Reilly, Robert F., and David H. Ellison. Mammalian Distal Tubule: Physiology, Pathophysiology, and Molecular Anatomy. Physiol. Rev. 80: 277–313, 2000.—The distal tubule of the mammalian kidney, defined as the region between the macula densa and the collecting duct, is morphologically and functionally heterogeneous. This heterogeneity has stymied attempts to define functional properties of individual cell types and has led to controversy concerning mechanisms and regulation of ion transport. Recently, molecular techniques have been used to identify and localize ion transport pathways along the distal tubule and to identify human diseases that result from abnormal distal tubule function. Results of these studies have clarified the roles of individual distal cell types. They suggest that the basic molecular architecture of the distal nephron is surprisingly similar in mammalian species investigated to date. The results have also reemphasized the role played by the distal tubule in regulating urinary potassium excretion. They have clarified how both peptide and steroid hormones, including aldosterone and estrogen, regulate ion transport by distal convoluted tubule cells. Furthermore, they highlight the central role that the distal tubule plays in systemic calcium homeostasis. Disorders of distal nephron function, such as Gitelman’s syndrome, nephrolithiasis, and adaptation to diuretic drug administration, emphasize the importance of this relatively short nephron segment to human physiology. This review integrates molecular and functional results to provide a contemporary picture of distal tubule function in mammals.

I. INTRODUCTION

The distal tubule of the mammalian kidney, defined as the nephron segment interposed between the macula densa region and the first confluence with another nephron to form the cortical collecting tubule, comprises several morphologically and functionally heterogeneous subsegments (see Fig. 1A). It reabsorbs 5–10% of the filtered sodium and chloride under normal conditions and participates importantly in net K⁺ secretion. It plays a central role in systemic calcium homeostasis, plays an important role in systemic magnesium homeostasis, and participates in net acid secretion. Inherited disorders of distal cell function lead to systemic abnormalities of extracellular fluid volume and potassium, calcium, and magnesium balance, confirming the importance of the distal tubule to human physiology and human disease (225). Yet, until recently, properties of individual distal cell types had not been identified conclusively. This led both from the cytological heterogeneity of the segment and from appar-
ent differences between properties of distal cells in different species. During the past several years, molecular tools have permitted investigators to assign functional properties to specific nephron segments and cell types, clarifying the molecular basis of physiological function. Coupled with the identification of human diseases, both genetic and acquired, that arise from dysfunction of distal tubule cells, these developments have renewed interest in the structure and function of the mammalian distal tubule.

II. MORPHOLOGICAL SEGMENTATION

The variable nomenclature applied to the distal portion of the mammalian nephron and segmental and functional differences between mammalian species have led to confusion about anatomical and functional properties of the distal tubule. This variability is summarized in Figure 2, which is adapted from a scheme presented by the Renal Commission of the International Union of Physiological Sciences (155). Although this scheme was intended to encourage a uniform nomenclature for nephron segments, the diversity of terms and definitions has persisted. As might be expected, based on nosological inconsistencies, attribution of function to particular segments may depend on the definition that is employed. One goal of this review is to provide precise definitions of cell types and segments with which to correlate functional properties.

Although the definition of “distal tubule” (Fig. 2, see
### Table: Segmentation of Mammalian Nephron

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**FIG. 2.** Segmentation of mammalian nephron. Scheme modified from that proposed by Renal Commission of International Union of Physiological Sciences (155). Note that definitions used in this review correspond to those listed as “preferred terms” except that the term distal tubule (see asterisk) is used to denote nephron segment between region of macula densa and confluence with another tubule to form collecting duct.

Asterisk) given in section I is precise, this term has also been used by anatomists (see Fig. 2, main divisions) to denote the segment comprising the thick ascending limb (or distal straight tubule) and distal convoluted tubule (see below). According to this anatomical definition, the connecting tubule and cortical and medullary collecting ducts form the collecting system. Early anatomists and physiologists, however, also described a “distal convolution” (see Fig. 2, microanatomical terms), a segment that is distinct from both the proximal convolution and the straight tubules (112). This distal convolution corresponds to the distal tubule of the micropuncture literature and comprises primarily the distal convoluted tubule and connecting tubule (253, 296). Some authors have referred to the entire region between the macula densa and the confluence as the “distal convoluted tubule” (190, 191); this region has then been divided into bright, granular, and light portions, according to its appearance during dissection in vitro. In this review, the term distal convoluted tubule is restricted to the segment comprising distal convoluted tubule cells.

Careful analysis of cell types along the renal distal tubule and of the segment’s physiological properties, its hormonal responsiveness, and its response to physiological perturbation indicates that the renal distal tubule comprises four anatomically discrete subsegments (59, 129, 155). These include a short region of thick ascending limb (TAL), the distal convoluted tubule (DCT), the connecting tubule (CNT), and the initial portion of the cortical collecting tubule (CCT). These terms (preferred terms...
posed between the nucleus and apical membrane but do fill the perinuclear region. The apical membrane is characterized by numerous short microvilli. Corresponding to the high density of mitochondria and to the extensive basolateral membrane amplification, the Na\(^{+}\)-K\(^{+}\)-ATPase activity is the highest in the DCT of any nephron segment (141, 229).

The CNT lies just distal to the DCT, arising abruptly in rabbits and gradually in most other species (133, 155). The existence of a connecting portion or Verbindungsstück was first suggested by Schweigger-Seidel (59). In all species, it comprises predominantly two cell types, CNT cells and intercalated cells. The microanatomical organization of CNT differs between superficial and deeper nephrons (see Fig. 1A). In superficial nephrons, the CNT is an unbranched segment that flows into the initial portion of the CCT (the initial collecting tubule) before draining into a collecting duct. In the rat, the CNT of superficial nephrons was shown to be 0.49 ± 0.03 mm in length (67). In contrast, as shown in Figure 1A, the CNT of midcortical and juxtamedullary nephrons frequently form branched structures (termed “arcades”) that ascend through the cortical labyrinth. These arcades usually run close to an interlobular artery (Fig. 1B; Ref. 148). Only after one or more CNT have joined do principal cells appear, indicating transition to the CCT. It has been suggested that some arcades probably exist in all mammalian species, but the percentage of nephrons emptying into the collecting duct via arcades, versus emptying directly, varies. In rat, rabbit, and pig, the majority of deep nephrons drain via arcades (67, 148, 155). In humans, most nephrons drain individually (155). In all species, the entire renal distal tubule lies within the cortical labyrinth (Fig. 1B).

The appearance of CNT cells (see Fig. 3) is the same whether in superficial distal tubules or in arcades. Connecting tubule cells have been characterized as appearing intermediate between DCT and principal cells. In species such as the rat, where transitions from DCT to CNT and CCT are gradual, distinguishing DCT, CNT, and principal cells may be difficult, especially near the junctions between segments. However, it is now clear that CNT cells are distinct morphologically, functionally, and at the molecular level (129, 133, 155, 177, 268). Connecting tubule cells demonstrate basolateral cell membrane amplification, as do DCT cells. However, in CNT cells, more of the amplification results from infolding of the basal membrane rather than amplification of lateral cell processes (as in DCT cells). Thus, in the CNT, there is less cell-cell contact than in the DCT. Mitochondria appear between the basal infoldings, but their number is significantly reduced compared with the DCT. The nucleus of CNT cells is apically oriented, but unlike in DCT cells, mitochondria may be observed between the nucleus and the apical membrane. The apical membrane of CNT cells exhibits
fewer apical projections than does the apical membrane of DCT cells.

The transition to the initial collecting tubule is abrupt in the rabbit but appears gradual in the rat, mouse, and human (58, 59, 129, 155). In the rat, the initial collecting tubule may be as long as 0.71 ± 0.12 mm in superficial nephrons, but it is shorter (0.22 mm) in deeper nephrons (67). The epithelium of the initial CNT is indistinguishable

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**FIG. 3.** Ion transport pathways and cellular morphology of rat distal tubule. Schematic cell models are shown at left. Transepithelial voltage ($V_{TE}$) in most proximal portion of DCT is near zero. Magnitude of voltage becomes larger in more distal segments. Ion transport pathways that have been identified by functional and molecular techniques are shown with solid black arrows. Ion transport pathways for which functional evidence is present but which lack molecular confirmation are shown with gray arrows. Each cloned pathway that has been localized to this segment is identified by a letter. DCT-1 and DCT-2 identify segments of DCT in which Na$^+$/Ca$^{2+}$ is (DCT-2) or is not (DCT-1) expressed. For details see text. At right are shown DCT and CNT cells. DCT cells are taller with more densely packed mitochondria. [Morphological drawings from Kriz and Kaissling (155).]
from that observed in true cortical collecting ducts (CNT). It comprises principal and intercalated cells. Principal cells are organized in a manner similar to that of CNT cells, but they are lower. Basal infoldings are generally restricted to the basal third of the cells, and the infoldings do not contain mitochondria. The apical membrane has few microvilli but does contain a prominent central cilium. Below the apical membrane, a dense meshwork is formed by microtubules and microfilaments; included in this network are aggrephores, which contain aquaporin for insertion into the apical membrane under the control of antidiuretic hormone (287).

