Anatomy of the Kidney

Knowledge of the complex structure of the mammalian kidney provides a basis for understanding the multitude of functional characteristics of this organ in both healthy and diseased states. In this chapter, gross observations coupled with light microscopic and ultrastructural information and examples of immunohistochemical localization of selected channels, transporters, and regulatory proteins are presented using illustrative material derived from a variety of laboratory animals and humans.

GROSS FEATURES

Kidneys are paired retroperitoneal organs situated in the posterior part of the abdomen on each side of the vertebral column. In the human, the upper pole of each kidney lies opposite the twelfth thoracic vertebra, and the lower pole lies opposite the third lumbar vertebra. The right kidney is usually slightly more caudal in position. The weight of each kidney ranges from 125 g to 170 g in the adult male and from 115 g to 155 g in the adult female. The human kidney is approximately 11 cm to 12 cm in length, 5.0 cm to 7.5 cm in width, and 2.5 cm to 3.0 cm in thickness. Located on the medial or concave surface of each kidney is a slit, called the hilus, through which the renal pelvis, the renal artery and vein, the lymphatics, and a nerve plexus pass into the sinus of the kidney. The organ is surrounded by a tough fibrous capsule, which is smooth and easily removable under normal conditions.

In the human, as in most mammals, each kidney is supplied normally by a single renal artery, although the presence of one or more accessory renal arteries is not uncommon. The renal artery enters the hilar region and usually divides to form an anterior and a posterior branch. Three segmental or lobar arteries arise from the anterior branch and supply the upper, middle, and lower thirds of the anterior surface of the kidney (Fig. 2-1). The posterior branch supplies more than half of the posterior surface and occasionally gives rise to a small apical segmental branch. However, the apical segmental or lobar branch arises most commonly from the anterior division. No collateral circulation has been demonstrated between individual segmental or lobar arteries or their subdivisions. Not uncommonly, the kidneys receive aberrant arteries from the superior mesenteric, suprarenal, testicular, or ovarian arteries. True accessory arteries that arise from the abdominal aorta usually supply the lower pole of the kidney. The arterial and venous circulations in the kidney are described in detail in Chapter 3 and are not discussed further in this chapter.
FIGURE 2-1 Diagram of the vascular supply of the human kidney. The anterior half of the kidney can be divided into upper (U), middle (M), and lower (L) segments, each supplied by a segmental branch of the anterior division of the renal artery. A small apical segment (A) is usually supplied by a division from the anterior segmental branch. The posterior half of the kidney is divided into apical (A), posterior (P), and lower (L) segments, each supplied by branches of the posterior division of the renal artery. (Modified from Graves FT: The anatomy of the intrarenal arteries and its application to segmental resection of the kidney. Br J Surg 42:132, 1954.)

Two distinct regions can be identified on the cut surface of a bisected kidney: a pale outer region, the cortex, and a darker inner region, the medulla (Fig. 2-2). In humans, the medulla is divided into 8 to 18 striated conical masses, the renal pyramids. The base of each pyramid is positioned at the corticomedullary boundary, and the apex extends toward the renal pelvis to form a papilla. On the tip of each papilla are 10 to 25 small openings that represent the distal ends of the collecting ducts (of Bellini). These openings form the area cribrosa (Fig. 2-3). In contrast to the human kidney, the kidney of the rat and of many other laboratory animals has a single renal pyramid and is therefore termed “unipapillate.” Otherwise, these kidneys resemble the human kidney in their gross appearance. In humans, the renal cortex is about 1 cm in thickness, forms a cap over the base of each renal pyramid, and extends downward between the individual pyramids to form the renal columns of Bertin (Fig. 2-4; see Fig. 2-2). From the base of the renal pyramid, at the corticomedullary junction, longitudinal elements termed the “medullary rays of Ferrein” extend into
the cortex. Despite their name, the medullary rays are actually considered a part of the cortex and are formed by the collecting ducts and the straight segments of the proximal and distal tubules.

FIGURE 2-2 Bisected kidney from a 4-year-old child, demonstrating the difference in appearance between the light-staining cortex and the dark-staining outer medulla. The inner medulla and papillae are less dense than the outer medulla. The columns of Bertin can be seen extending downward to separate the papillae.
The renal pelvis is lined by transitional epithelium and represents the expanded portion of the upper urinary tract. In humans, two and sometimes three outpouchings, the major calyces, extend outward from the upper dilated end of the renal pelvis. From each of the major calyces, several minor calyces extend toward the papillae of the pyramids and drain the urine formed by each pyramidal unit. In mammals possessing a unipapillate kidney, the papilla is directly surrounded by the renal pelvis. The ureters originate from the lower portion of the renal pelvis at the ureteropelvic junction, and in humans they descend a distance of approximately 28 cm to 34 cm to open into the fundus of the bladder. The walls of the calyces, pelvis, and ureters contain smooth muscle that contracts rhythmically to propel the urine to the bladder.
THE NEPHRON

The functional unit of the kidney is the nephron. Each human kidney contains about 0.6 × 10^6 to 1.4 × 10^6 nephrons, which contrasts with the approximately 30,000 nephrons in each adult rat kidney. The essential components of the nephron include the renal or malpighian corpuscle (glomerulus and Bowman’s capsule), the proximal tubule, the thin limbs, the distal tubule, and the connecting tubule. The origin of the nephron is the metanephric blastema. Although there has not been universal agreement on the origin of the connecting tubule, it is now generally believed to derive from the metanephric blastema. The collecting duct system, which includes the initial collecting tubule, the cortical collecting duct (CCD) in the medullary ray, the outer medullary collecting duct (OMCD), and the inner medullary collecting duct (IMCD), is not, strictly speaking, considered part of the nephron because embryologically it arises from the ureteric bud. However, all of the components of the nephron and the collecting duct system are interrelated functionally.

Two main populations of nephrons are recognizable in the kidney: those possessing a short loop of Henle and those with a long loop of Henle (Fig. 2-5). The loop of Henle is composed of the straight portion of the proximal tubule (pars recta), the thin limb segments, and the straight portion of the distal tubule (thick ascending limb, or pars recta). The length of the loop of Henle is generally related to the position of its parent glomerulus in the cortex. Most nephrons originating from superficial and midcortical locations have short loops of Henle that bend within the inner stripe of the outer medulla close to the inner medulla. A few species, including humans, also possess cortical nephrons with extremely short loops that never enter the medulla but turn back within the cortex. Nephrons originating from the juxtamedullary region near the corticomedullary boundary have long loops of Henle with long descending and ascending thin limb segments that enter the inner medulla. Many variations exist, however, between the two basic types of nephrons, depending on their relative position in the cortex. The ratio between long and short loops varies among species. Humans and most rodents have a larger number of short-looped than long-looped nephrons.
On the basis of the segmentation of the renal tubule, the medulla can be divided into an inner and an outer zone, with the outer zone further subdivided into an inner and an outer stripe (see Fig. 2-5). The inner medulla contains both descending and ascending thin limbs and large collecting
ducts, including the ducts of Bellini. In the inner stripe of the outer medulla, thick ascending limbs are present in addition to descending thin limbs and collecting ducts. The outer stripe of the outer medulla contains the terminal segments of the pars recta of the proximal tubule, the thick ascending limbs (partes rectae of the distal tubule), and collecting ducts. The division of the kidney into cortical and medullary zones and the further subdivision of the medulla into inner and outer zones are of considerable importance in relating renal structure to the ability of an animal to form a maximally concentrated urine.

Renal Corpuscle (Glomerulus)

The renal corpuscle is composed of a capillary network lined by a thin layer of endothelial cells; a central region of mesangial cells with surrounding mesangial matrix material; the visceral epithelial cells and the associated basement membrane; and the parietal layer of Bowman's capsule with its basement membrane (Figs. 2-6 through 2-8 [3] [4] [5]). Between the two epithelial layers is a narrow cavity called Bowman's space, or the urinary space. Although the term renal corpuscle is more precise anatomically than the term glomerulus when referring to that portion of the nephron composed of the glomerular tuft and Bowman's capsule, the term glomerulus is employed throughout this chapter because of its common use. The visceral epithelium is continuous with the parietal epithelium at the vascular pole, where the afferent arteriole enters and the efferent arteriole exits the glomerulus. The parietal layer of Bowman's capsule continues into the epithelium of the proximal tubule at the so-called urinary pole. The average diameter of the glomerulus is approximately 200 mm in the human kidney and 120 mm in the rat kidney. However, glomerular number and size vary significantly with age and gender as well as birth weight. The average glomerular volume has been reported to be 3 to 7 million mm$^3$ in humans [2] [3] [4] and 0.6 to 1 million mm$^3$ in the rat. In the rat, juxtamedullary glomeruli are larger than glomeruli in the superficial cortex. However, this is not the case in the human kidney.
FIGURE 2-6 Light micrograph of a normal glomerulus from a rat, demonstrating the four major cellular components: mesangial cell (M), endothelial cell (E), visceral epithelial cell (V), and parietal epithelial cell (P). MD, macula densa. (Magnification, ×750.)
FIGURE 2-7 Scanning electron micrograph of a cast of a glomerulus with its many capillary loops (CL) and adjacent renal vessels. The afferent arteriole (A) takes its origin from an interlobular artery at lower left. The efferent arteriole (E) branches to form the peritubular capillary plexus (upper left). (Magnification, ×300.) (Courtesy of Waykin Nopanitaya, PhD.)
FIGURE 2-8  Electron micrograph of a portion of a glomerulus from normal human kidney in which segments of three capillary loops (CL) are evident. The relationship between mesangial cells (M), endothelial cells (E), and visceral epithelial cells (V) is demonstrated. Several electron-dense erythrocytes lie in the capillary lumens. BS, Bowman's space. (Magnification, ×6700.)

The glomerulus is responsible for the production of an ultrafiltrate of plasma. The filtration barrier between the blood and the urinary space is composed of a fenestrated endothelium, the peripheral glomerular basement membrane (GBM), and the slit pores between the foot processes of the
visceral epithelial cells (Fig. 2-9). The mean area of filtration surface per glomerulus has been reported to be 0.203 mm$^2$ in the human kidney$^8$ and 0.184 mm$^2$ in the rat kidney.$^9$

**FIGURE 2-9** Electron micrograph of normal rat glomerulus fixed in a 1% glutaraldehyde solution containing tannic acid. Note the relationship among the three layers of the glomerular basement membrane and the presence of the pedicels (P) embedded in the lamina rara externa (arrowhead). The filtration slit diaphragm with the central dense spot (arrow) is especially evident between the individual pedicels. The fenestrated endothelial lining of the capillary loop is shown below the basement membrane. A portion of an erythrocyte is located in the extreme lower right corner. BS, Bowman's space; CL, capillary lumen. (Magnification, ×120,000.)

**Endothelial Cells**

The glomerular capillaries are lined by a thin fenestrated endothelium (Fig. 2-10; see Fig. 2-9). The endothelial cell nucleus usually lies adjacent to the mesangium, away from the urinary space,
and the remainder of the cell is irregularly attenuated around the capillary lumen (see Fig. 2-8).

The endothelium is perforated by pores or fenestrae, which in the human kidney range from 70 nm to 100 nm in diameter (see Fig. 2-10). Thin diaphragms have been observed extending across these fenestrae and electron microscopic studies using a modified fixation method reported the presence of filamentous sieve plugs in the fenestrae. The function of these plugs remains to be established and it is not known whether they represent a significant barrier to the passage of macromolecules. Recent studies have confirmed the presence of electron-dense filamentous material in the fenestrae and also demonstrated a thick filamentous surface layer on the endothelial cells. Nonfenestrated, ridge-like structures termed cytofolds are found near the cell borders.

FIGURE 2-10 Scanning electron micrograph demonstrating the endothelial surface of a glomerular capillary from the kidney of a normal rat. Numerous endothelial pores, or fenestrae, are evident. The ridge-like structures (arrows) represent localized thickenings of the endothelial cells. (Magnification, ×21,400.)
In both human and rat kidney, an extensive network of intermediate filaments and microtubules is present in the endothelial cells, and microfilaments surround the endothelial fenestrations. Recent studies have suggested that the endothelial cell glycocalyx contributes to the charge-selective properties of the glomerular capillary wall and thus may constitute an important part of the filtration barrier. The glomerular endothelial cells synthesize both nitric oxide (NO), previously called endothelium-derived relaxing factor, and endothelin-1, a vasoconstrictor. The synthesis of NO is catalyzed by endothelial nitric oxide synthase (eNOS), which is expressed in glomerular endothelial cells.

Receptors for vascular endothelial growth factor (VEGF) are expressed on the surface of the glomerular endothelial cells. VEGF is produced by the glomerular visceral epithelial cells and is an important regulator of microvascular permeability. In vitro studies in endothelial cells of different origins have demonstrated that VEGF increases endothelial cell permeability and induces the formation of endothelial fenestrations, and VEGF-induced formation of fenestrae has also been demonstrated in renal microvascular endothelial cells. Gene deletion studies in mice have demonstrated that VEGF is required for normal differentiation of glomerular endothelial cells and there is evidence that VEGF is important for endothelial cell survival and repair in glomerular diseases characterized by endothelial cell damage. Thus, VEGF produced by the visceral epithelial cells plays a critical role in the differentiation and maintenance of glomerular endothelial cells and is an important regulator of endothelial cell permeability.

The endothelial cells form the initial barrier to the passage of blood constituents from the capillary lumen to Bowman space and they contribute to the charge-selective properties of the glomerular capillary wall through their negatively charged glycocalyx. Under normal conditions, the formed elements of the blood, including erythrocytes, leukocytes, and platelets, do not gain access to the subendothelial space.

Visceral Epithelial Cells

The visceral epithelial cells, also called podocytes, are the largest cells in the glomerulus. They have long cytoplasmic processes, or trabeculae, that extend from the main cell body and divide into individual foot processes, or pedicels, that come into direct contact with the lamina rara externa of the GBM. By scanning electron microscopy, it is apparent that adjacent foot processes are derived from different podocytes. The podocytes contain a well-developed Golgi complex, and lysosomes are often observed. Large numbers of microtubules, microfilaments, and intermediate filaments are present in the cytoplasm and actin filaments are especially abundant in the foot processes where they connect the slit membrane with the GBM.
In the normal glomerulus, the distance between adjacent foot processes near the GBM varies from 25 nm to 60 nm (see Fig. 2-9). This gap, referred to as the filtration slit or slit pore, is bridged by a thin membrane called the filtration slit membrane or slit diaphragm, which is located approximately 60 nm from the GBM. A continuous central filament with a diameter of approximately 11 nm can be seen in the filtration slit diaphragm. Detailed studies of the slit diaphragm in the rat, mouse, and human glomerulus have revealed that the 11-nm-wide central filament is connected to the cell membrane of the adjacent foot processes by regularly spaced cross bridges approximately 7 nm in diameter and 14 nm in length, giving the slit diaphragm a zipper-like configuration (Fig. 2-12). The dimensions of the pores between the cross bridges are approximately 4 × 14 nm. The slit diaphragm has the morphologic features of an adherens junction and the ZO-1 protein that is specific to tight junctions has been localized to the sites where the slit diaphragm is connected to the plasma membrane of the foot processes.
FIGURE 2-12  Electron micrograph showing the epithelial foot processes of normal rat glomerulus preserved in a 1% glutaraldehyde solution containing tannic acid. In several areas, the slit diaphragm has been sectioned parallel to the plane of the basement membrane, revealing a highly organized substructure. The thin central filament corresponding to the central dot observed on cross section (see Fig. 2-9) is indicated by the arrows. (Magnification, ×52,000.)

The molecular structure of the slit diaphragm has long escaped identification, and its role in establishing the perm-selective properties of the filtration barrier has been a matter of dispute. However, there is evidence that a newly identified protein, nephrin, may constitute a key component of the filtration barrier. Nephrin is the product of the gene that is mutated in
congenital nephrotic syndrome of the Finnish type (NPHS1). Based on the deduced amino acid sequence, nephrin is a transmembrane protein that belongs to the immunoglobulin family of adhesion molecules. Nephrin is expressed in the visceral epithelial cells in the glomerulus, where it is located exclusively in the slit diaphragm. This suggests that nephrin and the slit diaphragm are essential components of the glomerular filtration barrier, as illustrated in the hypothetical model of the filter in Figure 2-13. A second protein, CD2-associated protein (CD2AP) has recently been identified in the slit diaphragm. CD2AP is an adapter molecule that binds to the cytoplasmic domain of nephrin and is believed to connect nephrin to the cytoskeleton. Deletion of CD2AP is also associated with congenital nephrotic syndrome and morphologically with effacement or fusion of foot processes. Therefore, both nephrin and CD2AP appear to be required for normal filtration to occur. The gene responsible for familial steroid-resistant nephrotic syndrome has also been identified. Its product, podocin, is an integral membrane protein that is expressed in the foot process membrane at the site of insertion of the slit diaphragm. Podocin is connected to both nephrin and CD2AP.

FIGURE 2-13 Diagram illustrating hypothetical assembly of nephrin forming the filter of the podocyte slit diaphragm. Nephrin molecules from adjacent interdigitating foot processes are shown in different shades of purple. X indicates proteins interacting with nephrin and connecting with the plasma membrane. (From Tryggvason K: Unraveling the mechanisms of glomerular ultrafiltration: Nephrin, a key component of the slit diaphragm. J Am Soc Nephrol 10:2440, 1999.)

Various membrane components have been identified on the surface of the visceral epithelial cells. Kerjaschki and co-workers identified and characterized the principal sialoprotein on the urinary surface of the podocytes and termed it "podocalyxin." The human glomerular C3b receptor and
the Heymann nephritis antigen (gp330 or megalin) \(^1\) have also been identified in the plasma membrane of the visceral epithelial cell. In Heymann nephritis induced in the Lewis rat, this antigen reacts with an antibody directed against a constituent of the microvilli that form the brush border of the proximal tubule. \(^2\) The visceral epithelial cells then undergo capping on their surface and the antigen-antibody complex is shed into the subepithelial space adjacent to the GBM. There is evidence that pathogenic epitopes are present in all four clusters of ligand-binding repeats in megalin. Injection of domain-specific antibodies against each of the four megalin fragments produced glomerular immune deposits indicative of passive Heymann nephritis in rats. \(^3\)

In many diseases associated with proteinuria, the foot processes are replaced by a continuous cytoplasmic band along the GBM. This process is commonly referred to as foot process fusion or effacement. Similar ultrastructural changes have been described in the rat kidney after intra-arterial infusion of protamine sulfate, a polycationic substance that interacts with anionic sites on the cell membrane. \(^4\) Furthermore, perfusion of rat kidneys with neuraminidase, which removes sialic acid, causes a detachment of both endothelial and epithelial cells from the GBM, \(^5\) suggesting that negatively charged sites on these cells are important for the maintenance of normal structure and function of the filtration barrier. More recent evidence has assigned a possible role for the plasma membrane protein, podoplanin, in the maintenance of podocyte shape. A single intravenous injection of antipodoplanin immunoglobulin G antibodies into rats induced rapid and reversible flattening of foot processes and heavy albeit transient proteinuria. \(^6\) It has also been demonstrated that podoplanin is transcriptionally down-regulated in puromycin aminonucleoside nephrosis, in which there is flattening of foot processes and proteinuria. \(^7\) Therefore, anionic sites on the podocytes as well as the presence of an intact slit diaphragm are important in establishing the permselective properties of the filtration barrier. The visceral epithelial cells are capable of endocytosis, and the heterogeneous content of their lysosomes most likely reflects the uptake of proteins and other components from the ultrafiltrate. In conditions associated with heavy proteinuria, an increase occurs in the number of protein droplets in the cytoplasm of the podocytes.

For a detailed discussion of the structure, cell biology, and function of the glomerular podocyte, the reader is referred to a recent review article by Pavenstadt and colleagues. \(^8\)

**Mesangial Cells**

The mesangial cells and their surrounding matrix material constitute the mesangium, which is separated from the capillary lumen by the endothelium (see Figs. 2-6 and 2-8 \(^9\) \(^10\)). Zimmermann provided the first detailed description of the mesangium by light microscopy in 1933 and proposed the current nomenclature based on his theory of the development of the glomerulus by invagination. \(^11\) It was not until the advent of the electron microscope, however, that the mesangial cell was distinguished clearly from the endothelial cell and described in detail. \(^12\) The mesangial cell is irregular in shape, with a dense nucleus and elongated cytoplasmic processes that can extend around the capillary lumen and insinuate themselves between the GBM and the overlying endothelium (see *Fig. 2-8*). In addition to the usual complement of subcellular organelles, mesangial cells possess an extensive array of microfilaments composed at least in part of actin, α-actinin, and myosin. \(^13\) There is an especially heavy concentration of microfilaments situated along the paramesangial region and within the mesangial cell processes adjacent to the glomerular
The contractile mesangial cell processes appear to bridge the gap in the GBM encircling the capillary, and bundles of microfilaments interconnect opposing parts of the GBM, an arrangement that is believed to prevent capillary wall distention secondary to elevation of the intracapillary hydraulic pressure.

The mesangial cell is surrounded by a matrix material that is similar to but not identical with the GBM; the mesangial matrix is more coarsely fibrillar and slightly less electron dense. The mesangial matrix contains sulfated glycosaminoglycans as well as large amounts of fibronectin, laminin, and various collagens. Several cell surface receptors of the β-integrin family have been identified on the mesangial cells, including α1b1, α3b1, and the fibronectin receptor, α5b1. An additional α-chain, α8, has been identified on mesangial cells in human as well as rat and mouse kidneys. The α8β1 integrin receptor can also serve as a receptor for fibronectin. The integrin receptors mediate attachment of the mesangial cells to specific molecules in the extracellular mesangial matrix and link the matrix to the cytoskeleton. The attachment to the mesangial matrix is important for cell anchorage, contraction, and migration, and ligand-integrin binding also serves as a signal transduction mechanism that regulates the production of extracellular matrix as well as the synthesis of various vasoactive mediators, growth factors, and cytokines.

