops of Henle, all aligned in parallel. We would expect that all these loops, by their combined action, would create in the medullary tissue as a whole a dramatic gradient of increasing osmotic pressure from the outer side of the tissue (next to the cortex) to the inner side of the tissue (farthest from the cortex). The classic data that originally confirmed this expectation are shown in **FIGURE 29.13**.

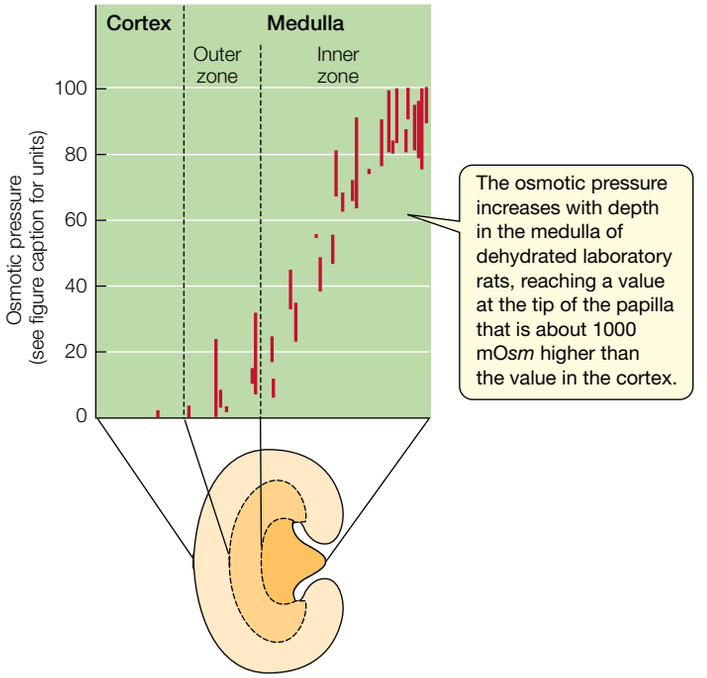


FIGURE 29.13 Osmotic pressure increases with depth in the medulla Each vertical red line shows the range of osmotic pressures measured at a particular depth in the cortex or medulla of kidneys taken from five dehydrated laboratory rats. All the rats, in addition to being sampled at various places, were deliberately sampled at the tip of the papilla, accounting for the cluster of data there. On the *y* axis, 0 represents an osmotic pressure equal to that of the blood plasma in the general circulation, whereas 100 represents the highest osmotic pressure measured (about 1000 m*Osm* greater). Intermediate osmotic pressures are scaled relative to the two extremes; specifically, any particular measured osmotic pressure (OP) is expressed as 100 × (measured OP – plasma OP)/(maximum OP – plasma OP). Throughout the cortex, the osmotic pressure is equivalent to the osmotic pressure of plasma in the general circulation. The increase in osmotic pressure with depth in the medulla is attributable both to an increase in NaCl concentration and to an increase in urea concentration. (After Wirz et al. 1951.)

CONCLUDING POINTS ON THE MECHANISM OF CONCENTRATING NONUREA SOLUTES **FIGURE 29.14A** summarizes the changes in the total concentration of nonurea solutes in the tubular fluid of nephrons and collecting ducts when the kidney of a mammal is producing concentrated urine. As fluid in a nephron travels down the descending limb of the loop of Henle, its concentration of nonurea solutes rises, reaching a high level at the hairpin bend of the loop. Thereafter, as the fluid comes back out of the medulla in the ascending limb of the loop of Henle, its concentration of nonurea solutes falls, so that by the time the fluid exits the loop, it is actually *more dilute* than when it started and more dilute than the blood plasma. Then, however, the fluid makes a final, crucial pass through the medulla, traveling down a collecting duct to be discharged into the renal pelvis. On this pass, final concentration of the nonurea solutes occurs.

The *total concentration of nonurea solutes* in the definitive urine depends on the *NaCl concentration* of the interstitial fluids of the innermost medulla, because in a kidney producing concentrated urine, the urine osmotically equilibrates with those interstitial fluids just before leaving the kidney (see Figure 29.14A). In turn, the inner-medullary NaCl concentration itself depends on the

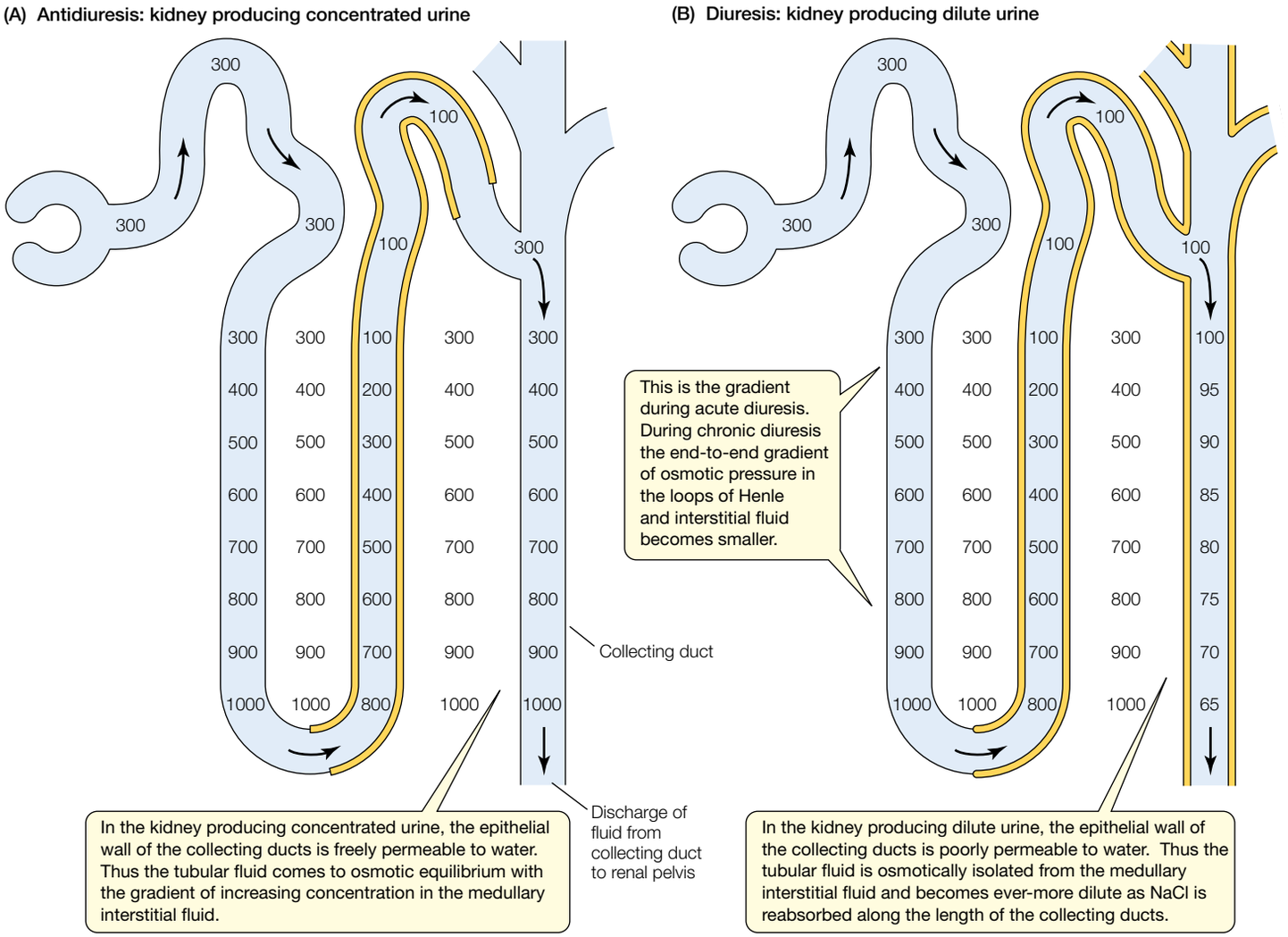


FIGURE 29.14 Osmotic pressures attributable to nonurea solutes in the nephrons and collecting ducts during antidiuresis and diuresis Thick yellow borders symbolize tubules that are poorly permeable to water. Tubules without yellow borders are permeable to water. The change in the water permeability of the collecting ducts between antidiuresis (A) and diuresis (B) is mediated by insertion and removal of aquaporins in apical cell membranes of the collecting-duct epithelium, as discussed later. The interstitial fluids (white areas) exhibit similar gradients of osmotic pressure throughout the medulla. The numbers, expressed in units of milliosmolarity, are approximate and intended only to illustrate general trends.

properties of the countercurrent multiplier system, including the size of the single effect, the rate of fluid flow through the loops of Henle, and the lengths of the loops. Lengthening of the loops tends to increase the end-to-end gradient of NaCl concentration that can be maintained by the loops and thus tends to raise the inner-medullary NaCl concentration. This explains why, among related species of similar body size, the species with a relatively thick medulla and prominent renal papilla tend to be capable of producing relatively concentrated urine (see Figures 29.7 and 29.8).[14]

CONCENTRATION OF UREA The mechanisms that concentrate urea differ from those that concentrate the nonurea solutes. Whereas the walls of the collecting ducts block most nonurea solutes in the urine and medullary interstitial fluid from diffusing to electrochemical equilibrium, they permit urea to do so. Urea is present at high concentration in the medullary interstitial fluid, and when mammals are in an antidiuretic state, the walls of the collecting ducts *in the inner medulla* permit urea to diffuse freely between the urine inside the ducts and the inner-medullary interstitial fluid (this diffusion is mediated by a facilitated-diffusion *urea transporter [UT] protein* that is dramatically upregulated by ADH). Basically, therefore, high urea concentrations in the urine reflect the diffusion of urea to concentration equilibrium across the walls of the inner-medullary collecting ducts.

How does urea come to be present at high concentration in the medulla? Put simply, much more urea is filtered than is excreted, and some of the urea reabsorbed along the nephrons accumulates in the medulla. The thick ascending segment of the loop of Henle, the distal convoluted tubule, and the cortical and outer-medullary

[14] When comparing species that cover a wide range of body sizes, *relative* loop length—estimated as relative medullary thickness—is a far better predictor of concentrating ability than *absolute* loop length (see Figure 29.8). Factors other than absolute length thus clearly play major roles in kidney concentrating function, but these additional factors are not yet understood.

parts of the collecting duct are poorly permeable to urea. During antidiuresis, at least in the cortical and outer-medullary collecting duct, water is drawn out of the tubular fluid by osmosis.[15] Because the permeability to urea in these tubular regions is low, urea—trapped inside—becomes concentrated in the tubular fluid as water is lost. The important net result is that the tubular fluid has a high urea concentration by the time it flows into the inner-medullary collecting duct, which is highly permeable to urea during antidiuresis. Urea therefore diffuses from the tubular fluid into the interstitial fluid in the inner medulla, a process that charges the inner-medullary interstitial fluid with urea. According to present thinking, this entire sequence of events is *self-reinforcing* because urea enters the tubular fluid in the loop of Henle. That is, as tubular fluid flows through the loop, it picks up urea from the inner-medullary interstitial fluid, and soon afterward that tubular fluid flows into the inner-medullary collecting duct. Because of this process (sometimes called *urea recycling*) the urea concentration of tubular fluid arriving in the inner-medullary collecting duct tends automatically to rise in parallel with the urea concentration of the interstitial fluid. Thus, with a steady influx of new urea from filtration, a gradient favorable for diffusion of urea *into* the interstitial fluid from the inner-medullary collecting duct is maintained, even though the interstitial-fluid concentration may rise to a high level. A high concentration of urea in the interstitial fluid promotes a high urinary concentration of urea because diffusive outflux of urea from the collecting-duct fluid continues only to the point of concentration equilibrium with the medullary interstitial fluid.

How does the process of urea concentration relate to the process by which nonurea solutes are concentrated? Although the answer is intricate, two important global points should be made. First, because urea and nonurea solutes are concentrated by rather separate mechanisms, a high urea concentration in the urine does not in any simple mathematical fashion displace nonurea solutes or reduce the concentration of nonurea solutes that is possible. The urine of a mammal can *simultaneously* contain high concentrations of both urea and nonurea solutes. The second point to be made is that the osmotic reabsorption of *water* from the urine in the inner medulla is controlled by the processing of the *nonurea* solutes. Because urea diffuses to concentration equilibrium across the walls of the inner-medullary collecting ducts, it does not (except transiently) make a direct contribution to the *difference* in osmotic pressure between the collecting-duct fluid and the interstitial fluid. The *difference* in osmotic pressure—which is responsible for concentrating nonurea solutes in the urine by governing the osmotic reabsorption of water—is a consequence of different concentrations of the nonurea solutes.

THE BLOOD SUPPLY OF THE MEDULLA: THE VASA RECTA The blood capillaries of the medulla form hairpin loops—known as **vasa recta**—that parallel the loops of Henle. This arrangement, diagrammed in Figure 29.6B, is vividly evident in Figure 29.10A–C, in which the structures visualized are the blood vessels.

The looped shape of the vasa recta prevents the circulation of blood to the medulla from destroying the concentration gradients of NaCl and urea in the medullary interstitium. To see this, consider what would happen if blood, after flowing into the medulla from the cortex, simply exited the medulla on the inner (i.e., deep, pelvic) side. The walls of blood capillaries are freely permeable to water and small solutes. Thus, as blood flowed from the cortex, deeper and deeper into the medulla—encountering ever-more-concentrated interstitial fluids—it would lose water to the interstitial fluids by osmosis and take up NaCl and urea by diffusion. Exiting on the inner (deep) side of the medulla, the blood would leave all that water behind and take the solutes away, diluting the medullary interstitial fluids in both ways. Instead, after flowing from the cortex to the inner medulla, the blood reverses direction and flows back to the cortex. On its way out, as it encounters ever-more-dilute interstitial fluids, it reabsorbs water and yields NaCl and urea, reversing the processes that occurred on the way in. The familiar tendency of countercurrent flow to preserve gradients oriented parallel to the axis of flow is once again evident. The vasa recta act as *countercurrent diffusion exchangers* (see Box 29.3).

An important function of blood flow through the vasa recta is to remove water from the medullary interstitial fluids. The final process of concentrating the urine, as we have seen, entails osmotic movement of water from the collecting ducts into the inner-medullary interstitial fluid. This water, if allowed to accumulate, would itself dilute the inner-medullary fluid and thereby diminish the medullary concentration gradient. The flow of blood through the vasa recta carries the water away. Evidently, as the blood dynamically loses water during its passage into the medulla and regains water during its passage out, the colloid osmotic pressure resulting from the blood proteins introduces a bias for the gains of water by the blood to exceed losses.