Intercalated cells appear in the CNT and collecting duct of all species. In the rabbit, they are not found in the DCT, but in most other species, including rat, mouse, and human, they appear in the latter third of the DCT. In the rat, Dorup (67) showed that intercalated cells appear ~1 mm beyond the macula densa, ~500 μm before DCT cells disappear (67). In humans, intercalated cells appear along the latter half of the DCT (268). Along the CNT, intercalated cells comprise from 25–30% (in rat) to 40–45% (in rabbit) of cells. In the CNT, intercalated cells comprise ~40% of cells (155). Unlike CNT and principal cells, which appear polygonal when viewed by scanning electron microscopy, intercalated cells have a round appearance. Their apical membranes are densely adorned with microprojections. Their lateral surfaces adhere to adjacent connecting and principal cells by desmosomes. The nucleus of these cells is at the basal pole (133, 155). Two types of intercalated cells are recognized morphologically. Type A cells manifest extensive apical microvilli and numerous small mitochondria within the subapical region. Type B cells have fewer apical microvilli, and mitochondria tend to accumulate in the basal portion of the cell. Although the ratio of type A to type B cells may vary depending on the physiological condition of the animal, type A cells are more numerous in the CNT and type B cells in the CCD (146, 147, 155).

III. EMBRYOLOGICAL DEVELOPMENT

The human kidney arises from two embryonic tissues, the metanephric blastema and the ureteric bud. During the process of renal development, the ureteric bud elongates and branches. At the tips of these branches the metanephric mesenchyme condenses to form renal vesicles. The renal vesicle elongates to become a comma-shaped body, then subsequently an S-shaped body before it fuses with a branch of the ureteric bud to form a complete nephron (262). After the S-shaped body fuses with the ampulla of the ureteric bud, this portion of the nephron undergoes marked elongation, and this structure is called the connecting piece. The connecting piece runs from near the glomerulus distal to the macula densa and enters the collecting duct at a right angle. The site that corresponds to the junction of the S-shaped body and the ureteric bud within the nephron is controversial, as is the embryonic tissue of origin of the connecting piece.

Initial studies that were based on microdissection concluded that the connecting piece originated from the metanephric blastema (202). They stated that the CNT was derived from the S-shaped body and therefore the metanephric blastema and that the site of union of the metanephric blastema and the ureteric bud was the junction of the CNT and the collecting duct.

Howie et al. (121) examined this issue using a series of markers against blood group antigens and cytokeratins in human kidney. They employed monoclonal antibody FC 10.2 directed against the type 1 precursor chain of ABO antigens, a lectin UEA-1 that recognizes the H antigen of the O blood group, monoclonal antibody PKK2 directed against cytokeratins 7, 16, 17, and 19, and monoclonal antibody AE1 generated against cytokeratins 14, 15, 16, and 19. All of these antibodies and lectins stained both the ureteric bud and the connecting piece, indicating that the connecting piece had arisen from the ureteric bud. The authors conclude that the junction of the ureteric bud and the metanephric blastema may be at the boundary of the TAL and the DCT.

Schmitt et al. (230) recently examined this same question in the newborn rat using a variety of antibodies directed against proteins involved either directly or indirectly in ion transport. These workers suggested a third alternative; the CNT arises as a product of the mutual induction of the metanephric blastema and the ureteric bud that creates a unique hybrid epithelium (230).

IV. PHYSIOLOGY OF ION TRANSPORT

A. Electrophysiology

Electrical properties of the DCT, CNT, and initial portion of the CCD were examined first in vivo using micropuncture techniques in rats. Subsequently, rabbit, mouse, and rat kidney tubules have been microperfused in vitro. Distal convoluted tubules dissected from rabbits and grown in cell culture have also been studied. There have been differences between results obtained in different mammalian species and using different experimental techniques. Although these differences have been emphasized, when data are analyzed in light of more recent physiological and molecular results, the differences are outweighed by similarities.

In the rat, experiments performed in vivo showed that the transepithelial voltage throughout most of the superficial renal distal tubule is oriented with the lumen negative with respect to the blood (48, 140, 181, 182, 211, 248). Wright (296) first showed that the mag-
mammalian distal tubule 283

FIG. 4. Top: transepithelial voltage ($V_T$, in mV) along rat distal tubule. Data obtained during in vivo microperfusion are indicated by solid symbols and plotted as a percentage of total distal tubule length (X, Ref. 296; ●, Ref. 179; ■, Ref. 10; ▼, Ref. 117; ▼, Ref. 55). Data obtained by micropumping cortical collecting ducts in vitro are presented as open or gray symbols within 2 ovals. Data collected without mineralecticoid hormone treatment are indicated with open circles (−MA; ○, Ref. 270; □, Ref. 228). Data collected in presence of mineralecticoid hormone treatment are indicated by shaded symbols (+MA; gray circle, Ref. 270; ■, Ref. 212). Location of DCT, CNT, and CCT is inferred from percentage length along distal tubule (67). Bottom: transepithelial resistance ($R_T$) along rat renal distal tubule. Data obtained during in vivo microperfusion are indicated by solid symbols (references as in top panel). Data obtained by micropumping in vitro are indicated by open symbols (references as in top panel).

The magnitude of the transepithelial voltage, measured in vivo, increases from the most proximal accessible segment to the most distal segment. This result was subsequently confirmed by others (see Fig. 4) (2, 9, 10, 36, 55, 179, 296). One concern about these experiments is that they utilized small-tipped Ling-Gerard-type microelectrodes. In contrast, Barratt and colleagues (2, 9, 10) reported that the transepithelial voltage, measured in the earliest portions of the superficial distal tubule, is oriented with the lumen positive compared with the blood. Those experiments utilized large-tipped electrodes (2, 9, 10). Although differences in electrode size or filling solution (3, 117) or differences in rat strain (2, 9, 10) may have contributed to differences in results from different laboratories, Figure 4 suggests a reasonably consistent picture. Whether measured using small-tipped or larger electrodes, the transepithelial voltage along the superficial rat distal tubule is oriented with the lumen negative, with respect to the blood, throughout most of its length. The transepithelial voltage of the most proximal segments is near to zero, but the voltage becomes negative as fluid courses along the DCT into the CNT and remains negative into the initial portion of the CCD.

With the use of new information about structural and molecular properties of rat superficial distal tubules, it is now possible to correlate electrophysiological with molecular results showing expression of specific transport proteins. The transepithelial voltage of the TAL is oriented with the lumen positive with respect to the interstitium. It typically ranges from 5 to 15 mV (107). Thick ascending limb cells extend 100–300 μm beyond the macula densa in both rabbit and rat (133). All investigators have reported that voltage of the CNT is oriented with the lumen negative with respect to the interstitium. Thus the transepithelial voltage must change from a lumen positive to lumen negative orientation between the region of the macula densa and the CNT. Schermann et al. (231) showed that the first 300 μm of rat DCT secretes NaCl in situ. They suggested that NaCl secretion creates a lumen-positive diffusion potential, owing to the higher permeability of these segments to Na$^+$ than to Cl$^-$. Thus the most proximal portion of DCT epithelium may be oriented with the lumen positive with respect to the interstitium. Interestingly, when Wright (296) plotted transepithelial voltage versus percent length along the superficial distal tubule, a sigmoidal relation was obtained (see Fig. 4). The voltage was very low along the first 40% of the tubule length, rapidly increased in the region between 40 and 60%, and remained constant along the terminal 30% of tubule length. The region between 40–60% of distal length was shown by Dorup (67) to be the site at which intercalated cells appear in the rat and to comprise primarily DCT and CNT cells. This region is also the site at which all three subunits of the epithelial Na$^+$ channel first appear (283). Thus the transepithelial voltage becomes negative in the region in which molecular pathways that mediate electrogenic Na$^+$ transport appear at the apical membrane.

Figure 4A also shows results of transepithelial voltage measurements of rat CCD, perfused in vitro. It is beyond the scope of this review to discuss properties of the CCD in detail, but it should be noted that the magnitude of the transepithelial voltage is smaller in collecting ducts perfused in vitro than in “late” distal tubules, perfused in vivo. This may reflect true differences in properties between collecting ducts and CNT or initial collecting tubules. A component of the difference is likely to reflect the environment in which the tubules are studied. Factors may be present in vivo, such as aldosterone and arginine vasopressin, that stimulate ion transport and that are removed during in vitro microperfusion. This is consistent with the observation that collecting ducts from rats...
treated with mineralocorticoid hormones and perfused in vitro display voltages nearly as large as those observed along the distal tubule in vivo (see Fig. 4A).

Figure 4B shows measurements of the transepithelial resistance of rat distal tubules, measured both in vivo and in vitro. Of note, the resistance is correlated inversely with the magnitude of the transepithelial voltage and therefore with the distance along the tubule. De Bermudez and Windhager (61) showed that the resistance of the distal tubule is reduced by arginine vasopressin, an effect that is more pronounced along the last 50% of distal tubule length than along the first half of distal tubule length. The decline in transepithelial resistance along the distal tubule probably reflects the larger component of electrogenic transport in more distal regions. Ion transport across the apical membrane of DCT cells appears to occur predominantly via electroneutral pathways, whereas transport across the apical membrane of CNT cells and principal cells is mediated in large part by electrogenic pathways. Both arginine vasopressin and aldosterone increase the luminal conductance for Na⁺ along the rat collecting duct (116, 218). Although the transepithelial resistance also reflects paracellular resistance, the profile observed along the distal tubule and the response of that profile to hormonal stimuli suggest that the axial differences in resistance may reflect cellular properties.