As reviewed by Schlondorff, the mesangial cell in all likelihood represents a specialized pericyte and possesses many of the functional properties of smooth muscle cells. In addition to providing structural support for the glomerular capillary loops, the mesangial cell has contractile properties and is believed to play a role in the regulation of glomerular filtration. Mesangial cells also exhibit phagocytic properties and participate in the clearance or disposal of macro-molecules from the mesangium. Finally, mesangial cells are involved in the generation and metabolism of the extracellular mesangial matrix and participate in various forms of glomerular injury.

The contractile properties of cultured mesangial cells are well established. Studies employing antibodies against actin, α-actinin, and myosin documented their colocalization with microfilaments in the rat mesangial cell. Cell contraction is stimulated by a variety of vasoactive agents, including angiotensin II, vasopressin, norepinephrine, thromboxane, leukotrienes, and platelet-activating factor. In contrast, such agents as PGE2, atrial peptides, and dopamine cause relaxation of cultured mesangial cells, in most instances by increasing intracellular levels of cyclic guanosine monophosphate (cGMP). Furthermore, receptors for angiotensin II, vasopressin, and platelet-activating factor have been demonstrated on the mesangial cell. The location of the mesangial cell in the intercapillary or centrilobular stalk region, combined with its contractile and relaxant properties, makes this cell an ideal candidate to participate in the control of glomerular filtration. It is possible that mesangial cell contraction decreases glomerular filtration by reducing blood flow through selected capillary loops, thereby eliminating their contribution to the process of filtration. The local generation of autacoids, such as PGE2, by the mesangial cell, may provide a counterregulatory mechanism to oppose the effect of the vasoconstrictors.

Morphologic aspects of the phagocytic properties of the mesangial cells are well documented. Uptake of tracers such as ferritin, colloidal carbon, aggregated proteins, and immune complexes has been described, and investigators have suggested that phagocytosed material may
be cleared from the mesangium by cell-to-cell transport to the extraglomerular mesangial region at the vascular pole of the glomerular tuft. Although some have reported that much of the phagocytic capability of the mesangium resides in the bone marrow-derived resident monocyte-macrophages, a population of cells possessing immune region-associated antigens (Ia), there is evidence that the mesangial cell is also capable of phagocytosis. Studies in cultured mesangial cells have demonstrated phagocytosis of opsonized zymosan, which was associated with the production of prostaglandins, reactive oxygen species, and lipoxygenase products. Endocytosis of immune complexes by mesangial cells was found to be associated with stimulation of PGE₂ and platelet-activating factor and was dependent on Fc receptor activity. Thus, there is evidence that both the Ia-bearing cell and the mesangial cell possess phagocytic capability.

Mesangial cells produce prostaglandins, and several vasoactive substances are known to influence this production. In addition to their proposed role in counter-regulating the effect of vasoconstrictors, prostaglandins can influence local cell proliferation and the production of cytokines, including platelet-derived growth factor, interleukin-1, and epithelial growth factor. This interaction among cytokines, mesangial cells, and prostaglandins may be important for understanding the mechanisms of the glomerular injury that is associated with mesangial cell proliferation and mesangial expansion in a host of kidney diseases.

Glomerular Basement Membrane

The GBM is composed of a central dense layer, the lamina densa, and two thinner, more electron-lucent layers, the lamina rara externa and the lamina rara interna (see Fig. 2-9). The latter two layers measure approximately 20 nm to 40 nm in thickness. The layered configuration of the GBM results in part from the fusion of endothelial and epithelial basement membranes during development. Several investigators have provided estimates of the width of the GBM of peripheral glomerular capillary loops. Jørgensen and Bentzon reported a geometric mean of 329 nm in 24 patients who showed no clinical evidence of renal disease. In a quantitative study of the GBM in five healthy individuals, Østerby calculated a mean width of 310 nm. Steffes and co-workers determined the GBM width in a large group of donor kidneys for transplantation and found a significantly thicker basement membrane in men (373 nm) than in women (326 nm). For the purpose of comparison with the human, the thickness of the GBM in the rat was found to be 132 nm.

Like other basement membranes in the body, the GBM is composed primarily of collagen IV, laminin, entactin/nidogen, and sulfated proteoglycans. In addition, the GBM contains specific components, such as laminin 11, distinct collagen IV α chains, and the proteoglycans agrin and perlecan, that most likely reflect its specialized function as part of the glomerular filtration barrier. Collagen IV is the major constituent of the GBM. As reviewed by Kashtan, six isomeric chains, designated α through α6 (IV), comprise the type IV collagen family of proteins. Of these six chains, α1 through α5 have been identified in the normal GBM. The six chains, α1 through α6 (IV), are encoded by genes located on human chromosomes 2, 13, and X. There is evidence that distinct networks of type IV collagen exist in different basement membranes. Although networks of α1/α2 (IV) chains are ubiquitous in all basement membranes, the GBM also contains networks of α3/α4/α5 (IV) chains, which are restricted in distribution. The exact
significance of the restricted networks remains unclear, but they may reflect specialization of function. Mutations in the genes encoding α3, α4, and α5 (IV) chains are known to cause Alport syndrome, a hereditary basement membrane disorder associated with progressive glomerulopathy.

The GBM possesses fixed, negatively charged sites that may influence the filtration of macromolecules. Caulfield and Farquhar demonstrated the existence of anionic sites in all three layers of the GBM with use of the cationic protein lysozyme. Additional studies employing cationic ferritin and ruthenium red, a cationic dye, revealed a lattice of anionic sites with a spacing of approximately 60 nm (Fig. 2-14) throughout the lamina rara interna and lamina rara externa, which might contribute to the formation of the charge barrier. Kanwar and Farquhar demonstrated that the anionic sites in the GBM consist of glycosaminoglycans rich in heparan sulfate. Their removal by enzymatic digestion resulted in an increase in permeability of the GBM to ferritin and to bovine serum albumin, suggesting that glycosaminoglycans play a role in establishing the permeability properties of the GBM to plasma proteins (see Fig. 2-14). It has also been suggested that the glycosaminoglycans might serve as anticlogging agents in the GBM.
FIGURE 2-14  Transmission electron micrographs of glomerular filtration barrier in normal rats perfused with native anionic ferritin (A) or cationic ferritin (C) and in rats treated with heparitinase before perfusion with anionic (B) or cationic (D) ferritin. In normal animals, anionic ferritin is present in the capillary (Cap) but does not enter the glomerular basement membrane (GBM), as shown in A. In contrast, cationic ferritin binds to the negatively charged sites in the lamina rara interna (LRI) and lamina rara externa (LRE) of the GBM (see C). After treatment with heparitinase, both anionic (B) and cationic (D) ferritin penetrates into the GBM, but there is no labeling of negatively charged sites by cationic ferritin. En, endothelial fenestrae; fp, foot processes; LD, lamina densa; US, urinary space. (Magnification, ×80,000.)  (From Kanwar YS: Biophysiology of glomerular filtration and proteinuria. Lab Invest 51:7, 1984.)
The glomerular capillary wall functions as a sieve or filter that allows the passage of small molecules but almost completely restricts the passage of molecules the size of albumin or larger. Physiologic studies have established that the glomerular capillary wall possesses both size-selective and charge-selective properties. To cross the capillary wall, a molecule must pass sequentially through the fenestrated endothelium, the GBM, and the epithelial slit diaphragm. The fenestrated endothelium, with its negatively charged glycocalyx, excludes formed elements of the blood and probably plays a role in determining the access of proteins to the GBM.

The exact role of the GBM in establishing the glomerular filtration barrier remains somewhat controversial. Ultrastructural tracer studies have provided evidence that the GBM constitutes both a size-selective and a charge-selective barrier. Caulfield and Farquhar infused dextrans of different molecular weights into rats and demonstrated that filtration depended on the size of the molecule and that the GBM was the main barrier to filtration. Rennke and co-workers used ultrastructural tracers such as ferritin and horseradish peroxidase with isoelectric points varying from 4.5 to 11.5 to examine the effect of molecular charge on the filtration of macromolecules. These studies demonstrated that the clearance of cationic molecules greatly exceeded that of neutral and anionic molecules. Furthermore, the electrostatic barrier appeared to be located mainly in the GBM. Subsequent studies reported that removal of negatively charged glycosaminoglycans resulted in increased permeability of the GBM to ferritin and albumin. Taken together these studies provided convincing experimental evidence that the GBM plays a major role in establishing a charge-selective filter in the glomerulus. However, the role of the GBM as the main determinant of charge selectivity was challenged subsequently because studies in the isolated GBM failed to demonstrate charge selectivity in vitro.

Because of the unique structure of the negatively charged filtration slit diaphragm and recent advances in its molecular characterization demonstrating that lack of distinct proteins associated with the slit diaphragm leads to massive proteinuria, it is now generally accepted that this structure plays a major role in establishing the ultrafiltration characteristics of the glomerular capillary wall. Most investigators, however, believe that the existence of all of the three structural components of the filtration barrier placed in series is important for the normal permeability properties of the glomerulus. A detailed discussion of glomerular permeability and the filtration barrier is provided in a recent review article by Deen and colleagues.

Parietal Epithelial Cells

The parietal epithelium, which forms the outer wall of Bowman's capsule, is continuous with the visceral epithelium at the vascular pole. The parietal epithelial cells are squamous in character, but at the urinary pole there is an abrupt transition to the taller cuboidal cells of the proximal tubule, which have a well-developed brush border (Figs. 2-15 and 2-16). The parietal epithelium of the capsule was described in detail by Jørgensen. The cells are 0.1 mm to 0.3 mm in height, except at the nucleus, where they increase to 2.0 mm to 3.5 mm. Each cell has a long cilium and occasional microvilli up to 600 nm in length. Cell organelles are generally sparse and include small mitochondria, numerous vesicles that are 40 nm to 90 nm in diameter, and the Golgi apparatus. Large vacuoles and multivesicular bodies are rarely, if ever, seen. The thickness of the basement membrane of Bowman's capsule is variable but has been found to range from 1200 nm to 1500
The basement membrane is composed of multiple layers, or lamellae, which increase in thickness with many disease processes. At both the vascular pole and the urinary pole, the thickness of Bowman's capsule decreases markedly. In certain disease processes, such as rapidly progressive glomerulonephritis, the parietal epithelial cells proliferate to contribute to the formation of crescents.

**FIGURE 2-16** Scanning electron micrograph illustrating the appearance of the surface of the parietal epithelial cells adjacent to the early proximal tubule at the urinary pole (lower left). Parietal epithelial cells possess a single cilium, and their lateral cell margins are accentuated by short microvilli (arrowheads). (Magnification, ×12,500.)  
(Courtesy of Jill W. Verlander, DVM.)

**Peripolar Cells**

Ryan and colleagues have described a peripolar cell that they believe is a component of the juxtaglomerular apparatus. It is located at the origin of the glomerular tuft in Bowman's space and is interposed between the visceral and parietal epithelial cells. The base of these cells rests on the basement membrane of Bowman's capsule, and the opposite surface is exposed to the urinary
space. They contain multiple membrane-bound electron-dense granules and are separated from
the afferent arteriole only by the basement membrane of Bowman's capsule. 16 The peripolar cells
are especially prominent in sheep, but they have also been identified in other species, including
humans, and have been localized predominantly in glomeruli in the outer cortex. 16 21

Juxtaglomerular Apparatus

The juxtaglomerular apparatus is located at the vascular pole of the glomerulus, where a portion
of the distal nephron comes into contact with its parent glomerulus. It has a vascular and a tubule
component. The vascular component is composed of the terminal portion of the afferent arteriole,
the initial portion of the efferent arteriole, and the extraglomerular mesangial region. The tubule
component is the macula densa, which is that portion of the thick ascending limb that is in contact
with the vascular component. 18 19 20 21 The extraglomerular mesangial region, which has also
been referred to as the polar cushion (polkissen) or the lacis, is bounded by the cells of the macula
densa, the specialized regions of the afferent and efferent glomerular arterioles, and the mesangial
cells of the glomerular tuft (the intraglomerular mesangial cells). Within the vascular component of
the juxtaglomerular apparatus, two distinct cell types can be distinguished: the juxtaglomerular
granular cells, also called epithelioid or the myoepithelial cells, and the agranular extraglomerular
mesangial cells, which are also referred to as the lacis cells or pseudo-meissnerian cells of
Goormaghtigh.

Juxtaglomerular Granular Cells

The granular cells are located primarily in the walls of the afferent and efferent arterioles, but they
are also present in the extraglomerular mesangial region. 16 18 20 21 They exhibit features of both
smooth muscle cells and secretory epithelial cells and therefore have been called myoepithelial
cells. 18 The juxtaglomerular granular cells are believed to represent modified smooth muscle cells.
They contain myofilaments in the cytoplasm and, except for the presence of granules, are
indistinguishable from the neighboring arteriolar smooth muscle cells. They also exhibit features
suggestive of secretory activity, including a well-developed endoplasmic reticulum and a Golgi
complex containing small granules with a crystalline substructure. 15 16

The juxtaglomerular granular cells are characterized by the presence of numerous
membrane-bound granules of variable size and shape (Fig. 2-17). 21 Some of these granules,
termed protogranules, have a crystalline substructure and are believed to represent precursors that
fuse to form the larger mature granules. 17 18 In addition to these so-called specific granules,
lipofuscin-like granules are commonly observed in the human kidney. 17 18
There is convincing evidence that the specific granules represent renin or its precursor. As early as 1945, Goormaghtigh proposed that the granular cells were the source of renin. That hypothesis was later proven correct by immunohistochemical and in situ hybridization studies as well as biochemical studies demonstrating renin enzyme activity in the juxtaglomerular apparatus.\cite{1}\cite{2}\cite{3} \cite{4} Immunohistochemical studies demonstrated the presence of both renin and angiotensin II in the juxtaglomerular granular cells, with activities being highest in the afferent arteriole.\cite{5} Through use of the immunogold technique in combination with electron microscopy, renin and angiotensin II were found to coexist in the same granules.\cite{6} Studies using in situ hybridization techniques demonstrated renin messenger RNA (mRNA) in the juxtaglomerular cells in normal kidneys, thus providing evidence that these cells produce renin.\cite{7} Histochemical and immunocytochemical studies also have demonstrated the presence of lysosomal enzymes, including acid phosphatase.
and cathepsin B, in renin-containing granules of the juxtaglomerular epithelioid cells, suggesting that these granules may represent modified lysosomes.

Extraglomerular Mesangium

The extraglomerular mesangium is also called the lacis or the cells of Goormaghtigh. It is located between the afferent and efferent arterioles in close contact with the macula densa (see Fig. 2-17). The extraglomerular mesangium is continuous with the intraglomerular mesangium and is composed of cells that are similar in ultrastructure to the mesangial cells. The extraglomerular mesangial cells possess long, thin cytoplasmic processes that are separated by basement membrane material. Under normal conditions, these cells do not contain granules; however, juxtaglomerular granular cells are occasionally observed in the extraglomerular mesangium. The extraglomerular mesangial cells are in contact with the arterioles and the macula densa, and gap junctions are commonly observed between the various cells of the vascular portion of the juxtaglomerular apparatus. Gap junctions have also been described between extraglomerular and intraglomerular mesangial cells, suggesting that the extraglomerular mesangium may serve as a functional link between the macula densa and the glomerular arterioles and mesangium. Moreover, there is evidence that mesangial cell damage and selective disruption of gap junctions eliminate the tubuloglomerular feedback response.

Macula Densa

The macula densa is a specialized region of the thick ascending limb adjacent to the hilus of the glomerulus (see Fig. 2-17). Only those cells immediately adjacent to the hilus are morphologically distinctive and form the macula densa. They are low columnar cells and exhibit an apically placed nucleus. With electron microscopy, the cell base is seen to interdigitate with the adjacent extraglomerular mesangial cells to form a complex relationship. Although mitochondria are numerous, their orientation is not perpendicular to the base of the cell, and they are rarely enclosed within plications of the basolateral plasma membrane. The position of the Golgi apparatus is lateral to and beneath the cell nucleus. In addition, other cell organelles, including lysosomes, autophagic vacuoles, ribosomes, and profiles of smooth and granular endoplasmic reticulum, are located principally beneath the cell nucleus. The basement membrane of the macula densa is continuous with that surrounding the granular and agranular cells of the extraglomerular mesangial region, which in turn is continuous with the matrix material surrounding the mesangial cells within the glomerular tuft. The macula densa cells lack the lateral cell processes and interdigitations that are characteristic of the thick ascending limb. Ultrastructural studies have provided evidence that the widths of the lateral intercellular spaces in the macula densa vary, depending on the physiologic status of the animal. This finding, coupled with the demonstrated absence of Tamm-Horsfall protein in the macula densa, has prompted some investigators to suggest that, in contrast to the thick ascending limb, where the presence of this glycoprotein may contribute to the water impermeability, the macula densa may be relatively permeable to water. Furthermore, direct visualization of the isolated perfused macula densa by use of differential interference contrast microscopy has revealed reversible dilatation of the lateral intercellular spaces between the macula densa cells with reduction of luminal osmolality.
Morphologic evidence suggests that the autonomic nervous system is involved in the regulation of the function of the juxtaglomerular apparatus. Electron microscopic studies have demonstrated the existence of synapses between granular and agranular cells of the juxtaglomerular apparatus and autonomic nerve endings. On serial sections of the rat juxtaglomerular apparatus, Barajas and Müller analyzed the frequency of contacts between axons and the various cellular components of the juxtaglomerular apparatus. Nerve endings, principally adrenergic in type, were observed to be in contact with approximately one third of the cells of the efferent arteriole and with somewhat less than one third of the cells of the afferent arteriole in the region of the juxtaglomerular apparatus. The frequency of innervation of the tubule component of the juxtaglomerular apparatus was far less. Electron microscopic autoradiography demonstrated uptake of tritiated norepinephrine in axons in contact with afferent and efferent arterioles, which suggests that the nerves are adrenergic in character. Extensive studies by Kopp and DiBona provided convincing evidence that renin secretion is modulated by renal sympathetic nerve activity, which is consistent with the existence of neuroeffector junctions on renin-positive granular cells of the juxtaglomerular apparatus.

The juxtaglomerular apparatus represents a major structural component of the renin-angiotensin system. The role of the juxtaglomerular apparatus is to regulate glomerular arteriolar resistance and glomerular filtration and to control the synthesis and secretion of renin. The cells of the macula densa sense changes in the luminal concentrations of sodium and chloride, presumably via absorption of these ions across the luminal membrane by the Na\(^+\)-K\(^+\)-2Cl cotransporter, which is expressed in the macula densa. This initiates the tubuloglomerular feedback response by which signals generated by acute changes in sodium chloride concentration are transferred via the macula densa cells to the glomerular arterioles to control the glomerular filtration rate. Signals from the macula densa, in response to changes in luminal sodium and chloride, are also transmitted to the renin-secreting cells in the afferent arteriole.

Renin synthesis and secretion by the juxtaglomerular granular cells are controlled by several factors, including neurotransmitters of the sympathetic nervous system, glomerular perfusion pressure (presumably through arteriolar baroreceptors), and mediators in the macula densa. There is increasing evidence that the macula densa control of renin secretion is mediated by NO, cyclooxygenase products such as PGE\(_2\), and adenosine.

At least two immunologically distinct isoforms of nitric oxide synthase (NOS) are present in the juxtaglomerular apparatus, a fact that, when coupled with complementary physiologic observations, indicates that NO may be an important regulator of the functions of the juxtaglomerular apparatus. Several investigators have demonstrated immunostaining of macula densa cells with antibodies directed against the neuronal isoform of nitric oxide synthase (nNOS), and the mRNA for nNOS has also been demonstrated in macula densa cells by in situ hybridization. In addition, the endothelial isoform of nitric oxide synthase (eNOS) is expressed in endothelial cells of both the glomerular arterioles and the glomerular capillary tuft. The localization of NOS in the macula densa and glomerular endothelial cells was confirmed by use of an independent histochemical technique to detect reduced nicotinamide adenine dinucleotide phosphate (NADPH)–dependent diaphorase activity, which has served as a marker for NOS.
functional studies, it has been reported that NO regulates renin release both in vivo and in vitro. Macula densa-controlled renin secretion plays an important role in the adaptation of tubuloglomerular feedback that occurs during long-term perturbations of macula densa sodium chloride concentration. Several investigators have provided evidence that NO modulates the tubuloglomerular feedback response. NO is believed to cause a resetting of the feedback mechanism, probably via its effect on renin secretion and by reducing ecto-5'-nucleotidase (CD73) activity (discussed later), but it is not a mediator of the response. Therefore, although NO blunts glomerular arteriolar constriction and is important for regulation of the feedback mechanism, it is not required for the feedback response to occur. In contrast, there is evidence that adenosine may play a role as a mediator of the tubuloglomerular feedback response, as discussed in detail by Schnermann and Levine. Mice lacking the type 1 adenosine receptor had a completely abolished feedback response, supporting a role for adenosine as a physiological mediator in this process. Moreover, mice with a genetic deletion of the ecto-5'-nucleotidase (CD73), an enzyme converting extracellular AMP to adenosine, has a markedly impaired tubuloglomerular feedback response. The stimulation of renin secretion in response to a decrease in macula densa sodium chloride concentration is abolished by inhibition of NOS, indicating that NO produced in the macula densa stimulates renin secretion. There is also evidence that prostaglandins generated by the cyclooxygenase (COX) enzymes are involved in macula densa-controlled renin secretion. Harris and co-workers using both in situ hybridization and immunohistochemical techniques, have demonstrated that COX-2 is expressed in the macula densa and that its expression is up-regulated in animals receiving a low-salt diet. Inhibition of COX-2 prevents macula densa-mediated stimulation of renin secretion and causes a decrease in the expression of renin in the kidney. Studies in COX-2 knockout mice demonstrated a significant decrease in renin expression and activity compared with wild-type animals, and the increase in renin mRNA expression observed in wild-type mice in response to a low-salt diet was abolished in COX-2 knockout animals. These studies indicate that COX-2 products such as PGE₂ are involved in the regulation of renin production and secretion. Therefore, there is both structural and functional evidence that both NO and COX-2-generated prostaglandins participate in the signaling pathway between the macula densa and the renin-producing cells in the afferent arteriole. The exact relationship between the two mediators remains to be established. However, studies suggest that the increase in COX-2 expression in the macula densa in response to a low-salt diet is mediated by NO. In addition to serving as a mediator of the tubuloglomerular feedback response, adenosine appears to be required for the inhibition of renin secretion that occurs in response to an increased NaCl concentration at the macula densa.