CELL-VOLUME REGULATION, COMPATIBLE SOLUTES, AND COUNTERACTING SOLUTES IN THE MEDULLA The cells in the medulla of the kidney—such as those in the walls of the loops of Henle and vasa recta—are unique among the cells in a mammal's body in that they must tolerate exposure to very high solute concentrations and osmotic pressures in the interstitial fluids that bathe them. The medullary cells must have high levels of intracellular solutes to maintain normal cell volumes rather than being shriveled by osmotic water loss, as explained in Figures 27.8 and 27.9. In comparison with all other cells in a mammal's body, the renal medullary cells are noted for having exceptionally high intracellular concentrations of organic osmolytes of metabolic origin, notably polyhydric alcohols and methylamines (**FIGURE 29.15**). These organic compounds serve as *compatible solutes* (see page 738): They balance the high extracellular NaCl concentration while having relatively small effects on cell macromolecules.[16]

The regulatory roles of the kidney tubules in overview: The concentrating and diluting kidney and the control of transitions

Thus far we have focused on how the mammalian kidney can produce urine more concentrated than the blood plasma. The mammalian kidney resembles other kidneys, however, in that it

[15] NaCl is actively transported out of the tubular fluid in these parts of the collecting duct, causing water to move out by osmosis.

[16] The high concentrations of urea in the renal medulla are themselves a challenge to the function of medullary cells because urea can perturb enzymes and other proteins. There is some evidence that the methylamines accumulated in medullary cells (see Figure 29.15) help to offset the perturbing effects of urea. That is, the methylamines act as counteracting solutes (see page 739).

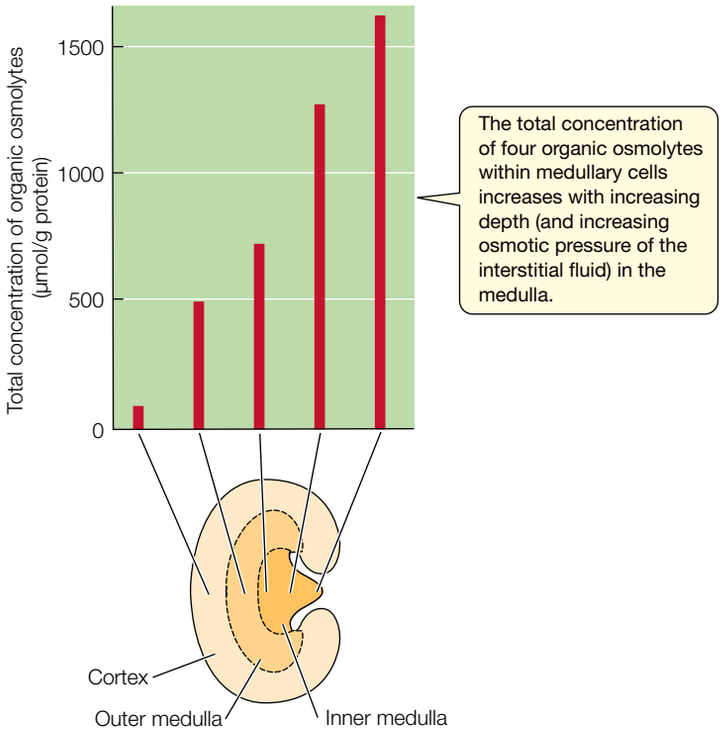


FIGURE 29.15 Cell-volume regulation by organic osmolytes in the medulla of the kidney The cells in the renal medulla produce high intracellular concentrations of organic osmolytes as a means of regulating cell volume in the face of the high osmotic pressures in the interstitial fluids bathing them. The data shown are for normally hydrated laboratory rats. Each bar represents, at the designated anatomical location, the sum of the four principal organic osmolytes. The four osmolytes are two polyhydric alcohols (sorbitol and *myo*-inositol) and two trimethylamines (glycine betaine and glycerophosphorylcholine). Concentration is expressed as total micromoles of osmolytes per gram of tissue protein. (After Beck et al. 1998.)

carries out many processes simultaneously as it performs its overall function of regulating the composition and volume of the blood plasma and other body fluids. In this section we take more of an overview of nephron and collecting-duct function in mammals. A useful way to approach this task is, first, to discuss how multiple solutes and water are processed when the kidney is producing a concentrated urine, and then discuss—in a synthetic way—how the kidney functions when producing dilute urine and how the switch between concentration and dilution is regulated.

AN OVERVIEW OF EVENTS IN THE CONCENTRATING KIDNEY

Glomerular filtration is, of course, the first step in forming urine. In comparison with other vertebrates, mammals—with some known exceptions (e.g., dromedary camels)—tend to maintain relatively stable GFRs and adjust their rate of urine production principally by adjusting the fraction of filtered fluid that they ultimately reabsorb and return to the blood prior to excretion. The fluid introduced into the Bowman's capsule of a nephron by filtration is approximately isosmotic to the blood plasma and contains similar concentrations of inorganic ions, glucose, and amino acids. A major function of the proximal convoluted tubule is net reabsorption of NaCl and water—net return of NaCl and water to the body fluids. In fact, 60%–80% of the filtered amounts of NaCl and water are reabsorbed by the time the tubular fluid reaches the beginning of the loop of Henle. The cells of the epithelial walls of the proximal tubule are freely permeable to water because of aquaporins (discussed shortly), so water exits osmotically as NaCl and other solutes are reabsorbed, and the tubular fluid stays isosmotic to the blood plasma. Glucose, many amino acids, and HCO_3^- (bicarbonate ion) are almost completely reabsorbed and returned to the blood plasma in the proximal tubule.

A major contemporary area for research is establishing the molecular basis for the function of the proximal convoluted tubule and all other segments of the kidney tubules. The ultimate goal of this research is to understand every aspect of reabsorption and secretion along all parts of the renal tubules in terms of the specific transporter proteins, channel proteins, and other molecules that mediate the processes. **FIGURE 29.16A** summarizes the major molecular ion-transport mechanisms in the epithelial cells of the wall of the early proximal convoluted tubule. Na^+ reabsorption from the urine is driven by primary active transport carried out by Na^+–K^+-ATPase (see page 115) in the basolateral membrane, which creates a Na^+ electrochemical gradient across the apical membrane favoring Na^+ uptake from the tubular fluid. The reabsorption of glucose and amino acids occurs by secondary active transport (see page 115).

Aquaporins, as earlier mentioned, provide the molecular basis for the high water permeability of the proximal tubule epithelium. The aquaporins in cell membranes of the epithelial cells of the proximal tubule are classified as *constitutive* because they are always present in the cell membranes; their levels are not much affected by external agents.

After fluid leaves the proximal convoluted tubule, its next step is to travel through the loop of Henle. Although the tubular fluid enters the loop isosmotic to plasma (~300 m*Osm*), it exits the loop hyposmotic to plasma (perhaps 100–150 m*Osm*), as we have seen (see Figure 29.14A). In the ascending thick segment of the loop of Henle, active NaCl transport out of the tubular fluid is a key process that both creates the single effect for countercurrent multiplication, and accounts for the hyposmotic state of the tubular fluid as it leaves the loop of Henle. **FIGURE 29.16B** presents a current model of the molecular biology of the active NaCl transport out of the tubular fluid in the ascending thick segment. **Loop diuretics**—medications employed to treat hypertension (high blood pressure)—are targeted at the Na–K–2Cl cotransporter. These medications inhibit NaCl transport out of the tubular fluid by inhibiting the cotransporter, resulting in increased Na^+ excretion and water excretion, which tend to decrease the volume of the blood plasma (see page 777).

After exiting the loop of Henle, the tubular fluid passes through the distal convoluted tubule. The epithelial walls of much or all of the distal convoluted tubule are poorly permeable to water and actively transport NaCl out. Thus the tubular fluid remains strongly hyposmotic to the blood plasma (see Figure 29.14A). Potassium (K^+) is added to the tubular fluid (partly passively, partly actively) in the distal convoluted tubule and cortical collecting duct. This addition of K^+ controls the amount of K^+ that is removed from the body fluids and eliminated in the urine because most K^+ from filtration was reabsorbed from the tubular fluid in earlier parts of the nephron.

Perhaps 5% or less of the originally filtered volume reaches the collecting duct. In the concentrating kidney, the collecting duct is permeable to water because of an aquaporin-based mechanism discussed in the next section.[17] Thus dilute tubular fluid arriving in the collecting duct promptly comes to isosmoticity with the cortical

[17] The terminal distal tubule may also be permeable to water.

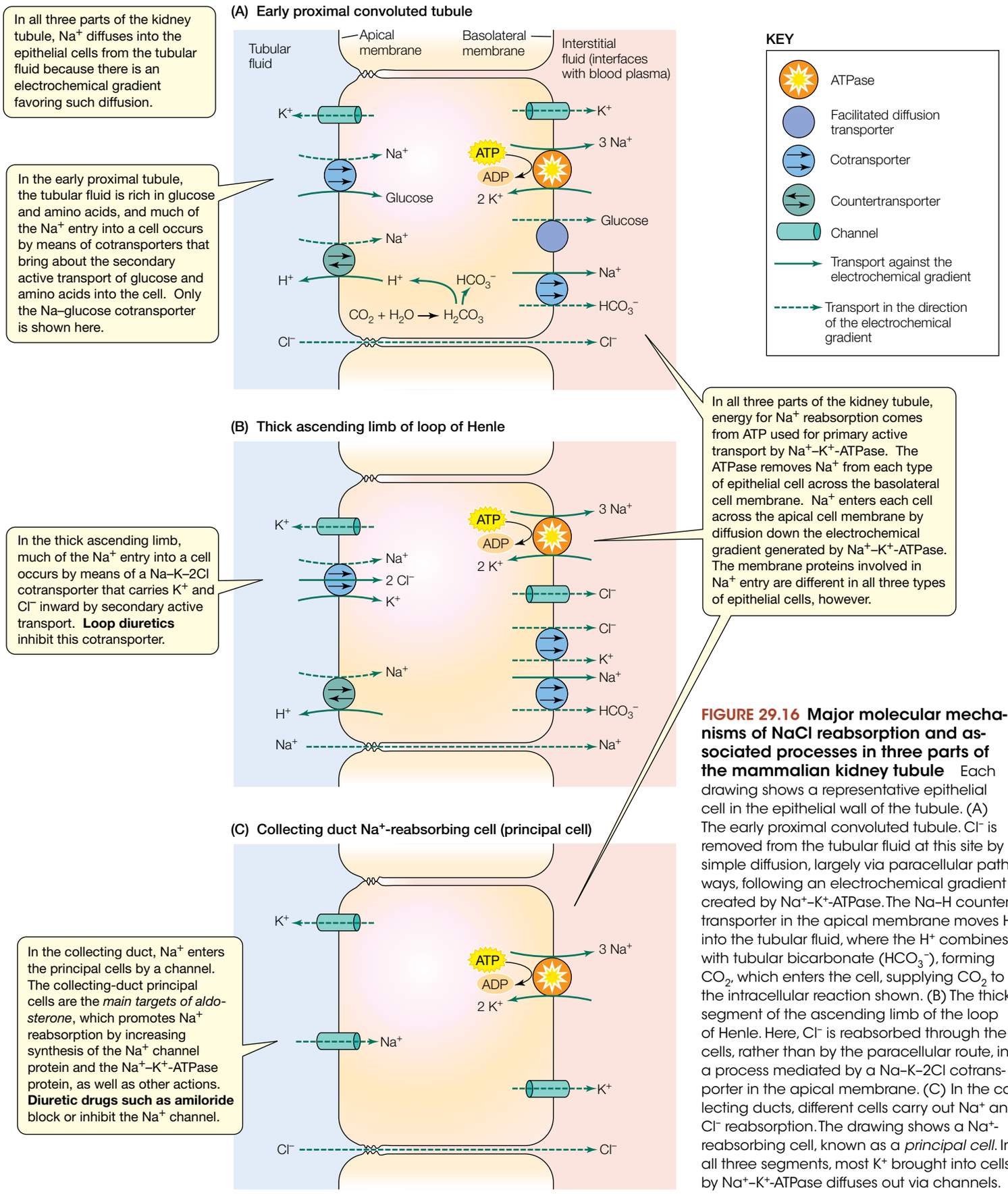


FIGURE 29.16 Major molecular mechanisms of NaCl reabsorption and associated processes in three parts of the mammalian kidney tubule Each drawing shows a representative epithelial cell in the epithelial wall of the tubule. (A) The early proximal convoluted tubule. Cl^- is removed from the tubular fluid at this site by simple diffusion, largely via paracellular pathways, following an electrochemical gradient created by Na^+–K^+-ATPase. The Na–H countertransporter in the apical membrane moves H^+ into the tubular fluid, where the H^+ combines with tubular bicarbonate (HCO_3^-), forming CO_2, which enters the cell, supplying CO_2 to the intracellular reaction shown. (B) The thick segment of the ascending limb of the loop of Henle. Here, Cl^- is reabsorbed through the cells, rather than by the paracellular route, in a process mediated by a Na–K–2Cl cotransporter in the apical membrane. (C) In the collecting ducts, different cells carry out Na^+ and Cl^- reabsorption. The drawing shows a Na^+-reabsorbing cell, known as a *principal cell*. In all three segments, most K^+ brought into cells by Na^+–K^+-ATPase diffuses out via channels.

interstitial fluid (~300 m*Osm*) by osmotic outflux of water (see Figure 29.14A). The tubular fluid then descends deeper and deeper into the medulla, from the cortical to the pelvic end of the collecting duct. As it does so, it encounters an ever-higher interstitial NaCl concentration and attains higher concentrations of urea and nonurea solutes by the mechanisms we have discussed. Water is reabsorbed osmotically and returned to the blood plasma in the vasa recta. Especially in the cortical part of the collecting duct, but also in the inner-medullary part, NaCl is actively reabsorbed (**FIGURE 29.16C**). This reabsorption of NaCl in the collecting duct determines the final amount of NaCl that is removed from the body fluids and excreted. It also plays a key role in controlling urine volume—the amount of water removed from the body fluids—because by reducing the amount of nonurea solute in the urine, it enhances osmotic return of water from the urine to the blood. In the end, mammals in antidiuresis typically excrete only 1% or less of the filtered NaCl and water.