Transitions between segments of the rabbit distal nephron are abrupt, making it possible to isolate specific nephron segments for study in vitro. Data concerning the transepithelial voltage of rabbit distal tubules in vivo are not available, so a direct comparison with rat data is not possible. Unfortunately, despite discrete segmentation of the rabbit distal nephron, a great deal of variability has been observed in studies of rabbit distal segments perfused in vitro. This has led to confusion about electrical and physiological properties of rat and rabbit distal tubules. Figure 5A shows results of voltage measurements in rabbit DCT, CNT, and collecting ducts. Two studies during the 1970s indicated that the voltage of the rabbit DCT is large and oriented with the lumen negative, with respect to the bath (114, 124). Subsequently, studies conducted during the 1980s and 1990s have obtained voltages that are much lower (195, 239, 240, 283, 303). There are several explanations for the differences in transepithelial voltage between studies performed before 1980 and those performed subsequently. In the early experiments, the perfused DCT segments were as long as 800 μm (114). As noted by Imai and colleagues (241, 243), DCT segments are usually <500 μm in length in rabbits, and it is likely that the longer DCT segments contained CNT cells as well as DCT cells. Velázquez et al. (283) recently harvested DCT from rabbits in the usual manner by cutting at the transition from TAL to DCT and at the junction with the CNT. Each DCT segment was then transected a second time at its midpoint. When the proximal segments were perfused in vitro, the transepithelial voltage was 0 mV and was amiloride resistant. In contrast, when the more distal segments (still “DCT” segments) were perfused in vitro, the transepithelial voltage was lumen negative (−4 mV) and could be inhibited by amiloride (283). These results suggest that the large transepithelial voltages obtained from early studies resulted, in part, from contamination by CNT cells. A second cause for variability of membrane voltages is the tubule perfusion rate. The transepithelial voltage of distal nephron segments varies inversely with tubule perfusion rate and with perfusion pressure (114, 124, 235). Although the mechanisms by which perfusion pressure and flow rate affect voltage are not understood fully, the same effect is observed in vivo, during perfusion of distal tubules (111). In vivo, the effect appears to result predominantly from flow-dependent changes in luminal ion concentration (108). Regardless of the mechanisms by which luminal flow rate affects the transepithelial voltage, it appears that most measurements of transepithelial voltage in earlier studies were obtained using lower perfusion pressures. If one, therefore, uses data from four more recent studies performed in two different laboratories,
remarkably similar results are obtained. These indicate that the transepithelial voltage of the rabbit DCT is quite low when perfused in vitro (Fig. 5A), a result similar to that obtained from rat DCT segments perfused in vivo.

When CNT are perfused in vitro, they are usually obtained from arcade segments (see Fig. 1A), not from superficial distal tubules. Thus data on the properties of rabbit CNT cannot be compared directly with data on properties of “mid” or “late” segments of rat distal tubules. Nevertheless, both morphological and molecular information suggest that CNT from arcades are similar to CNT from superficial distal tubules (7, 50, 76, 199). The transepithelial voltage of CNT from rabbits perfused in vitro has ranged between 5 and 27 mV, oriented with the lumen negative with respect to the bath (see Fig. 5A). As is the case for other tubule segments, the voltage of CNT varies inversely with perfusion pressure; the highest voltage (~27 mV) reported for this segment was obtained at a very low perfusion rate (124).

The voltage of the rabbit CCT perfused in vitro is oriented in the lumen-negative direction and is low (average ~10 mV). The voltage is higher when animals or tubules have been treated with mineralocorticoid hormones (235); it is lower when animals have consumed a high-sodium low-potassium diet or after adrenalectomy (194). As is the case for the CNT and DCT, the transepithelial voltage varies inversely with fluid flow rate and perfusion pressure (235).

Differences between properties of rabbit and rat distal tubules have been emphasized (152), yet several of these differences may not be as great as previously suspected. Figure 5A might be taken to suggest that the transepithelial voltage does not change along the distal tubule of the rabbit. However, if one uses only the results from studies performed after 1980, and if one plots the data from collecting ducts of aldosterone-treated animals, the voltage profile is quite similar to that obtained in rat distal nephrons. One indication that mineralocorticoid treatment is necessary for in vitro data to more closely approximate the voltage profile observed in rat. The proposed similarities in electrical profiles between rat and rabbit distal tubules are strongly supported by recent observations on expression of electroneutral and electrogenic transport proteins by rabbit tubules (7, 199).

Figure 5B shows the transepithelial resistance of rabbit distal segments, perfused in vitro. Although the transepithelial resistance is highest in the CCT, this value reflects both transcellular and paracellular resistances.

Figure 5B also shows the fractional apical resistance of cells along the distal tubule (ignoring intercalated cells). The fractional apical resistance declines from DCT to CCT, and then further in the CCT. This indicates that the apical membrane of principal cells and CNT cells is more conductive than that of DCT cells. This conclusion is consistent with results of molecular studies.

Rabbit DCT cells have also been grown in culture as primary cultures. Whether grown for 15 or 30 days, cells exhibited a transepithelial voltage oriented with the apical surface negative with respect to the basolateral surface (77, 189). The magnitude of the voltage increased from 3 to 22 mV from 15 to 30 days. This voltage was inhibited by the Na⁺ channel blocker amiloride. The resistance of the epithelial monolayers was 500 Ω·cm² at 15–20 days and ~1,800 Ω·cm² at 30 days (189).

B. Na⁺ and Cl⁻ Transport

1. NaCl transport by the distal tubule

Information about the quantitative contribution of the distal tubule to renal NaCl transport derives primarily from micropuncture experiments in rats. When volume status ranges from low to high, Na⁺ delivery to the superficial distal tubule ranges from 4 to 20% (122, 144, 180). Because solute but not water is reabsorbed by TAL cells, the Na⁺ concentration in the lumen of the first accessible segments of distal tubules ranges from 35 to 77 mM (108). It should be recalled, however, that solute delivery to distal segments accessible to micropuncture reflects the actions of the post macula densa TAL and the proximal portion of the DCT. These sites lie proximal to the “earliest” distal puncture site in typical rats. Using Munich Wistar rats, which have post macula densa segments access to the kidney surface, Schnermann et al. (231) showed that luminal Na⁺ and Cl⁻ concentrations rise as fluid flows from the macula densa into the DCT, owing to Na⁺ and Cl⁻ secretion (231). Thus solute delivery to the most proximal portions of the DCT may be overestimated by measurements taken in superficial distal tubules.

When Na⁺ delivery to the DCT is varied acutely, Na⁺ transport varies directly. Khuri et al. (144) and Kunau et al. (158) increased distal Na⁺ delivery by infusing saline or urea-saline into hydropenic animals. Khuri et al. (144) found that 80% of the delivered Na⁺ load was reabsorbed along the distal tubule across a wide range of delivered loads. Kunau et al. (158) reported a similar relation between delivered load and transport when hydropenic animals were volume expanded slightly. When volume expansion was more extreme, however, these investigators reported that fractional Na⁺ reabsorption declined to 40% (158). Although these data suggest that the distal tubule responds to extracellular volume expansion by reducing the fractional NaCl reabsorption, Diezi et al. (63) showed...
that acute volume expansion does not alter Na\(^+\) transport along the distal tubule when Na\(^+\) and Cl\(^-\) delivery rates are controlled by microperfusion. The bulk of the increased NaCl transport along the distal tubule that occurs when luminal delivery increases results from enhanced transcellular transport via the thiazide-sensitive Na\(^+\)-Cl\(^-\) cotransporter (NCC). In microperfused rat distal tubules, raising the luminal NaCl concentration twofold increased transepithelial Na\(^+\) transport by a factor of 3; this increase could be blocked entirely by luminal chlorothiazide (79). The dependence of transepithelial NaCl transport on luminal NaCl concentration probably results from a dependence of the thiazide-sensitive NCC on extracellular Na\(^+\) and Cl\(^-\) concentrations (95).

Very recently, a numerical model of the renal distal tubule has been presented (41). The model incorporated 40 initial parameters and could successfully predict the behavior of the tubule during exposure to thiazide diuretics and amiloride.

2. Mechanisms of Na\(^+\) and Cl\(^-\) transport

A) DCT. Pathways for Na\(^+\) and Cl\(^-\) transport across the mammalian distal tubule have been investigated using functional, optical, electrophysiological, immunological, radioligand, and molecular techniques. These pathways are shown in Figure 3. Sodium-potassium-ATPase is expressed at the basolateral membrane of DCT cells, CNT cells, and principal cells (139). It provides the primary driving force for Na\(^+\) transport across all three segments by keeping the cellular Na\(^+\) concentration low and the cellular K\(^+\) concentration high. The Na\(^+\)-K\(^+\)-ATPase also contributes to making the inside of the cell electronegative with respect to the outside, both because it is electrogenic (moves 3 Na\(^+\) out and 2 K\(^+\) in) and because it generates large ion concentration gradients. Distal convoluted tubule cells express the highest Na\(^+\)-K\(^+\)-ATPase activity of any nephron segment (69, 80, 96, 141, 166), an observation that mirrors the extensive amplification of mitochondrial Na\(^+\) transport protein is postulated to possess two ion binding sites: one for anions and DCT diuretics and one for cations. Rate constants can be devised that permit the behavior of the model system to correspond to the behavior of the protein under a variety of physiological conditions.