**Proximal Tubule**

The proximal tubule begins abruptly at the urinary pole of the glomerulus (see Fig. 2-15). It consists of an initial convoluted portion, the pars convoluta, which is a direct continuation of the parietal epithelium of Bowman's capsule, and a straight portion, the pars recta, which is located in the medullary ray (see Fig. 2-5). The length of the proximal tubule is approximately 10 mm in the
rabbit, 8 mm in the rat, and 4 mm to 5 mm in the mouse, compared with approximately 14 mm in the human. The outside diameter of the proximal tubule is about 40 mm. In the rat, three morphologically distinct segments—S1, S2, and S3—have been identified. The S1 segment is the initial portion of the proximal tubule; it begins at the glomerulus and constitutes approximately two thirds of the pars convoluta. The S2 segment consists of the remainder of the pars convoluta and the initial portion of the pars recta. The S3 segment represents the remainder of the proximal tubule, located in the deep inner cortex and the outer stripe of the outer medulla.

FIGURE 2-15 Scanning electron micrograph depicting the transition from the parietal epithelial cells of Bowman’s capsule (foreground) to the proximal tubule cells, with their well-developed brush border, in the kidney of a rat. (Magnification, ×3200.)

The structural features that distinguish the cells in the three segments in the rat have been described in detail by Maunsbach and are illustrated in Figures 2-18 through 2-20.
The S1 segment has a tall brush border and a well-developed vacuolar-lysosomal system. The basolateral plasma membrane forms extensive lateral invaginations, and lateral cell processes extending from the apical to the basal surface interdigitate with similar processes from adjacent cells. Elongated mitochondria are located in the lateral cell processes in proximity to the plasma membrane. The ultrastructure of the S2 segment is similar to that of the S1 segment; however, the brush border is shorter, the basolateral invaginations are less prominent, and the mitochondria are smaller. Numerous small lateral processes are located close to the base of the cell. The endocytic compartment is less prominent than in the S1 segment. However, the number and size of the lysosomes vary among species and between males and females, and numerous large lysosomes are often observed in the S2 segment of the male rat. In the S3 segment, lateral cell processes and invaginations are essentially absent, and mitochondria are small and randomly distributed within the cell. The length of the brush border in the S3 segment varies among species. It is tall in the rat, fairly short in the rabbit, and intermediate in length in the human kidney. Considerable species variation is also observed in the vacuolar-lysosomal compartment in the S3 segment. In the rat and the human, endocytic vacuoles and lysosomes are small and sparse, whereas in the rabbit, large endocytic vacuoles and numerous small lysosomes are present in the S3 segment. Peroxisomes are present throughout the proximal tubule. They increase in number along the length of the proximal tubule and are most prominent in the S3 segment.
FIGURE 2-18  Transmission electron micrograph of S1 segment of rat proximal tubule. The cells are characterized by a tall brush border, a prominent endocytic-lysosomal apparatus, and extensive invaginations of the basolateral plasma membrane. (Magnification, ×10,600.)
FIGURE 2-19 Transmission electron micrograph of S2 segment of rat proximal tubule. The brush border is less prominent than in the S1 segment. Note numerous small lateral processes at the base of the cell. (Magnification, ×10,600.)
Three segments have also been described in the proximal tubule of the rabbit and the rhesus monkey. However, according to Kaisling and Kriz, in the rabbit the S2 segment is not clearly demarcated morphologically and represents a transition between the S1 and S3 segments. Interestingly, a recent ultrastructural study found no evidence of structural segmentation along the proximal tubule of the mouse. Only the pars convoluta and the pars recta have been positively identified and described in the nondiseased human kidney. Because most functional studies have distinguished between the convoluted and the straight portions of the proximal tubule rather than the S1, S2, and S3 segments, the former distinction is used in the following description.

Pars Convoluta
The individual cells of the pars convoluta are extremely complex in shape as described for the S1 segment of the rat proximal tubule (Fig. 2-21). From the main cell body, large primary ridges extend laterally from the apical to the basal surface of the cells. Lateral processes large enough to contain mitochondria extend outward from the primary ridges and interdigitate with similar processes from adjacent cells. These lateral processes can be demonstrated by scanning electron microscopy (Fig. 2-22). At the luminal surface of the cells, smaller apical lateral processes extend outward from the primary ridges to interdigitate with those of adjacent cells. Small basal villi that do not contain mitochondria are found along the basal cell surface (see Fig. 2-21).

FIGURE 2-21 Schematic drawing illustrating three-dimensional configuration of proximal convoluted tubule cell. (From Welling LW, Welling DJ: Shape of epithelial cells and intercellular channels in the rabbit proximal nephron. Kidney Int 9:385, 1976.)

As a result of the extensive interdigitations of lateral and basal processes between adjacent cells, a complex extracellular compartment is formed. It is referred to as the basolateral intercellular space (Fig. 2-23; see Fig. 2-22). This space is separated from the tubule lumen by a specialization of the plasma membrane called the zonula occludens, or tight junction. The zonula occludens forms a continuous band around the luminal surface of each cell, where the outer leaflets of the
plasma membrane of adjacent cells appear to be fused, resulting in a pentilaminar appearance of the tight junction. Early tracer studies employing high-molecular-weight substances such as ferritin, peroxidase, and hemoglobin failed to provide morphologic evidence of a pathway between proximal tubule cells. However, physiologic and electrophysiologic studies have revealed the presence of a low-resistance shunt pathway in parallel with a high-resistance pathway across the apical and basal plasma membranes of the proximal tubule cell. The low-resistance pathway is believed to be formed by the tight junction and the lateral intercellular space. In ultrastructural studies of in vivo perfused rat proximal tubules, Tisher and Yarger demonstrated that both ionic and colloidal lanthanum were capable of penetrating the entire region of the tight junction from either the luminal or the peritubular surface of the cell. These results were confirmed by Martinez-Palomo and Erij thus providing further support for the existence of a paracellular pathway in the proximal tubule.

FIGURE 2-23 Electron micrograph of the pars convoluta of the proximal tubule from a normal
human kidney. The mitochondria (M) are elongated and tortuous, occasionally doubling back on themselves. The endocytic apparatus, composed of apical vacuoles (AV), apical vesicles (V), and apical dense tubules (arrows), is well developed. G, Golgi apparatus; IS, intercellular space; L, lysosome; Mv, microvilli forming the brush border; TL, tubule lumen. (Magnification, ×15,000.)

Freeze-fracture electron microscopy of proximal convoluted tubules of mouse, several other species of animals including rat and rabbit, and human kidney has revealed that the tight junction is formed by one or two strands. These strands are equivalent to ridges on the P face (that half of the plasma membrane adjacent to the cytoplasm of the cell) and grooves on the E face (that half of the plasma membrane adjacent to the intercellular space). In the proximal convoluted tubule of the rat, up to 10% of the strands forming the tight junction are discontinuous, which may explain the ability of lanthanum to penetrate the tight junction of the proximal convoluted tubule. Thus, morphologic as well as physiologic data provide evidence for the presence of a paracellular shunt pathway between cells of the mammalian proximal convoluted tubule.

Immediately beneath the tight junction is a second specialized region of the plasma membrane, termed the intermediate junction or zonula adherens. It is a seven-layered structure formed by the two adjacent, triple-layered plasma membranes separated by a narrow upper extension of the intercellular space. Dense condensations of cytoplasm are located adjacent to the regions of the plasma membranes that form the intermediate junction. Desmosomes, or maculae adherentes, are also found in the proximal convoluted tubule, distributed randomly at variable distances beneath the intermediate junction. These seven-layered structures are also formed by the two adjacent plasma membranes and the intervening intercellular space. However, they are disk-shaped rather than belt-like in configuration and they are responsible for cell-cell adhesion. Gap junctions are present in small numbers in mammalian and invertebrate renal proximal tubules. They are specialized connections between adjacent cells where the plasma membranes are separated by a 2-nm gap that contains characteristic subunits. The gap junction is believed to provide a pathway for the movement of ions between cells.

The intercellular space is open toward the basement membrane, which separates it from the peritubular interstitium and capillaries. The thickness of the basement membrane gradually decreases along the proximal tubule. In the rhesus monkey, it decreases in thickness from approximately 250 nm in the S1 segment to 145 nm in the S2 segment and to only 70 nm in the S3 segment. In the rat, the basement membrane of the proximal convoluted tubule was found to be 143 nm in thickness.

The lateral cell processes and invaginations of the plasma membrane serve to increase the intercellular space and the area of the basolateral plasma membrane, where the sodium-potassium adenosine triphosphatase (Na\(^+\),K\(^+\)-ATPase) or Na\(^+\) pump is located. Morphometric studies of the proximal convoluted tubule in the rabbit have demonstrated that the area of the lateral surface equals that of the luminal surface and amounts to 2.9 mm\(^2\)/mm tubule. Elongated mitochondria
are located in the lateral cell processes in close proximity to the plasma membrane (see Fig. 2-23), an arrangement that is characteristic of epithelia involved in active ion transport. With standard transmission electron microscopy, these organelles appear rod-shaped and tortuous; however, studies using high-voltage electron microscopy of 0.5- to 1.0-mm-thick sections have revealed that many mitochondria in the proximal tubule are branched and anastomose with one another. A system of smooth membranes, called the paramembranous cisternal system, is often observed between the plasma membrane and the mitochondria. The function of the paramembranous cisternal system is not known, but studies suggest that it is in continuity with the smooth endoplasmic reticulum.

The cells throughout the proximal tubule contain large quantities of smooth and rough endoplasmic reticulum, and free ribosomes are also abundant in the cytoplasm. A well-developed Golgi apparatus is located above and lateral to the nucleus in the midregion of the cell. It is composed of four basic elements: smooth-surfaced sacs or cisternae, coated vesicles, uncoated vesicles, and larger vacuoles. The cisternae form parallel stacks that possess a convex surface, the cis side, and a concave surface, the trans side, from which small, coated vesicles appear to bud off (Fig. 2-24). An extensive system of microtubules is located throughout the cytoplasm of the proximal tubule cells.
A well-developed brush border forms the apical or luminal surface of the proximal convoluted tubule. It is formed by numerous finger-like projections of the apical plasma membrane, the microvilli (see Figs. 2-18 through 2-20 [18] [19] [20]). Morphometric studies performed on isolated segments of rabbit proximal convoluted tubules found that the brush border increases the apical cell surface 36-fold. On cross section, 6 to 10 filaments, approximately 6 nm in diameter, can be
seen within individual microvilli, often extending downward into the apical region of the cell for considerable distances. A network of filaments, called the terminal web, is located in the apical cytoplasm just beneath and perpendicular to the microvilli. The filaments of the microvilli are actin filaments. Immunocytochemical studies have demonstrated the presence of the cytoskeletal proteins, villin and fimbrin, in the microvillar core, whereas myosin and spectrin were found in the terminal web. It is well established that the protein composition of the brush border membrane is different from that of the basolateral membrane. Biochemical studies have reported the presence of alkaline phosphatase, aminopeptidase, 5′-nucleotidase, and Mg^2+ -ATPase activity within brush border membranes from the kidney cortex while Na^+ -K^+ -ATPase is present in the basolateral plasma membrane. Furthermore, immunocytochemical studies have demonstrated microdomains with different glycoproteins in brush border membranes. The Heymann nephritis antigen (gp330 or megalin) is located mainly in the apical invaginations between the microvilli, whereas maltase, a disaccharidase, is concentrated on the microvilli. Ecto-5′-nucleotidase, which is involved in the generation of adenosine, is also expressed in the brush border of the proximal tubule.

The pars convoluta of the proximal tubule contains a well-developed endocytic-lysosomal apparatus that is involved in the reabsorption and degradation of macromolecules from the ultrafiltrate. The endocytic compartment consists of an extensive system of coated pits, small coated vesicles, apical dense tubules, and larger endocytic vacuoles without a cytoplasmic coat (Fig. 2-25). The coated pits are invaginations of the apical plasma membrane at the base of the microvilli that form the brush border. The cytoplasmic coat of the small vesicles is similar in ultrastructure to the coat that is present on the cytoplasmic side of the coated pits. Immunocytochemical studies have demonstrated that this coat contains clathrin, a protein believed to play a role in receptor-mediated endocytosis. The Heymann nephritis antigen demonstrated in brush border membranes from the proximal tubule is also located mainly in the clathrin-coated pits and clathrin-coated vesicles.
A large number of lysosomes are present in the proximal convoluted tubule. Lysosomes are membrane-bound, heterogeneous organelles that contain a variety of acid hydrolases, including acid phosphatase, and various proteases, lipases, and glycosidases (Fig. 2-26). They vary considerably in size, shape, and ultrastructural appearance. Lysosomes are involved in the degradation of material absorbed by endocytosis (heterophagocytosis), and they often contain multiple electron-dense deposits that are believed to represent reabsorbed substances such as proteins (see Fig. 2-26). Lysosomes also play a role in the normal turnover of intracellular...
constituents by autophagocytosis, and autophagic vacuoles containing fragments of cell organelles are often seen in the proximal tubule (see Fig. 2-26). Lysosomes containing nondigestible residues are called residual bodies, and they can empty their contents into the tubule lumen by exocytosis. Multivesicular bodies that are part of the vacuolar-lysosomal system are often observed in the cytoplasm of the proximal convoluted tubule. They are believed to be involved in membrane retrieval or membrane disposal (or both).

**FIGURE 2-26** Electron micrographs illustrating the appearance of different types of lysosomes from human proximal tubules. A, Lysosomes. Several mitochondria (M) are also shown. (Magnification, ×15,500.) B, Early stage of formation of an autophagic vacuole. (Magnification, ×23,500.) C, Fully formed autolysosome containing a mitochondrion undergoing digestion. (Magnification, ×28,500.) D, Autolysosome, containing a microbody undergoing digestion. A multivesicular body (arrow) is also shown. (Magnification, ×29,250.) (From Tisher CC, Bulger RE, Trump BF: Human renal ultrastructure. I. Proximal
Studies using the weak base N-(3-((2,4-dinitrophenyl)amino)propyl)-N-(3-aminopropyl) methylamine dihydrochloride (DAMP), in combination with immunocytochemical techniques at the ultrastructural level, to identify intracellular acidic compartments in the proximal tubule of the rat and rabbit found that lysosomes and a population of endosomes were acidic. In agreement with these observations, biochemical studies demonstrated the presence of an electrogenic H⁺ pump in endosomes isolated from the kidney cortex. Furthermore, immunocytochemical studies using antibodies to the vacuolar H⁺ pump demonstrated labeling of both endosomes and the apical plasma membrane at the base of the microvilli, thus confirming that an H⁺-ATPase exists in these structures. Polybasic cationic drugs, such as aminoglycoside antibiotics, are absorbed and accumulate in acidic organelles in the proximal tubule. Certain heavy metals also accumulate in renal lysosomes, probably because they are bound to protein in the tubule fluid and undergo endocytosis. Long-term exposure to mercuric chloride was found to cause both structural and functional changes in the lysosomal system of the proximal tubule of the rat.

The vacuolar-lysosomal system plays an important role in the reabsorption and degradation of albumin and low-molecular-weight plasma proteins from the glomerular filtrate. Proteins are absorbed from the tubule lumen by endocytosis or pinocytosis (Fig. 2-27). By this process, the protein becomes located in invaginations of the apical plasma membrane—the so-called coated pits—which pinch off to form small coated vesicles. The coated vesicles fuse with endosomes and the absorbed protein is transferred through the endosomal compartment to the lysosomes, where it is catabolized by proteolytic enzymes. The apical dense tubules are part of the vacuolar system and are believed to be involved in the recycling of membrane back to the apical plasma membrane. Interestingly, binding sites for insulin are present on both the apical and basolateral plasma membrane. However, the uptake of insulin is much greater from the luminal side than from the peritubular side.
FIGURE 2-27  Schematic drawing of the endocytic-lysosomal system in a proximal tubule cell.

Under normal conditions, the vacuolar-lysosomal system is most prominent in the S1 segment. In proteinuric states, however, with large amounts of protein being presented to the proximal tubule cells, large vacuoles—the so-called protein droplets—and lysosomes can be observed in the proximal tubule, especially in the S2 segment. In agreement with these ultrastructural observations, biochemical studies on individual tubule segments have demonstrated that under normal nonproteinuric conditions, the activity of the lysosomal proteolytic enzymes, cathepsins B and L, is significantly higher in the S1 segment than in the S2 and S3 segments of the proximal tubule. In proteinuric conditions, the activity of cathepsins B and L in the proximal tubule is increased, and activity is highest in the S2 segment.

Studies in the isolated perfused rat kidney, in vivo micropuncture studies, and studies using isolated perfused rabbit proximal tubule have provided evidence that the absorption of protein by the proximal tubule is a selective process determined by the net charge, size, and configuration of the protein molecule and possibly by the presence of preferential endocytic sites on the apical plasma membrane. Based on studies by Farquhar and co-workers and by Christensen and Birn, it is now generally accepted that the reabsorption of numerous proteins...
and polypeptides by the proximal tubule is mediated by megalin, a multiligand endocytic receptor. Kerjaschki and Farquhar purified megalin (gp330) from rat kidney brush border membrane and demonstrated that it represents the antigen for rat Heymann nephritis. Megalin was subsequently cloned and found to be a 600-kD glycoprotein belonging to the low-density-lipoprotein receptor gene family. The receptor-associated protein, RAP, which binds to megalin, is also an antigen for Heymann nephritis. RAP is believed to function as a chaperone for megalin.

Immunocytochemical studies have demonstrated that megalin is located in the brush border, coated pits, endocytic vesicles, and apical dense tubules in the proximal tubule, particularly in the S2 segment (Fig. 2-28).

**FIGURE 2-28** Transmission electron micrograph (magnification, ×35,000) illustrating immunogold localization of megalin in a proximal tubule cell from normal mouse kidney. Labeling of megalin is seen on microvilli (MV), coated pits (arrows), and the apical endocytic apparatus. Inset is a higher-magnification (×53,000) micrograph illustrating the labeling of coated pits (large arrowheads), apical endosomes (arrows), and apical dense tubules (small arrowheads). (From Christensen EI, Willnow TE: Essential role of megalin in renal proximal tubule for vitamin homeostasis. J Am Soc Nephrol 10:2224, 1999.)

As reviewed in detail by Christensen and Birn, megalin serves as a receptor for numerous ligands, including low-molecular-weight proteins, polypeptide hormones, albumin, vitamin-binding proteins, and polybasic drugs such as aminoglycosides. Orlando and co-workers demonstrated that megalin binds and internalizes insulin, and evidence was also provided from ligand blotting assays that megalin serves as a receptor for various low-molecular-weight polypeptides including β₂-microglobulin, lysozyme, prolactin, cytochrome C, and epidermal growth factor. Moestrup, Christensen, Nykjaer, and their co-workers
demonstrated that megalin serves as the principal receptor for the carrier proteins of various vitamins, including vitamin B₁₂, vitamin D, and retinol, suggesting that megalin may play a role in vitamin metabolism and homeostasis. Other studies demonstrated a loss of components of the endocytic apparatus and increased excretion of low-molecular-weight proteins in the urine of megalin-deficient mice, providing further support for the role of megalin in proximal tubule reabsorption of protein.

A second multiligand endocytic receptor, cubilin, which is identical to the intestinal intrinsic factor-cobalamin receptor, has been identified in the proximal tubule. Cubilin is a 460 kD glycoprotein that binds to megalin. It is expressed in the brush border and in the endocytic compartment in a pattern similar to that of megalin. Studies by Christensen, Birn, and their co-workers demonstrated that cubilin binds several ligands present in the glomerular ultrafiltrate, including albumin and various vitamin-binding proteins, and that both megalin and cubilin are essential for the reabsorption of these proteins in the proximal tubule.

The proximal convoluted tubule plays a major role in the reabsorption of Na⁺, HCO₃⁻, Cl⁻, K⁺, Ca²⁺, PO₄³⁻, water, and organic solutes such as glucose and amino acids. Approximately half of the ultrafiltrate is reabsorbed in the proximal tubule. Fluid reabsorption is coupled to the active transport of Na⁺, and little change occurs in the osmolality or in the Na⁺ concentration of the tubule fluid along the proximal tubule, indicating that fluid reabsorption in this segment is almost isosmotic. The rate of fluid absorption from the proximal tubule to the peritubular capillaries is influenced by the hydraulic and oncotic pressures across the tubule and capillary wall. Changes in these parameters cause significant ultrastructural changes in the proximal tubule, especially in the configuration of the lateral intercellular spaces.

In the early 1990s Preston and co-workers cloned a water channel protein, CHIP28 or aquaporin-1 (AQP1), from human erythrocytes. AQP1 is believed to mediate osmotic water permeability in red blood cells as well as in renal tubule cells. Immunocytochemical studies using antibodies to AQP1 demonstrated the presence of this water channel protein in both the proximal tubule (Fig. 2-29) and the descending thin limb, segments known to have high osmotic water permeability. Labeling was observed in both the apical and the basolateral plasma membrane, which indicates that water is reabsorbed across the epithelium through these channels in response to the existent osmotic gradient. AQP8 is also expressed in the renal proximal tubule where it is located mainly in intracellular structures with little or no expression at the plasma membrane. Biophysical studies in Xenopus oocytes and yeast have revealed that AQP8 conducts water as well as ammonia/ammonium and formamide. AQP8 gene knockout mice have no major phenotype. Thus, the role of AQP8 remains to be established.
Aquaporin-11 (AQP11) is a channel protein with unusual pore-forming NPA (asparagine-proline-alanine) boxes. Immunocytochemical studies have demonstrated intracellular expression of AQP11 in the proximal tubule as well as in AQP11-transfected CHO-K1 cells. A predominantly intracellular expression has also been reported in other cell types.
including cells in the brain. AQP11-null mice exhibit vacuolization and cyst formation of the proximal tubule. The mice appeared normal at birth, but developed polycystic kidneys and died before weaning due to advanced renal failure. Interestingly, primary cultured proximal tubule cells from AQP11-null mice exhibited an endosomal acidification defect. However, the physiological role of AQP11 in the proximal tubule remains to be identified.