THE DILUTING KIDNEY AND THE REGULATION OF SWITCHES BETWEEN CONCENTRATION AND DILUTION Individual mammals are typically capable of adjusting the concentration and volume of their urine over broad ranges, thereby modulating the effects of urine production on the concentration and volume of the blood plasma and other body fluids. A person in antidiuresis might produce urine that is as concentrated as about 1200 m*Osm* (U/P = 4) and limited in volume to less than 1% of the filtered amount. In diuresis, by contrast, that person might produce urine as dilute as about 50 m*Osm* (U/P = 0.2) and increase the volume to about 15% of the filtered amount. The effects on the body fluids vary commensurately. *In antidiuresis the high osmotic concentration of the urine tends to dilute the body fluids (see Figure 27.7), and the low urine volume has the synergistic effect of conserving water. Conversely, in diuresis, the low urine osmotic concentration tends to concentrate the body fluids (see Figure 27.7), and the high urine volume voids water.*

The principal agent of control of switches between antidiuresis and diureses is antidiuretic hormone (ADH). The ADH of most mammals is *arginine vasopressin*; therefore ADH is often called **vasopressin** in books on mammalian physiology and medicine. As knowledge advances, the known effects of ADH become more extensive and complex. The action of ADH that is of most central importance in mammals is that it modulates the permeability of the collecting ducts to water.

The effect of ADH on the permeability of the collecting-duct epithelium is mediated by a specific molecular form of aquaporin, AQP-2, that is inserted into and retrieved from the apical cell membranes of the collecting-duct epithelial cells; Figure 16.16 shows this process in detail. The presence of ADH causes insertion of aquaporin molecules into the apical cell membranes and an increase in epithelial permeability to water (**FIGURE 29.17**). When ADH levels fall, the aquaporin molecules are retrieved from the apical cell membranes and epithelial permeability to water decreases.

In the concentrating kidney, although we have not said so heretofore, the high permeability of the collecting ducts to water is elicited by high blood levels of ADH. This high water permeability permits water to leave the collecting ducts by following the osmotic gradient between the collecting-duct fluid and medullary interstitial fluid. The osmotic exit of water, as previously stressed, accounts for both the *low volume* and *high concentration* of the urine produced during antidiuresis.

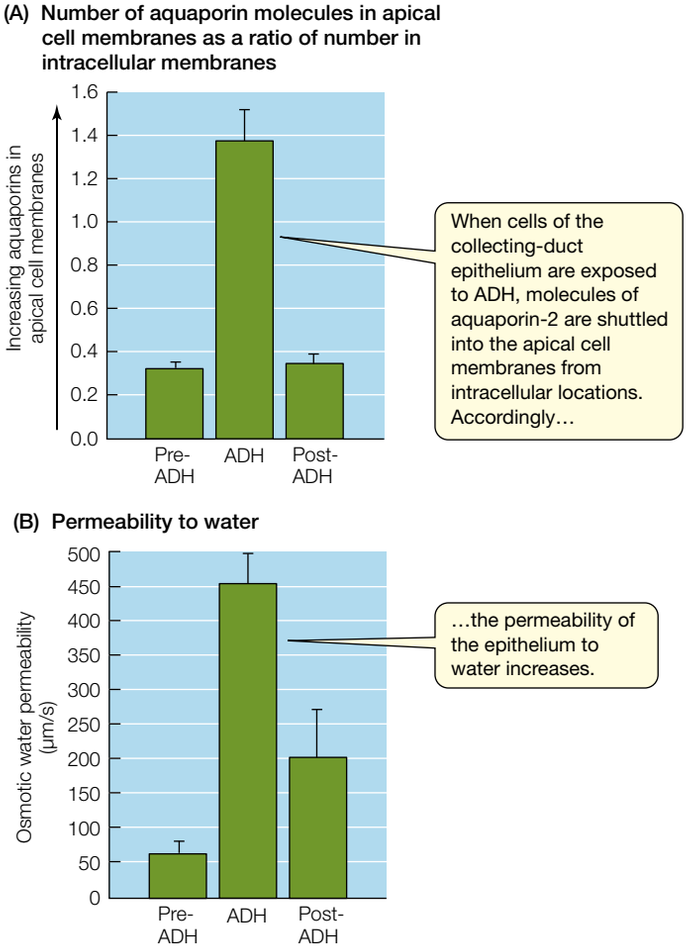


FIGURE 29.17 The collecting-duct epithelium: Cellular position of molecules of aquaporin-2 (AQP-2), and permeability to water, when ADH is present or absent Studies were carried out on collecting ducts from the inner medulla. The distribution of AQP-2 molecules was determined by visualizing and directly counting the molecules by means of immunological labels and electron microscopy. According to the shuttle hypothesis, the AQP-2 molecules in each epithelial cell are shuttled back and forth between the apical cell membrane and intracellular vesicular membranes. (A) The number of AQP-2 molecules in apical cell membranes as a ratio of the number in intracellular vesicular membranes. (B) Permeability of the collecting-duct epithelium to water. (After Knepper et al. 1996.)

When blood levels of ADH are low and aquaporins are retrieved from the apical cell membranes of the collecting-duct epithelium, the collecting ducts are poorly permeable to water. The distal convoluted tubules are also poorly permeable. Thus, during diuresis, from the time the tubular fluid exits the loops of Henle to the time it is discharged into the renal pelvis, it is blocked from coming freely to osmotic equilibrium with the surrounding cortical and medullary interstitial fluids. Recall that the tubular fluid is hyposmotic to plasma when it exits the loops; therefore it would lose water osmotically if it could. However, the low water permeability of the walls of the distal tubules and collecting ducts in diuresis—when ADH levels are low—impedes such water loss. **FIGURE 29.14B** shows a further consequence of the low water permeability: As NaCl is actively reabsorbed in the distal tubules and collecting ducts during diuresis, the tubular fluid becomes ever-more hyposmotic to the plasma. The urine produced is both *abundant* (because of little water reabsorption) and *dilute* (because of the diluting process just described).

Notice how fundamentally similar the action of ADH is in mammals and in amphibians. In both groups—and indeed, in all groups of tetrapod vertebrates—the primary effect of ADH on the renal tubules is to increase the permeability to water of tubular epithelia that otherwise are poorly permeable.[18] This increase in permeability to water has the important consequence that it allows the tubular fluids to come to osmotic equilibrium with the fluids surrounding the tubules. In amphibians, the fluids surrounding the distal tubules and collecting ducts are osmotically similar to the blood; thus the presence of ADH causes production of urine that approaches isosmoticity with the blood. In mammals, the collecting ducts are surrounded by fluids that are hyperosmotic to the blood. Consequently, ADH causes production of hyperosmotic urine. In both mammals and amphibians, ADH principally controls the excretion of *water* and thus controls the removal of *water* from the body fluids.[19] Although the amount of each nonurea *solute* excreted is adjusted by solute-specific tubular mechanisms (e.g., active reabsorption or secretion), the concentration of ADH determines the amount of water that is extracted from the body fluids and excreted with the solutes.

When an individual mammal switches between chronic antidiuresis and chronic diuresis, an additional change besides the permeability adjustments occurs: The magnitude of the osmotic gradient in the medullary interstitial fluids—the gradient between the cortex and inner medulla—diminishes. For example, in a dog shifted from chronic antidiuresis to chronic diuresis, the osmotic pressure of the inner-medullary interstitial fluid might change from 2400 m*Osm* (about 2100 m*Osm* higher than the cortical osmotic pressure) to 500 m*Osm* (about 200 m*Osm* higher). Periods of hours or days are required for such changes to be fully realized.

ADH is not the only hormone that controls kidney function. As discussed in Chapter 28 (see page 777), aldosterone and natriuretic hormones help control the reabsorption and secretion of Na^+ and K^+. In addition, calcitonin affects renal function, and the kidneys themselves employ paracrines, such as eicosanoids and kinins, as *local* chemical messengers.

Modern molecular and genomic methods create new frontiers in the study of kidney function

Modern molecular and genomic methods are enabling kidney researchers to study subjects that seemed utterly beyond reach 25 years ago. A stunning example is provided by recent studies of the fine structure of the mammalian renal medulla. Different parts of the tubules in the medulla often differ categorically in one or more cell-membrane proteins. When this is the case, elements can be distinguished by immunological labels. For the immunocytochemical study in **FIGURE 29.18A**, fluorescent antibodies were prepared against three distinguishing proteins. A blue-fluorescing antibody was prepared against aquaporin-2, which is found only in collecting ducts. Red- and green-fluorescing antibodies were prepared against aquaporin-1 and a urea transporter found, respectively,

[18] This is postulated to occur in all cases by aquaporin insertion.

[19] See Chapter 28 (page 776) for a full explanation of this point and Figure 29.5 for a diagram of how changes in tubular permeability to water can alter the amount of water excreted in the urine.

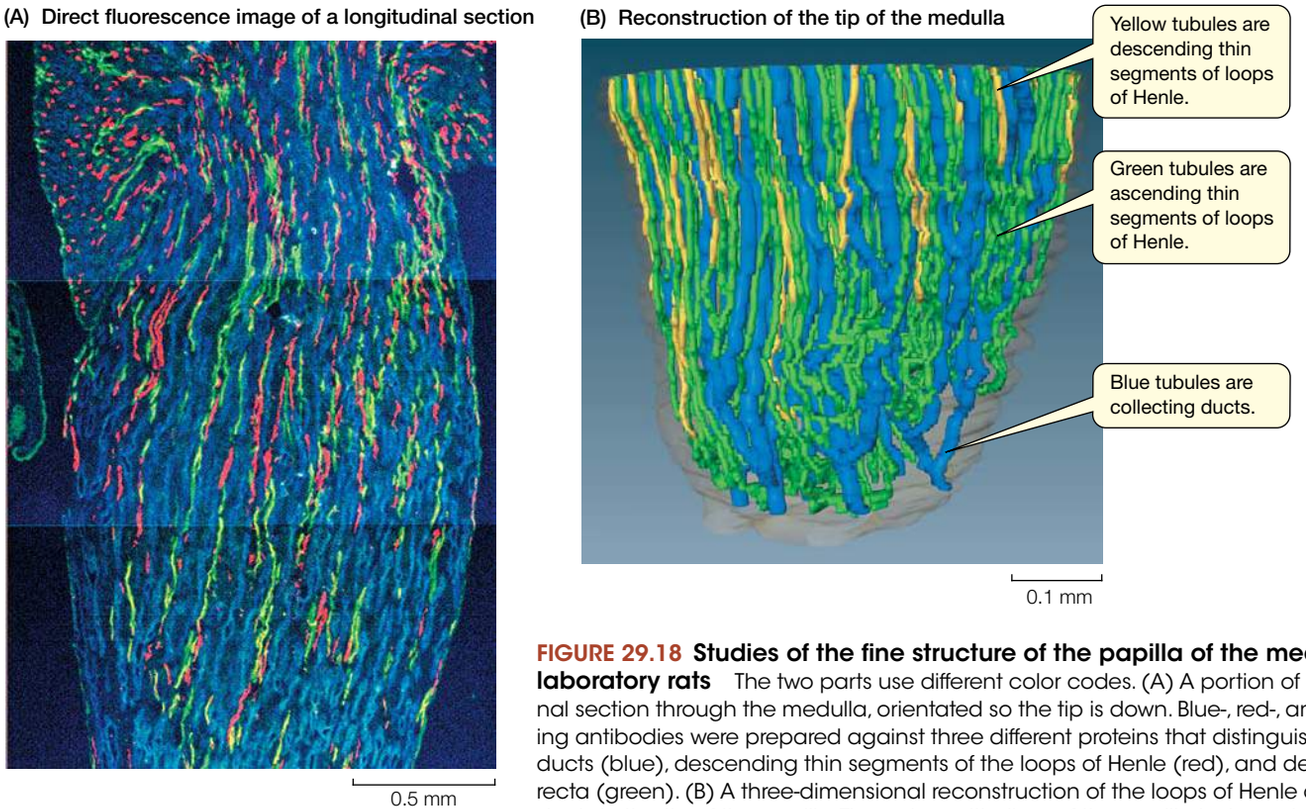


FIGURE 29.18 Studies of the fine structure of the papilla of the medulla in young laboratory rats The two parts use different color codes. (A) A portion of a single longitudinal section through the medulla, orientated so the tip is down. Blue-, red-, and green-fluorescing antibodies were prepared against three different proteins that distinguish the collecting ducts (blue), descending thin segments of the loops of Henle (red), and descending vasa recta (green). (B) A three-dimensional reconstruction of the loops of Henle and collecting ducts in the tip of the papilla. The outer epithelium of the papilla is shown in gray. (Images courtesy of Thomas Pannabecker; from Pannabecker and Dantzler 2007.)

TABLE 29.1 Results of a comprehensive transcriptomic study of the inner-medullary collecting duct: Ten highly expressed genes and the proteins for which they code[a]

Gene	Protein
Actb	Beta actin (a form of nonmuscle actin)
Actg1	Gamma actin (a form of nonmuscle actin)
Aqp2	Aquaporin-2 water channel
Aqp3	Aquaporin-3 water channel
Aqp4	Aquaporin-4 water channel
Atp1b1	Na^+–K^+-ATPase β1 polypeptide
Atp1a1	Na^+–K^+-ATPase α1 polypeptide
Clic1	Chloride channel
Sgk1	Serum/glucocorticoid regulated protein kinase
Slc14a2	Solute carrier family 14 urea transporter

Source: After Uawithya et al. 2007.

[a]Animals studied were pathogen-free laboratory rats (*Rattus norvegicus*).

in the descending thin segments of the loops of Henle and the descending vasa recta. Figure 29.18A shows just a single section through the kidney. By synthesizing information from many sections, the three-dimensional fine structure can be reconstructed. **FIGURE 29.18B** shows the collecting ducts and the ascending and descending thin segments of loops of Henle in just the very tip—*only 0.5 mm long!*—of the medullary papilla. One discovery from this detailed study of structure is that there is far more contact among the loops of Henle and the collecting ducts in the medullary tip than would be predicted from a random arrangement of the two types of tubules relative to each other.

Genomic studies are being used to develop comprehensive lists of all the proteins present in specific parts of the kidneys. For example, a recent transcriptomic study of the inner-medullary collecting duct identified almost 8000 proteins. The genes for some are highly expressed (**TABLE 29.1**). These genes encode proteins that, for the most part, are already known to play important roles in kidney function. For example, the genes for aquaporins, Na^+–K^+-ATPase, and a urea transporter are among the most highly expressed. The protein kinase coded by another highly expressed gene, *Sgk1*, is well known to be under aldosterone control (see Figure 29.16C). By contrast, the proteins coded by some highly expressed genes do not have fully documented roles in the inner-medullary collecting duct. The nonmuscle actins (see Table 29.1), for example, seem likely to have roles in transducing ADH signals to control aquaporins, but these roles are not fully documented in the inner-medullary collecting duct. With almost 8000 proteins identified, there are many—including, for example, many transcription factors—about which almost nothing is known. Identifying the proteins present in the collecting duct through a comprehensive genomic survey helps set the stage for future functional research.