A major pathway by which Na\(^+\) enters DCT cells across the apical membrane is the thiazide-sensitive NCC (see Fig. 3) (7, 23, 199, 206, 301). Thiazide diuretics were shown more than 30 years ago to inhibit Na\(^+\) transport along the superficial distal tubule (159), but the nature of the transport pathway(s) inhibited by these diuretics remained obscure. Velázquez et al. (278) showed that Na\(^+\) transport by distal tubules is dependent on the luminal Cl\(^-\) concentration and that Cl\(^-\) transport is dependent on the luminal Na\(^+\) concentration (278). These data suggested close coupling between Na\(^+\) and Cl\(^-\) transport by distal tubules. Stokes (260) reported the existence of a thiazide-sensitive electroneutral Na\(^+\)-Cl\(^-\) cotransport pathway in the bladder of the winter flounder. Ellison et al. (78) reported that a thiazide-sensitive electroneutral NCC mediates the majority of Na\(^+\) and Cl\(^-\) transport by DCT. Gamba et al. (95) used an expression cloning strategy to isolate a NCC from the bladder of the winter flounder. Northern blot analysis, using the probe from the flounder bladder, indicated that a related transcript is present in rat and mouse renal cortex. The thiazide-sensitive NCC was subsequently cloned from rat (94), human (246), mouse (160), and rabbit (281).

The mammalian renal thiazide-sensitive NCC has a core size of 110 kDa, but because it is glycosylated runs between 125 and 180 kDa on Western blots (23, 206). The protein is believed to comprise 12 membrane-spanning domains, with amino and carboxy termini within the cytoplasm. The protein is glycosylated on two asparagine residues on the fourth extracellular loop (198); deletion of both glycosylation sites (but not either one alone) creates a protein that is not functional. The sites on the cloned protein at which thiazide diuretics, Na\(^+\), and Cl\(^-\) bind have not been identified. Tran et al. (271) showed that Cl\(^-\) competitively inhibits [\(^3\)H]metolazone binding to kidney membranes, suggesting that the diuretic binds to the anion site on the transporter. The NCC is homologous to the bumetanide-sensitive Na\(^+\)-K\(^+\)-2Cl\(^-\) cotransporters (both secretory and absorptive isoforms, Ref. 125), and to the more recently described K\(^+\)-Cl\(^-\) cotransporter (105). These transporters are part of a gene family, the cation chloride cotransporters (118); the genes for this family are identified as SLC12, and the thiazide-sensitive NCC is SLC12A3.

Recently, Chang and Fujita (40) proposed a kinetic model of the thiazide-sensitive NCC. According to this model, mechanisms underlying the binding of ions and diuretics can be approximated by a state diagram. The transport protein is postulated to possess two ion binding sites: one for anions and DCT diuretics and one for cations. Rate constants can be devised that permit the behavior of the model system to correspond to the behavior of the protein under a variety of physiological conditions.

Functional data from rat microperfusion studies in vivo (52, 78) and isotopic studies of mouse DCT cells grown in vitro (100) indicate that the thiazide-sensitive NCC is expressed predominantly by DCT cells. In contrast, some results of experiments using microperfusion of rabbit distal segments in vitro have suggested expression by other cell types. In rabbit nephron segments, dissected and perfused in vitro, thiazide-sensitive Na\(^+\)-Cl\(^-\) cotransport was detected in CNT but not in DCT (238, 240, 303) by some, but not all (280), investigators. Some (267) but not other (220) investigators detected thiazide-sensitive Na\(^+\)-Cl\(^-\) transport in rat CCD perfused in vitro. In those experiments, pretreatment of animals with min-
eralocorticoid hormones was necessary to induce the thiazide-sensitive Na\(^+\) and Cl\(^-\) transport.

The cloning of the mammalian NCC has permitted sites of NCC expression to be determined at the molecular level. Whether assessed by in situ hybridization (7, 199), nephron-segment PCR (281, 301), or immunocytochemistry (23, 145, 206), the thiazide-sensitive NCC is expressed predominantly, if not exclusively, by DCT cells. This pattern of expression has been observed in rat, mouse, rabbit, and human. Thus, from a molecular standpoint, the NCC is expressed by DCT cells in all mammalian species examined to date. In rabbit, NCC expression is limited to DCT cells and ends abruptly at the transition to CNT. In human, rat, and mouse, expression of NCC extends from the proximal end of the DCT into a transitional segment. This transitional segment, referred to as the DCT-2 (199), shares properties of the distal convoluted and CNT and will be described more fully below (23, 199).

Cloning of the thiazide-sensitive NCC has provided definitive proof that a single protein couples Na\(^+\) and Cl\(^-\) transport. Data obtained by in vivo microperfusion of rat distal tubules indicated that the half-maximal luminal concentrations for transport were 9 mM for Na\(^+\) and 12 mM for Cl\(^-\) (278). These values contrast with Michaelis constant values of 25 for Na\(^+\) and 14 for Cl\(^-\), obtained by Eadie-Hofstee plots of data from Xenopus oocytes injected with the flounder transport protein (95). It is not clear whether the difference in mean affinity constant for Na\(^+\) reflects genuine physiological differences or differences in experimental technique.

There is evidence for other apical Na\(^+\) and Cl\(^-\) entry pathways in DCT cells. Wang et al. (289) showed that the addition of formate or oxalate to the lumen of distal tubule segments increases Na\(^+\) and volume absorption. They showed that this effect occurs in the “early” part of the distal tubule, presumably in the DCT. The effect could be inhibited by DIDS and by ethylisopropylamiloride, but not by thiazide diuretics. They interpreted these data as indicating that rat DCT cells express a Na\(^+\)/H\(^+\) exchanger, a Cl\(^-\)/formate exchanger, a Cl\(^-\)/oxalate exchanger, and a carbonate/oxalate exchanger. Recently, molecular confirmation that a Na\(^+\)/H\(^+\) exchanger is expressed at the apical membrane of rat DCT cells was presented. NHE-2 was shown to be expressed at the apical membrane of rat DCT cells (103), where it is coexpressed with the NCC (39). Although NHE-3 has been detected at the apical surface of TAL cells, it does not appear to be expressed by DCT (6).

The possibility that DCT cells express amiloride-sensitive Na\(^+\) channels has been a subject of controversy. Shortly after the epithelial Na\(^+\) channel (ENaC) was cloned, Duc et al. (71) studied its expression in rat kidney using in situ hybridization and immunocytochemistry. Those studies detected expression of \(\alpha\)-, \(\beta\)-, and \(\gamma\)-ENaC by the DCT, but the method for identifying this segment was not stated explicitly. Subsequent experiments utilizing in situ PCR showed expression of the \(\alpha\)-subunit of ENaC along the rat TAL and DCT (47); the same subunit was detected by PCR of immortalized mouse DCT cells (47). Other data, however, suggest that ENaC is not expressed uniformly by DCT cells. First, experiments in developing (230) and mature rats (76), using antibodies generated by Duc et al. (71), did not detect ENaC expression along the proximal portion of the DCT (the DCT-1). In the rabbit, nephron-segment PCR detected only the \(\alpha\)-subunit of ENaC in DCT cells; \(\beta\) and \(\gamma\) were not detected (283). The physiological significance of \(\alpha\)-ENaC expression alone by DCT cells remains unclear. The \(\alpha\)-subunit of ENaC is sufficient to generate a small but finite Na\(^+\) conductance, but conductance is increased severalfold by coexpression of the \(\beta\)- and \(\gamma\)-subunits. Noncoordinated expression of ENaC subunits has also been detected in the lung (87), and it is possible that expression of \(\beta\) and \(\gamma\) is regulated physiologically. Evidence against an important component of apical Na\(^+\) entry via Na\(^+\)/Pi transport (99, 102). The majority of Na\(^+\) uptake by these cells, however, is mediated by the thiazide-sensitive NCC and amiloride-sensitive Na\(^+\) channels.

Sodium appears to exit DCT cells across the basolateral membrane via the ubiquitous Na\(^+\)/K\(^+\)/ATPase, discussed above (see Fig. 3). Until recently, mechanisms of Cl\(^-\) exit remained obscure. Chloride is raised above electrochemical equilibrium within the DCT cell by the NCC. Recent data suggest that one important pathway for Cl\(^-\) to exit the cell across the basolateral cell membrane is a Cl\(^-\) channel (or channels) of the CLC family. Vandewalle et al. (285a) detected both CLC-K1 and CLC-K2 in microdissected Sprague-Dawley rat DCT segments. Immunochemical analysis, using an antibody that recognizes both CLC-K1 and CLC-K2, demonstrated abundant labeling of the DCT basolateral membrane (285a). In contrast, Uchida et al. (272) did not detect expression of the CLC-K by DCT segments using a different antibody (272). Very recently, Yoshikawa et al. (302) used in situ hybridization,
combined with immunocytochemistry with known markers, to identify sites of CLC-K expression. Whereas CLC-K1 was expressed in the medulla, CLC-K2 was expressed throughout the TAL, DCT, and CNT (302).

Another pathway by which Cl⁻ may exit from DCT cells is a K⁺/Cl⁻ cotransporter. Functional evidence for K⁺/Cl⁻ cotransport across DCT cells is discussed in section nC2, but there is also molecular evidence for renal expression of K⁺/Cl⁻ cotransporters (105, 120, 168). One K⁺/Cl⁻ cotransporter isoform, KCC1, was recently detected in DCT segments of rat kidney by in situ hybridization (168).