Sodium reabsorption by the proximal tubule is an active process driven by Na⁺/K⁺-ATPase, which is located in the basolateral plasma membrane, as demonstrated by both histochemical and immunocytochemical studies. The active transport of Na⁺ out of the cell across the basolateral membrane creates a lumen-to-cell concentration gradient for Na⁺. The transport of various anions and organic solutes is coupled with the transport of Na⁺ down its concentration gradient. The main anions transported together with Na⁺ are HCO₃⁻ and Cl⁻. HCO₃⁻ reabsorption takes place primarily in the early proximal tubule and is secondary to H⁺ secretion, which is mediated predominantly by an Na⁺/H⁺ exchange mechanism located in the brush border membrane. Studies in several laboratories have demonstrated expression of the Na⁺/H⁺ exchanger isoform, NHE3, in the brush border of the proximal tubule. In addition, there is evidence that active H⁺ secretion mediated by an H⁺-ATPase occurs in the proximal tubule. An H⁺-ATPase has been demonstrated in brush border membrane vesicles as well as in endosome vesicles isolated from the kidney cortex. Immunocytochemical studies using antibodies to a renal H⁺-ATPase have revealed labeling of both endosomes and coated pits at the base of the brush border microvilli, which supports the presence of H⁺-ATPase at both sites. Studies in mice lacking the NHE3 have confirmed the importance of NHE3 for bicarbonate and fluid reabsorption in the proximal convoluted tubule. The expression and activity of NHE3 is regulated by various hormones, including angiotensin II and aldosterone. The regulation involves multiple signaling pathways and molecules such as PKA/cAMP and EPAC, NHERF-1, and Rho GTPases. It has also been suggested that mechanosensory pathways involving the microvilli may play a role in the regulation of NHE3 in the proximal tubule.

Bicarbonate reabsorption in the proximal tubule is mediated by an electrogenic Na⁺/HCO₃⁻ co-transporter, NBC1, with a stoichiometry of 3 HCO₃⁻ for each Na⁺. NBC1 belongs to a superfamily of bicarbonate transporters that includes the anion exchangers (AE) and the sodium bicarbonate co-transporters (NBC). NBC1 in the proximal tubule was the first sodium bicarbonate co-transporter to be identified. It was cloned initially from Ambystoma and later from both human and rat kidney. In situ hybridization studies in the rabbit kidney demonstrated that NBC1 mRNA was present only in the renal cortex where it was localized to the proximal tubule. Subsequent immunofluorescence studies in rat and rabbit and high-resolution electron microscopic studies in the rat kidney revealed that NBC1 was expressed exclusively in the basolateral plasma membrane of the S1 and S2 segments of the proximal tubule (Fig. 2-30). This is in agreement with the results of physiologic studies demonstrating that bicarbonate is reabsorbed primarily in the initial part of the proximal tubule. Humans with mutations in NBC1 develop permanent proximal renal tubular acidosis with associated ocular abnormalities consistent with NBC1 being the primary pathway for basolateral bicarbonate transport.
The proximal tubule is a major site of ammonia production in the kidney. Ammonia is produced in the mitochondria from the metabolism of glutamine. At the pH that exists in the proximal tubule cells, ammonia combines with H⁺ to form NH₄⁺, which is secreted into the tubule lumen.
Pars Recta

The pars recta of the proximal tubule consists of the terminal portion of the S2 segment and the entire S3 segment. The epithelium of the S3 segment is simpler than that of the S1 and S2 segments. Basolateral invaginations of the plasma membrane are virtually absent, mitochondria are small and randomly scattered throughout the cytoplasm, and the intercellular spaces are smaller and less complex (Fig. 2-31; see Fig. 2-20). These morphologic characteristics are in agreement with results of biochemical studies demonstrating that Na⁺,K⁺-ATPase activity is significantly lower in the pars recta than in the pars convoluta. In addition, studies examining transport parameters in individual segments of the proximal tubule have demonstrated that fluid reabsorption in the S3 segment is significantly less than in the S1 and S2 segments.

FIGURE 2-31 Low-magnification electron micrograph of a segment of the pars recta of a proximal tubule from a human kidney. The microvilli on the convex apical cell surface are not as long as those from the pars recta of the rat. The lysosomes are extremely electron-dense.
The clear, single-membrane-limited structures at the base of the cell to the right represent lipid droplets. (Magnification, ×10,400.) (Courtesy of R.E. Bulger, PhD.)

The morphologic appearance of the pars recta varies considerably among species. In the rat, the microvilli of the brush border measure up to 4 mm in length, whereas in the rabbit and human kidney they are much shorter. The vacuolar-lysosomal system is less prominent in the S3 segment of the proximal tubule. However, in both rabbit and human, many small lysosomes with electron-dense membrane-like material are common in the S3 segment. The specific role of lysosomes in this segment is not known.

Peroxisomes are common in the pars recta. In contrast to lysosomes, the peroxisomes are surrounded by a 6.5-nm-thick membrane and do not contain acid hydrolases. Peroxisomes are irregular in shape and vary considerably in appearance among species. In the rat, small, circular profiles can be observed just inside the limiting membrane, and rod-shaped structures often project outward from the organelle. In addition, a small nucleoid is often present in peroxisomes in the rat proximal tubule. In the proximal tubule of both rabbit and human, electron-dense structures called marginal plates are located at the periphery of the organelle (see Fig. 2-31). The functional significance of the peroxisomes in the kidney is not known with certainty; however, they are believed to be involved in lipid metabolism and to play a role in fatty acid oxidation. They have a high content of catalase, which is involved in the degradation of hydrogen peroxide, and of various oxidative enzymes, including L-α-hydroxy-acid oxidase and d-amino acid oxidase.

The pars recta of the proximal tubule is involved in the secretion of organic anions and cations and it is a portion of the nephron that is often damaged by nephrotoxic compounds, including various drugs and heavy metals. Woodhall and colleagues examined the secretion of p-aminohippuric acid, an organic anion, in individual S1, S2, and S3 segments of superficial and juxtamedullary proximal tubules of the rabbit and found that secretion was significantly higher in the S2 segment of both nephron populations. In similar studies of organic cation transport, McKinney demonstrated that the secretion of procainamide was greatest in S1 segments of superficial nephrons and in S1 and S2 segments of juxtamedullary nephrons.

Recent studies from several laboratories have identified a family of transporters involved in the uptake of organic anions and cations across the basolateral membrane into the cells of the proximal tubule. The organic ion transporters play an important role in the excretion of numerous commonly used drugs, including various antibiotics, nonsteroidal anti-inflammatory drugs, loop diuretics, and the immunosuppressive drug cyclosporine. The uptake of organic anions, including p-aminohippuric acid, from the blood into the proximal tubule cells is mediated by an organic anion transporter, OAT1, which has been cloned and found to be expressed in the proximal tubule by both in situ hybridization and immunohistochemistry. In the kidney, OAT1 is present only in the basolateral plasma membrane of the proximal tubule, and studies in the rat have demonstrated that OAT1 is expressed predominantly in the S2 segment of
the proximal tubule. Organic cation transporters, OCT1 and OCT2, have also been demonstrated in the proximal tubule of the rat. By in situ hybridization, expression of both OCT1 and OCT2 was detected in all three segments of the proximal tubule. By immunohistochemistry, OCT1 was observed mainly in S1 and S2 segments, whereas OCT2 was expressed in S2 and S3 segments.

The aquaglyceroporin AQP7, which is abundantly expressed in the testis has also been demonstrated in the proximal tubule brush border but exclusively in the part recta. AQP7 knockout mice fail to reabsorb glycerol and exhibit marked glyceroluria indicating a role for AQP7 in glycerol metabolism.

Thin Limbs of the Loop of Henle

The transition from the proximal tubule to the descending thin limb of the loop of Henle is abrupt (Figs. 2-32 and 2-33) and marks the boundary between the outer and inner stripes of the outer medulla. Short-looped nephrons originating from superficial and midcortical glomeruli have a short descending thin limb located in the inner stripe of the outer medulla. Close to the hairpin turn of the short loops of Henle, the thin limb continues into the thick ascending limb. Long-looped nephrons originating from juxtamedullary glomeruli have a long descending thin limb that extends into the inner medulla and a long ascending thin limb that continues into the thick ascending limb. The transition from the thin to the thick ascending limb forms the boundary between the outer and inner medulla (see Fig. 2-5). Nephrons arising in the extreme outer cortex may possess short cortical loops that do not extend into the medulla.
FIGURE 2-32 Transmission electron micrograph from rabbit kidney, illustrating the transition from the pars recta of the proximal tubule to the descending thin limb of the loop of Henle. (Magnification, ×4500.) (From Madsen KM, Park CH: Lysosome distribution and cathepsin B and L activity along the rabbit proximal tubule. Am J Physiol 253:F1290, 1987.)
FIGURE 2-33 Scanning electron micrograph from a normal rat kidney depicting the transition from the terminal S3 segment of the proximal tubule (above) to the early descending thin limb of Henle (below). Note the elongated cilia projecting into the lumen from cells of the proximal tubule and the thin limb of Henle. (Magnification, ×4500.)

Considerable effort has been expended toward identification of the histotopographic organization of the renal medulla in several laboratory animals. Most mammals, including rabbits, guinea pigs, prairie dogs, cats, dogs, pigs, and humans, have a simple medulla in which the vascular bundles contain only descending and ascending vasa recta. The thin limb segments of both short- and long-looped nephrons are located outside the vascular bundles. In contrast, most animals with a high urine concentrating ability, such as the rat, mouse, and desert sand rat (Psammomys
obesus), have a complex medulla in which the descending thin limbs of the short-looped nephrons are incorporated into the vascular bundles in the outer medulla together with the vasa recta. Descending thin limbs from long-looped nephrons descend through the outer medulla outside the vascular bundles, in the so-called interbundle regions. In most species studied thus far, the inner medulla appears to lack the discrete compartmentalization that is characteristic of the outer medulla. Recent 3-D reconstruction studies of the mouse nephron indicate that a highly complex structural relationship exists between the thin limb segments and the thick ascending limbs in the outer medulla of the mouse.

Early ultrastructural studies demonstrated that the cells of the initial part of the descending thin limb of Henle were complex because of extensive interdigitation with one another, whereas the cells of the ascending thin limb near the transition with the thick ascending limb, and thin limb cells in the inner medulla, were less complex in configuration. Dieterich and associates later described the presence of four types of epithelia (types I through IV) in the thin limbs of the mouse kidney and devised a classification based on the ultrastructural characteristics of the cells and their location within the different regions of the medulla. Subsequent studies in other species, including rat, rabbit, hamster, and P. obesus, confirmed the existence of four morphologically distinct segments in the thin limb of Henle (Fig. 2-34). In these animals, type I epithelium is found exclusively in the descending thin limb of short-looped nephrons. Type II epithelium forms the descending thin limb of long-looped nephrons in the outer medulla. This epithelium gives way to type III epithelium in the inner medulla. Type IV epithelium forms the bends of the long loops and the entire ascending thin limb to the transition into the thick ascending limb at the boundary between the inner and outer medulla.
In all animals studied thus far, type I epithelium is extremely thin and has few basal or luminal surface specializations, the latter in the form of microvilli (see Fig. 2-34). There is a virtual absence of lateral interdigitations with adjacent cells, and cellular organelles are relatively sparse. Microbodies have not been identified in the thin limbs of the loop of Henle. Tight junctions between cells are intermediate in depth with several junctional strands, which suggests a tight epithelium.

Type II epithelium is taller and exhibits considerable species differences. In the rat, mouse, P. obesus, and hamster, the type II epithelium is complex and characterized by extensive lateral and basal interdigitations and a well-developed paracellular pathway (Fig. 2-35).
The tight junctions are extremely shallow and contain a single junctional strand, which is characteristic of a leaky epithelium. The luminal surface is covered by short blunt microvilli, and cell organelles, including mitochondria, are more prominent than in other segments of the thin limb. In the rabbit, the type II epithelium is less complex. Lateral interdigitations and paracellular pathways are less prominent, and tight junctions are deeper. As in the rat and mouse, the luminal surface is covered with short microvilli, and many small mitochondria are present in the cytoplasm. In comparison with type II epithelium, type III epithelium is lower and has a simpler structure. The cells do not interdigitate, the tight junctions are intermediate in depth, and fewer microvilli cover the luminal surface. Type IV epithelium (see Fig. 2-34) is generally low and flattened and possesses relatively few organelles. It is characterized by an absence of surface microvilli but has an abundance of lateral cell processes and interdigitations as well as prominent paracellular pathways. The tight junctions are shallow and are characteristic of a leaky epithelium. The basement membrane of the thin limb segments varies greatly in thickness from species to species and in many animals is multilayered. A secreted phosphoprotein, osteopontin, is constitutively expressed in the type I and especially the type II epithelium of the descending thin limb in the inner stripe of the outer medulla. The function of osteopontin in the descending thin limb is unknown.

FIGURE 2-35  Transmission electron micrograph of type II epithelium of the thin limb of the loop of Henle in the inner stripe of the outer medulla of rat kidney. (Magnification, ×11,800.)

Freeze-fracture studies have confirmed the structural heterogeneity along the thin limb of the loop of Henle in the rat, rabbit, and P. obesus. Segmental as well as species differences were found in the number of strands and the depth of the tight junctions. The most striking finding in these studies was an extremely high density of intramembrane particles in both the luminal and the basolateral membrane of type II epithelium in all animals studied. Biochemical studies in isolated
nephron segments, as well as histochemical studies, demonstrated significant levels of Na\(^{+}\), K\(^{-}\)-ATPase activity in type II epithelium of the thin limb in the rat. Little or no activity was present in other segments of the rat thin limb or in any segment of the rabbit thin limb. The functional significance of these observations is not known. However, physiologic studies determining the permeability properties of isolated perfused segments of descending thin limbs from different species have demonstrated that the permeability of the type II epithelium to Na\(^{+}\) and K\(^{-}\) is higher in the rat and hamster than in the rabbit, supporting the described ultrastructural and biochemical differences among species in this epithelium.

Studies of salt and water permeability in descending thin limb segments from the hamster have demonstrated that both type I and type II epithelia are highly permeable to water, whereas the permeability to Na\(^{+}\) and Cl\(^{-}\) is significantly higher in type II than in type I epithelium. In contrast, urea permeability is higher in the type I epithelium. No evidence has been found for active transport of Na\(^{+}\) or Cl\(^{-}\) in the thin limb of the loop of Henle. In support of the reported permeability characteristics of the different thin limb segments, immunohistochemical studies have demonstrated high levels of expression of the water channel protein, AQP1, in the descending thin limb and especially in the type II epithelium of long-looped nephrons in the outer medulla (Fig. 2-36). There is no AQP1 immunoreactivity in the ascending thin limb and AQP1 is not expressed in the innermost part of the type I epithelium of short-looped nephrons. Detailed studies by Pannabecker and Dantzler have described the heterogeneity in the expression of AQP1 in descending thin limbs in the inner medulla. Those investigators reported that descending thin limbs that form loops more than 1 mm below the base of the inner medulla express AQP1 only in the initial portion of the segment, leaving almost 60% of the inner medullary segment of long-looped thin descending limbs with no detectable AQP1 expression. Moreover, AQP1 stainable descending thin limbs and collecting ducts were separated into two distinct lateral compartments, suggesting that water transport may occur between these compartments.

Interestingly, the innermost part of the thin descending limb of short-looped nephrons that lacks AQP1 does express high levels of the urea transporter, UT-A2, as demonstrated at both the mRNA and protein level (Fig. 2-37). There is also weak expression of UT-A2 mRNA and protein in the type III epithelium of the descending thin limb of long-looped nephrons in the outer portion of the inner medulla. The expression of UT-A2 in the descending thin limb is up-regulated by vasopressin, although specific receptors for vasopressin have not been detected in this segment. The urea transporter is believed to be involved in urea recycling in the renal medulla, a process that is important for the maintenance of medullary hypertonicity.
FIGURE 2-37  Light micrograph illustrating immunoperoxidase staining of the urea transporters UT-A2 in the descending thin limb of short-looped nephrons (closed arrows) and UT-A1 in the inner medullary collecting duct (arrowhead) in normal rat kidney. Open arrow indicates weak UT-A2 immunolabeling in descending thin limb segments of long-looped nephrons in the outer part of the inner medulla. (Magnification, ×20.)  (Courtesy of Young-Hee Kim, PhD.)

The thin limbs of the loop of Henle play an important role in the countercurrent multiplication process that is responsible for the maintenance of a hypertonic medullary interstitium and for the dilution and concentration of the urine. According to the passive model of the countercurrent
multiplier mechanism described by Kokko and Rector, the descending thin limb epithelium is permeable to water but has a low permeability to Na and Cl; this allows water to be extracted from the tubule fluid as the thin limb descends through the hypertonic interstitium of the medulla. In contrast, the ascending thin limb is largely impermeable to water but highly permeable to Na and Cl, which causes salt to diffuse out of the tubule. A kidney-specific chloride channel, ClC-K1, was cloned from rat kidney and was found to be expressed in the ascending thin limb. Immunohistochemical studies revealed that ClC-K1 is expressed exclusively in this segment, where it is located in both the apical and basolateral plasma membrane. Expression of ClC-K1 in the ascending thin limb has also been demonstrated in the human kidney. The presence of ClC-K1 in the ascending thin limb is in agreement with results of physiologic studies demonstrating that this segment is highly permeable to chloride. Mice lacking ClC-K1 became dehydrated after water deprivation and were not able to concentrate their urine in response to vasopressin, indicating that ClC-K1 is essential for the urinary concentrating mechanism.

The passive model for the countercurrent multiplier system is supported by both functional and immunohistochemical studies. However, for this model to work the permeability of the ascending thin limb to outward movement of Na and Cl must be considerably higher than the permeability to inward movement of urea from the interstitium. Evidence from studies in isolated thin limb segments from hamster and chinchilla suggests that the difference between the permeabilities of Na and urea in the ascending thin limb may not be sufficient to account for the osmolality gradient in the inner medulla. Various mathematical models (reviewed by Knepper and Rector) have also failed to explain the countercurrent multiplication process and the concentration of solutes in the inner medulla on the basis of purely passive mechanisms. However, it is generally accepted that the permeability properties of the thin limb epithelium are extremely important for the maintenance of a hypertonic interstitium and for the urine concentration mechanism.

The cells in the renal medulla are exposed to a hypertonic environment. To protect the cells against extreme changes in tonicity and allow them to maintain cell volume, intracellular organic osmolytes, including sorbitol, myoinositol, glycerophosphorylcholine, and betaine, are generated and accumulate in the cells in the renal medulla. Several genes are known to be regulated in response to hyperosmotic stimuli. Some of these osmoprotective genes include the sodium/myo-inositol cotransporter, aldose reductase (catalyzes the enzymatic conversion of glucose to sorbitol), and heat shock protein 70. The regulation of the osmoprotective genes appears to be mediated via the tonicity-responsive element (TonE)/TonE-binding protein (TonEBP) pathway, which is activated during hyperosmotic stimuli. Mice lacking the TonEBP gene suffer from increased mortality and exhibit an altered medullary morphology with atrophy of the renal medulla. Consistently, mice lacking the TonEBP gene have a reduced renal expression of osmoprotective genes.

Distal Tubule

The distal tubule is composed of three morphologically distinct segments: the thick ascending limb of the loop of Henle (pars recta), the macula densa, and the distal convoluted tubule (pars convoluta). Studies of rat kidney and rabbit kidney revealed that the cortical thick ascending
limb extends beyond the vicinity of the macula densa and forms an abrupt transition with the distal convoluted tubule. Therefore, the macula densa is a specialized region of the thick ascending limb.

Thick Ascending Limb

The thick ascending limb, or pars recta, represents the initial portion of the distal tubule and can be divided into a medullary and a cortical segment (see Fig. 2-5). In long-looped nephrons, there is an abrupt transition from the thin ascending limb to the thick ascending limb, which marks the boundary between the inner medulla and the inner stripe of the outer medulla. In short-looped nephrons, the transition to the thick ascending limb occurs shortly before the hairpin turn. From its transition with the thin limb, the thick ascending limb extends upward through the outer medulla and the cortex to the glomerulus of the nephron of origin, where the macula densa is formed. At the point of contact with the extraglomerular mesangial region, only the immediately contiguous portion of the wall of the tubule actually forms the macula densa. The transition to the distal convoluted tubule occurs shortly after the macula densa. The cells forming the medullary segment in the inner stripe of the outer medulla measure approximately 7 ũm to 8 ũm in height. As the tubule ascends toward the cortex, cell height gradually decreases to approximately 5 ũm in the cortical thick ascending limb of the rat and to 2 ũm in the terminal part of the cortical thick ascending limb of the rabbit. Welling and co-workers reported an average cell height of 4.5 ũm in the cortical thick ascending limb of the rabbit kidney.