SUMMARY

Urine Formation in Mammals

- The loops of Henle, collecting ducts, and vasa recta form parallel arrays in the medulla of the mammalian kidney, creating the structural basis for the ability to form urine hyperosmotic to the blood plasma. Among species of mammals of a particular body size, the species with long loops of Henle tend to be able to produce more concentrated urine than those with shorter loops.
- The proximal convoluted tubule reabsorbs—and returns to the body fluids—much of the NaCl and water from the filtrate by processes that do not alter the osmotic pressure of the tubular fluid. It also fully reabsorbs glucose and amino acids, returning them to the body fluids.
- After the tubular fluid passes through the loop of Henle, it is less concentrated than when it entered. Nonetheless, processes in the loop of Henle create the gradients of osmotic pressure and NaCl concentration in the medullary interstitial fluid that are responsible for the ultimate concentration of the urine. In the part of the loop where the ascending limb is thick, active NaCl transport creates a single-effect difference in osmotic pressure and NaCl concentration between adjacent parts of the ascending and descending limbs. By acting as a countercurrent multiplication system, the loop generates a difference in osmotic pressure and NaCl concentration from end to end that is much larger than the single effect.
- During antidiuresis, as tubular fluid makes its last pass through the medulla in the collecting ducts, nonurea solutes are concentrated because the collecting-duct walls are freely permeable to water, permitting osmotic equilibration between the tubular fluid and the medullary interstitial fluid. The high permeability of the collecting-duct epithelial walls to water results from insertion of aquaporin-2 molecules into cell membranes in response to ADH (vasopressin).
- During diuresis, the collecting-duct walls are poorly permeable to water, so tubular fluid is osmotically isolated from the medullary interstitial fluid and can be diluted by solute reabsorption.

Urine Formation in Other Vertebrates

Freshwater and marine teleost fish differ in nephron structure and function

The sort of nephrons we described in amphibians apparently evolved in their freshwater progenitors, because the nephrons of nearly all freshwater teleost (bony) fish are structurally similar to those of amphibians. In freshwater fish, as in amphibians, the distal convoluted tubule plays a key role in diluting the urine. The walls of the tubule are nearly impermeable to water. Thus, as NaCl is reabsorbed and returned to the body fluids, water remains behind in the tubule, and a dilute urine is produced. The effect is to help keep the blood osmotic pressure high (see Figure 27.7) despite the water overload that occurs in freshwater fish because of inward osmosis from the environment.

Marine teleost fish commonly lack the distal convoluted tubule. If they are descended from freshwater ancestors, as is usually thought

(see Box 28.2), the absence of the distal tubule probably represents a secondary loss rather than a primitive condition. The reason for the loss seems straightforward: Marine teleosts are hyposmotic to their seawater environment and thereby face continuous osmotic desiccation. They have no need of a nephron segment specialized for the production of a voluminous, dilute urine rich in osmotically free water.

In addition to differing in the presence or absence of the distal convoluted tubule, freshwater and marine teleosts differ in other ways. Freshwater teleosts typically have relatively large numbers of nephrons and well-developed glomeruli. Their GFRs are relatively high, as suits animals that have excesses of water that must be voided in urine. In contrast, marine teleosts tend to have relatively few nephrons and small glomeruli. They have low GFRs, a condition that seems adaptive for animals that face desiccation and produce relatively little urine.

Many marine teleosts—according to present evidence—do not form their primary urine entirely by ultrafiltration. Instead, they form their primary urine partly by *secretion* into the proximal tubules. The mechanism of secretion is that ions—including Na^+, Cl^-, Mg^{2+}, and SO_4^{2-}—are actively transported into the proximal tubules, and water and other solutes follow (see Figure 29.2).

In about 30 known species of marine teleosts—described as **aglomerular**—the trend toward small glomeruli in the marine environment is carried to its logical extreme, and the nephrons lack glomeruli. These aglomerular species form their primary urine entirely by secretion. Aglomerularism has evolved on three independent occasions, suggesting it is adaptive under some circumstances. Some seahorses and pipefish, some Antarctic fish, and the oyster toadfish (*Opsanus tau*) are aglomerular.

Some of the most interesting fish from the viewpoint of kidney function are the euryhaline teleost species that can live in either freshwater or seawater, such as salmon and migratory eels. The control of kidney function in teleosts has in fact been most thoroughly studied in some of these species. When a euryhaline fish is transferred from seawater to freshwater, it typically undergoes a large increase in GFR, mediated for the most part by an increase in the number of filtering nephrons. Active secretion of Mg^{2+} and SO_4^{2-} into the urine, which is vigorous in seawater, is curtailed in freshwater (where ambient Mg^{2+} and SO_4^{2-} concentrations are vastly lower). Moreover, when fish are transferred from seawater to freshwater, the nephrons—and sometimes other excretory structures (e.g., the bladder)—undergo decreases in their overall permeability to water, a change that favors water excretion. Prolactin, arginine vasotocin (the "ADH" of fish; see Table 16.2), and angiotensin II are implicated in controlling these changes, but the controls are not well understood.

The reptiles other than birds have nephrons like those of amphibians, but birds have some mammalian-type nephrons

The nephrons of lizards, snakes, turtles, and crocodilians are broadly similar to those of amphibians. Birds, by contrast, have a range of nephron forms, which are usually categorized into two major types (**FIGURE 29.19**). Some of the nephrons of birds have short, uncomplicated proximal and distal tubules, and they lack loops of Henle. These nephrons superficially resemble the nephrons of nonavian reptiles in structure, and they are called **loopless nephrons**. Other avian nephrons have a loop of Henle interposed between the proximal and distal convoluted tubules and are called **looped nephrons** or **mammalian-type nephrons**. These nephrons have relatively large glomeruli and elaborate proximal tubules. Approximately 10%–30% of the nephrons in a bird's kidney are typically of the mammalian type; the remainder are of the loopless type. The mammalian-type nephrons are organized into sets. Among the nephrons of a set, the Bowman's capsules and proximal and distal convoluted tubules are all positioned near the same part of the kidney that houses the loopless nephrons, but the loops of Henle all project in a compact parallel array toward the direction of the ureter. Each parallel array of loops of Henle is called a medullary cone (see Figure 29.19). Each kidney includes many cones. Collecting ducts carrying the outflow from both the loopless and the mammalian-type nephrons run through the medullary cones on their way to the ureter.

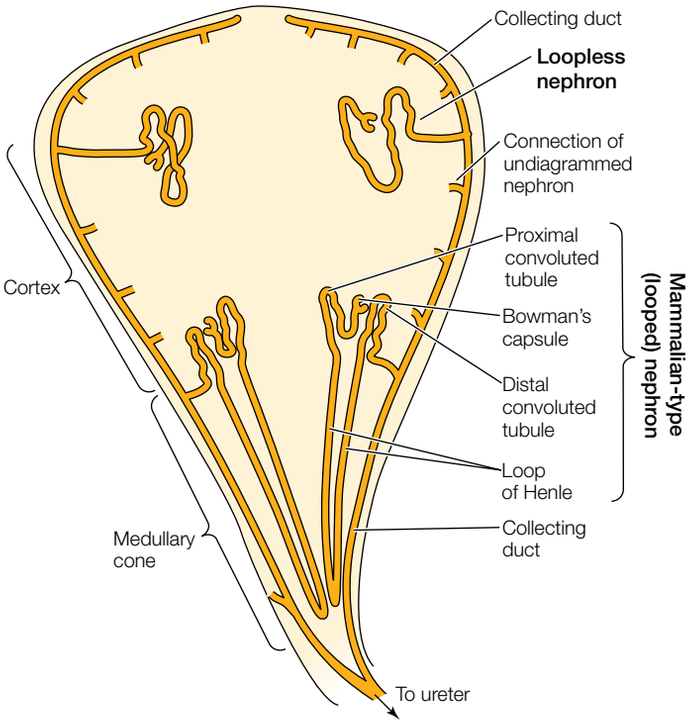


FIGURE 29.19 One lobe of a bird's kidney in cross section
(After Willoughby and Peaker 1979.)

Neither the nephrons in the kidneys of nonavian reptiles nor the loopless nephrons in a bird's kidneys can produce urine that is hyperosmotic to blood. However, the loops of Henle of the mammalian-type nephrons in a bird's kidney carry out countercurrent multiplication and can raise the urine osmotic pressure above the blood osmotic pressure. Details of the countercurrent mechanism in birds are probably different from those in mammals. For most species of birds, the maximum urine osmotic pressure is no more than about 2.5 times blood osmotic pressure.

Uric acid, the principal nitrogenous end product of birds and most other reptiles, is introduced into the nephrons by filtration and secretion. It is actively secreted into the urine as the urine flows through the nephrons, and this secretion accounts for the greater part of the excreted amount.

In both birds and nonavian reptiles, the ureters do not discharge directly to the outside of the body but instead discharge into the cloaca. From the cloaca, the urine is often moved by reverse peristalsis into the lower intestine. Because of these attributes, both the cloaca and the intestine may reclaim constituents from the urine and modify the composition and volume of the urine before the urine is excreted. At least four categories of cloaca–intestine processing are recognized, correlated to some extent with the life histories of the birds and other reptiles. When the urine enters the cloaca from the ureters, the uric acid and urates in it are often present largely in the form of supersaturated colloidal suspensions stabilized by specific proteins. The uric acid and urates are then precipitated into the form of tiny solid particles in the cloaca–intestine, forming the thick white paste that is often seen mixed with the feces of a bird or other reptile. Precipitation *after* the urine has left the ureters helps prevent clogging of the renal tubules with the precipitate.

SUMMARY

Urine Formation in Other Vertebrates

- Freshwater teleost fish have nephrons structurally similar to amphibian nephrons. Marine teleost fish, however, usually lack the distal convoluted tubule and have a relatively poorly developed glomerular filtration apparatus that seems often to be supplemented by active solute secretion. A few marine fish are aglomerular and depend entirely on secretion.
- Birds and other reptiles have nephrons structurally similar to amphibian nephrons. Birds, in addition, have mammalian-type nephrons (with loops of Henle) organized into parallel arrays—the medullary cones—in which urine hyperosmotic to blood plasma can be made.

Urine Formation in Decapod Crustaceans

An adult crayfish, crab, lobster, or other decapod crustacean has two renal organs, known as **antennal glands** or **green glands**, which are located in its head and open to the outside independently near the bases of its second antennae (**FIGURE 29.20A**). Each antennal gland is basically a single tube, sometimes loosely described as resembling "a single giant nephron." In a freshwater crayfish (**FIGURE 29.20B**), each antennal gland begins with a closed **end sac** or **coelomosac** lying to the side of the esophagus. Following the end sac is the **labyrinth** (or green body), a sheet of spongy tissue consisting of a channel that branches and anastomoses extensively along its length. The **nephridial canal**, which also has a spongy internal morphology, leads from the labyrinth to the expanded **bladder**, and the bladder empties to the outside. The nephridial canal is found only in certain freshwater decapod crustaceans.

The walls of the end sac are thin, and arteries from the heart supply a network of small vessels or lacunae on the outer surface of the end sac (see Figure 25.23). This morphological evidence has long suggested that fluid enters the end sac by filtration under the force of blood pressure. Additional morphological evidence for this concept is provided by the presence of cells resembling podocytes

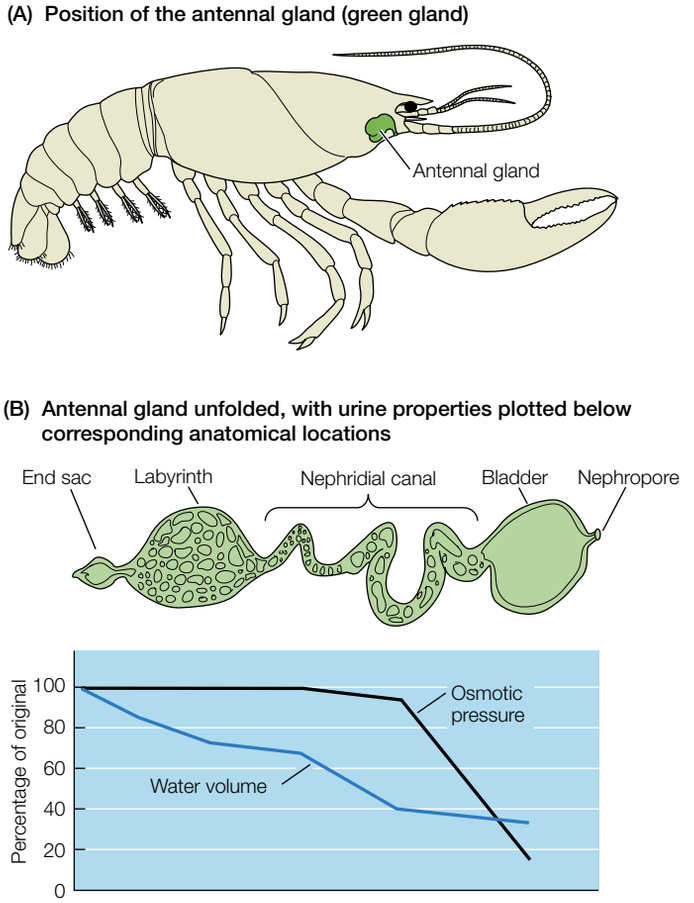


FIGURE 29.20 The antennal gland and urine formation in a freshwater crayfish (A) The position of the antennal gland on the right side of a crayfish's body. (B) Although the nephridial canal is in fact tightly convoluted and partly enveloped by the sheetlike labyrinth, the antennal gland can be stretched out to reveal its parts. The graph shows measured changes in the osmotic pressure and water content of the urine as it passes through the parts of the antennal gland in crayfish (*Austropotamobius pallipes* and *Orconectes virilis*) living in freshwater. Values are plotted immediately below the anatomical locations where they were measured and are expressed as percentages of the values in the end sac. (After Riegel 1977.)