Other Na⁺ entry pathways have been detected in DCT cells in some species. The "housekeeping" isoform of the Na⁺/H⁺ exchanger, NHE-1, has been localized by immunocytochemistry to the basolateral surface of DCT cells in rabbit (17). Immortalized mouse DCT cells grown in vitro express a Na⁺/Ca²⁺ exchanger (102, 292; see below), which has also been detected in rat DCT-2 segments in situ (199). Rabbit DCT cells do not express the Na⁺/Ca²⁺ exchanger (7), but mouse (173) and probably human (199) DCT cells resemble those in rat.

Distal convoluted tubule cells do not express aquaporin-2, the vasopressin-sensitive water channel (50). The DCT therefore functions as the most distal portion of the diluting segment.

b) CNT. Mechanisms of Na⁺ and Cl⁻ transport by CNT cells have been studied using in vitro microperfusion, optical techniques, electrophysiological techniques, and molecular techniques. The major Na⁺ and Cl⁻ transport pathways are shown in Figure 3. Early studies showed that rabbit CNT isolated and perfused in vitro transport Na⁺ and K⁺ and exhibit a transepithelial voltage oriented in the lumen-negative direction (4, 124). Rates of Na⁺ transport were reported to be ~20 pmol·cm⁻²·s⁻¹, which is three or four times as great as rates in isolated CCD but similar to rates in isolated DCT segments perfused in vitro (4). Like DCT segments, CNT express the Na⁺/K⁺-ATPase at their basolateral surface. Although Na⁺/K⁺-ATPase expression levels are lower than in the DCT, they are higher than in the CCT (141).

Net Cl⁻ transport by rabbit CNT perfused in vitro was reduced 31% by 10⁻⁴ M trimethiazide, suggesting that the thiazide-sensitive NCC is expressed by this segment (240). Ouabain-induced swelling of CNT cells was prevented by the combination of trimethiazide and amiloride but not by either agent alone (237). These data were interpreted as a further indication that rabbit CNT cells express thiazide-sensitive NCC activity. Surprisingly, levels of NCC expression by rabbit CNT segments were either very low and variable (when detected by nephron segment PCR; H. Velázquez, personal communication) or absent (when detected by in situ hybridization). This is in contrast to NCC. This protein demonstrated robust expression in the rabbit DCT, whether detected by in situ hybridization (7) or nephron segment PCR (281). These results suggest that the component of thiazide-sensitive Cl⁻ transport detected when rabbit CNT are microperfused in vitro does not reflect high-level expression of the NCC. They indicate that the major site of NCC expression in rabbit, as in rat, is the DCT and not the CNT. The functional effects of thiazides in rabbit CNT may reflect low-level NCC expression or expression of thiazide-sensitive transporters that are structurally distinct from the NCC. One electroneutral Na⁺/H⁺ transporter shown recently to be expressed by CNT cells is NHE-2 (39). Its functional significance in this segment is, at this time, unclear. The Na⁺/H⁺ exchanger NHE-1 has been detected at the basolateral membrane of rabbit CNT cells (17).

 Whereas data suggesting expression of electroneutral NCC by rabbit CNT cells have been contradictory, evidence for expression of Na⁺ channels at the apical membrane of CNT has been more consistent. Rabbit CNT segments demonstrate a luminal-negative transepithelial voltage and electrogenic Na⁺ transport when perfused in vitro (4, 124, 239–241). Although it has not been possible to isolate and perfuse CNT from rats, micropuncture data, reviewed above, suggest that the magnitude of the transepithelial voltage increases along the superficial CNT in rat. Furthermore, both rabbit (283) and rat CNT segments have been shown to express ENaC at the apical membrane (71, 76, 230).

A role of CNT cells in mediating transepithelial chloride transport has not been well established. As discussed above, data from rabbit CNT perfused in vitro suggest that a component of transepithelial Na⁺ and Cl⁻ transport by this segment is sensitive to thiazide diuretics (240), yet expression of NCC by CNT cells (or intercalated cells) has been minimal in both rabbit or rat (7, 199, 283). The chloride channel CLC-K2 is expressed at the basolateral cell membrane of CNT (285a, 285b) (302). Connecting tubule cells, whether in the superficial distal tubule or in arcade segments, also express the vasopressin-regulated water channel and the vasopressin receptor (50, 148). Thus the junction between the DCT and CNT represents the transition between the diluting segment and the vasopressin-sensitive portion of the nephron.

3. Regulation of Na⁺ and Cl⁻ transport

As discussed in section nBI, the superficial distal tubule reabsorbs ~5–10% of the filtered NaCl load under many conditions. This means that NaCl transport by the superficial distal tubule is load dependent. In fact, luminal NaCl delivery appears to be the predominant regulator of NaCl cotransport acutely. Sodium reabsorption by distal tubules does not change during acute volume expansion if ion delivery is maintained constant (63); in contrast, extracellular fluid volume expansion depresses proximal solute and volume reabsorption independent of changes
in luminal delivery. Because it was difficult to demonstrate acute regulation of NaCl reabsorption by distal tubules, few studies were conducted to examine the chronic regulation of ion transport by the superficial distal tubule. More recent data, however, indicate that ion transport by the distal tubule is regulated by physiological control systems. These physiological regulators include steroid and peptide hormones, metabolic and dietary factors, and pathological events, such as ischemia. Factors purported to regulate NCC function are shown in Table 1.

TABLE 1. Regulation of NaCl transport by the distal convoluted tubule

<table>
<thead>
<tr>
<th></th>
<th>NaCl Transport</th>
<th>[3H]metolazone Binding</th>
<th>NCC Message</th>
<th>NCC Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hormones</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aldosterone</td>
<td>↑(275)</td>
<td>↑(43, 275)</td>
<td>↔ (Ellison, unpublished data)</td>
<td>↑(145)</td>
</tr>
<tr>
<td>Glucocorticoids</td>
<td>↑(275)</td>
<td>↑(43)</td>
<td>↑(286)</td>
<td></td>
</tr>
<tr>
<td>Estrogen</td>
<td>↑(286)</td>
<td>↑(43)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adrenomedullin</td>
<td>↑(290)</td>
<td>↑(21)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Angiotensin II</td>
<td></td>
<td>↑(21)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amylin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcitonin</td>
<td>↑(74)</td>
<td>↑(20)</td>
<td></td>
<td></td>
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<tr>
<td><strong>Diuretics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Furosemide</td>
<td>↑(79, 255)</td>
<td>↑(45, 199)</td>
<td>↑(199)</td>
<td>↑(Ellison, unpublished data)</td>
</tr>
<tr>
<td>HCTZ</td>
<td>↓(193)</td>
<td>↑(45, 193)</td>
<td>↓(172)</td>
<td>↔ (172)</td>
</tr>
<tr>
<td><strong>Metabolic and dietary factors</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metabolic alkalosis</td>
<td></td>
<td>↑(83)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metabolic acidosis</td>
<td></td>
<td>↓(83)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low NaCl intake</td>
<td>↑(79)</td>
<td>↔ (45)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Ischemia</strong></td>
<td></td>
<td>↓(12)</td>
<td>↓(291)</td>
<td>↓(73)</td>
</tr>
<tr>
<td><strong>Other factors</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Renal nerves</td>
<td>↑(15)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-Adrenergic receptors</td>
<td>↑(99)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

↑, Increased; ↓, decreased; ↔, unchanged. Unless otherwise noted, these factors appear to increase thiazide-sensitive component of Na\(^+\) and Cl\(^-\) transport by distal tubules. Other hormones that have been examined include glucagon (and somatostatin) and parathyroid hormone. Neither of these has altered the thiazide-sensitive component of Na\(^+\) or Cl\(^-\) transport (56, 187). NCC, Na\(^+\)-Cl\(^-\) cotransport; HCTZ, hydrochlorothiazide.

A) DIETARY NaCl INTAKE AND ADRENAL STEROID HORMONES. Mineralocorticoid hormones contribute importantly to Na\(^+\) homeostasis under both normal and pathological conditions. Although glucocorticoid hormone receptors are present throughout the nephron (269), mineralocorticoid receptors are expressed primarily by distal nephron segments (269). Coupled with clearly evident effects of mineralocorticoids on principal cells, these results have been used to suggest that mineralocorticoids act predominantly along the collecting duct and not in the DCT (70, 219, 249). This view, however, is now being modified in light of more recent results. These more recent results are in agreement with very early studies that were later ignored. An example is the report of Hierholzer et al. (119) who studied the effects of adrenal steroids on Na\(^+\) and K\(^+\) transport by superficial distal tubules of rats. Their data indicated that adrenalectomy increases the tubule fluid-to-plasma Na\(^+\) ratio in distal but not proximal tubules (see Fig. 6A). Although these changes were later ascribed to effects on connecting and collecting duct cells, inspection of the data reveals that the mineralocorticoid effects are as large along the first 40% of tubule length (which would comprise exclusively DCT cells, Ref. 67) as they are along the last 40%. The effects of adrenalectomy could be reversed completely by infusing aldosterone, indicating that they resulted from mineralocorticoid action. These changes in luminal NaCl concentration occurred without any change in transepithelial voltage. When...
viewed in light of more recent information (reviewed below), these results are consistent with an effect of adrenalectomy and mineralocorticoid hormones on activity of the NCC in DCT cells.