The cells of the thick ascending limb are characterized by extensive invaginations of the basolateral plasma membrane and interdigitations between adjacent cells. The lateral invaginations often extend a distance of two thirds or more from the base to the luminal border of the cell. This arrangement is most prominent in the thick ascending limb of the inner stripe of the outer medulla (Fig. 2-38). Numerous elongated mitochondria are located in lateral cell processes, and their orientation is perpendicular to the basement membrane. The mitochondria resemble those in the proximal tubule but contain very prominent granules in the matrix. Other subcellular organelles in this segment of the nephron include a well-developed Golgi complex, multivesicular bodies and lysosomes, and abundant quantities of smooth- and rough-surfaced endoplasmic reticulum. Numerous small vesicles are commonly observed in the apical portion of the cytoplasm. The cells are attached to one another via tight junctions that are 0.1 ũm to 0.2 ũm in depth in the rat. Intermediate junctions are also present, but desmosomes appear to be lacking. In the rat, ultrastructural tracer studies using colloidal lanthanum have demonstrated that the tracer readily penetrates the tight junction, which suggests the presence of a potential paracellular shunt pathway for movement of solute and fluid.
Scanning electron microscopy of the thick ascending limb of the rat kidney has revealed the existence of two distinct surface configurations of the luminal membrane. Some cells have a rough surface because of the presence of numerous small microprojections, whereas others have a smooth surface that is largely devoid of microprojections except along the apical cell margins (Fig. 2-39). Most cells possess one cilium; some have two cilia. The rough-surfaced cells possess more extensive lateral processes radiating from the main cell body than do the smooth-surfaced cells. In contrast, small vesicles and tubulovesicular profiles are more numerous in the apical region of the smooth-surfaced cells. A predominance of cells with the smooth-surface pattern is observed in the medullary segment. As the thick limb ascends toward the cortex, the number of cells with a rough surface pattern increases, and luminal microprojections and apical lateral invaginations become more prominent. Consequently, the surface area of the luminal plasma membrane is significantly greater in the cortical than in the medullary thick ascending limb. The functional significance of the two surface configurations is not known; however, physiologic studies have provided evidence for the presence of two functionally distinct cell types in the medullary thick ascending limb of the hamster. One cell type has a low Cl− conductance in
the basolateral membrane, and the other has a high basolateral Cl conductance. Immunohistochemical studies of the localization of ROMK channels in the rat kidney have also demonstrated cellular heterogeneity along the thick ascending limb. Some cells exhibited strong ROMK immunoreactivity in the apical plasma membrane, whereas other cells showed little or no labeling for ROMK. Tamm-Horsfall protein is a glycoprotein that is produced and secreted by the thick ascending limb. It has been demonstrated along the luminal membrane of the thick ascending limb of both the rat and human kidney, and by use of the immunogold technique, labeling was found mainly over apical vesicles. The functional significance of Tamm-Horsfall protein in the distal tubule is not known.

FIGURE 2-39  Scanning electron micrograph illustrating the luminal surface of rat medullary thick ascending limb. The white asterisk denotes smooth-surfaced cells; the black asterisk identifies rough-surfaced cells. (Magnification, ×4300.) (From Madsen KM, Verlander JW, Tisher CC: Relationship between structure and function in distal tubule and collecting duct. J
The thick ascending limb is involved in active transport of NaCl from the lumen to the surrounding interstitium. Because this epithelium is almost impermeable to water, the reabsorption of salt contributes to the formation of a hypertonic medullary interstitium and the delivery of a dilute tubule fluid to the distal convoluted tubule. The reabsorption of NaCl in both the medullary and the cortical segments of the thick ascending limb is mediated by a Na\(^{+}\)-K\(^{+}\)-2Cl\(^{-}\) cotransport mechanism, which is inhibited by loop diuretics such as furosemide and bumetanide. The cDNA sequences for a kidney-specific bumetanide-sensitive Na\(^{+}\)-K\(^{+}\)-2Cl\(^{-}\) cotransporter (BSC-1 or NKCC2) have been cloned from rat, rabbit, and mouse. The expression of the transporter in the cortical and medullary thick ascending limb was subsequently confirmed by reverse transcription polymerase chain reaction on microdissected tubule segments as well as by in situ hybridization. Immunohistochemical studies using antibodies against a fusion protein or peptides based on the sequence of the cloned cDNAs demonstrated the presence of BSC-1 in the apical plasma membrane of the thick ascending limb in the rat.

Different splice variants of NKCC2 have been located in rat kidney. They are distributed spatially throughout the thick ascending limb. In situ hybridization revealed that the F isoform was most highly expressed in the inner stripe of the outer medulla, isoform A was mostly expressed in the outer stripe of the outer medulla and cortex, and isoform B was expressed mainly in the macula densa. An AF splice variant has also been detected in the thick ascending limb. Flux studies investigating three of the splice variants (A, B, and F) have demonstrated marked differences in their kinetics, especially in their affinity for Na\(^{+}\), K\(^{+}\), and Cl\(^{-}\). The function of the AF splice variant remains obscure and it does not appear to be functional when expressed in Xenopus oocytes. Moreover, it exerts a dominant-negative effect on the A or F splice variants by forming nonfunctional heterodimers when expressed together in Xenopus oocytes. In addition, the A, F, and AF variants are regulated differently in response to water loading and furosemide administration.

Three phosphorylation sites have been described in NKCC2 corresponding to Thr\(^{99}\), Thr\(^{104}\), and Thr\(^{117}\) in rabbit NKCC2. Gimenez and Forbush have provided evidence for the involvement of these phospho-sites in the regulation of NKCC2 cotransport activity in the oocyte expression system. They found that deletion of all three phosphorylation sites abolished the increase in NKCC2 transport normally observed during exposure to hypertonic media and that the phospho-sites worked in concert to increase transport during hypertonic stimulation. Moreover, the A, B, and F splice variants all experienced the same amount of activation when expressed in Xenopus oocytes and exposed to hypertonic media.

Chronic vasopressin administration strongly up-regulates the expression of the Na\(^{-}\)-K\(^{-}\)-2Cl\(^{-}\) cotransporter in the thick ascending limb. The energy for the reabsorptive process is provided by the Na\(^{-}\),K\(^{-}\)-ATPase that is located in the basolateral plasma membrane. Biochemical and histochemical studies have demonstrated that Na\(^{-}\),K\(^{-}\)-ATPase activity is greatest in that segment.
of the thick ascending limb that is located in the inner stripe of the outer medulla, which also has a larger basolateral membrane area and more mitochondria than does the remainder of the thick ascending limb. In agreement with these observations, physiologic studies using the isolated perfused tubule technique have demonstrated that NaCl transport is greater in the medullary segment than in the cortical segment of the thick ascending limb. However, the cortical segment can create a steeper concentration gradient and therefore can achieve a lower NaCl concentration and a lower osmolality in the tubule fluid. Thus, an excellent correlation exists between the structural and functional properties of the thick ascending limb.

Studies by Good and colleagues provided evidence that the thick ascending limb is involved in HCO₃⁻ reabsorption in the rat. The reabsorption of HCO₃⁻ is Na⁺ dependent and is inhibited by amiloride, which indicates that it is mediated by an Na⁺/H⁺ exchanger. The identity of the Na⁺/H⁺ exchanger isoform responsible for HCO₃⁻ reabsorption in the thick ascending limb was established by immunohistochemical studies demonstrating strong expression of NHE3 in the apical plasma membrane. Immunohistochemical studies also demonstrated labeling for carbonic anhydrase IV in the rat thick ascending limb, thus providing additional support for a role of this part of the nephron in acid-base transport in the rat. Activation of adenylate cyclase by various peptide hormones was shown to inhibit HCO₃⁻ reabsorption in the thick ascending limb. There is no evidence for HCO₃⁻ reabsorption or carbonic anhydrase activity in the thick ascending limb of the rabbit, and NHE3 is not expressed in this segment in the rabbit.

The mechanism of base exit across the basolateral plasma membrane is not known with certainty. However, studies in rat, mouse, and human kidneys have demonstrated that a Cl⁻/HCO₃⁻ exchanger, AE2, is expressed in the thick ascending limb, suggesting that this transporter may be, at least in part, responsible for HCO₃⁻ exit from the cells. Moreover, a recent immunohistochemical study revealed that an electroneutral sodium bicarbonate cotransporter, NBCn1, is also expressed in the basolateral membrane of the medullary thick ascending limb in the rat. NBCn1 was expressed only in the renal medulla, and there was no labeling of the cortical thick ascending limb.

In addition to its role in producing a dilute tubule fluid and a hypertonic interstitium and in the reabsorption of bicarbonate, the thick ascending limb is involved in the transport of divalent cations such as Ca²⁺ and Mg²⁺. The extracellular Ca²⁺/polyvalent cation-sensing receptor, which was originally cloned from bovine parathyroid gland and subsequently from rat kidney, is strongly expressed in the distal tubule, particularly in the cortical thick ascending limb. It modulates various cellular functions in response to changes in extracellular Ca²⁺ and other polyvalent cations and is believed to play a central role in mineral ion homeostasis.

The function of the thick ascending limb is regulated by a variety of hormones, including vasopressin, parathyroid hormone, and calcitonin, which exert their effects through activation of the adenylate cyclase system. However, significant differences exist between segments, as well as between species, in the response to these hormones. Vasopressin stimulates adenylate cyclase activity and NaCl reabsorption in the medullary thick ascending limb of both the rat and the mouse but has little or no effect on this segment in the rabbit. In contrast, vasopressin has less effect on adenylate cyclase activity in the cortical thick ascending limb and does not
stimulate NaCl transport in this segment. In mice, acute vasopressin administration increases the phosphorylation of the Na K-2Cl co-transporter (BSC-1/NKCC2) in the thick ascending limb in the inner stripe of the outer medulla, whereas the cortical thick ascending limb appears unresponsive. Recent data has provided direct evidence for shuttling of NKCC2 in response to cAMP administration. Addition of dibutyryl cAMP to rat medullary thick ascending limb suspensions increases surface expression of NKCC2, whereas preincubation of suspensions with tetanus toxin (which inactivates VAMP-2 and VAMP-3), blocks the effect of dibutyryl cAMP on NKCC2 surface expression and transport. Prolonged administration of vasopressin to Brattleboro rats with hereditary diabetes insipidus was found to cause hypertrophy of the cells of the medullary thick ascending limb. Furthermore, vasopressin stimulated Cl reabsorption in the isolated perfused medullary thick ascending limb from Brattleboro rats, providing further support for the role of vasopressin in the regulation of ion transport in this segment. Drug-induced hypothyroidism in rats is associated with a decrease in cell height and basolateral membrane area in the medullary thick ascending limb. Given that a decrease in vasopressin-stimulated adenylate cyclase activity has been reported in the medullary thick ascending limb in a similar model of hypothyroidism, it is conceivable that the reduction in cell size observed in this segment was caused by an impairment of vasopressin-stimulated ion transport.

Segmental differences in the response of the thick ascending limb to parathyroid hormone have also been demonstrated. Whereas parathyroid hormone stimulates adenylate cyclase activity in the cortical thick ascending limb of all species studied, it has no effect in the medullary segment. In agreement with these findings, physiologic studies demonstrated that parathyroid hormone stimulates the reabsorption of Ca\(^{2+}\) and Mg\(^{2+}\) in the cortical thick ascending limb but has no effect on ion transport in the medullary segment. In contrast, calcitonin and glucagon stimulate adenylate cyclase activity and ion transport in both the cortical and the medullary segments of the thick ascending limb.

**Distal Convoluted Tubule**

The distal convoluted tubule, or pars convoluta, measures approximately 1 mm in length. It begins at a variable distance beyond the macula densa and extends to the connecting tubule that connects the nephron with the collecting duct. The transition from the thick ascending limb is abrupt. The cells of the distal convoluted tubule resemble those of the thick ascending limb but are considerably taller. By light microscopy, the cells appear tall and cuboid, and they contain numerous mitochondria. The cell nuclei occupy a middle to apical position. The distal convoluted tubule lacks the well-developed brush border and the extensive endocytic apparatus that are characteristic of the pars convoluta of the proximal tubule.
Scanning electron microscopy has demonstrated that the luminal surface of the distal convoluted tubule differs substantially from that of the thick ascending limb (Fig. 2-41; compare with Fig. 2-39). The distal convoluted tubule is covered with numerous small microprojections, and the lateral cell margins are straight, without the apical interdigitations that are characteristic of the thick ascending limb. The individual cells possess one centrally placed cilium, and occasionally two cilia are observed. The epithelium of the distal convoluted tubule is characterized by extensive
invaginations of the basolateral plasma membrane and by interdigitations between adjacent cells similar to the arrangement in the thick ascending limb. Transmission electron microscopy reveals numerous elongated mitochondria that are located in lateral cell processes and are closely aligned with the plasma membrane. They are oriented perpendicular to the basement membrane and often extend from the basal to the apical cell surface (Fig. 2-42). The junctional complex in this segment of the nephron is composed of a tight junction, or zonula occludens, which is approximately 0.3 mm in depth, and an intermediate junction, or zonula adherens. Tracer studies have demonstrated that the tight junction is freely permeable to both colloidal and ionic lanthanum. These observations, together with the demonstration of a relatively low transepithelial electrical resistance, suggest the existence of a potential paracellular shunt pathway for solute and water movement in this segment of the nephron.

FIGURE 2-41 Scanning electron micrograph illustrating the appearance of the luminal surface of a distal convoluted tubule from rat kidney. Microvilli are prominent, but there is a
marked absence of lateral interdigitations in the apical region of the cells. The cell boundaries are accentuated by taller microvilli. (Magnification, ×3000.) (Modified from Madsen KM, Verlander JW, Tisher CC: Relationship between structure and function in distal tubule and collecting duct. J Electron Microsc Tech 9:187, 1988.)

FIGURE 2-42  Transmission electron micrograph illustrating a typical portion of the pars convoluta segment of the distal tubule of a rat. The ultrastructural features closely resemble those of the pars recta of the distal tubule (see Fig. 2-38). (Magnification, ×10,000.)

Lysosomes and multivesicular bodies are common in the cells of the distal convoluted tubule, but microbodies are absent. The Golgi complex is well developed, and its location is lateral to the cell nucleus. The cells contain numerous microtubules and abundant quantities of rough- and smooth-surfaced endoplasmic reticulum and free ribosomes. The basement membrane is complex, often multilayered, and frequently irregular in configuration. Numerous small vesicles are located in the apical region of the cells. Electron microscopic studies examining the uptake of electron-dense tracer from the luminal side showed no uptake of anionic ferritin in the distal convoluted tubule. However, cationic ferritin was absorbed into small apical vesicles and multivesicular bodies, suggesting that these vesicles may be involved in some form of transport or in membrane recycling in the distal convoluted tubule.  

Investigators working with micropuncture techniques arbitrarily defined the distal tubule as that region of the nephron that begins just after the macula densa and extends to the first junction with another renal tubule. With that definition, however, the distal tubule can be formed by as many as
four different types of epithelia. In general, the “early” distal tubule corresponds largely to the distal convoluted tubule and the short segment of the thick ascending limb that extends beyond the macula densa, whereas the “late” distal tubule actually represents the connecting tubule and the first portion of the collecting duct, which is usually referred to as the initial collecting tubule (Fig. 2-43). (A more detailed discussion of the anatomy of this region of the renal tubule can be found in the next section, which describes the connecting segment or connecting tubule.)

**FIGURE 2-43** Light micrograph of initial collecting tubules (asterisks) of a cortical collecting duct in a rat kidney. One tubule is situated just beneath the surface of the capsule (top of picture) and hence is easily accessible to micropuncture. This segment of the cortical collecting duct corresponds to the so-called late distal tubule as defined at the micropuncture table. (Magnification, ×360.)

Micropuncture studies in the rat have demonstrated net NaCl reabsorption and K⁺ secretion in the distal tubule. Because the microscopic anatomy of the segments under study was not carefully defined at the time, it was not possible to state with certainty whether these functional properties were limited to the early distal tubule or whether they occurred along the entire “distal tubule.” Stanton and Giebisch, using in vivo microperfusion, succeeded in perfusing short segments of the rat distal tubule. They demonstrated that Na⁺ is reabsorbed in both early and late segments,
whereas $K^+$ is secreted only in the late segment of the distal tubule, corresponding to the connecting segment and the initial collecting tubule. These findings agree with the results of a combined structural-functional study from the same laboratory, which demonstrated significant morphologic changes in the connecting segment and initial collecting tubule of the rat during $K^+$ loading but no changes in the distal convoluted tubule. Ultrastructural changes were also seen in the connecting tubule and cortical collecting duct of the rabbit after ingestion of a high-potassium, low-sodium diet, whereas no changes occurred in the distal convoluted tubule. After ingestion of a high-sodium, low-potassium diet, an increase occurred in cell height and in basolateral membrane area in the rabbit distal convoluted tubule.

The distal convoluted tubule has a higher $Na^+,K^+-ATPase$ activity than any other segment of the nephron. As in the thick ascending limb, $Na^+,K^+-ATPase$ is located in the basolateral membrane and provides the driving force for ion transport in this segment. Reabsorption of sodium and chloride is a main function of the distal convoluted tubule. It is mediated by a thiazide-sensitive $Na^+/Cl^-$ cotransporter, TSC or NCC, that is distinct from the $Na^+ K^+ 2Cl^-$ co-transporter, BSC-1, present in the thick ascending limb. The TSC mRNA and protein are expressed exclusively in the distal convoluted tubule, as revealed by in situ hybridization and immunohistochemistry. By immunoelectron microscopy, TSC was localized to the apical plasma membrane and apical cytoplasmic vesicles.

The expression of the thiazide-sensitive cotransporter in the rat distal convoluted tubule is up-regulated by the mineralocorticoid, aldosterone. In agreement with this observation, immunohistochemical and in situ hybridization studies demonstrated that the mineralocorticoid receptor as well as the enzyme that confers mineralocorticoid specificity, 11-b-hydroxysteroid dehydrogenase 2, are expressed in the distal convoluted tubule of the rat. Studies in the rat have distinguished between the early segment, DCT1, and the late segment, DCT2, of the distal convoluted tubule. The thiazide-sensitive cotransporter was expressed throughout the distal convoluted tubule, whereas a $Na^+/Ca^{2+}$ exchanger and a calcium-binding protein, calbindin—proteins normally expressed only in the connecting segment—were found in DCT2 but not in DCT1. A similar distinction between DCT1 and DCT2 could not be established in the mouse kidney, where both the $Na^+/Ca^{2+}$ exchanger and calbindin were expressed in most of the distal convoluted tubule. The sodium transport-related proteins in the distal tubule have been reviewed in detail by Bachmann and colleagues.

Detailed studies by Kaissling and colleagues demonstrated that the ultrastructure and the functional capacity of the distal convoluted tubule and connecting tubule are highly dependent on the delivery and uptake of sodium. Animals treated with a loop diuretic, furosemide, and given sodium chloride in their drinking water exhibited a striking increase in epithelial cell volume and in basolateral membrane area in the distal convoluted tubule and connecting tubule, and in vivo microperfusion studies demonstrated increased rates of sodium reabsorption in these nephron segments. The observed structural and functional changes were independent of changes in extracellular fluid volume and levels of aldosterone and vasopressin.

Gitelman syndrome, an autosomal recessive renal tubulopathy caused by loss-of-function mutations in NCC/TSC, is characterized by mild renal sodium wasting, hypocalciuria,
hypomagnesemia, and hypokalemic alkalosis. Studies in NCC-deficient mice demonstrated that these animals have significantly elevated plasma aldosterone levels and exhibit hypocalciuria, hypomagnesemia, and compensated alkalosis. Immunofluorescent detection of distal tubule marker proteins and ultrastructural analysis revealed that the early DCT, which physiologically lacks epithelial Na\(^+\) (ENaC) and Ca\(^{2+}\) (TRPV5) channels, was virtually absent in NCC-deficient mice. In contrast, the late DCT appeared intact with normal expression of ENaC, TRPV5, and the Na\(^-\)Ca\(^{2+}\) exchanger. The connecting tubule exhibited a marked epithelial hypertrophy accompanied by an increased apical abundance of ENaC. Reduced glomerular filtration and enhanced fractional reabsorption of Na\(^+\) and Ca\(^{2+}\) upstream and of Na\(^+\) downstream of the DCT provided some compensation for the Na\(^+\) transport defect in the DCT. Thus, loss of NCC leads to major structural remodeling of the renal distal tubule that goes along with marked changes in glomerular and tubular function, which may explain some of the clinical features of Gitelman syndrome. A modest reduction of dietary potassium induced a marked reduction in plasma potassium and elevated renal potassium excretion in NCC null mice, which was associated with a pronounced polydipsia and polyuria of central origin.

The distal convoluted tubule is also involved in the reabsorption of Ca\(^{2+}\) and has a higher Ca\(^{2+}\), Mg\(^{2+}\)-ATPase activity than any other segment of the nephron. Immunohistochemical studies using antibodies to the erythrocyte Ca\(^{2+}\), Mg\(^{2+}\)-ATPase demonstrated labeling of the basolateral plasma membrane of the distal convoluted tubule cells, and immunoreactivity for a vitamin D-dependent Ca\(^{2+}\)-binding protein has also been observed in this segment.

Because of the heterogeneity of the distal tubule, there has been some confusion regarding the functional properties of this segment of the nephron. Early micropuncture studies in the rat demonstrated that a hypotonic tubule fluid was delivered to the early distal tubule. In the presence of vasopressin, the tubule fluid approached isotonicity somewhere beyond the midportion of the distal tubule. From these data, investigators concluded that vasopressin-induced osmotic water flow occurs along the entire length of the distal tubule that is accessible to micropuncture. In a combined morphologic-physiologic study performed in rats with hereditary hypothalamic diabetes insipidus, Woodhall and Tisher could not find morphologic evidence, in the form of cell swelling and intercellular space dilatation, of transepithelial water flow in the early distal tubule (distal convoluted tubule). Vasopressin-induced osmotic water flow was evident, however, in the late distal tubule (connecting segment and initial collecting tubule). Subsequently, studies using the isolated perfused tubule technique demonstrated that osmotic water permeability in the distal convoluted tubule of the rabbit was low and unaffected by exposure of the tubule to exogenous vasopressin. These data agree with the demonstration that adenylate cyclase activity in the distal convoluted tubule is not increased after vasopressin stimulation. The combined data suggest that the distal convoluted tubule, like the thick ascending limb, is relatively impermeable to water.

**Connecting Tubule**

The connecting tubule (or connecting segment) represents a transitional region between the distal nephron and the collecting duct, and it constitutes the main portion of the late distal tubule as defined in the micropuncture literature. The connecting tubules of superficial nephrons continue
directly into initial collecting tubules, whereas connecting tubules from midcortical and juxtamedullary nephrons join to form arcades that ascend in the cortex and continue into initial collecting tubules (Fig. 2-44; see Fig. 2-43). In the rabbit, the connecting tubule is a well-defined segment composed of two cell types: the connecting tubule cell and the intercalated cell. In most other species, however, including rat, mouse, and human, there is a gradual transition from the distal convoluted tubule to the cortical collecting duct, and the connecting tubule is not clearly demarcated because of intermingling of cells from neighboring segments.