(see Figure 29.1) in the end-sac epithelium. The physiological evidence that is available supports the hypothesis that primary urine is formed in the end sac by filtration from the blood. The composition of the urine is modified in all the structures through which it flows as it passes through the antennal gland. The labyrinth of American lobsters, for example, is known to reclaim glucose, and probably reclaims amino acids, from the filtrate. In marine crabs, the bladder is an important site where glucose is reabsorbed from the urine and Mg^{2+} is secreted into the urine. Unfortunately, a truly synthetic understanding of the handling of solutes by all parts of an antennal gland is not yet available for any species.

Modifications of the osmotic pressure of the urine have been a major focus of study in freshwater decapods. The labyrinth is by all accounts incapable of rendering the urine hyposmotic to the blood. There is a much-emphasized correlation between the presence of a nephridial canal and the ability to produce urine hyposmotic to the blood. Freshwater crayfish, which can make dilute urine, have a nephridial canal, which is often hypothesized to function in ways analogous to the vertebrate distal convoluted tubule. The nephridial

canal, however, is absent in ocean decapods, which produce urine isosmotic to their blood plasma; and it is also absent in freshwater crabs, such as *Eriocheir sinensis* (see page 748), which are recent immigrants to freshwater and unable to make dilute urine. Some studies on crayfish indicate that the bladder helps produce dilute urine (see Figure 29.20B). Excretion of dilute urine by a crayfish helps keep the osmotic pressure of its body fluids high (see Figure 27.7). Moreover, because crayfish actively reabsorb NaCl from the primary urine when they are producing a dilute definitive urine, the process helps retain NaCl in the body fluids.

Urine Formation in Molluscs

The renal organs of molluscs are tubular or saccular structures, called **nephridia**, or **kidneys**, that empty into the mantle cavity or directly to the outside. Bivalves, most cephalopods (octopuses and squids), and some gastropods have two kidneys, but most gastropods have only one. The kidneys are closely associated with the heart or hearts. In fact, the pericardial fluid in the pericardial cavity that surrounds the heart is the primary urine in most molluscs! The pericardial fluid flows into a canal called the **renopericardial canal**, which is part of the kidney.

The most thoroughly understood molluscan kidney is that of the giant octopus *Enteroctopus dofleini*, an ocean mollusc—isosmotic to seawater—found along the Pacific coast of North America. The kidneys of octopuses and squids are associated with the branchial (gill) hearts rather than the systemic heart (**FIGURE 29.21**). Each branchial heart bears a thin-walled protuberance, the *branchial heart appendage*, which communicates with the lumen of the heart. In *Enteroctopus*, the pericardial cavity of each branchial heart encloses only the side of the heart bearing the heart appendage, as seen in Figure 29.21. A kidney connects to each pericardial cavity. Compelling evidence exists in *Enteroctopus* and certain other cephalopods that the pericardial fluid is an ultrafiltrate of the blood, forced into the pericardial cavity across the branchial heart appendage under the force of pressure developed in the heart. In each kidney, this filtrate flows through a long renopericardial canal and then an enlarged **renal sac** before being discharged into the mantle cavity. Studies have shown that the renopericardial canal alters the composition of the urine. Glucose and amino acids are promptly reabsorbed there and returned to the body fluids, for example—a process reminiscent of their prompt reclamation in vertebrate nephrons.

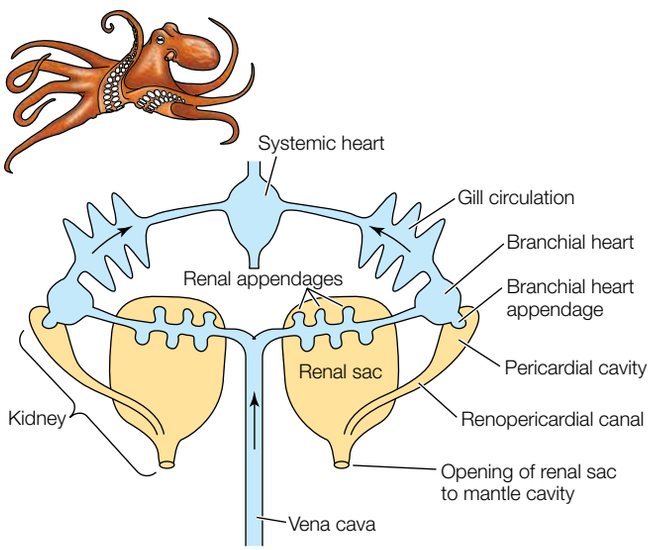


FIGURE 29.21 The kidneys of an octopus (*Enteroctopus*) and their relations to the circulatory system The kidneys are yellow, and the circulatory system is blue. After the principal vein returning blood from the systemic tissues, the vena cava, branches, each branch passes by one of the renal sacs, and there it bears many glandular diverticula, called *renal appendages*, which are closely juxtaposed to the walls of the sac. In *Enteroctopus*, ammonia is believed to be secreted into the renal sacs across the renal appendages. Blood then travels to the branchial hearts, where ultrafiltration occurs. (After Martin and Harrison 1966.)

Urine Formation in Insects

The formation of urine has been much more thoroughly studied in insects than in any other group of invertebrates. Most insects possess Malpighian tubules,[20] and these tubules are often called the *excretory tubules*. A point to be stressed from the outset, however, is that the hindgut is as important as the Malpighian tubules in the formation of urine.

The **Malpighian tubules** are long, slender, blind-ended structures that typically arise from the junction of the midgut and hindgut (**FIGURE 29.22**). They number from 2 to more than 200, depending on the species. Projecting into the hemocoel, they are bathed by the blood (hemolymph). The walls of the tubules consist of a single layer of epithelial cells, surrounded on the outside by

[20] The tubules are named after Marcello Malpighi (1628–1694), one of the great early microscopists, who was the first to describe the blood capillaries and renal corpuscles of vertebrates as well as the Malpighian tubules and tracheae of insects.

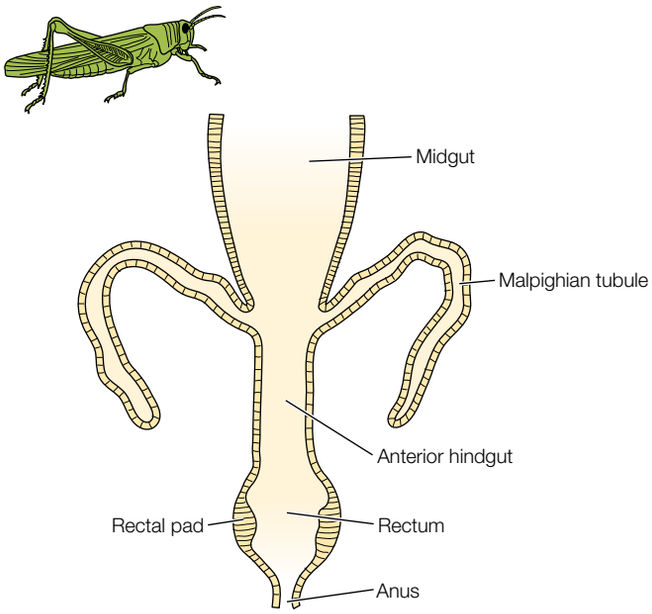


FIGURE 29.22 The posterior gut and Malpighian tubules of an insect The Malpighian tubules empty into the gut at the junction of the midgut and hindgut.

a thin basement membrane. Although the tubules exhibit little histological differentiation along their length in some species, they are differentiated into two to six (or possibly more) distinct regions in numerous others. In many species, the various tubules within an individual are morphologically similar, but in others, two or more types of tubules are present.

The hindgut, which is lined with cuticle (see Box 6.3), typically consists of a relatively small-diameter **anterior hindgut** (*ileum* or *intestine*) and an expanded posterior part, the **rectum** (see Figure 29.22). The walls of the anterior hindgut usually consist of a single layer of cuboidal or squamous epithelial cells. The walls of the rectum are similar to those of the anterior hindgut in some insects, but in many other species, parts of the rectal wall consist of thick columnar epithelial cells, sometimes associated with secondary cell layers. These thickened parts of the rectal wall are termed **rectal pads** or **rectal papillae** (see Figure 29.22), depending on their gross morphology.

The Malpighian tubules form and sometimes modify the primary urine

The function shared by the Malpighian tubules of all insects studied is the formation of the primary urine. The tubules are not supplied with blood vessels, and filtration does not occur. Instead, the primary urine is formed by a secretory mechanism in insects (see Figure 29.2). In the most common scenario, potassium chloride (KCl) is secreted vigorously by the Malpighian tubule epithelium into the lumen of the tubule from the blood bathing the tubule—so vigorously that the K^+ concentration in the tubular fluid is 6–30 times higher than the blood K^+ concentration. According to theories that have rapidly matured in the last 30 years, the K^+ secretion occurs by secondary active transport; a H^+-ATPase uses ATP bond energy to create electrochemical gradients that drive the secondary active transport of K^+. The secretion of K^+ into a Malpighian tubule is electrogenic, and Cl^- accompanies the K^+ passively by following the electrical gradient (inside positive) set up by K^+ secretion. The result is that KCl is secreted at a cost of ATP. The flux of KCl into the lumen of a Malpighian tubule drives osmotic entry of water, which typically occurs briskly enough that the tubular fluid remains approximately isosmotic to the blood (a case of near-isosmotic water transfer). Many additional solutes then enter the tubular fluid passively, as by following solute concentration gradients set up by the osmotic influx of water; these solutes include amino acids, sugars, diverse organic wastes and toxins, and several inorganic ions. Proteins are largely excluded from the tubular fluid because of their molecular size. Certain organic compounds—notably some detoxification products plus secondary compounds synthesized by plants to deter herbivory (e.g., alkaloids)—are actively secreted into the primary urine by some insects.

Although KCl is most commonly the principal salt secreted to initiate primary-urine formation, NaCl plays this role in some species. There are also species in which KCl predominates under some conditions, whereas NaCl does under other conditions.

As primary urine flows through the Malpighian tubules toward the gut, the tubular epithelium may reabsorb salts, water, or other molecules such as glucose—returning them to the blood. In the end, the fluid that enters the hindgut from the Malpighian tubules is approximately isosmotic to the blood and contains numerous solutes. Its solute composition is quite unlike that of the blood, however. In particular, the fluid that enters the hindgut is typically far richer in KCl than the blood is—a consequence of the secretory mechanism of primary-urine formation.

The rate at which primary urine is formed can be strikingly high relative to the total volume of the insect's body fluids. Current estimates, for example, indicate that in a female yellow-fever mosquito (*Aedes aegypti*), during an ordinary 24-h day when she has *not* taken a blood meal, the Malpighian tubules produce primary urine equivalent to 12 times her total body volume of extracellular fluid! If this sounds strange, it is really not; recall the very high rate at which human kidneys produce primary urine—also equivalent to about 12 times the entire extracellular fluid volume every day. In insects, as in vertebrates, most primary urine is reabsorbed (mostly by the hindgut) rather than being excreted. The overall process—a high rate of primary-urine formation followed by a high rate of reabsorption—gives the excretory system intimate access to the blood to carry out its regulatory functions, as already stressed.

A noteworthy aspect of the reabsorption process in insects is the reclamation of KCl. KCl must be secreted into the Malpghian tubules at a high rate to drive the production of primary urine, but it could not be lost from the body fluids at that rate. Instead, most KCl is reabsorbed back into the blood and recycled to produce more primary urine.

The reabsorption of KCl and water from the primary urine sometimes starts in the lower parts of the Malpighian tubules. However, the reabsorption occurs predominantly in the hindgut, especially in the rectum.

The hindgut modulates urine volume and composition in regulatory ways

After urine is discharged from the Malpighian tubules, it flows with the feces through the hindgut, where its composition, concentration, and volume are modified, resulting in the definitive urine, which is excreted. The rectum (which is far better understood than the anterior hindgut) not only reabsorbs—and returns to the blood—most of the water, K^+, Na^+, and Cl^- introduced into the hindgut by the Malpighian tubules, but also often reabsorbs amino acids, acetate, and phosphate. The rectum also has some secretory functions. For example, H^+ is secreted from the blood into the urine in the rectum, and the resulting acidification contributes to the precipitation of uric acid and urates there.

Research has increasingly clarified that the insect rectum has impressive *regulatory* abilities. It can modify the volume, composition, and osmotic pressure of the urine in ways that serve to regulate the volume, composition, and osmotic pressure of the blood. The rectum adjusts the osmotic pressure of the urine by varying the relative rates of reabsorption of water and total solutes. It also adjusts the ionic composition of the urine. In one set of experiments (**TABLE 29.2**), for example, fasting locusts were permitted to drink either tap water or a saline solution containing K^+, Na^+, Cl^-, and other ions. The rectum in the water-fed locusts reclaimed ions, returning them to the blood: It *lowered* ion concentrations in the rectal fluid (soon to be excreted as urine), compared with concentrations in the anterior-hindgut fluid. However, the rectum in the saline-fed locusts *raised* ion concentrations in the rectal fluid, compared with the anterior-hindgut fluid. The saline-fed locusts also accumulated

TABLE 29.2 Average composition of the rectal fluid and other body fluids in locusts (*Schistocerca gregaria*) that were deprived of food and provided with either tap water or a saline solution to drink

Experimental treatment	Fluid	Osmotic pressure (m*Osm*)	Ion concentration (m*M*)		
			Cl^-	Na^+	K^+
Water-fed	Rectal fluid	820[a]	5	1	22
	Anterior-hindgut fluid	420	93	20	139
	Blood	400	115	108	11
Saline-fed	Rectal fluid	1870	569	405	241
	Anterior-hindgut fluid	—	192	67	186
	Blood	520	163	158	19

Source: After Phillips 1964.
[a]The high osmotic pressure in the scanty rectal fluid of water-fed animals is presumed to be caused by organic solutes.

greater volumes of urine in the rectum, so that overall, the quantities of ions excreted in their urine were hundreds of times greater than those excreted by the water-fed animals. In this way the rectum played a major role in helping to regulate blood ion concentrations.

The study of the hormonal control of urine production in insects is a burgeoning field at present, in part because of the expectation that the next generation of controls for insect pests might include procedures that defeat vital control mechanisms. Numerous diuretic and antidiuretic neurohormones—which affect both Malpighian-tubule and rectal function—have been identified in various species.

PRODUCTION OF URINE HYPEROSMOTIC TO THE BLOOD Insects are one of the three major groups of animals that can produce urine that is hyperosmotic to their blood plasma (mammals and birds are the other two groups). When insects produce hyperosmotic urine (see page 768), the process of concentration usually occurs in the rectum. At least three different mechanisms of concentrating the urine have evolved.