Studies using isolated dissected rabbit kidney tubules detected minimal binding of $[^3H]$aldosterone to DCT segments (70, 84–86). When binding was examined in situ by autoradiography, however, binding along the DCT (and TAL) was detected, albeit at higher aldosterone concentrations (1.5–20 nM) (85). Similar results were obtained in rats, in which binding to DCT segments was detectable at 2 but not 0.2 nM $[^3H]$aldosterone (85). More recently, after the cloning of steroid receptors, it has become possible to localize mineralocorticoid receptors at the molecular level. Antibodies generated against the mineralocorticoid receptor were found to localize to the DCT, the CNT, and the CCT in rat (23, 156), human (247), and rabbit (174). In the only experiments to use nephron-segment PCR to detect mineralocorticoid receptors, neither DCT nor CNT segments were tested (269). Thus, in all species in which molecular evidence has been obtained, mineralocorticoid receptors were shown to be expressed by DCT cells (and CNT cells) as well as principal cells.

More recent functional and molecular data confirm the importance of mineralocorticoid hormones in regulating NaCl transport by DCT (see Table 1). Stanton et al. (250) reported that low physiological concentrations of mineralocorticoid hormones increased Na$^+$ transport by the superficial distal tubule, but this was attributed to an effect on the late distal tubule (250). Ellison et al. (79) reported that dietary NaCl restriction increased thiazide-sensitive Na$^+$-Cl$^-$ cotransport capacity in rats. Chen et al. (43) and Velázquez et al. (275) reported that mineralocorticoid and glucocorticoid hormones both increase the number of $[^3H]$metolazone binding sites when administered to adrenalectomized animals (see Fig. 6D). The number of $[^3H]$metolazone binding sites was taken as an indication of the number of thiazide-sensitive NCC. The combination of glucocorticoid and mineralocorticoid hormones increased the number of receptors more than did either hormone alone. Velázquez et al. (275) showed that mineralocorticoid hormones strongly stimulate thiazide-sensitive NCC activity (see Fig. 6C). In those experiments, thiazide-sensitive Na$^+$-Cl$^-$ cotransport was barely detectable in adrenalectomized rats. High physiological doses of either aldosterone or dexamethasone increased transport capacity by up to 20-fold (275). The increase in ion transport did not result only from increases in luminal NaCl delivery, because they persisted when the luminal NaCl delivery was controlled by microperfusion. Recently, Kim et al. (145) showed that aldosterone increased expression of thiazide-sensitive NCC (see Fig. 6B), as determined by Western blot and immunocytochemistry of rat kidney cortex. The effects of aldosterone to increase expression of the NCC protein were observed whether in the setting of high (aldosterone infusion) or low (NaCl restriction) extracellular fluid volume. Taken together, these results indicate that both the activity and the abundance of the NCC are regulated by adrenal steroid hormones. It appears that high levels of either glucocorticoid hormones or mineralocorticoid hormones increase thiazide-sensitive transport activity.

Aldosterone specificity is maintained in target tissues because the metabolic enzyme 11$\beta$-hydroxysteroid dehydrogenase is expressed. Endogenous glucocorticoid and mineralocorticoid hormones bind to the mineralocorticoid receptor with equal affinity. Glucocorticoid hormones typically circulate at concentrations that are 100- to 1,000-fold higher than mineralocorticoid hormone concentrations. 11$\beta$-Hydroxysteroid dehydrogenase metabolizes glucocorticoid hormones in mineralocorticoid target cells, leaving mineralocorticoids intact. Recently, Bostanjoglo et al. (23) showed that 11$\beta$-hydroxysteroid dehydrogenase colocalizes with the NCC in rat distal tubules. In rabbit, Veláquez et al. (281) showed that 11$\beta$-hydroxysteroid dehydrogenase message colocalizes with message for the NCC in DCT segments (281). In the rat, although 11$\beta$-hydroxysteroid dehydrogenase was expressed by DCT cells, expression was not uniform (23). It was present at low to undetectable levels in the DCT-1 (the most proximal portion of the the DCT) and at higher levels along the DCT-2 (the more distal portion of the DCT). Even along the DCT-2, however, expression of 11$\beta$-hydroxysteroid dehydrogenase was lower than expression along the CNT.

Dietary NaCl intake is a powerful determinant of circulating aldosterone levels. Although the effects of aldosterone on NCC abundance and activity suggest that dietary NaCl intake may regulate NCC abundance and activity, these effects have not been as consistent. In rats, dietary NaCl restriction has been reported to increase thiazide-sensitive Na$^+$-Cl$^-$ transport by threefold (79) and to increase NCC protein expression twofold (145). Dietary NaCl restriction, however, was not shown to increase the number of $[^3H]$metolazone binding sites in kidney cortex (82) or to increase NCC message expression (192). In the rabbit, dietary NaCl loading, which suppresses aldosterone secretion, increases the fractional volume of DCT cells and decreases basolateral cell membrane amplification (134) and Na$^+$-K$^+$-ATPase activity (166). These morphological effects of high dietary NaCl intake are accompanied by increases in the transport capacity of DCT cells, perfused in vitro (243).

These data can be used to construct the following hypothesis concerning regulation of ion transport by the distal tubule of the rat kidney. Adrenal steroid hormones increase NCC activity at least in part by increasing NCC protein expression. This action of mineralocorticoid hormones may be predominantly along the DCT-2, a segment that expresses significant levels of 11$\beta$-hydroxysteroid
dehydrogenase. As in the collecting duct (134), the effect of mineralocorticoid hormones may be enhanced when luminal NaCl delivery is high. Thus the effects of dietary NaCl restriction (high aldosterone/low distal NaCl delivery) on NCC abundance are blunted, compared with the effects of exogenous mineralocorticoid administration (high aldosterone/high distal NaCl delivery). Based on the absence of effects of dietary NaCl restriction on NCC mRNA levels (192), these effects of mineralocorticoid hormones on NCC protein abundance may occur predominantly at the level of protein synthesis or protein stability. Glucocorticoid hormones may act predominantly along the DCT-1, a segment that expresses very low levels of 11β-hydroxysteroid dehydrogenase. Nevertheless, the rat DCT may be more promiscuous than either the CNT or CCT with respect to steroid hormone specificity because levels of 11β-hydroxysteroid dehydrogenase expression are intermediate, lower than in the CNT, and higher than in more proximal segments. These variations may have physiological relevance. When aldosterone concentrations are increased moderately, electrogenic Na^{+} transport along the CNT and CCT may be stimulated via the action of mineralocorticoids. When volume depletion is more extreme, circulating mineralocorticoid levels may be sufficient to stimulate Na^{+}-Cl⁻ cotransport along the more distal portions of the DCT. When volume status is severely reduced, stress-induced glucocorticoid secretion may stimulate Na^{+} reabsorption along the entire distal tubule and in more proximal segments. Based on molecular and morphological similarities, regulation of DCT cells in humans may be similar to that in rodents.

The CNT is the site at which the enzyme 11β-hydroxysteroid dehydrogenase is expressed at its highest level (23). Mineralocorticoid receptors are also expressed by CNT cells (23). These data suggest that aldosterone participates importantly in regulating ion transport by CNT (130, 131, 134, 166).

It is more difficult to construct a unifying hypothesis that explains all the results from experiments in rabbit. In the rabbit, high dietary NaCl intake increases DCT cell size, probably by increasing distal NaCl delivery and NaCl transport. Nevertheless, rabbit DCT cells coexpress the thiazide-sensitive NCC with 11β-hydroxysteroid dehydrogenase. One possibility is that dietary NaCl loading drives high-level transepithelial Na^{+} transport along the DCT even in the absence of aldosterone. This leads to morphological and functional changes. When low dietary NaCl intake raises aldosterone in the setting of low NaCl delivery, aldosterone may preserve NaCl reabsorption, preventing volume depletion. It appears, however, that transepithelial transport of salt is the primary stimulus to cell growth.

b) Sex steroids. Chen et al. (44) reported that the densities of [³H]metolazone receptors were higher in kidneys from female than male rats (see Table 1). This observation correlated with an increased sensitivity to thiazide diuretics in female animals. They noted that ovariectomy reduced the number of [³H]metolazone receptors, suggesting that estrogens increase expression of activated NCC. Verlander et al. (286) reported that estrogen increases the amount of NCC protein in rat kidney cortex and increases the complexity of the apical plasma membrane of the DCT. In preliminary results, Verlander and colleagues (271) showed that estrogens increase expression of the NCC in immortalized mouse DCT cells, grown in vitro. These data were interpreted as suggesting that the effects of estrogen to increase NCC expression are a direct effect of the hormone on receptors in distal tubule cells.

c) Peptide hormones. Three peptide hormones have been reported to increase the density of [³H]metolazone binding sites in rat kidney. Amylin is a peptide secreted by β-cells of the pancreas in response to nutrient stimuli. Administration of this peptide increased the density of [³H]metolazone binding sites 32–58% (21). Administration of the structurally related peptide adrenomedullin also increased [³H]metolazone density by ~30% (21). The physiological significance of these observations remains unclear.