FIGURE 2-44 Diagram of the various anatomic arrangements of the distal tubule and cortical collecting duct in superficial and juxtamedullary nephrons. (See text for detailed explanation.) ATL, ascending thick limb (of Henle); CS, connecting segment; DCT, distal convoluted tubule; G, glomerulus; ICT, initial collecting tubule; MD, macula densa; MRCT, medullary ray collecting tubule.

The connecting tubule in the rat measures 150 mm to 200 mm in length. It is composed of four different cell types: connecting tubule cells, intercalated cells, distal convoluted tubule cells, and principal cells, which are similar to principal cells in the cortical collecting duct. The connecting
tubule cell is characteristic of this segment. It is intermediate in ultrastructure between the distal convoluted tubule cell and the principal cell and exhibits a mixture of lateral invaginations and basal infoldings of the plasma membrane. Connecting tubule cells are taller than principal cells and have an apically located nucleus. Mitochondria are fewer and more randomly distributed than in the distal tubule. A main function of the connecting tubule cells is potassium secretion (see later discussion).

At least two configurations of intercalated cells are present in the connecting tubule. In the rabbit, a “black” form and a “gray” form have been described. Investigators found both configurations to be rich in mitochondria, but the flat and spherical vesicles characteristic of intercalated cells were much more common in the gray cell. In the rat, variations were also reported in the density of the cytoplasm of intercalated cells in the connecting tubule. Two configurations of intercalated cells, type A and type B, were described in both the connecting tubule and the cortical collecting duct of the rat. In the connecting tubule, type A cells were more numerous than type B cells, and they resembled the gray cells described in the rabbit. More recently, a third type of intercalated cell was identified in the connecting tubule of both rat and mouse. This cell has been referred to as the nonA-nonB type of intercalated cell. In the mouse, this is the most prevalent form of intercalated cell in the connecting tubule. The function of the intercalated cells in the connecting tubule is not known with certainty. However, ultrastructural changes have been demonstrated in the type A intercalated cells in both the connecting tubule and the cortical collecting duct of rats with acute respiratory acidosis, indicating that these cells may be involved in acid secretion, as are the intercalated cells in the outer medullary collecting duct. The functional properties and immunohistochemical features of the intercalated cells have been studied extensively and are described later.

Both morphologic and physiologic studies have provided evidence that the connecting tubule plays an important role in K+ secretion, which is at least in part regulated by mineralocorticoids. Free-flow micropuncture studies in rats demonstrated high levels of K+ secretion in the superficial distal tubule. In a combined structural-functional study, Stanton and co-workers demonstrated that chronic K+ loading, which stimulates aldosterone secretion, caused an increase in K+ secretion by the late distal tubule and a simultaneous increase in the surface area of the basolateral plasma membrane of connecting tubule cells and principal cells of both the connecting tubule and the initial collecting tubule, which indicates that these cells are responsible for K+ secretion. No changes were observed, however, in the cells of the distal convoluted tubule. Studies in the rabbit revealed a similar increase in the basolateral membrane area of the connecting tubule cells after ingestion of a high-potassium, low-sodium diet. Studies in adrenalectomized rats demonstrated a decrease in K+ secretion in the superficial distal tubule as well as a decrease in the surface area of the basolateral membrane of the principal cells in the initial collecting tubule. Both structural and functional changes could be prevented by aldosterone treatment, which indicates that K+ secretion in the connecting tubule and initial collecting tubule is regulated by mineralocorticoids.

Immunocytochemical studies have demonstrated staining for kallikrein in the connecting tubule cells in the vicinity of the juxtaglomerular apparatus at the vascular pole of the glomerulus. Immunostaining was observed in the endoplasmic reticulum, the Golgi apparatus, the cytoplasmic
vesicles, and the plasma membrane, suggesting that kallikrein may be both produced and secreted by the connecting tubule cells.

The connecting tubule is an important site of calcium reabsorption in the kidney. Immunohistochemical studies have demonstrated the presence of an Na\(^+\)/Ca\(^{2+}\) exchanger, as well as a Ca\(^{2+}\)-ATPase, in the basolateral plasma membrane of the connecting tubule cells. Strong expression of a vitamin D-dependent calcium-binding protein, calbindin D28K, has also been demonstrated in the connecting tubule cells. The exact role of calbindin in these cells is not known. Interestingly, treatment with cyclosporine is associated with a striking decrease in calbindin expression in the connecting tubule and with increased excretion of calcium in the urine.

Immunohistochemical studies in rats revealed that a subpopulation of cells in the late part of the distal convoluted tubule, at the transition to the connecting tubule, expresses both the thiazide-sensitive cotransporter, TSC, and the Na\(^+\)/Ca\(^{2+}\) exchanger, which are traditionally considered specific for distal convoluted tubule cells and connecting tubule cells, respectively. In the rat kidney, the vasopressin V\(_2\) receptor and the vasopressin-regulated water channel, AQP2, are also expressed in the connecting tubule, and AQP2 immunoreactivity has been demonstrated in connecting tubule cells in both rats and mice. In the rabbit the connecting tubule constitutes a distinct segment with respect to both structure and function, and there is no co-expression of TSC and the Na\(^+\)/Ca\(^{2+}\) exchanger in any cells in the distal convoluted tubule or connecting tubule. Furthermore, vasopressin has no effect on either adenylate cyclase activity or water permeability in the connecting tubule of the rabbit. Biochemical studies on isolated nephron segments have demonstrated that parathyroid hormone and isoproterenol stimulate adenylate cyclase activity in this segment.

Subunits of the amiloride-sensitive sodium channel, ENaC, which are responsible for sodium absorption in the collecting duct, are also highly expressed in the connecting tubule as well as in DCT2. Studies in collecting duct-selective gene knockout mice generated by exploiting the CRE/LoxP approach revealed that selective deletion of alpha ENaC in the collecting duct system with retained expression in the DCT2 and connecting tubule failed to produce major phenotypic changes. In contrast, global gene knockout of the alpha subunit was postnatally lethal. Thus, the DCT2 and connecting tubule represent the major aldosterone-sensitive pathway for sodium reabsorption. In contrast to these observations, collecting duct specific AQP2 gene-knock out animals developed using the same CRE/LoxP approach exhibited a dramatic phenotype with severe polyuria. Mice with global AQP2 gene knockout died postnatally within 2 weeks with severe hydronephrosis. Thus, in contrast to ENaC (with regard to sodium reabsorption), expression of AQP2 in the entire connecting tubule and collecting duct is essential for water balance regulation.

Recent studies have reported that in experimental forms of diabetes insipidus (either in vasopressin-deficient Brattleboro rats or in rats with lithium-induced nephrogenic DI), exogenous aldosterone markedly reduced remnant apical AQP2 with increased basolateral AQP2 targeting. This was paralleled by a marked further increase in urine production. Conversely, increased apical
AQP2 and reduced urine output were seen in response to treatment with spironolactone, a mineralocorticoid receptor antagonist.  

COLLECTING DUCT

The collecting duct extends from the connecting tubule in the cortex through the outer and inner medulla to the tip of the papilla. It can be divided into at least three regions, based primarily on their location in the kidney. These include the cortical collecting duct, the outer medullary collecting duct, and the inner medullary collecting duct. The inner medullary segments terminate as the papillary collecting ducts, or ducts of Bellini, which open on the surface of the papilla to form the area cribrosa (see Fig. 2-3). Traditionally, two types of cells have been described in the mammalian collecting duct: principal or light cells, and intercalated or dark cells.

Principal cells are the major cell type; they were originally believed to be present in the entire collecting duct, whereas intercalated cells disappear in the inner medulla. However, there is both structural and functional evidence that the cells in the terminal portion of the inner medullary collecting duct constitute a distinct cell population. Furthermore, at least two, and in certain species three, configurations of intercalated cells have been described in the cortical collecting duct. Therefore, significant structural axial heterogeneity exists along the collecting duct.

Cortical Collecting Duct

The cortical collecting duct can be further subdivided into two parts: the initial collecting tubule and the medullary ray portion (see Fig. 2-5). The cells of the initial collecting tubule are taller than those of the medullary ray segment, but otherwise no major morphologic differences exist between the two subsegments. The cortical collecting duct is composed of principal cells and intercalated cells, the latter constituting approximately one third of the cells in this segment in the rat, the mouse, and the rabbit. Principal cells have a light-staining cytoplasm and relatively few cell organelles (Fig. 2-45). They are characterized by numerous infoldings of the basal plasma membrane that are restricted to the basal region of the cell below the nucleus. The infoldings do not enclose mitochondria or other cell organelles, which causes the basal region to appear as a light rim by light microscopy. Lateral cell processes and interdigitations are virtually absent. Mitochondria are small and scattered randomly in the cytoplasm. A few lysosomes, autophagic vacuoles, and multivesicular bodies are also present, as are rough- and smooth-surfaced endoplasmic reticulum and free ribosomes. Scanning electron microscopy of the luminal surface of the principal cells reveals a relatively smooth membrane covered with short, stubby microvilli and a single cilium (Fig. 2-46).
FIGURE 2-45  Transmission electron micrograph of a principal cell from the cortical collecting duct of a normal rat kidney. Note the extensive infoldings of the basal plasma membrane. (Magnification, ×11,000.)  (From Madsen KM, Tisher CC: Structural-functional relationship along the distal nephron. Am J Physiol 250:F1, 1986.)
Intercalated cells in the cortical collecting duct have a dense-staining cytoplasm and therefore have been called dark cells (Fig. 2-47). They are characterized by the presence of various tubulovesicular membrane structures in the cytoplasm and prominent microprojections on the luminal surface.
luminal surface. In addition, numerous mitochondria and polyribosomes are located throughout the cells, which also contain a well-developed Golgi apparatus. Previous studies have described two distinct populations of intercalated cells, type A and type B, in the cortical collecting duct of the rat, each constituting approximately 50% of the intercalated cells in this segment (see Fig. 2-47). Type A intercalated cells are similar in ultrastructure to intercalated cells in the outer medullary collecting duct. They have a prominent tubulovesicular membrane compartment that includes both spherical and invaginated vesicles and flat saccules or cisternae that appear as tubular profiles on section (Fig. 2-48). The cytoplasmic face of these membrane structures is coated with characteristic club-shaped particles or studs, similar to the coat that lines the cytoplasmic face of the apical plasma membrane. The ultrastructural appearance of the apical region of type A intercalated cells can vary considerably, depending on the physiologic state. Some cells have numerous tubulovesicular structures and few microprojections on the luminal surface, whereas other cells have extensive microprojections on the surface but only a few tubulovesicular structures in the apical cytoplasm.

FIGURE 2-47 Transmission electron micrograph from rat cortical collecting duct illustrating type A (right) and type B (left) intercalated cells. Note differences in density of cytoplasm and in apical microprojections. (Magnification, ×5300.) (From Madsen KM, Verlander JW, Tisher CC: Relationship between structure and function in distal tubule and collecting duct. J Electron Microsc Tech 9:187, 1988.)
FIGURE 2-48  Higher-magnification transmission electron micrograph illustrating the apical region of an intercalated cell from rat kidney. Note especially the large number of tubulocisternal profiles (solid arrows), invaginated vesicles (open arrows), and small coated vesicles with the appearance of clathrin vesicles (arrowheads). (Magnification, ×38,000.)

The type B intercalated cell has a denser cytoplasm and more mitochondria than the type A cell, which gives it a darker appearance (see Fig. 2-47). Numerous vesicles are present throughout the cytoplasm, but tubular profiles and studded membrane structures are rare in the cytoplasm of type B cells. The apical membrane exhibits small, blunt microprojections, and often a band of dense cytoplasm without organelles is present just beneath the apical membrane. Morphometric analysis in the rat has demonstrated that type B intercalated cells have a smaller apical membrane area but a larger basolateral membrane area when compared with type A cells. By scanning electron microscopy, two different surface configurations have been described in the rat. Type A cells have a large luminal surface covered with microvilli or a mixture of microvilli and microvilli; type B cells have a smaller, angular surface with a few microprojections, mostly in the form of small microvilli (see Fig. 2-46).

Both type A and type B intercalated cells are present in the cortical collecting duct of the mouse. However, the type B cells are less common than in the rat. More recent studies have identified and characterized a third type of intercalated cell in both rat and mouse. This so-called nonA-nonB type of intercalated cell constitutes approximately 40% to 50% percent of the intercalated cells in the connecting tubule and initial collecting duct of the mouse but is fairly rare in the rat.
Kaissling and Kriz described both light and dark manifestations of intercalated cells in the collecting duct of the rabbit. The light form was most commonly observed in the outer medulla, whereas the dark form was observed mainly in the cortex. Flat and invaginated vesicles were present in both cell configurations. The two manifestations of intercalated cells in the rabbit possibly correspond to type A and type B intercalated cells in the rat. Scanning electron microscopy has also revealed different surface configurations of intercalated cells in the collecting duct of the rabbit. Cells with either microplicae or microvilli, or both, have been described, but their relationship to the two cell types has not been investigated. Cells with microvilli are prevalent in the cortex, however. Most intercalated cells in the cortical collecting duct of the rabbit bind peanut lectin on the luminal surface and are believed to correspond to the type B intercalated cells. In the rat, the binding of various lectins to intercalated cells varies between the cortical collecting duct and outer medullary collecting duct, and peanut lectin labels all cells in the collecting duct of the rat. Histochemical and immunocytochemical studies have demonstrated high levels of carbonic anhydrase in intercalated cells, which suggests that these cells are involved in tubule fluid acidification in the collecting duct. The cortical collecting duct is capable of both reabsorption and secretion of HCO₃⁻. Studies using the isolated perfused tubule technique have demonstrated that cortical collecting tubules from acid-loaded rats and rabbits reabsorb HCO₃⁻ (i.e., secrete H⁺), whereas tubules from HCO₃⁻-loaded or deoxycorticosterone-treated rats and rabbits secrete HCO₃⁻.

Both morphologic and immunocytochemical studies have provided evidence that the type A intercalated cells are involved in H⁺ secretion in the cortical collecting duct of the rat. In a study of the effect of acute respiratory acidosis on the cortical collecting duct of the rat, Verlander and colleagues demonstrated a significant increase in the surface area of the apical plasma membrane of type A intercalated cells. No ultrastructural changes were observed in type B intercalated cells. Similar ultrastructural findings were reported in intercalated cells in the outer cortex of rats with acute metabolic acidosis; however, no distinction was made between type A and type B cells. Immunocytochemical studies using antibodies to the vacuolar H⁺-ATPase and the erythrocyte anion exchanger, band 3 protein, (now known as AE1) have confirmed the presence of two types of intercalated cells in the cortical collecting duct of both mouse and rat. Type A intercalated cells have an apical H⁺-ATPase and a basolateral band 3-like Cl⁻/HCO₃⁻ exchanger, AE1, which indicates that they are involved in H⁺ secretion. In contrast, type B intercalated cells have the H⁺-ATPase in the basolateral plasma membrane and in cytoplasmic vesicles throughout the cell (see Fig. 2-49A), and they do not express AE1.
FIGURE 2-49 Light micrograph illustrating immunostaining for (A) the vacuolar H\(^+\)-ATPase and the anion exchanger, AE1, and (B) pendrin and AE1 in serial sections of the mouse cortical collecting duct with use of a horseradish peroxidase technique. In A, type A intercalated cells (arrows) have strong apical labeling for H\(^+\)-ATPase and basolateral labeling for AE1, whereas type B intercalated cells (arrowheads) have basolateral and diffuse labeling for H\(^+\)-ATPase and no AE1. In contrast, type B intercalated cells have apical labeling for pendrin (B). PT, proximal tubule. (Differential interference microscopy; magnification, ×800.) (Courtesy of Jin Kim, MD, Catholic University, Seoul, Korea.)

There is convincing evidence that type B cells secrete HCO\(_3^−\) by an apical Cl\(^-\)/HCO\(_3^−\) exchanger that is distinct from AE1, the anion exchanger in the type A cells. Recent studies have demonstrated that a novel anion exchanger, pendrin, is expressed in a subpopulation of cells in the
Pendrin is structurally unrelated to AE1, and mutations in the gene encoding pendrin cause Pendred syndrome, a genetic disorder associated with deafness and goiter. Immunohistochemical studies revealed that pendrin expression is restricted to the apical region of AE1-negative intercalated cells (see Fig. 2-49), suggesting that pendrin might represent the long sought-after apical Cl/HCO₃ exchanger of type B intercalated cells. Immunogold electron microscopy confirmed that pendrin is located in the apical plasma membrane and apical cytoplasmic vesicles of type B (Fig. 2-50) as well as in nonA-nonB intercalated cells in the connecting tubule and cortical collecting duct of both mouse and rat. Elegant microperfusion studies in isolated cortical collecting duct segments from alkali-loaded mice deficient in pendrin demonstrated a failure to secrete HCO₃, compared with tubules from wild-type mice, indicating that pendrin is important for HCO₃ secretion in the cortical collecting duct. This conclusion is also supported by studies in the rat demonstrating that the expression of pendrin in the renal cortex is significantly increased in chronic metabolic alkalosis and decreased in chronic metabolic acidosis. In addition, ultrastructural studies have demonstrated changes in type B intercalated cells during experimental conditions designed to stimulate HCO₃ secretion in the collecting duct. Recent studies have focused on the possible role of pendrin in hypertension. Deoxycorticosterone induces hypertension and metabolic alkalosis in mice. However, pendrin-deficient mice appear resistant to deoxycorticosterone-induced hypertension and more sensitive to deoxycorticosterone-induced metabolic alkalosis. In addition, Wall and co-workers found that during NaCl restriction, Cl excretion and urinary volume were increased in pendrin-deficient mice compared with wild-type animals, suggesting that pendrin may play a role in renal Cl conservation. Recent studies examining pendrin regulation in response to changes in chloride balance have demonstrated an inverse relationship between pendrin expression and the level of chloride loading and provided evidence that distal chloride delivery may be the chief regulator of pendrin expression. Taken together, these findings suggest a prominent role for pendrin in chloride transport in the connecting tubule and cortical collecting duct.
FIGURE 2-50  Transmission electron micrograph illustrating immunogold localization of pendrin in a type B intercalated cell from rat kidney. Labeling of pendrin is seen in the apical plasma membrane (arrows) and in small vesicles (arrowheads) in the apical cytoplasm. M, mitochondrion; N, nucleus. (Magnification, ×46,000.)  (Courtesy of Tae-Hwan Kwon, MD, University of Aarhus, Denmark.)

Recently researchers cloned the cDNA of a member of the AE family of anion exchangers, AE4, from rabbit collecting duct cells and demonstrated that the AE4 protein is expressed in the apical membrane of type B intercalated cells in the rabbit kidney. Whether AE4 is expressed in intercalated cells in rodents remains to be established.

In the rabbit cortical collecting duct, about 70% of the intercalated cells bind peanut lectin, which has been considered a marker of HCO₃⁻-secreting cells in the rabbit, and originally it was reported that the remainder of the intercalated cells were labeled with antibodies to band 3 protein. However, subsequent studies reported immunostaining for band 3 protein in about 40% of the intercalated cells, including some of the peanut lectin-positive cells, indicating that binding of peanut lectin may not be specific for type B intercalated cells. Finally, studies by Verlander and colleagues revealed that in the rabbit approximately 50% of the intercalated cells in the connecting tubule and 30% to 40% of the intercalated cells in the cortical collecting duct were band 3 positive. These investigators also demonstrated staining for peanut lectin in band 3–positive
intercalated cells in the outer medulla of acidotic rabbits, thus raising further doubt about the specificity of peanut lectin as a marker of type B intercalated cells.

Interestingly, in the cortical collecting duct of the rabbit, band 3 immunoreactivity is located mainly in intracellular vesicles and multivesicular bodies in a subpopulation of intercalated cells, and there is little labeling of the basolateral plasma membrane. Moreover, immunocytochemical studies have demonstrated that H^+\text{-ATPase} is located in intracellular vesicles in most intercalated cells in the rabbit cortical collecting duct, and only a minority of intercalated cells have H^+\text{-ATPase} immunoreactivity in the apical plasma membrane characteristic of type A intercalated cells. These observations suggest that, under normal conditions, most type A intercalated cells in the rabbit cortical collecting duct are not functionally active. However, after chronic ammonium chloride loading there is increased band 3 immunolabeling in the basolateral plasma membrane and increased labeling for H^+\text{-ATPase} in the apical plasma membrane of intercalated cells in the cortical collecting duct of the rabbit.

A subpopulation of intercalated cells in the rabbit cortical collecting duct exhibits H^+\text{-ATPase} immunolabeling in the basolateral plasma membrane, which is characteristic of type B intercalated cells. Physiologic studies using pH-sensitive dyes to monitor changes in intracellular pH have provided evidence for HCO_3^- secretion by an apical Cl^-/HCO_3^- exchange mechanism in peanut lectin-positive intercalated cells in the rabbit cortical collecting duct. Because these cells are more prevalent than cells with basolateral H^+\text{-ATPase}, it is likely that some of the cells with diffuse labeling for H^+\text{-ATPase} also represent HCO_3^- -secreting cells.

It has been suggested that the type A and type B configurations of intercalated cells could represent different functional states of the same cell population and that these cells may change polarity in response to changes in the acid-base status of the animal. Support for this hypothesis was provided by the presence of H^+\text{-ATPase} in the apical membrane of the acid-secreting type A cell and in the basolateral membrane of the type B cell, which might suggest a reversed polarity. However, there is no evidence that a reversal of intercalated cell polarity occurs in vivo, and AE1 and pendrin have never been observed in the same cell in the kidney. The observation that AE1 is expressed only in type A intercalated cells, whereas pendrin is expressed only in type B and nonA-nonB intercalated cells, is more consistent with the concept that type A and type B cells represent structurally and functionally distinct cell types. This concept is also supported by studies demonstrating that distinct members of the chloride channel gene family are expressed in the two types of intercalated cells. CIC-3 mRNA is present only in type B intercalated cells, whereas CIC-5 mRNA is expressed in type A intercalated cells.