In insects that have rectal pads or rectal papillae—such as cockroaches (*Periplaneta*), desert locusts (*Schistocerca*), and blowflies (*Calliphora*)—the urine in the rectal lumen is concentrated by *water reabsorption in excess of solute reabsorption*. This water reabsorption is highly intriguing because it can continue even when the osmotic pressure of the rectal contents has risen to be two or more times higher than the osmotic pressure of the blood bathing the rectum! The existence of this seemingly paradoxical process has been demonstrated in several ways. Perhaps the most compelling evidence comes from experiments in which the rectum was filled with a *pure* solution of a solute (e.g., trehalose) that is neither reabsorbed nor secreted across the rectal wall. The *amount* of such a solute in the rectum is constant during the course of an experiment. When locusts (*Schistocerca*) were treated in this way, they reabsorbed water from their rectal contents and the rectal-fluid osmotic pressure rose. After the rectal-fluid osmotic pressure had become twice as high as blood osmotic pressure, the rectal-fluid osmotic pressure *continued to rise* and reached nearly three times the blood osmotic pressure. Results of this sort show that the rectal wall can move water *against* a large, opposing osmotic gradient between the rectal fluid on the inside and blood on the outside. The results show, moreover, that in the short term, this water reabsorption can occur even in the absence of simultaneous solute reabsorption.

The mechanism of such water reabsorption was mystifying for many years. Now, however, a consensus exists that this water reabsorption is a case of osmosis on a microscopic scale: *local osmosis*. The mechanism depends in part on a complex microarchitecture in the rectal pads or papillae. The details of structure and possibly of function vary from species to species. Here we focus on the blowfly (*Calliphora*) as an example.

In the rectal papilla of a blowfly (**FIGURE 29.23A**), adjacent cells of the columnar epithelium are tightly joined on the side facing the rectal lumen and on the opposite (basal) side, but in between, the cells are separated by an elaborate network of minute channels and spaces, here termed the **intercellular spaces** (*intercellular*, "between cells"). The network of intercellular spaces communicates at the apex of the papilla with subepithelial spaces—here called **infundibular channels**—that are positioned under the basal side of the epithelial cell layer and connect with general blood spaces. Researchers hypothesize that the epithelial cells actively secrete solutes into the intercellular spaces, thereby rendering the fluid in the intercellular spaces strongly hyperosmotic to both the blood and the fluid in the rectal lumen (**FIGURE 29.23B**). Osmosis then carries water out of the rectal lumen into the intercellular spaces; that is, because of the *locally* high osmotic pressure in the intercellular spaces, water is osmotically withdrawn from the rectal fluid, even though the latter is thereby made increasingly hyperosmotic to the blood. Entry of water into the intercellular spaces adds volume to the fluid in the spaces and thereby causes fluid to flow in streams through the intercellular spaces toward the apex of the papilla and then through the infundibular channels toward the main blood cavity of the body (hemocoel). The fluid exiting the intercellular spaces is highly concentrated, but as it flows under the epithelial cells in the infundibular channels, solutes are believed to be actively or passively reabsorbed from the fluid into the cells across membranes poorly permeable to water, with two highly significant consequences. First, the fluid flowing through the infundibular channels is diluted, so that *in the end a fluid rich in water, rather than in solutes, is returned to the blood*—helping, for instance, to keep the osmotic pressure of the blood of a dehydrated insect from rising too high. Second,

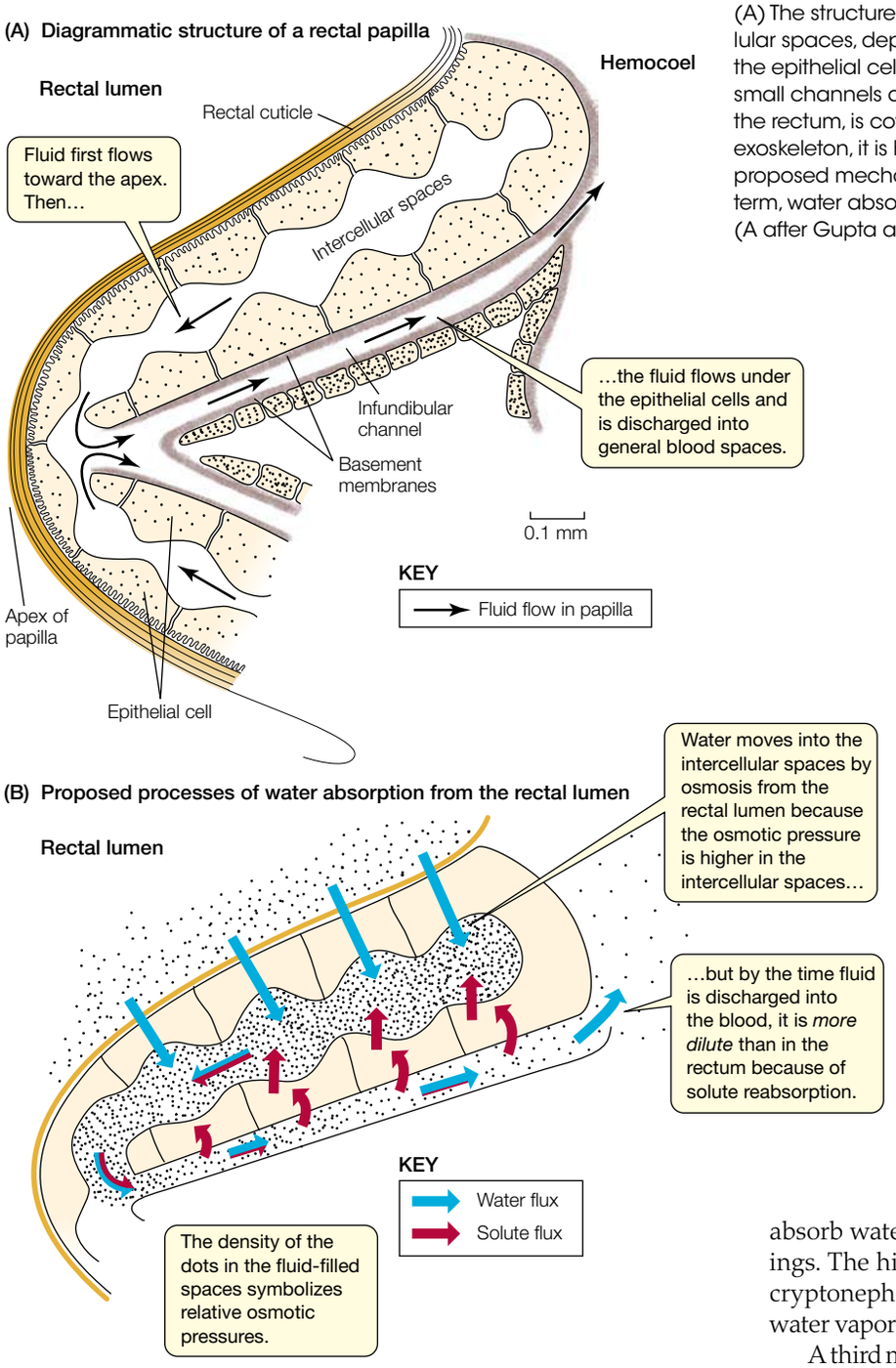


FIGURE 29.23 The structure and function of the blowfly rectal papilla Each blowfly (*Calliphora erythrocephala*) has four rectal papillae. (A) The structure of a papilla, shown highly diagrammatically. The intercellular spaces, depicted for simplicity as a single broad cavity running through the epithelial cells, actually consist of a complex, interconnecting network of small channels and spaces *between* the epithelial cells. The papilla, being in the rectum, is covered with a thin cuticle; although this material is part of the exoskeleton, it is highly permeable to water and to solutes of small size. (B) The proposed mechanism of water absorption from the rectal lumen. In the short term, water absorption can occur without solute absorption from the rectum. (A after Gupta and Berridge 1966.)

solutes are returned to the epithelial cells and thus can again be secreted into the intercellular spaces, permitting continued osmotic water absorption from the rectal fluid without great need for new solutes from any source. The nature of the solutes involved is not fully resolved, although Na^+, K^+, and Cl^- are strongly implicated; some organic solutes also play roles.

A second type of concentrating mechanism has been described in insects that have a *cryptonephridial complex*. These insects include mealworms (larval *Tenebrio molitor*), certain larval and adult coleopterans (beetles), and certain larval lepidopterans (butterflies and moths). Mealworms can produce pellets of excrement (feces and urine combined) that are particularly concentrated relative to the excrement of other insects, and ostensibly dry. The structural basis for concentrating and drying the excrement is a specialized association between the Malpighian tubules and rectum: the **cryptonephridial complex**. In this complex, the distal parts of the Malpighian tubules (the parts nearest the blind ends)—which float freely in the hemocoel of most insects—are closely associated with the outer rectal wall, and these parts of the tubules and the rectum are together enclosed by a **perinephric membrane**, which separates them from the hemocoel. KCl and NaCl are actively transported from the blood into the lumen of each of these cryptonephric Malpighian tubules. In a marked departure from the usual condition in insects, however, water is prevented from entering the tubular fluid from the blood (probably by water-impermeability of the perinephric membrane). Because the fluid in the cryptonephric Malpighian tubules is formed by the inward secretion of ions without water, it has a dramatically high osmotic pressure (far higher than blood osmotic pressure), creating a gradient that favors osmotic reabsorption of water from the closely juxtaposed rectal lumen. One advantage for the insects is that (probably) this arrangement allows them to take up water vapor from the air: an extremely unusual capability for animals. Mealworms and some of the other insects with a cryptonephridial complex are known to absorb water vapor from the atmosphere across their rectal linings. The high concentrations of salts in the tubular fluids of the cryptonephric Malpighian tubules—which give the fluids a low water vapor pressure—appear to be responsible (see page 732).

A third mechanism by which insects produce concentrated urine is known in the small subset of insects that live in saline waters. Some, at least, produce concentrated urine by secreting ions into their rectal fluid. Living in salty water, they face the challenge of keeping their body fluids from becoming too concentrated in ions. They respond by secreting ions out of their blood in abundance.

SUMMARY

Urine Formation in Insects

- Primary urine is introduced into the Malpighian tubules by a secretory process usually based on active transport of KCl into the tubular fluid. As the primary urine flows down the Malpighian tubules, it may be modified by reabsorption or secretion, but typically remains isosmotic to the blood.

- The Malpighian tubules empty into the hindgut at the junction of the midgut and hindgut.
- The rectum modifies the volume, composition, and osmotic pressure of the urine in ways that help regulate the volume, composition, and osmotic pressure of the blood. The production of hyposmotic urine occurs by reabsorption of solutes in excess of water. Two of the known mechanisms of producing hyperosmotic urine, however, enable insects to reabsorb water in excess of solutes. Some of the insects that produce hyperosmotic urine in this way do so by local osmosis and solute recycling in rectal pads or papillae; others do so with a cryptonephridial complex. Saline-water insects may form hyperosmotic urine by secretion of solutes into the rectum.

Nitrogen Disposition and Excretion

When animals catabolize organic molecules to release chemical energy, the atoms of the molecules appear in a variety of catabolic end products. During aerobic catabolism, the three most abundant atoms—carbon, hydrogen, and oxygen—appear in CO_2 and H_2O. The CO_2 is typically voided promptly into the environment across lungs, gills, or skin. The H_2O (metabolic water) simply becomes part of an animal's body water resources. The fourth most abundant atom is nitrogen, which is a characteristic constituent of proteins and nucleic acids. The disposition of nitrogen atoms from catabolism is not as simple as that of carbon, hydrogen, and oxygen.

Some of the compounds into which animals incorporate nitrogen during catabolism are shown in **FIGURE 29.24**. Each of these nitrogenous end products has advantages and disadvantages for the animals that synthesize it. For example, some of the compounds are relatively cheap to make, whereas others are low in toxicity. There is no single end product that is ideal in all ways. Moreover, whereas end products often represent wastes to be excreted, they sometimes can be used in adaptive ways and have value. With all these considerations in mind, we cannot be surprised that animals have evolved a variety of strategies for dealing with the nitrogen atoms released from organic molecules by catabolism.

The relation between nitrogen excretion and kidney function varies from one group of animals to another. Mammals, birds, and nonavian reptiles exemplify one end of the spectrum: They excrete nitrogenous end products entirely in their urine. At the other end of the spectrum, there are many aquatic animals in which nitrogenous end products are excreted mainly across the gills or skin, and the kidneys play little or no role.

Animals often produce two or more nitrogenous end products. There are several reasons for this. One is that nitrogen is a major constituent of nucleic acids as well as proteins, and often the catabolic pathways involved in breaking down nucleic acids produce a different nitrogenous end product than those responsible for breaking down proteins or amino acids. Humans and other primates, for example, synthesize uric acid from the nitrogen of nucleic acid purines, but they synthesize principally urea from protein nitrogen.[21] Protein catabolism dominates as a source of nitrogen. About 95% of waste nitrogen is from protein catabolism, and the products of protein catabolism therefore dominate.

[21] The affliction known as gout results from abnormal uric acid metabolism.

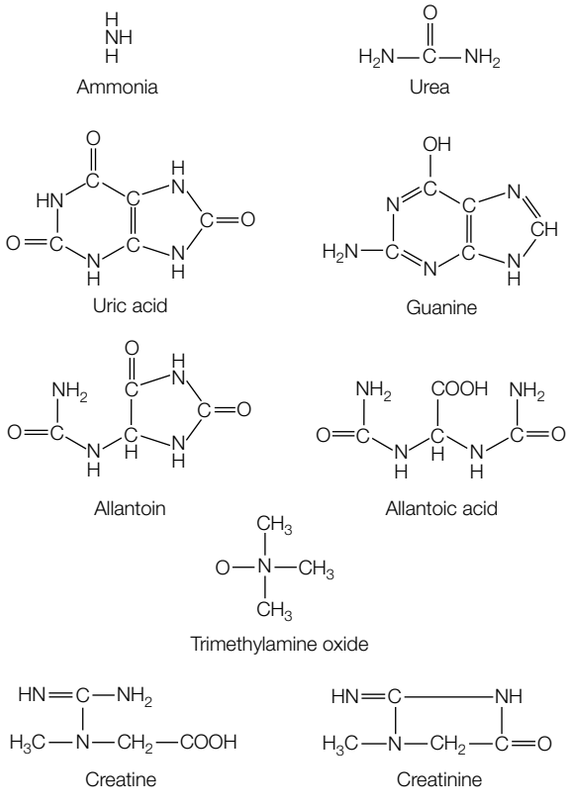


FIGURE 29.24 Some nitrogenous compounds excreted by animals Uric acid and guanine are purines. Allantoin and allantoic acid are poorly soluble breakdown products of uric acid. Trimethylamine oxide and its precursor, trimethylamine, are found in a variety of marine animals but do not occur in freshwater animals; both are highly soluble. Creatine and its internal anhydride, creatinine, occur as relatively minor excretory compounds in many vertebrates and some invertebrates. Some animals, mostly invertebrates, also lose significant amounts of amino acids to the environment.