Angiotensin II increases, and [Sar¹,Ile⁸]ANG II decreases, Na^{+}, fluid, and HCO₃⁻ transport along the superficial distal tubule (290). A component of this effect was attributed to cells of the early distal tubule, presumably DCT cells. It was suggested that the increase in Na⁺ transport reflected stimulation of Na⁺/H⁺ exchange, although data were not presented to support this directly. Along the late distal tubule, probably primarily the CNT, angiotensin II stimulation of Na⁺ transport was believed to reflect increases in amiloride-sensitive electrogenic Na⁺ transport. Some other peptide hormones have been shown not to affect Na⁺ transport along the distal tubule. These include glucagon, somatostatin, and parathyroid hormone (8, 56, 187).

d) Diuretic Drugs. Chronic diuretic treatment has profound effects on the structure and function of the DCT. Kaisling and colleagues (130–132) showed that chronic furosemide infusion in rats causes hypertrophy and hyperplasia of DCT cells. Diuretic-induced hypertrophy was associated with increases in basolateral cell membrane amplification (132) and Na⁺-K⁺-ATPase activity (226). The functional activity of the thiazide-sensitive NCC was increased approximately threefold after 1 wk of furosemide infusion (79). The effects of chronic diuretic infusion on ion transport and cell morphology were shown to occur even if circulating mineralocorticoid and glucocorticoid levels were maintained constant (135, 255). The effects were also shown to occur even if arginine vasopressin concentrations were maintained at high and constant levels by hormonal infusion. Based on earlier experiments in rabbits, wherein high dietary NaCl intake led to
hypertrophy of DCT cells, and based on the ability of furosemide to induce structural changes even when steroide hormones were controlled, Stanton and Kaissling (256) postulated that the effects resulted from increases in distal NaCl delivery and NaCl transport. These observations, however, do not exclude a role of steroid hormones in contributing to the changes observed during chronic diuretic administration. Chen et al. (43) and later Obermüller et al. (199) showed that chronic furosemide infusion increased the density of [3H]metolazone receptors by up to 100%, suggesting the chronic furosemide infusion increases expression of the NCC. Obermüller et al. (199) detected an increase in NCC mRNA by in situ hybridization after chronic diuretic infusion. Moreno et al. (192), however, did not detect a significant increase in NCC message after once-daily furosemide administration. Whether the difference in results reflected more intense stimulation in the setting of constant diuretic infusion is unknown. Of note, Moreno et al. (192) reported that NCC message levels increased after dehydration. The authors suggested that the results of Obermüller et al. (199) may have reflected effects of dehydration. Venkatachalam and co-workers (150) showed that chronic loop diuretic infusion increases expression of both insulin-like growth factor (IGF) and insulin-like growth factor binding protein (IGFBP) in distal tubules. These investigators postulated that the morphological changes that occur during chronic diuretic infusion result, at least in part, from activation of growth factors. Beck et al. (14) studied the effects of inhibiting angiotensin converting enzyme on diuretic-induced hypertrophy of the distal nephron. Administration of an angiotensin-converting enzyme inhibitor did not block the development of hypertrophy, but instead converted the predominant effect from one in which tubule cells thickened to one in which tubule length increased (14).

If increased transepithelial ion flux drives the structural and functional effects of chronic loop diuretic infusion, then administration of diuretics that block such transport would be expected to have opposite effects. Morsing et al. (193) administered thiazide diuretics chronically to rats and measured the functional activity of the DCT. After 10 days of thiazide infusion, the ability of the distal tubule to reabsorb Na⁺ and Cl⁻ was reduced significantly. Surprisingly, this reduction was associated with an increase in the number of [3H]metolazone binding sites. A similar result was obtained by Chen et al. (45), who found that chronic thiazide infusion increased the number of [3H]metolazone binding sites (45). Loffing et al. (172) showed that chronic thiazide infusion in rats led to apoptosis of DCT cells and destruction of the epithelium. This was associated with decreases in the amount of NCC protein, as determined by Western blot. They postulated that DCT cells are dependent on Na⁺ entry via the apical membrane to maintain their structural and functional integrity. They suggested that thiazide diuretics, by blocking the major Na⁺ entry pathway, disrupt this process leading to structural changes.

E) ISCHEMIA. Beaumont et al. (12) noted that warm ischemia reduced the number of [3H]metolazone binding sites in rat kidney cortex. As little as 10 min of warm ischemia was sufficient to reduce the number of receptors significantly, and 30 min reduced them close to background. Wang et al. (291) studied the effects of ischemia in situ on expression of message for NCC. They found reductions in NCC mRNA expression 24 h after an ischemic insult, but not before. Edelstein et al. (73) showed that ischemia and reperfusion led to reductions in NCC protein expression that began within 15 min and lasted more than 24 h. These findings suggest that decreases in NCC expression may contribute to the high fractional Na⁺ excretion that characterizes ischemic renal injury.

F) ACID-BASE ABNORMALITIES. Recently, Fanestil et al. (83) reported that chronic administration of NaHCO₃ increased and chronic NH₄Cl decreased the density of receptors for [3H]metolazone.

C. K⁺ Transport

1. K⁺ transport by the distal tubule

Filtered K⁺ is reabsorbed along the proximal tubule and loop of Henle; ~10% of filtered K⁺ reaches the DCT. Early micropuncture work established the superficial distal tubule as an important site of K⁺ secretion. Many studies indicated that K⁺ secretion along the superficial distal tubule accounted for most or all of urinary K⁺ excretion under a variety of conditions. In fact, Jamison et al. (126) summarized the existing literature as indicating that the “principal site of the regulation of K⁺ excretion is the distal tubule.” He noted that the collecting duct probably plays an important role as well because K⁺ delivery to the end of the superficial distal tubule could not fully account for K⁺ excretion under some conditions (126). Wright (297) reviewed data concerning sites of K⁺ secretion and emphasized the importance of the late distal tubule. He noted that this segment probably comprises two structurally distinct segments, the CNT and the initial portion of the CCD. He raised the possibility that ion transport mechanisms in the two segments might be different.

More recently, regulation of K⁺ excretion has been viewed as determined primarily by principal cells of the cortical (and initial) collecting tubule (104, 299). This view has resulted from morphological and functional data demonstrating conclusively that principal cells secrete K⁺. Also most of the factors that regulate K⁺ excretion have been shown to regulate K⁺ secretion by principal cells. This review does not address the important role of principal cells and of the CCD in renal K⁺ homeostasis,
nor will it provide a detailed description of factors that regulate K\(^+\) secretion by principal cells. Mechanisms and control of K\(^+\) excretion by the kidney and by the collecting duct have been reviewed extensively (104, 299). Instead, this review emphasizes the important role that DCT and CNT cells play in K\(^+\) homeostasis and discusses insights that derive from a synthesis of recent molecular results with earlier physiological results.

Micropuncture studies indicated that the luminal concentration of K\(^+\) along the first 20–30% of the superficial distal tubule is low (297). This portion of the tubule is lined by DCT cells (67). In one study, when early distal tubules were perfused separately from late distal tubules, rates of K\(^+\) secretion by early tubules were found to be low and did not reach statistical significance (251). These data suggested that the DCT does not contribute importantly to K\(^+\) secretion. More recently, however, this view has been revised. Velázquez et al. (277) microperfused segments of the DCT that lay within the initial 40% of tubule length. These segments, which would be expected to comprise only DCT cells (67), did secrete K\(^+\) at low but significant rates under control conditions. When the luminal Cl\(^-\) concentration was reduced, rates of K\(^+\) secretion rose substantially (see below).

A plot of the luminal K\(^+\) concentration along the length of the distal tubule juxtaposed with the axial distribution of cell types (see Fig. 7) shows that a large percentage of K\(^+\) secretion occurs between 20 and 80% of tubule length. This portion of the distal tubule comprises primarily DCT, CNT, and intercalated cells. As discussed in section IV C2, pathways that can participate in K\(^+\) secretion have been identified in both DCT-2 cells and in CNT cells. In view of the fact that deep nephrons...
join each other via arcades lined with CNT cells, it is likely that CNT cells play a central role in renal K\(^+\) homeostasis.

2. Mechanisms of K\(^+\) transport

A) DCT cells. Two mechanisms of K\(^+\) secretion across the apical membrane of DCT cells have been proposed (Fig. 3). Based on the effects of low luminal Cl\(^-\) concentrations to stimulate K\(^+\) secretion, Velázquez et al. (277) proposed that a K\(^+\)-Cl\(^-\) cotransport pathway is expressed at the luminal membrane of DCT cells. A second apical K\(^+\) secretory pathway is a K\(^+\) channel. Antibodies against ROMK show expression at the apical membrane of DCT-1, DCT-2, and CNT cells (see Fig. 3) (186, 300). It seems likely that the low rates of K\(^+\) secretion by the DCT-1 (the early distal tubule) that are observed in vivo under typical conditions reflect the absence of electrogenic Na\(^+\) reabsorption and the low transepithelial voltage in this segment (as discussed in sect. ivC1) rather than the absence of K\(^+\) secretory pathways.

Along the DCT-2, K\(^+\) secretion is more rapid and contributes importantly to net K\(^+\) excretion under typical conditions. This results because Na\(^+\) channels are expressed by DCT-2 cells at the apical membrane (47, 102, 230). These channels depolarize the membrane, driving K\(^+\) from cell to lumen. This group of cells also expresses the thiazide-sensitive NCC at the apical membrane as well as mineralocorticoid receptors and the enzyme 11β-hydroxysteroid dehydrogenase (23, 230).

B) CNT cells. Connecting tubule cells are distinguished at the molecular level by the absence of the thiazide-sensitive NCC at the apical membrane and the presence of the Na\(^+\)/Ca\(^2+\) exchanger at the basolateral membrane (see Fig. 3; Ref. 199). Connecting tubule cells are designed optimally to secrete K\(^+\), with both Na\(^+\) channels and K\(^+\) channels expressed at their apical membrane (71, 230, 300). Furthermore, their abundance of mitochondria and more amplified basolateral membrane, compared with principal cells, would be expected to confer a large ion transport capacity. Interestingly, recent immunocytochemical experiments have suggested that CNT cells express the highest levels of the enzyme 11β-hydroxysteroid dehydrogenase (23).