A major function of the cortical collecting duct is the secretion of K^+. This process is, at least in part, regulated by mineralocorticoids, which stimulate K^+ secretion and Na^+ reabsorption in the isolated perfused cortical collecting duct of the rabbit. Treatment with mineralocorticoids has also been shown to stimulate Na^+K^+\text{-ATPase} activity in individual segments of the cortical collecting duct of both rat and rabbit. Morphologic studies of the collecting duct of rabbits given a low-sodium, high-potassium diet and of rabbits treated with deoxycorticosterone demonstrated a significant increase in the surface area of the basolateral plasma membrane of the principal cells. The observed changes were similar to those reported in principal cells in the
connecting segment and in the initial collecting duct of rats on a high-potassium diet, indicating that these cells are responsible for K⁺ secretion in the connecting tubule and cortical collecting duct.

Sodium is absorbed through an amiloride-sensitive sodium channel, ENaC, which is located in the apical plasma membrane of connecting tubule cells and in principal cells along the entire collecting duct. The amiloride-sensitive sodium channel is composed of three homologous ENaC subunits, α, β, and γ, which together constitute the functional channel. Immunohistochemical and in situ hybridization studies have demonstrated that all three subunits are expressed in connecting tubule cells and principal cells in the collecting duct. However, high-resolution immunohistochemistry and immunogold electron microscopy revealed that α-ENaC was expressed in both the apical plasma membrane and apical cytoplasmic vesicles, whereas β-ENaC and γ-ENaC appeared to be located in small vesicles throughout the cytoplasm.

The activity of ENaC in the collecting duct is regulated by aldosterone and vasopressin as well as other hormonal systems via mechanisms that involve complex signaling pathways and incorporate changes in expression and subcellular trafficking of ENaC subunits. In mice receiving a high sodium diet, which is associated with a low plasma aldosterone level, α-ENaC was not detectable and β- and γ-ENaC were distributed throughout the cytoplasm. In mice given a low-sodium diet, which is associated with high plasma aldosterone levels, all three subunits of ENaC were expressed in the apical and subapical region of the connecting tubule cells and in principal cells of the cortical collecting duct. In the medullary collecting duct, however, cytoplasmic staining for β- and γ-ENaC was still observed. Vasopressin increases the abundance of all three ENaC subunits in the rat kidney and angiotensin II also plays a role in the regulation of ENaC.

Epithelial Na⁺ transport is regulated in large part through trafficking mechanisms that control ENaC expression at the apical cell surface. Delivery of ENaC to the cell surface is regulated by aldosterone (and corticosteroids) and vasopressin, which increase ENaC synthesis and exocytosis. Endocytosis and degradation is at least in part controlled by a PPPXYXXL motif in the C terminus of alpha, beta, and gamma ENaC that serves as a binding site for Nedd4-2, an E3 ubiquitin protein ligase that targets ENaC for degradation. Mutations that delete or disrupt this so-called PY motif cause accumulation of channels at the cell surface, resulting in Liddle syndrome. Nedd4-2 is central for ENaC regulation by aldosterone and vasopressin; both induce phosphorylation of Nedd4-2 residues, which blocks Nedd4-2 binding to ENaC. Thus, aldosterone and vasopressin regulate epithelial Na⁺ transport in part by altering ENaC trafficking to and from the cell surface (for recent review see Ref 479). An essential role of serum- and glucocorticoid-regulated kinase (sgk1) in the regulation of ENaC has also been established. A series of studies have also underscored a role of proteolytic processing in the activation and regulation of ENaC.

Recent immunohistochemical studies also provide evidence that aldosterone-mediated ENaC targeting may (at least in certain experimental conditions) occur via mineralocorticoid receptor-independent mechanisms. This conclusion is based on the observation that targeting of ENaC subunits is maintained in the presence of spironolactone, a mineralocorticoid receptor blocker, and it is consistent with previous findings regarding non-genomic effects of aldosterone. Abnormal regulation of ENaC is associated with salt retention or wasting by the collecting duct (reviewed by Shaefer). Certain single-gene defects affecting ENaC or its regulation by
Aldosterone cause severe hypertension, whereas others cause sodium wasting and hypotension. Such gene defects underscore the role of the collecting duct in maintaining normal extracellular volume and blood pressure. Changes in the regulatory actions of other hormones, such as vasopressin, and various autocrine and paracrine regulators, such as norepinephrine, dopamine, and prostaglandin E2, which inhibit the antinatriuretic effects of vasopressin, may also be associated with abnormal regulation of sodium reabsorption and lead to sodium retention or wasting, as seen in hypertension, congestive heart failure, or cirrhosis. Recent studies have provided evidence for dysregulation of ENaC via enhanced apical targeting in experimental conditions with severe sodium retention such as in various forms of nephrotic syndrome and in CCl4-induced liver cirrhosis. However, ENaC dysregulation in such conditions is only one of several factors because adrenalectomized animals also have sodium retention, suggesting that dysregulation of the alpha-1 subunit of the Na\(^+\),K\(^+\)-ATPase may also play a significant role.

### Outer Medullary Collecting Duct

The collecting duct segments in the outer and inner stripe of the outer medulla are abbreviated OMCDo and OMCDi, respectively. The outer medullary collecting duct is composed of principal cells and intercalated cells. In the rat and mouse, intercalated cells constitute approximately one third of the cells in both the OMCDo and the OMCDi, and a similar ratio between the two cell types is found in the OMCDo of the rabbit. In the rabbit OMCDi, however, the number of intercalated cells varies between animals. In some animals intercalated cells are present only in the outer half, where they represent 10% to 15% of the total cell population.

Principal cells in the outer medullary collecting duct are similar in ultrastructure to those in the cortical collecting duct. The cells become slightly taller, however, and the number of organelles and basal infoldings decreases as the collecting duct descends through the outer medulla. Whether principal cells in the outer medullary collecting duct are functionally similar to those in the cortical collecting duct is not known with certainty. They express Na\(^+\),K\(^+\)-ATPase in the basolateral plasma membrane and the amiloride-sensitive sodium channel in the apical plasma membrane, and they are believed to be involved in Na\(^+\) reabsorption; however, there is no evidence that they secrete K\(^-\) as in the cortical collecting duct. In fact, the OMCDi is a site of K\(^-\) reabsorption, at least in the rabbit. In the rat, intercalated cells in the outer medullary collecting duct are similar in ultrastructure to type A intercalated cells in the cortical collecting duct (Fig. 2-51).
In the OMCD, the cells become taller and less electron-dense, and little or no difference in the density of the cytoplasm exists between intercalated cells and principal cells in this segment. The main characteristics of the intercalated cells in the outer medulla include numerous tubulovesicular structures in the apical cytoplasm and prominent microprojections on the luminal surface. Scanning electron microscopy has revealed that intercalated cells are covered with microvilli and often bulge into the tubule lumen.

The outer medullary collecting duct plays an important role in urine acidification, which is believed to be a primary function of this segment. There is evidence that H⁺ secretion in the OMCD is an Na⁺-independent electrogenic process, similar to that described in the turtle urinary bladder. In the turtle bladder, H⁺ secretion is mediated by an N-ethylmaleimide-sensitive H⁺-translocating ATPase that has been isolated in a microsomal fraction from bladder epithelial cells. A similar H⁺-ATPase has been isolated from bovine and rat renal medulla. Furthermore, biochemical studies on isolated tubule segments have demonstrated the presence of N-ethylmaleimide-sensitive H⁺-ATPase activity in both the cortical collecting duct and the outer...
medullary collecting duct. The activity varies with the acid-base status of the animal, and it is stimulated by mineralocorticoids.

Morphologic studies have demonstrated characteristic ultrastructural changes in intercalated cells in the collecting duct after stimulation of H+ secretion. In rats with acute respiratory acidosis or chronic metabolic acidosis, an increase occurred in the surface area of the apical plasma membrane concomitant with a decrease in the number of tubulovesicular structures in the apical cytoplasm. These findings are similar to those reported in the carbonic anhydrase-rich cells in the turtle urinary bladder after stimulation of H+ secretion. On the basis of these observations, it was suggested that membrane containing the H+-ATPase is transferred from the tubulovesicular structures to the luminal membrane in intercalated cells in response to stimulation of H+ secretion.

Subsequent immunocytochemical studies using antibodies against the H+-ATPase from bovine renal medulla confirmed the presence of an H+-ATPase in the tubulovesicular structures and apical membrane of intercalated cells. These studies, together with the demonstration that antibodies against band 3 protein (AE1) label the basolateral membrane of intercalated cells, provided convincing evidence that these cells are involved in acid secretion in the collecting duct. Finally, there is now convincing evidence that mutations in the human H+-ATPase gene lead to renal tubular acidosis (extensively reviewed by Wagner and co-workers).

There is evidence that an H,K+-ATPase is present in the collecting duct, where it plays an important role in K+ reabsorption as well as H+ secretion. Biochemical studies have demonstrated H,K+-ATPase activity in individual segments of both the cortical collecting duct and the outer medullary collecting duct of the rat and the rabbit. The activity was inhibited by omeprazole and Sch-28080, both of which are potent inhibitors of the gastric H,K+-ATPase. In addition, H,K+-ATPase activity was found to increase in rats receiving a potassium-deficient diet, which suggests that the transporter may be involved in K+ reabsorption. Transport studies in the isolated perfused OMCD of rabbits receiving a potassium-deficient diet demonstrated K+ reabsorption and H+ secretion that were inhibited by omeprazole.

Immunohistochemical studies have revealed that antibodies to the gastric H,K+-ATPase label the intercalated cells in the collecting duct of both the rat and the rabbit. Subsequent in situ hybridization studies confirmed the expression of the gastric isoform of H,K+-ATPase in intercalated cells of rat and rabbit collecting duct. Therefore, intercalated cells are capable of both electrogenic H+ secretion, mediated by a vacuolar-type H+-ATPase, and electroneutral H+ secretion in exchange for K+, mediated by an H,K+-ATPase. However, the two processes seem to be regulated differently. A study examining the role of aldosterone and dietary potassium in the regulation of ATPase activity in the collecting duct reported that H+-ATPase activity is dependent on plasma aldosterone levels, whereas H,K+-ATPase activity varies with changes in dietary potassium. An isoform of the α-subunit of the colonic H,K+-ATPase, encoded by the HK-alpha 2 gene has been shown to be expressed in the kidney, where it mainly localizes to the outer medullary collecting duct principal cells. Additionally, transgenic mice expressing green fluorescent protein after the H,K-alpha 2 promoter, show fluorescence in the collecting duct system. The colonic H,K+-ATPase isoform has been shown to be regulated by chronic Na and K depletion and in IMCD-3 cells vasopressin and forskolin stimulates H,K-Alpha 2 mRNA abundance as does overexpression of cAMP/Ca2+-responsive elements binding protein.
Immunohistochemical studies have also demonstrated that a splice variant of the colonic isoform of the H⁺,K⁺-ATPase is expressed in the apical domain of both intercalated cells and principal cells in the rabbit collecting duct. Generation of colonic H⁺,K⁺-ATPase deficient mice showed no apparent renal phenotype in either normal or K⁺-depleted mice.

Secretion of ammonia/ammonium by the collecting duct is an important component of net acid secretion. However, NH₄⁺-specific transporters have not been identified with certainty in the kidney. Recent studies have reported that members of the Rhesus protein superfamily are expressed in the kidney and, based on sequence homology and structural similarities between the human Rhesus associated glycoprotein (RhAG) and the methylammonium and ammonium permease/ammonium transporters in yeast and bacteria, it was suggested that these proteins may function as NH₄⁺ transporters in the kidney. Recently two non-erythroid Rhesus glycoprotein homologs, RhBG and RhCG, were cloned from both mouse and human. Determination of their tissue-specific expression demonstrated high abundance in the kidney, adding further attention to their role in ammonium transport. Indeed, subsequent studies revealed that these proteins, when expressed in yeast or in Xenopus oocytes, were capable of ammonium transport, suggesting that they could play a major role in renal ammonium transport. Immunohistochemical analysis revealed that RhBG and RhCG are coexpressed in cells along the connecting tubule and collecting duct with strong immunoreactivity in intercalated cells. The RhCG protein is predominantly expressed in the apical plasma membrane whereas RhBG is expressed in the basolateral membrane. Moreover, RhCG appears to be regulated in response to chronic metabolic acidosis, whereas RhBG remained unchanged. The generation and detailed phenotyping of a RhBG deficient mice, did not reveal any phenotypical changes, suggesting that RhBG my not contribute significantly to distal tubular ammonium excretion. Further studies are needed to establish the exact role of these proteins in renal ammonium transport.

**Inner Medullary Collecting Duct**

The inner medullary collecting duct extends from the boundary between the outer and inner medulla to the tip of the papilla. As the collecting ducts descend through the inner medulla, they undergo successive fusions, which result in fewer tubules that have larger diameters (Fig. 2-52). The final ducts, the ducts of Bellini, open on the tip of the papilla to form the area cribrosa (see Fig. 2-3).
FIGURE 2-52  Scanning electron micrographs of normal papillary collecting duct of a rabbit.  

The epithelium of the ducts of Bellini is tall, columnar, and similar to that covering the tip of the papilla.  There are considerable species differences regarding the length of the papilla, the number of fusions of the collecting ducts, and the height of the cells.  In the rabbit, the height of the cells gradually increases from approximately 10 mm in the initial portion to approximately 50 mm close to the papillary tip, where the tubules form the ducts of Bellini.  In the rat the epithelium is considerably lower, and the increase in height occurs mainly in the inner half, from approximately 6 mm to 15 mm at the papillary tip.  

The inner medullary collecting duct has been subdivided arbitrarily into three portions: the outer third (IMCD₁), middle third (IMCD₂), and inner third (IMCD₃).  The IMCD₁ is similar in ultrastructure to the OMCD, but most of the IMCD₂ and the IMCD₃ appear to represent a distinct segment.  The inner medullary collecting duct was originally believed to be a functionally homogeneous segment.  However, transport studies have provided evidence that two functionally distinct segments exist in the inner medulla: an initial portion, the IMCD₁, which corresponds to the IMCD₁, and a terminal portion, the IMCD₄, which includes most of the IMCD₂ and the IMCD₃.  In the
following text, the terminology IMCD<sub>i</sub> and IMCD<sub>t</sub> is used to distinguish these two functionally
distinct segments of the inner medullary collecting duct.

In both rat and mouse, the IMCD<sub>i</sub> consists of principal cells (Fig. 2-53) and intercalated cells,
the latter constituting approximately 10% of the total cell population. Both cell types are similar in
ultrastructure to the cells in the OMCD, and are believed to have the same functional properties. In
the rabbit, the IMCD is often composed of only one cell type, similar in ultrastructure to the
predominant cell type in the OMCD. However, in some rabbits, intercalated cells can be found
in this segment.

FIGURE 2-53 Transmission electron micrograph of a principal cell from the initial portion of
the rat inner medullary collecting duct (IMCD<sub>i</sub>). Few organelles are present in the cytoplasm,
and apical microprojections are sparse. (Magnification, ×11,750.) (From Madsen KM, Clapp
34:441, 1988.)

In the rat, the transition from the IMCD<sub>i</sub> to the IMCD<sub>t</sub> is gradual and occurs in the outer part of the
IMCD<sub>t</sub>. The IMCD<sub>t</sub> consists mainly of one cell type, the inner medullary collecting duct cell. It
is cuboid to columnar with a light-staining cytoplasm and few cell organelles (Fig. 2-54). It
contains numerous ribosomes and many small, coated vesicles resembling clathrin-coated
vesicles. Small, electron-dense bodies representing lysosomes or lipid droplets are present in the
cytoplasm, often located beneath the nucleus. The luminal membrane has short, stubby microvilli
that are more numerous than on principal cells, and they are covered with an extensive glycocalyx.
Infoldings of the basal plasma membrane are sparse. By scanning electron microscopy, the luminal surface of inner medullary collecting duct cells is covered with numerous small microvilli (Figs. 2-55 and 2-56 [55] [56]). However, these cells lack the central cilium that is characteristic of principal cells.

FIGURE 2-54  Transmission electron micrograph of cells from the terminal portion of rabbit inner medullary collecting duct (IMCD). The cells are tall, possess few organelles, and exhibit small microprojections on the apical surface. Ribosomes and small coated vesicles are scattered throughout the cytoplasm. (Magnification, ×7000.)
FIGURE 2-55  Scanning electron micrograph from the middle portion of the rat inner medullary collecting duct. The luminal surface is covered with small microvilli, and some cells possess a single cilium. (Magnification, ×10,500.)  (From Madsen KM, Clapp WL, Verlander JW: Structure and function of the inner medullary collecting duct. Kidney Int 34:441, 1988.)
The functional properties of the inner medullary collecting duct have been studied mainly by in vivo micropuncture of the exposed rat papilla or by microcatheterization through a duct of Bellini. Use of these techniques has established that the inner medullary collecting duct is involved in the reabsorption of Na\(^{+}\), Cl\(^{-}\), K\(^{+}\), and urea and water. Only a few studies using the isolated perfused tubule technique had been performed until recently because of difficulties in dissection of this segment of the collecting duct. Sands and Knepper described an improved method of microdissection and perfusion of segments of the inner medullary collecting duct from both rat and rabbit. Use of this technique has revealed significant functional differences between the IMCD\(_i\) and the IMCD\(_t\). In the absence of the antidiuretic hormone, vasopressin, the IMCD\(_i\) is impermeable to both urea and water, whereas significant permeabilities for both urea and water were demonstrated in the IMCD\(_t\). Vasopressin stimulated water permeability in both segments of the inner medullary collecting duct, but urea permeability was stimulated only in the IMCD\(_t\). There is evidence that salt and water transport in the inner medullary collecting duct is regulated by atrial natriuretic peptides. Studies in the isolated perfused IMCD\(_t\) have demonstrated that atrial natriuretic peptides cause a significant decrease in vasopressin-stimulated osmotic water permeability but have no effect on urea permeability. Furthermore, evidence indicates that
cGMP is the second messenger mediating the effect of atrial natriuretic peptides on the collecting duct.

Urea transport in the IMCD is a facilitated process that is mediated by specific transport proteins located in the plasma membrane of the inner medullary collecting duct cells. The renal urea transporters, UTA, belong to a large family of urea transporters that also include the erythrocyte urea transporter, UT-B, which is expressed in the descending vasa recta. The UT-A1 and UT-A2 isoforms were first cloned from rabbit inner medulla and subsequently also from rat inner medulla. Studies of the segmental distribution of these transporters by in situ hybridization and immunohistochemistry revealed that UT-A1 was expressed exclusively in the IMCD, whereas UT-A2 was expressed in the descending thin limb of Henle’s loop (see Fig. 2-37). A third isoform, UT-A3, has also been identified in the IMCD. UT-A1 and UT-A3 are expressed exclusively in IMCD cells and immunocytochemistry has revealed that UT-A1 is present in the apical region of the IMCD, whereas UT-A3 is localized both intracellularly and in the basolateral membrane. To assess the role of inner medullary urea transport in kidney function, Fenton and colleagues developed a mouse model by deleting both UT-A1 and UT-A3 using standard gene targeting techniques (UT-A1/3-/- mice) and found that the animals exhibited a urinary concentrating defect. However, there were no differences in inner medullary Na and Cl concentrations between UT-A1/3-/- mice and wild-type control mice indicating that NaCl accumulation in the inner medulla was not dependent on either IMCD urea transport or accumulation of urea in the inner medullary interstitium. Thus, the passive countercurrent multiplier mechanism in the form originally proposed by Stephenson (see review in Ref 303) and by Kokko and Rector where NaCl reabsorption from Henle’s loop depends on a high IMCD urea permeability, cannot completely explain how NaCl is concentrated in the inner medulla.

Physiologic studies have provided evidence that the inner medullary collecting duct is involved in urine acidification. Microcatheterization experiments estimating in situ pH demonstrated a decrease in pH along the inner medullary collecting duct, and micropuncture of the papillary collecting duct revealed reabsorption of bicarbonate, which could be inhibited with acetazolamide. In addition, microcatheterization studies demonstrated an increase in net acid secretion in the inner medullary collecting duct of rats with acute and chronic metabolic acidosis. The mechanism of H+ secretion in this segment of the collecting duct is not known. In the rat, intercalated cells are present in the IMCD. They are similar in ultrastructure to intercalated cells in the outer medullary collecting duct, and they exhibit immunostaining for both H-ATPase and AE1, which suggests that they are involved in H+ secretion.

Urine acidification has been demonstrated along the papillary portion of the collecting duct, where there are no intercalated cells, which indicates that the inner medullary collecting duct cells must also be involved in H+ secretion; however, carbonic anhydrase has not been demonstrated in inner medullary collecting duct cells of adult animals, and these cells are also negative for AE1. An H-ATPase has been isolated from both bovine and rat renal medulla, but the exact cellular origin of this ATPase is not known. Although there is no immunoreactivity for either H-ATPase or H,K-ATPase in inner medullary collecting duct cells in vivo, studies in cultured inner medullary collecting duct cells have demonstrated acid secretion mediated by H-ATPase as well as H,K-ATPase. Moreover, acid secretion mediated by an H,K-ATPase was demonstrated in
isolated perfused IMCD segments from the rat kidney. Interestingly, studies in AQP1-deficient mice revealed strong H\(^{+}\) ATPase immunoreactivity in the apical plasma membrane of IMCD cells and increased H\(^{+}\) ATPase protein expression in the inner medulla of AQP1 null mice compared with wild-type mice. Recently studies have also demonstrated the presence of NBCn1 in IMCD cells implicating a role of this transporter in regulation of urinary acidification.