When ammonia (NH_3) or the ammonium ion (NH_4^+) is the principal nitrogenous end product of an animal, the animal is described as **ammonotelic**. If urea is the principal nitrogenous end product, an animal is termed **ureotelic**. If uric acid is the principal end product, an animal is **uricotelic**.

Ammonotelism is the primitive state

Ammonia (NH_3) reacts with hydrogen ions to form the ammonium ion (NH_4^+). At the ordinary pH values of animal body fluids and tissues, this reaction is shifted strongly toward the formation of NH_4^+. For simplicity, we use the word *ammonia* here to refer to either NH_3 or NH_4^+. Ammonia is clearly the primitive nitrogenous end product of animals. One piece of strong evidence for this view is the fact that the great majority of today's ocean invertebrates are ammonotelic.

Ammonia is highly toxic. For example, even at low concentrations, it can disrupt neuron function, the integrity of the blood–brain barrier, and gill permeability. Blood concentrations are ordinarily kept low: usually under 0.3 millimolar (m*M*) in vertebrates, for example.

Because of its toxicity, ammonia cannot ordinarily be allowed to accumulate in an animal's body. Thus, for an animal to be ammonotelic, it must have a means of unfailingly voiding ammonia as rapidly as it is formed by catabolism. Aquatic animals can meet this challenge because of the abundance of water in which they live; often, much of

the ammonia they produce is voided directly into the ambient water across their gills or other external body surfaces. Not only most ocean invertebrates, but also most other water-breathing aquatic animals, are ammonotelic. Both freshwater and marine teleost fish are typically ammonotelic, and both the aquatic tadpoles of amphibians and adult aquatic amphibians (e.g., mudpuppies) are ammonotelic.

Ammonotelism, however, is unusual on land. A terrestrial animal is likely to depend on excretion in urine to rid itself of nitrogenous wastes. Urinary excretion of ammonia requires the excretion of a great deal of water.[22] Because of this and because an animal often faces an urgent need to prevent ammonia accumulation in its body, excretion of ammonia in the urine might sometimes require a terrestrial animal to void large amounts of water when water itself is in short supply.

Although ammonotelism is unusual on land, some *humidic* terrestrial animals are either ammonotelic or at least produce substantial quantities of ammonia. Some of the earliest studies on this phenomenon were carried out on terrestrial isopod crustaceans (e.g., pillbugs), which are ammonotelic. They void much of their ammonia into the atmosphere as NH_3 gas! In this way, they avoid use of water to get rid of ammonia. Many terrestrial snails, although not ammonotelic, void substantial NH_3 gas as well. In both isopods and snails, the fundamental reason for ammonia production may be that ammonia is useful to them because it plays a role in the deposition of calcium carbonate in their exoskeleton or shell. Production of NH_3 gas is also known to occur in some land crabs and a few species of amphibious fish.

Ammonia is the cheapest nitrogenous end product to produce. This probably explains why ammonotelism is so common among aquatic animals, which can easily evade ammonia's toxicity by excreting it freely into the water they occupy. Ammonia is generally formed during the catabolism of proteins by way of reactions that have no ATP cost. Some of these, termed *transamination* reactions, move amino groups to particular amino acids for which deamination enzymes exist; then the latter amino acids are *deaminated* (see Figure 6.3B).

Urea is more costly to synthesize but less toxic than ammonia

Urea is highly soluble and generally diffuses readily across membranes. Although hardly benign in its effects on macromolecules, it is far less toxic than ammonia. In humans, blood concentrations are normally in the range of 3–7 m*M*, and much higher concentrations, although abnormal, can be tolerated. As discussed in Chapter 28, very high blood urea concentrations occur in marine elasmobranch fish (≥ 300 m*M*) and some other animals.

If urea is less toxic than ammonia, why are so many animals ammonotelic? There are probably several reasons. One, certainly, is that urea is more costly to make than ammonia. The synthesis of each urea molecule requires the energy from four or five high-energy phosphate bonds (equivalent to that released by converting four or five ATP molecules to ADP).[23] As is so often the case in biology, animals face trade-offs. Ammonia is toxic but cheap; urea is less toxic but more costly.

[22] This topic is discussed at greater length in the next section.

[23] Authorities differ in whether they estimate the cost to be four or five per molecule.

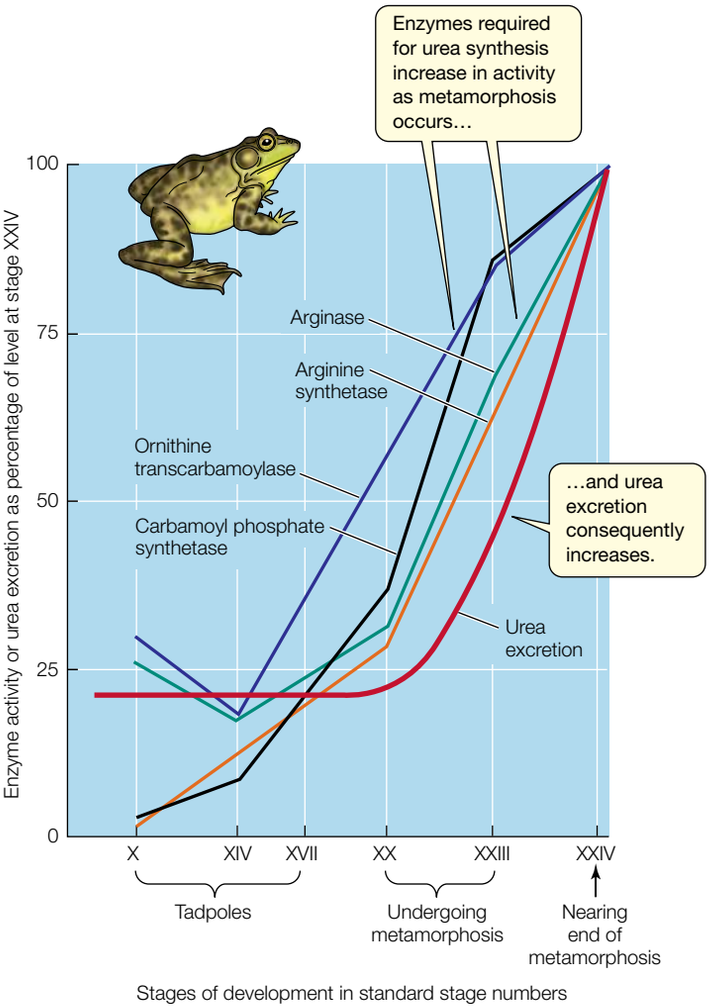


FIGURE 29.25 Bullfrogs shift from ammonotelism to ureotelism as they undergo metamorphosis American bullfrogs (*Lithobates catesbeianus*, until recently named *Rana catesbeiana*), which start life as aquatic tadpoles, metamorphose into semiterrestrial adults. Developmental biologists identify the successive stages of development using the stage numbers on the *x* axis. The activities in the liver of four enzymes of the metabolic pathway that synthesizes urea (the ornithine–urea cycle) are shown as functions of stage. The excretion of urea is also shown (red line). ("Arginine synthetase" is now recognized to represent the activity of two enzymes that together produce arginine.) (After Brown and Cohen 1958.)

Most of the animals that routinely employ urea as their principal nitrogenous end product are terrestrial vertebrates. Adult terrestrial amphibians are predominantly ureotelic. All mammals are ureotelic, as are some turtles. Terrestrial invertebrates have more often evolved uricotelism; only some flatworms and earthworms are at times ureotelic. For reasons that remain baffling in some cases, certain ocean aquatic animals are ureotelic, including some small, planktonic crustaceans and some larval fish.

There is persuasive evidence that vertebrates adopted ureotelism when they emerged onto land. One line of supportive evidence is the fact that modern terrestrial amphibians are ureotelic, in contrast to freshwater fish, which are nearly always ammonotelic. Another line of evidence is that, although the tadpoles of amphibians are usually ammonotelic, they express the enzymes for urea synthesis increasingly as they go through metamorphosis, eventually becoming ureotelic adults (**FIGURE 29.25**).

For terrestrial animals that excrete nitrogenous wastes in their urine, the advantage of ureotelism is that urea excretion requires less water than ammonia excretion. This is a direct consequence of the lower toxicity of urea. Because urea is less toxic than ammonia, steady-state blood concentrations of urea in ureotelic vertebrates—expressed in molar terms—are typically *at least* 20 times higher than steady-state blood concentrations of ammonia in ammonotelic vertebrates. Thus, if a urea-producing and an ammonia-producing species were both to excrete identical quantities of nitrogen in their urine at identical urine-to-plasma ratios, the water cost for the urea-producing species would be no more than 1/40th of that for the ammonia-producing species.[24] These considerations have no significance for aquatic animals that void nitrogen across general body surfaces. The considerations have great significance, however, for animals on land that excrete nitrogen in urine.

Some animals employ ureotelism for functions other than the simple, routine excretion of nitrogen, meaning that the evolution of ureotelism cannot always be interpreted strictly in terms of waste processing. Three cases in which ureotelism has an adaptive advantage are worthy of note:

1. Some ocean fish use urea as an osmolyte to aid osmoregulation in seawater—notably marine elasmobranchs, holocephalans, and the coelacanth *Latimeria*. This use of urea—which entails perpetually high urea concentrations in the body fluids—is discussed at length in Chapter 28 (see page 756).
2. Some aquatic, ammonotelic vertebrates switch to producing urea when they face crises that prevent them from being able to get rid of ammonia. For example, lungfish (see page 762) and some freshwater teleost species from stillwater environments switch from being ammonotelic to being ureotelic when they are confronted with drying of their habitat or other stresses. They then can stop voiding urine and allow their nitrogenous waste to accumulate in their body fluids, a strategy that would be impossible if they were synthesizing highly toxic ammonia.
3. Recently it was discovered that when toadfish living in the ocean excrete urea, the urea has value because it interferes with the ability of predators to find the toadfish by chemosensory processes.

The most thoroughly known biochemical mechanism for the synthesis of urea from protein nitrogen—the mechanism used by all the vertebrates that make urea and some invertebrates—is the **ornithine–urea cycle**, a set of biochemical reactions requiring five enzymes.[25] In the ornithine–urea cycle, one of the nitrogen atoms incorporated into urea originates from free ammonia derived from deamination reactions, especially deamination of glutamic acid. The second nitrogen incorporated into urea comes from the amino group of aspartic acid. Amino groups from most amino acids can be transferred to glutamic acid or aspartic acid by transamination reactions. In vertebrates that synthesize urea, the liver is the one tissue that expresses the full suite of enzymes of the ornithine–urea cycle.

The earliest vertebrates probably had all the genes required for the ornithine–urea cycle (see Box 28.4). A current working hypothesis, therefore, is that all living vertebrates have the genes, and that the existence of a functioning ornithine–urea cycle in a species depends on whether the genes are *expressed* in the species. If the ability to synthesize urea is such a "readily available" option, it would help explain why ureotelism occurs in a notably scattered and wide variety of vertebrates (see the cladogram in Box 28.4).

Uric acid and related compounds remove nitrogen from solution

An animal is classified as uricotelic if its primary nitrogenous end product is uric acid, the dihydrate of uric acid, urate salts, or a mix of these compounds, all of which are purines. These compounds all have low toxicities and solubilities. Uric acid itself is poorly soluble in water (0.4 millimoles—65 mg—dissolves in a liter at 37°C). The urate salts, although more soluble than uric acid, also have very low solubilities in comparison with urea or ammonia. Because of their low solubilities, uric acid and urates remove nitrogen from solution, reducing the water costs of excretion; they can be excreted as semisolid pastes or even as dry pellets or powders.

Experiments have shown that a variety of cations—including Na^+, K^+, NH_4^+, Ca^{2+}, and Mg^{2+}—can be incorporated into uric acid excrement in a poorly soluble state. Uncertainty exists over the chemical form assumed by these ions; they can be present as urate salts, but apparently they also can be bound in some manner to undissolved uric acid. Regardless, the cations are removed from solution, and this state can appreciably reduce the water demands of cation excretion. Calculations indicate, for example, that desert iguanas (*Dipsosaurus*) can void as much as 5000 milliequivalents of undissolved K^+ per liter of water when the K^+ is combined with uric acid—an effective concentration that is well above the highest K^+ concentration achieved by reptilian salt glands!

Focusing again on nitrogen, uric acid and urates not only permit nitrogen to be excreted with little water, but also have great advantages in times of water crisis when urine production is curtailed or stopped. If a ureotelic animal in water crisis stops producing urine, the urea concentration in its body fluids steadily rises because urea is so soluble that its solubility limits are never reached. This buildup cannot continue indefinitely because at high concentrations, urea becomes toxic. By contrast, if a uricotelic animal stops producing urine, uric acid and urates are deposited as precipitates within its body. Because the solubilities of these compounds are low, their concentrations in the body fluids cannot increase above low levels regardless of the amounts stored. Uric acid and urates, therefore, are suited to indefinite storage.

In addition to uric acid and urates, other purines or compounds derived from purines are sometimes employed as nitrogenous end products. The purine *guanine*—which is even less soluble than uric acid—is a primary nitrogenous end product in some spiders and other animals. *Allantoin* and *allantoic acid*—compounds formed by the partial breakdown of uric acid—may also be primary nitrogenous end products; although more soluble than uric acid, they have low solubilities.

[24] This is true because the urinary molar concentration of urea would be at least 20 times higher than that of ammonia, and each molecule of urea contains two atoms of nitrogen, rather than just one.

[25] Additional biochemical mechanisms that produce urea are known in some animals. The reactions of the ornithine–urea cycle are presented in any biochemistry text.

BOX 29.4 Why Are Mammals Not Uricotelic?

If birds, lizards, and snakes are uricotelic, why aren't mammals? An informed biologist knowing the patterns of nitrogen excretion in other animal groups, but knowing nothing about nitrogen excretion in mammals, would surely predict uricotelism in mammals, at least in desert species. Yet without known exception, all mammals are ureotelic. We do not know the answer to this riddle. However, a point worth stressing is that mammals are able to do things with urea that are unique.