The epithelium of the collecting duct is tight and relatively impermeable to water in the absence of the antidiuretic hormone, vasopressin. Freeze-fracture examination of the cortical collecting duct and outer medullary collecting duct has revealed relatively complex tight junctions with 6 to 10 individual strands or ridges that form an anastomosing network in all species studied. As the medullary collecting duct descends toward the papillary tip, both the depth and the number of strands that form the tight junction gradually decrease. This observation is in agreement with the studies of Tisher and Yarger, who demonstrated that the tight junctions of the cortical and outer medullary segments of the collecting duct resist penetration by the extracellular tracer lanthanum, whereas those of the inner medullary collecting duct are permeable to lanthanum.

In the presence of vasopressin, all segments of the collecting duct become permeable to water. Morphologic changes, including cell swelling, dilatation of intercellular spaces, and an increased number of intracellular vacuoles, have been demonstrated along the entire collecting duct in association with vasopressin-induced osmotic water reabsorption. Vasopressin binds to specific receptors on the surface of the basolateral plasma membrane, which stimulates adenylate cyclase to generate cyclic adenosine monophosphate (cAMP), the second messenger for activation of the water transport system in the apical membrane. Freeze-fracture studies have demonstrated characteristic intramembrane particle aggregates in the apical membrane of vasopressin-responsive cells in the collecting duct. Demonstration of a correlation between the frequency of particle aggregates in the apical membrane and water permeability of the epithelium suggested that these aggregates represent water channels. Subsequent studies demonstrated that similar particle aggregates exist in the membrane of tubulovesicles, the so-called aggrephores, in the apical cytoplasm, and it was hypothesized that vasopressin increases water permeability by stimulating the fusion of vesicles containing water channels with the apical membrane.
In the collecting duct the intramembrane particle clusters are present in the principal cells and in the inner medullary collecting duct cells. Furthermore, specific vasopressin receptors have been demonstrated on the basolateral membrane of the principal cells. Transmission electron microscopy revealed the presence of coated pits in the luminal plasma membrane of principal cells; these coated pits correspond to the location of intramembrane particle clusters, which suggests that endocytosis plays a role in the regulation of water permeability. Studies using horseradish peroxidase as a marker of endocytosis demonstrated that removal of vasopressin stimulates endocytosis in principal cells of the collecting duct in the rabbit, which suggests that water channels are internalized from the luminal membrane. Similar studies in Brattleboro rats with hereditary diabetes insipidus also indirectly suggested that insertion and retrieval of water channels in the principal cells are regulated by vasopressin.

Since the cloning and characterization of AQP1, the water channel protein in the proximal tubule and descending thin limb, an entire family of aquaporins has been cloned and sequenced. The complementary DNA for the vasopressin-regulated water channel, AQP2, was cloned from a rat.
kidney library by polymerase chain reaction amplification using degenerate oligonucleotide probes based on the published sequence of AQP1. Using antibodies generated to synthetic peptides based on the sequence for AQP2, Nielsen and co-workers demonstrated selective labeling of principal cells and inner medullary collecting duct cells along the entire collecting duct of the rat (Fig. 2-58). Immunolabeling was localized to the apical plasma membrane and small subapical vesicles. There was no labeling of other cells in the kidney. Subsequent immunohistochemical studies demonstrated that AQP3 and AQP4 are expressed in the basolateral plasma membrane representing exit pathways for water reabsorbed through AQP2 channels. Thus, the water channels demonstrated in the collecting duct are distinct from the water channel responsible for the high water permeability in the proximal tubule and descending thin limb of the loop of Henle. The regulation of AQP2 involves both short-term and long-term mechanisms and is described in detail in this volume (see Chapter 8). Briefly, studies in isolated perfused tubules and in whole animals revealed that vasopressin acutely regulates collecting duct water permeability, and hence body water balance, by inducing a translocation of AQP2 from vesicles to the apical plasma membrane. On a longer-term basis vasopressin regulates the expression of AQP2. A series of detailed studies have been devoted to elucidate and characterize the regulation of AQP2, including signaling pathways, cytoskeletal elements, targeting receptors, and associated regulatory proteins (see Chapter 8). Mutations in AQP2 or in vasopressin V2-receptors lead to very severe forms of nephrogenic diabetes insipidus. Dysregulation of AQP2 expression, as well as of AQP3 expression is associated with a number of acquired forms of nephrogenic diabetes insipidus such as lithium-induced polyuria, hypokalemia, hypercalcemia, and post-obstructive polyuria. Conversely up-regulation of AQP2 expression has been seen in conditions with water and sodium retention such as congestive heart failure and CCl4-induced liver cirrhosis.

FIGURE 2-58  A, Light micrograph of 50-mm vibratome section of rat kidney illustrating
immunolocalization of the vasopressin-sensitive aquaporin-2 water channel in the collecting duct using a horseradish peroxidase procedure. B, Immunoelectron microscopy of the vasopressin-sensitive aquaporin-2 water channel in the collecting duct using an immunogold procedure. The labeling of aquaporin-2 is seen in the apical plasma membrane of principal cells in the inner medullary collecting duct and the labeling is associated with the apical plasma membrane and intracellular vesicles (×20,000). (A, Courtesy of Jin Kim, MD, Catholic University, Seoul, Korea.)

INTERSTITIUM

The renal interstitium is composed of interstitial cells and a loose, flocculent extracellular matrix material consisting of sulfated and nonsulfated glucosaminoglycans. The amount of interstitial tissue in the cortex is limited, and the tubules and capillaries are often directly opposed to each other. The interstitium constitutes 7% to 9% of the cortical volume in the rat. Three percent of the 7% represents the interstitial cells, and the remaining 4% represents the extracellular space. In humans, the relative volume of cortical interstitial tissue has been found to be 11.7% ± 5.5% in kidneys from patients younger than 36 years of age and 15.7% ± 3.0% in older patients. Bohle and associates reported a mean cortical interstitial volume of 9% with a range of 5% to 11% in 20 kidneys, but the age of the patients was not given. Hestbech and co-workers reported similar values, with a mean of 13.6% and a range of 6.3% to 21.3%, in 13 kidneys from patients 33 to 65 years of age. Kappel and Olsen evaluated 54 donor kidneys that were unsuitable for transplantation and reported a range of 7.1% to 37.1%, with values dependent on age.

In the medulla, a gradual increase occurs in interstitial volume, from 10% to 20% in the outer medulla to approximately 30% to 40% at the papillary tip in both the rat and the rabbit. In a study using the volume of distribution of inulin and similar extracellular markers, the interstitial volume in the rat kidney was found to constitute approximately 13% of the total kidney volume; in the rabbit kidney, the value was 17.5%.

Cortical Interstitium

The cortical interstitium can be divided into a wide interstitial space, located between two or more adjacent renal tubules, and a narrow or slit-like interstitial space, located between the basement membrane of a single tubule and the adjacent peritubular capillary. Whether such a division has any functional significance is unknown; however, it is of interest that approximately two thirds of the total peritubular capillary wall faces the narrow compartment and that this portion of the vessel wall is fenestrated. This relationship might facilitate the control of fluid reabsorption across the basolateral membrane of the proximal tubule by Starling forces.

There are two types of interstitial cells in the cortex: one that resembles a fibroblast (type 1 cortical interstitial cell) and another, less common mononuclear or lymphocyte-like cell (type 2
Type 1 cells are positioned between the basement membranes of adjacent tubules and peritubular capillaries. They have a stellate appearance and contain an irregularly shaped nucleus and a well-developed rough- and smooth-surfaced endoplasmic reticulum. Type 2 cells are usually round, with sparse cytoplasm and few cell organelles. Studies by Kaissling and Le Hir demonstrated antigen-presenting dendritic cells among the fibroblasts in the peritubular interstitium in both cortex and outer medulla of the normal rat kidney. The interstitial space contains a loose, flocculent material of low density and small bundles of collagen fibrils. Immunocytochemical staining of immature and mature human kidney has revealed types I and III collagen and fibronectin in the interstitium of the cortex. Type V collagen has been described in the cortical interstitium of the rat.

![FIGURE 2-59](image)

**FIGURE 2-59** Transmission electron micrograph of type 1 cortical interstitial cell (asterisk) from rat. A peritubular capillary is located at right center. (Magnification, ×9300.)

There is evidence that the peritubular, fibroblast-like interstitial cells are the site of erythropoietin production in the kidney. In situ hybridization studies using sulfur 35–labeled probes detected erythropoietin mRNA in peritubular cells in the kidney cortex of anemic mice. This localization was confirmed by Bachmann and co-workers, who demonstrated colocalization of erythropoietin...
mRNA and immunoreactivity for ecto-5'-nucleotidase, a marker of the fibroblast-like interstitial cells in the renal cortex; thus identifying the erythropoietin-producing cells in the renal cortex as being interstitial cells. The lymphocyte-like interstitial cells in the cortex are believed to represent bone marrow-derived cells.

Medullary Interstitium

Bohman described three types of interstitial cells in the rat renal medulla. Type 1 cells are the prominent, lipid-containing interstitial cells and resemble the type 1 cells in the cortex. However, they do not express erythropoietin mRNA and do not contain ecto-5'-nucleotidase. They are present throughout the inner medulla and are also found in the inner stripe of the outer medulla. The type 2 medullary interstitial cell is a lymphocyte-like cell that is virtually identical to the mononuclear cell (type 2 interstitial cell) described previously in the cortex. It is present in the outer medulla and in the outer part of the inner medulla. It is free of lipid droplets, but lysosome-like bodies are often observed. Type 2 cells are sometimes found together with type 1 cells. The type 3 cell is a pericyte that is located in the outer medulla and the outer portion of the inner medulla. It is closely related to the descending vasa recta, where it is found between two leaflets of the basement membrane. These three types of interstitial cells are also found in the rabbit.

Most interstitial cells in the inner medulla are the lipid-containing type 1 interstitial cells, which are often referred to as the renomedullary interstitial cells. They have long cytoplasmic projections that give them an irregular, star-shaped appearance. The cells are often arranged in rows between the loops of Henle and vasa recta, with their long axes perpendicular to those of adjacent tubules and vessels, thus resembling the rungs of a ladder. The elongated cell processes are in close contact with the thin limbs of Henle and the vasa recta, but direct contact with collecting ducts is observed only rarely. Often, a single cell is in contact with several vessels and thin limbs. The long cytoplasmic processes from different cells are often connected by specialized cell junctions that vary in both size and shape and contain elements of tight junctions, intermediate junctions, and gap junctions.
FIGURE 2-60  Light micrograph of the renal medullary interstitium from a normal rat. The lipid-laden type 1 interstitial cells bridge the interstitial space between adjacent thin limbs of Henle (TL) and vasa recta (VR). (Magnification, ×830.)

Several investigators have described the ultrastructure of the type 1 medullary interstitial cells in rat, rabbit, and human kidney. They contain numerous lipid inclusions or droplets in the cytoplasm that vary considerably in both size and number (Fig. 2-61). An average diameter of 0.4 mm to 0.5 mm has been reported in the rat, but profiles of up to 1 mm in diameter were also observed. The droplets have a homogeneous content, but they have no limiting membrane; however, they are often surrounded by whorls of smooth cytomembranes with a thickness of 6 nm to 7 nm. The cells contain large amounts of rough endoplasmic reticulum that often is continuous
with elements of the smooth cytoplasmic membranes. Mitochondria are sparse and scattered randomly in the cytoplasm. A small number of lysosomes are present, but endocytic vacuoles are sparse, although interstitial cells are capable of endocytosis of particulate material. Cavallo reported that the type 1 interstitial cells in the inner medulla of the rat kidney contain endogenous peroxidase activity in the endoplasmic reticulum, in the perinuclear cisterna, and in small cytoplasmic vesicles. In contrast, no activity was observed in interstitial cells in the outer medulla or the cortex.

**FIGURE 2-61** Higher-magnification electron micrograph illustrating the relationship between the electron-dense lipid droplets, which almost fill the type 1 interstitial cells, and the granular endoplasmic reticulum (arrows). Wisps of basement membrane-like material adjacent to the surface of the cells are contiguous with the basement membrane of the adjacent tubules (lower right). (Magnification, ×12,000.)
An unusual type of cylindrical body, measuring 0.1 mm to 0.2 mm in diameter and up to 11 mm in length and believed to be derived from the endoplasmic reticulum, has been described in the type 1 interstitial cells. These structures were observed originally in dehydrated rats and were believed to represent a response to severe dehydration, but subsequent studies demonstrated their presence under normal conditions. The wall of the cylinders consists of two triple-layered membranes, each measuring 6 nm in thickness, and connections between the walls and the membranes of the endoplasmic reticulum have been observed. The functional significance of these cylindrical structures remains unknown.

The number and size of the lipid inclusions in the type 1 medullary interstitial cells vary considerably, depending on the physiologic state of the animal and on the species. In the rat, lipid droplets constitute 2% to 4% of the interstitial cell volume, and the volume depends largely on the physiologic state of the animal. The lipid droplets were originally reported to decrease in both size and number after 24 hours of dehydration, but in a later study Bohman and Jensen were unable to confirm these findings. However, an increase occurred in both the size and the number of lipid droplets in water-loaded rats, whereas the interstitial cells were almost depleted of lipid inclusions in water-loaded rabbits. Although the lipid droplets and the diuretic state of an animal seem to be related, the exact nature of this relationship remains to be established.

The function of type 1 interstitial cells (renomedullary interstitial cells) is not known. They probably provide structural support in the medulla because of their special arrangement that is perpendicular to the tubules and vessels. The close relationship between these cells and the thin limbs and capillaries also suggests a possible interaction with these structures. Because of the presence of a well-developed endoplasmic reticulum and prominent lipid droplets, researchers have suggested that the type 1 interstitial cells may also be secretory in nature. The lipid droplets are not secretory granules in the usual sense, however, because they have no limiting membrane and there is no evidence that they are secreted by the cell. The droplets have been isolated from homogenates of the renal medulla of both rat and rabbit. They consist mainly of triglycerides and small amounts of cholesterol esters and phospholipids. The triglycerides are rich in unsaturated fatty acids, including arachidonic acid. The renomedullary interstitial cells are a major site of prostaglandin synthesis. Studies using tissue cultures of renal medullary interstitial cells demonstrated synthesis of prostaglandins by these cells, with the major product being PGE$_2$. However, prostaglandin synthetase activity has been found in rabbit collecting duct cells, which suggests that prostaglandin synthesis is not limited to the interstitial cells.

Prostaglandin synthesis in the renomedullary interstitial cells is mediated by COX-2. The expression of COX-2 in these cells increases in response to water deprivation and hypertonicity. Binding sites for several vasoactive peptides, including angiotensin II, are present in renomedullary interstitial cells, and there is evidence that angiotensin may be involved in the regulation of prostaglandin production in the renal medulla. Using histochemical techniques, Kugler demonstrated aminopeptidase A (EC 3.4.11.7), an angiotensinase that is capable of
degrading angiotensin, in the type 1 renomedullary interstitial cells of rat, rabbit, golden hamster, and guinea pig. Therefore, another possible mechanism to control angiotensin stimulation of prostaglandin production seems to be present in the medulla.

The type 1 renomedullary interstitial cells may have an endocrine antihypertensive function. Muirhead and co-workers demonstrated that transplantation of renal papillary tissue and cultured renomedullary interstitial cells into hypertensive animals was followed by a decrease in blood pressure. The lipid droplets may be related to the antihypertensive function of the interstitial cells, but the precise mechanism has yet to be established. Finally, the interstitial cells are responsible for the synthesis of the glycosaminoglycans, in particular hyaluronic acid, which are present in the matrix material of the interstitium.

Little is known about the function of the type 2 and type 3 medullary interstitial cells. Bohman suggested that the type 2 cells are probably phagocytic, but the function of type 3 cells remains unknown.

LYMPHATICS

Interstitial fluid can leave the kidney by two different lymphatic networks, a superficial capsular system and a deeper hilar system. Our knowledge of the distribution of lymphatics within the kidney, however, is restricted. Intrarenal lymphatics are embedded in the periarterial loose connective tissue around the renal arteries and are distributed primarily along the interlobular and arcuate arteries in the cortex. Kríž and Dieterich believed that the cortical lymphatics begin as lymphatic capillaries in the area of the interlobular arteries and that these capillaries drain into the arcuate lymphatic vessels at the region of the corticomedullary junction (Fig. 2-62). The arcuate lymphatic vessels drain to hilar lymphatic vessels through interlobar lymphatics. Numerous valves have been described within the interlobar and hilar lymphatic channels. Similar findings were reported by Bell and associates in both calves and dogs. In the horse, glomeruli are often completely surrounded by lymphatic channels, whereas in the dog, only a portion of the glomerulus is surrounded by lymphatics. Electron microscopic studies in the dog kidney after injection of India ink into capsular lymphatic vessels revealed the presence of small lymphatic channels, in close apposition to both proximal and distal tubules, in addition to the interlobular arteries. An electron microscopic study of the lymphatic system in the dog kidney demonstrated the existence of cortical intralobular lymphatics closely associated with terminal arteries, arterioles, renal corpuscles, and tubule elements. Morphometric analysis revealed that the cross-sectional area of interlobular lymphatics was almost twice that of intralobular lymphatics in the cortex. The volume density of renal cortical lymphatics was 0.17%. Similar morphometric studies in the rat, hamster, and rabbit revealed volume densities of cortical lymphatics of 0.11%, 0.37%, and 0.02%, respectively.
FIGURE 2-62 Diagram of the lymphatic circulation in the mammalian kidney. (Modified from Kriz W, Dieterich HJ: [The lymphatic system of the kidney in some mammals. Light and electron microscopic investigations]. Z Anat Entwicklungsgesch 131:111, 1970.)

A less extensive system of lymphatic vessels is present within and immediately beneath the renal capsule. The lymphatic vessels of the renal capsule drain into subcapsular lymphatic channels that lie adjacent to interlobular arteries located just beneath the renal capsule. These lymphatic vessels appear to provide continuity between the major intrarenal lymphatic vessels within the cortex (interlobular and arcuate lymphatic vessels) and the capsular lymphatic vessels; thus, in some animals, a continuous system of lymphatic drainage has been observed from the renal capsule, through the cortex, and into the hilar region (Fig. 2-63). In the dog kidney, two
types of tributaries have been described in association with the surface lymphatics. So-called communicating lymphatic channels were found in small numbers, usually in association with an interlobular artery and vein; these lymphatics penetrated the capsule and appeared to represent a connection between the hilar and capsular systems. The second type of vessel, the so-called perforating lymphatic channel, penetrated the capsule alone or in association with a small vein; these channels appeared to represent a primary pathway for lymph drainage from the superficial cortex. From a study in the dog kidney, investigators concluded that intramedullary lymphatics do not exist in this species, and they suggested that interstitial fluid from the medulla may drain to the arcuate or interlobar lymphatics. It has also been suggested that plasma proteins are cleared from the medullary interstitium through the ascending vasa recta.

**FIGURE 2-63** Light micrograph of a sagittal section through the cortex and outer medulla of a dog kidney. A capsular lymphatic (C) was injected with India ink. Intrarenal lymphatics (arrows) follow the distribution of the interlobular arteries in the cortex. (Magnification, ×10.) (From Bell
On microscopic examination, the wall of the interlobular lymphatic vessel is formed by a single endothelial layer and does not have the support of a basement membrane. The arcuate and interlobar lymphatic vessels are similar in appearance, although the latter possess valves.

**INNERVATION**

The efferent nerve supply to the kidney arises largely from the celiac plexus, with additional contributions originating from the greater splanchnic nerve, the intermesenteric plexus, and the superior hypogastric plexus. The postganglionic sympathetic nerve fiber distribution generally follows the arterial vessels throughout the cortex and outer stripe of the outer medulla. Adrenergic fibers have been observed lying adjacent to smooth muscle cells of arcuate and interlobular arteries and afferent arterioles. An extensive innervation of the efferent arteriolar vessels of the juxtamedullary glomeruli, which eventually divide to form the afferent vasa recta, has been described. However, quantitation of monoaminergic innervation by autoradiography revealed a higher density of norepinephrine-containing nerves associated with the afferent rather than the efferent arteriole. Newstead and Munkacsi reported the existence of large bundles of unmyelinated nerve fibers that accompanied the efferent arterioles from the region of the juxtamedullary glomeruli to the level of the inner stripe of the outer medulla. Nerve fibers and nerve endings were no longer present at the site at which the smooth muscle layer of the efferent arterioles and arteriolae rectae gave way to the pericytes surrounding the arterial vasa recta, which begin in the deep inner stripe of the outer medulla. There is also evidence for the presence of transitory adrenergic fibers in the inner medulla of the cat kidney.

For some time, controversy has existed regarding the presence of direct tubule innervation in the renal cortex. Nerve bundles arising from perivascular nerves have been described in proximity to both proximal and distal tubules. Structures termed varicosities, which are believed to represent nerve endings, have been described as being in close contact with proximal and distal tubules, often in the vicinity of the hilus of the glomerulus and the juxtaglomerular apparatus, and in the connecting segment and the cortical collecting duct. Autoradiographic studies have also revealed that injected tritiated norepinephrine is associated with both proximal and distal convoluted tubules, which indicates monoaminergic innervation of these tubules. The thick ascending limb of Henle receives the largest nerve supply. Both myelinated and unmyelinated nerve fibers have been demonstrated in the corticomedullary region and in perivascular connective tissue. Electron microscopic autoradiography revealed that tritiated norepinephrine is concentrated mainly on unmyelinated fibers, suggesting that these fibers are adrenergic in nature. There is evidence that renal nerves possess fibers containing neuropeptide Y, a potent vasoconstrictor, as well as immunoreactive somatostatin and neurotensin. Vasoactive intestinal polypeptide immunoreactive nerve fibers are also well documented in the kidney.
Earlier studies describing cholinergic nerve fibers within the renal parenchyma have fallen into disrepute because the conclusions were based largely on the presence of acetylcholinesterase.

The afferent renal nerves are found principally in the pelvic region, the major vessels, and the corticomedullary connective tissue. Most, although not all, afferent renal nerves are unmyelinated. Largely on the basis of immunocytochemical localization of calcitonin gene-related peptide, a marker of afferent nerve fibers, Barajas and co-workers suggested that these immunoreactive nerve fibers may be involved in baroreceptor and afferent nerve responses to changes in arterial, venous, interstitial, or intrapelvic pressure.