The mammalian kidney is in a class by itself in its ability to concentrate urea. The maximum U/P ratio for urea in mammalian urine is typically higher than that for any other solute and can greatly exceed the maximum osmotic U/P ratio—signifying that urea can be concentrated to a much greater extent than solutes as a whole. In humans, for example, the maximum osmotic U/P ratio is about 4.2, and the maximum Cl^- U/P ratio is about 3.5, but the maximum urea U/P ratio is about 170. Many desert rodents can achieve urinary urea concentrations of 2.5–5.0 molar (*M*), corresponding to 70–140 g of nitrogen per liter! Because the urinary urea concentrations achieved by mammals are much greater than those attained by other animals, the water losses obligated by nitrogen excretion in mammals are exceptionally low in comparison with other ureotelic groups. In fact, urinary nitrogen-to-water ratios attained by desert rodents equal or exceed those observed in some of the uricotelic vertebrates that void their uric acid in a relatively fluid mix (e.g., certain birds). However, some birds and other reptiles that void uric acid in the form of relatively dry pellets achieve nitrogen-to-water ratios that are several times higher than the highest mammalian values.

Some researchers have argued that mammals have remained ureotelic because the elaborately developed countercurrent multiplication system of the mammalian kidney provides such great potential for concentrating urea that the selective advantage of uricotelism has been blunted. The reverse argument is that mammals for some reason were unable to evolve the biochemical and physiological attributes required for uricotelism. Being tied to ureotelism, so this argument goes, the mammals experienced great selective pressures to evolve exceptional urea-concentrating abilities.

Uricotelism—or the production of other purines as principal nitrogenous end products—is the most common state in terrestrial animals. This striking generalization is true even though uric acid probably requires considerably more energy per nitrogen atom for its synthesis than urea.[26] Birds, lizards, and snakes are uricotelic (**BOX 29.4**). (The white matter in bird droppings is uric acid.) As already mentioned, turtles that inhabit dry terrestrial habitats tend toward uricotelism. Most terrestrial invertebrates that live in the open air employ purines or purine derivatives as their primary nitrogenous end products. Most terrestrial insects, for example, employ uric acid, allantoin, or allantoic acid as their principal nitrogenous excretion. Spiders, scorpions, and certain ticks excrete mostly guanine. Temporary or permanent storage of purines in the body has been observed in many insects and snails and in certain land crabs. Stepping back from all these details, an important point to recognize is that several terrestrial *phyla* have converged on uricotelism, and this convergent evolution testifies to the advantages of poorly soluble nitrogenous end products for terrestrial existence.

The biochemical pathways employed for the synthesis of uric acid or related compounds from protein nitrogen are complicated.[27] However, they in fact are only relatively small modifications of very ancient and universal pathways for the synthesis of the purine constituents of DNA. This origin of the pathways helps explain how uricotelism (or "purinotelism") could have evolved independently in several phyla on land.

[26] Although some biochemists calculate that the synthesis of uric acid from protein nitrogen costs about the same amount of ATP-bond energy per nitrogen atom as urea synthesis (2–2.5 high-energy phosphate bonds per atom), others calculate that each nitrogen atom costs as much as 6 high-energy phosphate bonds to be incorporated into uric acid. Regardless of that consideration, uric acid probably has a higher overall cost than urea in at least some animals because of extra processes that must be carried out to prevent it from precipitating prematurely.

[27] These pathways are reviewed in biochemistry texts.

SUMMARY
Nitrogen Disposition and Excretion

- Animals that synthesize ammonia or urea as their primary nitrogenous end product are termed, respectively, ammonotelic or ureotelic. Animals that synthesize mainly uric acid or urates are uricotelic.
- Ammonotelism is the primitive condition and is seen in most water-breathing aquatic animals. Ammonia has the advantage of costing no extra ATP to produce. It is toxic, however. Thus, for an animal to be ammonotelic, the animal must have a means to void ammonia reliably as fast as it is produced so that blood levels are kept low. Aquatic animals void ammonia into the ambient water across their gills or general body surfaces.
- Ureotelism is more costly than ammonotelism because producing urea has an ATP cost. Urea is far less toxic than ammonia, however. Ureotelism has evolved principally in certain groups of vertebrates, in which it usually serves one or more of three possible functions: reducing the water requirement of routine nitrogen excretion (e.g., terrestrial amphibians and mammals), adjusting the blood osmotic pressure in advantageous ways (e.g., elasmobranch fish), and detoxification of waste nitrogen during periods when water-stressed animals cease urine production.

■ Although uricotelism is even more costly per nitrogen atom than ureotelism, uric acid and related compounds have the advantage that they are so poorly soluble that they are low in toxicity, can be excreted in little water, and can be accumulated in the body indefinitely. Most groups of terrestrial animals, including invertebrates (e.g., insects) and vertebrates (e.g., birds, lizards, and snakes), are uricotelic or primarily produce other purines (e.g., guanine) or purine derivatives.

STUDY QUESTIONS

1. We have seen that animals often encounter problems in dealing with waste nitrogen. Considering ammonotelic, ureotelic, and uricotelic types of animals, explain why these problems are often greatest in carnivores (as compared with omnivores or herbivores).
2. Considering the distal convoluted tubule of the amphibian nephron, explain how changes in the permeability of the tubule wall to water affect the amount of pure, osmotically free water excreted in the urine. Define what is meant by pure, osmotically free water.
3. Outline how the orientation of nephrons relative to each other imparts gross structure to the kidneys of mammals and birds.
4. If you were attempting to tell whether an animal produces its primary urine by ultrafiltration or secretion, what measurements would you make *on the primary urine*? If your measurements indicated that ultrafiltration might be occurring, what other types of measurements would you make to determine whether physical and physicochemical conditions favorable to ultrafiltration exist? Explain.
5. When researchers first proposed the countercurrent multiplication hypothesis for concentration of urine in the mammalian kidney, there was great resistance to its acceptance in certain quarters. The anatomist Ivar Sperber, whose comparative morphological studies originally helped draw attention to the loops of Henle, pointed out that there were certain rodents in which the anatomy of the kidney should make it relatively simple to sample blood from the hairpin bends of the vasa recta deep in the medulla. Samples of such blood were obtained, and the osmotic pressure of this blood proved to be far higher than the osmotic pressure of blood in the general circulation. This research convinced doubters of the validity of the countercurrent multiplication process. Why does blood at the hairpin bends of the vasa recta have a high osmotic pressure, and why would knowing its osmotic pressure in the cases described provide strong support for the countercurrent multiplication hypothesis?
6. Production of any sort of nitrogenous waste other than ammonia costs energy. Name at least three distinctly different advantages an animal might gain by investing in production of urea or uric acid.
7. Explain how primary urine is introduced into the Malpighian tubules of an insect.
8. The immediate effect of ADH on the renal tubules of frogs and mice is the same, yet when ADH is secreted, frogs produce urine that is approximately isosmotic to their blood plasma, whereas mice produce urine far more concentrated than their blood plasma. Explain this difference in terms of the factors affecting osmosis in the kidneys of frogs and mice.
9. Drugs that increase urine flow (diuretic drugs) are often employed in the treatment of hypertension (high blood pressure) or other disease states. Three physiological categories of such drugs are ones that (i) function as loop diuretics, (ii) inhibit the action of aldosterone, and (iii) block Na^+ channels in the collecting ducts. Explain why each of these categories would be expected to increase Na^+ excretion and urine flow. (Hint: Rereading the section on hormones at the end of Chapter 28 might prove helpful.)
10. In mammals, the kidneys are the only organs that regulate routine excretion of water, salts, and nitrogenous wastes from the blood. As logical as this may sound to us, it is unique among vertebrates. For each of the other groups of vertebrates, describe the functions of as many organs as you can—in addition to the kidneys—that participate in these processes. Consider the discussions of these groups of animals in Chapter 28 as well as in this chapter.
11. Whenever the concentrating ability of mammalian kidneys has been studied in relation to the lengths of the loops of Henle in various species, a clear correlation between the two has been found—indicating that loop length matters—but in addition, there has been a great deal of scatter in the data (e.g., see Figure 29.8). Fifty years from now, physiologists will probably understand the mechanistic reasons *why* loop length is not a perfect predictor of concentrating ability. Suppose a government agency has decided to give you all the resources you need to study whatever you desire. As a brainstorming exercise, what specific aspects of mammalian kidney function other than loop length would you investigate to try to account better for differences among species in concentrating ability?

Go to **sites.sinauer.com/animalphys4e** for box extensions, quizzes, flashcards, and other resources.

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See also **Additional References** *and* **Figure and Table Citations**

BOX 28.1 Fish Mitochondria-Rich Cells and Their Diversity

Mitochondria-rich cells (**MRCs**)—also called **mitochondrion-rich cells**, **chloride cells**, or **ionocytes**—have two distinctive morphological features, both indicative of high metabolic activity: They contain large numbers of mitochondria and an elaborate system of intracellular membranes (this system is continuous with the basolateral cell membrane). MRCs are typically also strikingly rich in Na^+–K^+-ATPase by comparison with most cells—another sign of high metabolic activity. Certain MRCs contain more than 100 million molecules of Na^+–K^+-ATPase per cell, one of the highest abundances known. MRCs are in general believed to be the principal sites of active ion transport in the gills of teleost fish.

A discovery of great significance—which has emerged with full clarity in just the last 20 years—is that there are *multiple types of MRCs*. For example, largely owing to the revolution in immunocytochemistry, researchers now recognize types of MRCs that differ biochemically: These MRCs can differ in their *quantities* of key ion-transport proteins and in their *molecular forms* of the proteins. MRCs with different molecular forms of Na^+–K^+-ATPase occur.

Based on the latest evidence, a fish capable of living in both freshwater and seawater typically has different types of MRCs in its gills—dubbed *freshwater and seawater types*—in the two environments. When the fish is transferred from one environment to the other, it switches types by replacing or transforming its MRCs. Moreover, a fish may have two types of MRCs present in its gills in one environment. For example, rainbow trout (*Oncorhynchus mykiss*) living in freshwater have at least two types.

As yet researchers have not created a standardized nomenclature for the types of MRCs. Reading the research literature published prior to about 1995 can be confusing because, at the time, physiologists tended to think of MRCs as being relatively homogeneous and in general spoke of them as if they are all the same.

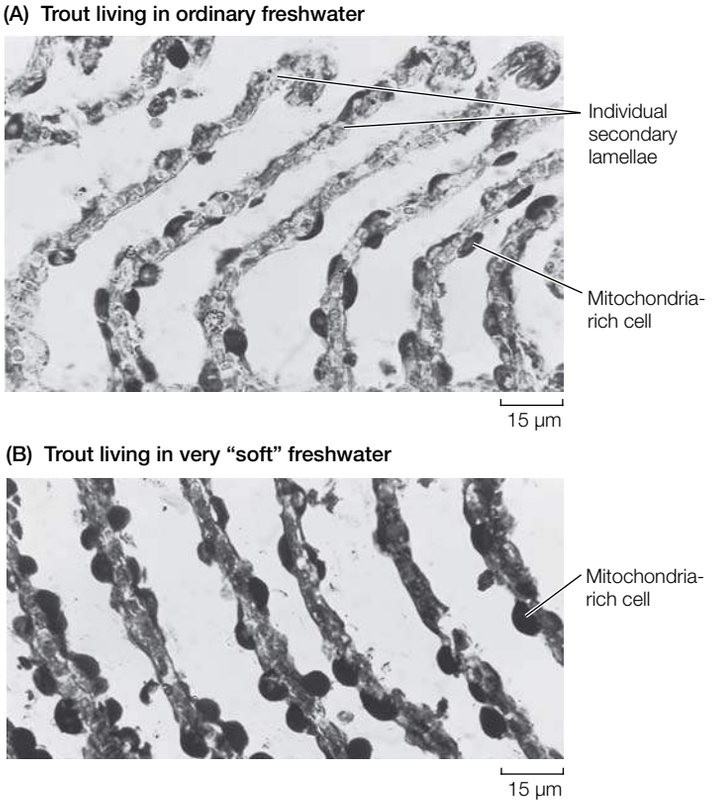

FIGURE 28.5 Cellular acclimation to living in two types of water in the gill epithelium of freshwater fish Seen here are sections of the secondary lamellae in the gills of rainbow trout (*Oncorhynchus mykiss*), viewed using light microscopy and stained to show mitochondria-rich cells. (A) Tissue section from a fish that had been living in ordinary freshwater with a Ca^{2+} concentration of 0.4 millimolar (mmol/L). (B) Tissue section from a fish that had been living for 2 weeks in very "soft" freshwater with a Ca^{2+} concentration of 0.05 mmol/L. (Photographs courtesy of Steve Perry; from Perry 1998.)

the MRCs, besides becoming more numerous, also modify their cell proteins—upregulating a key Cl^-/HCO_3^- countertransport protein that exports HCO_3^- from the body fluids in exchange for Cl^- (see Figure B in Box Extension 5.2).

A second, and fascinating, condition that leads to increased numbers of MRCs in freshwater fish is life in very "soft" water: water of exceptionally low Ca^{2+} concentration (**FIGURE 28.5**). Freshwater fish acquire most of their Ca^{2+} from the water in which they live, rather than from their food. The MRCs (or a subset of them) are the sites of active Ca^{2+} uptake. When fish are living in Ca^{2+}-poor waters, an increase in the number of MRCs is believed to help them acquire sufficient Ca^{2+}. However, increasing the number of MRCs can also interfere with uptake of O_2! Recent research on several species has shown that in fish living in very soft water, the replacement of pavement cells by MRCs in the secondary lamellae can double the average diffusion distance between blood and water in the gills, because MRCs are thick (see Figure 28.5)—thicker than the pavement cells they replace. This doubling of the diffusion distance measurably interferes with O_2 uptake. *Thus freshwater fish exhibit a trade-off between their ability to take up Ca^{2+} and their ability to take up O_2; increasing one ability decreases the other.*

The concept of trade-offs is a major theme in modern ecology and evolutionary biology. The situation in freshwater fish just described is one of the physiological trade-offs that, considering all of animal physiology, is best understood at a cellular level.

FOOD AND DRINKING WATER Freshwater animals of all types—fish, crayfish, and so forth—gain ions from their food, in addition to acquiring them by active uptake from the ambient water. The role of food in meeting ion needs is not well understood, although inputs of ions by active transport are generally thought to exceed those from food. In addition to eating food, freshwater animals also have the opportunity to drink water. But do they? Freshwater animals typically must produce urine at a very high