C) PRINCIPAL CELLS. Clearly, principal cells of the initial and CCD contribute importantly to systemic K\(^+\) homeostasis. Their role has been reviewed recently (104) and is not emphasized here.

3. Regulation of K\(^+\) transport

Many factors regulate K\(^+\) secretion along the distal tubule. These have been reviewed recently (104, 299). Factors that act from the peritubular side to increase K\(^+\) secretion along the distal tubule include elevations of K\(^+\) (253), aldosterone (88), vasopressin (89), and angiotensin II (290). A low H\(^+\) concentration also stimulates K\(^+\) secretion (252). Basolateral factors that inhibit K\(^+\) secretion along the distal tubule include metabolic acidosis (252) and hypokalemia (253).

Factors that influence K\(^+\) secretion along the distal tubule from the luminal side include the luminal concentrations of Na\(^+\), K\(^+\), and Cl\(^-\) and the luminal fluid flow rate (299). Luminal inhibitors of K\(^+\) secretion include organic cations, such as amiloride, triamterene, trimethoprim, and pentamidine, as well as inorganic cations, such as Ca\(^2+\) (see Fig. 8). Because these factors have received less attention, but appear to be potent and clinically significant, they are discussed in more detail in section ivC3.

Increases in distal flow rate and luminal Na\(^+\) concentration both stimulate K\(^+\) secretion by distal tubules. It is likely that reducing the luminal Na\(^+\) concentration below a critical level inhibits K\(^+\) secretion by its effects on cellular Na\(^+\) concentration and activity of the Na\(^+\)-K\(^+\)-ATPase pump (108). This is supported by the parallel reductions in K\(^+\) secretion and transepithelial voltage that occur when luminal Na\(^+\) concentration is reduced below 40 mM (108). As noted by Good et al. (108), however, the luminal Na\(^+\) concentration in fluid entering the distal tubule usually ranges from 38 to 77 mM, even under conditions of low dietary NaCl intake. Thus the luminal Na\(^+\) concentration would not limit K\(^+\) secretion along the distal tubule unless severe extracellular fluid volume contraction reduced the luminal Na\(^+\) concentration below 40 mM. It should be emphasized that the luminal Na\(^+\) concentration can and does frequently decline below 40 mM.
along the collecting duct. If the same relation between Na\(^+\) concentration and K\(^+\) secretion holds in that segment, then luminal Na\(^+\) may be limiting to K\(^+\) excretion, even under conditions of mild extracellular fluid contraction.

Fluid flow rate also affects K\(^+\) secretion along the distal tubule (110, 143). In a series of microperfusion experiments, when the luminal flow rate was increased from 6 to 26 nl/min, K\(^+\) secretion nearly doubled (110). In contrast to the case of luminal Na\(^+\) concentration, discussed above, this range of flow rates is well within the range observed under free flow conditions of hydropenia and mild volume expansion. Thus the flow rate of fluid entering the distal tubule is likely to have a large effect on K\(^+\) secretion on a day-to-day basis. An important mechanism mediating the flow-induced increase in K\(^+\) secretion results from changes in luminal K\(^+\) concentration. When the flow rate is low, luminal K\(^+\) concentrations increase dramatically along the length of the distal tubule, owing to continued secretion. In contrast, when the flow rate is high, luminal K\(^+\) concentrations remain lower. The lower luminal K\(^+\) concentration permits continued K\(^+\) secretion along the length of the distal tubule (298).

Another factor that alters K\(^+\) excretion in humans and animals is diuretic drugs. Loop and DCT diuretics (thiazides and others) increase urinary K\(^+\) excretion and can cause hypokalemia. A factor that contributes to diuretic-induced K\(^+\) wasting is contraction of the extracellular fluid volume and hyperaldosteronism (293). Another factor is the increase in distal flow rate. If distal Na\(^+\) delivery is markedly reduced, increases in distal Na\(^+\) delivery may also contribute. Loop diuretics inhibit K\(^+\) reabsorption along the TAL and increase K\(^+\) delivery to the DCT (122, 284). Thus a component of the K\(^+\) wasting that results from loop diuretic administration reflects direct actions of the drugs, yet DCT diuretics such as the thiazides do not alter K\(^+\) secretion directly (284), at least under normal conditions. However, DCT diuretics are potent kaliuretic drugs. One factor contributing to this effect is that DCT diuretics increase Ca\(^{2+}\) absorption by the DCT. This reduces the luminal concentration of Ca\(^{2+}\) in the late distal tubule. Calcium acts in the distal tubule to inhibit K\(^+\) secretion indirectly, by blocking Na\(^+\) channels (see Fig. 8, Ref. 201). This observation may explain part of the difference in kaliuretic potency of loop and DCT diuretics. In contrast to the effects of DCT diuretics, loop diuretics increase distal Ca\(^{2+}\) delivery, which would be expected to inhibit K\(^+\) secretion.

Under some conditions, however, thiazide diuretics may inhibit K\(^+\) secretion by the distal tubule. When the luminal concentration of Cl\(^-\) in the distal tubule is low, the relation between luminal Na\(^+\) concentration and K\(^+\) secretion changes. Velázquez et al. (285) showed that raising luminal Na\(^+\) concentration from 40 to 150 mM stimulates K\(^+\) secretion by distal tubules perfused with a Cl\(^-\)-free solution (285). They showed that this effect occurred primarily along the early distal tubule and suggested that it reflected transport via an apical K\(^+\)-Cl\(^-\) cotransport pathway. Figure 8 shows a model apical membrane of a DCT cell (a DCT-2 cell). According to this hypothesis, the K\(^+\)-Cl\(^-\) cotransport pathway is activated only when the luminal Cl\(^-\) concentration is low. In this case, Cl\(^-\) recycles back into the cell via the thiazide-sensitive NCC. Under conditions of low luminal Cl\(^-\) concentrations, DCT diuretics should inhibit K\(^+\) secretion by reducing the availability of Cl\(^-\) in the cell. This hypothesis was supported by data showing that chlorothiazide inhibited K\(^+\) secretion during perfusion with a Cl\(^-\)-free solution. Chlorothiazide was also shown to reduce K\(^+\) excretion during infusion of sodium sulfate (which reduces urinary Cl\(^-\) concentrations) (276).

Organic cations may also affect K\(^+\) secretion by the distal tubule. Amiloride blocks epithelial Na\(^+\) channels and thereby reduces K\(^+\) secretion along the distal tubule and collecting duct. Triamterene appears to act in much the same manner (37). More recently, it has been recognized that other organic cations, used primarily as antimicrobial substances, can also block epithelial Na\(^+\) channels and cause hyperkalemia in patients. It is postulated that these drugs, like amiloride, enter the pore region of Na\(^+\) channels (203) leading to channel blockade. It seems likely that other organic cations may have similar “amiloride-like” effects on the distal tubule.

D. Ca\(^{2+}\) Transport

1. Ca\(^{2+}\) transport by the distal tubule

Total body Ca\(^{2+}\) homeostasis is maintained by the interplay of three processes: intestinal absorption, bone resorption and formation, and urinary excretion. Net intestinal absorption of Ca\(^{2+}\) amounts to ~200 mg of the normal dietary intake of 1,000 mg. In the steady state, this net absorption is matched by urinary excretion. As a result, 10,600 mg of Ca\(^{2+}\) filtered daily must be reabsorbed by the kidney (57). Thereby, renal Ca\(^{2+}\) reabsorption plays an important role in Ca\(^{2+}\) homeostasis.

It is generally accepted that the bulk of the filtered load of Ca\(^{2+}\) (60–70%) is reabsorbed in the proximal tubule largely by passive means through the paracellular pathway and only ~10–15% of the filtered load reaches the distal tubule. The mammalian distal nephron, despite reabsorbing only ~10% of the filtered load of Ca\(^{2+}\), is the primary target site for all of the major Ca\(^{2+}\) regulatory hormones (57). In addition, most or all of the systemic factors that affect renal Ca\(^{2+}\) excretion also affect Ca\(^{2+}\)
reabsorption by the distal nephron. Micropuncture studies have shown that Ca\(^{2+}\) reabsorption in the distal nephron must be active given that the luminal concentration of Ca\(^{2+}\) is below that of blood and the transepithelial voltage is lumen negative (164). The passive permeability of the distal nephron for Ca\(^{2+}\) is very low. As the amount of Ca\(^{2+}\) delivered to the distal tubule increases there is a proportionate increase in reabsorption (55, 113). The primary nephron site of this adaptive response is the early distal tubule in the rat.

As discussed in section ii, there are appreciable species differences in the molecular structure of the distal nephron. In addition, a variety of functional differences have been observed. In the rabbit, calcitonin (1 nM) significantly increased Ca\(^{2+}\) flux from 1.58 ± 0.29 to 4.45 ± 1.01 pmol/min but had no effect in either the CNT or the CCD. Whereas parathyroid hormone (PTH; 1 nM) increased Ca\(^{2+}\) flux in the CNT from 2.28 ± 0.35 to 9.44 ± 1.13 pmol/min but had no effect in either the DCT or the collecting duct (242). In the rabbit, the CNT is the predominant site of both PTH-stimulated adenylyl cyclase and Ca\(^{2+}\) reabsorption. In contrast, in humans and rats, PTH stimulates adenylyl cyclase in the DCT. In isolated perfused rabbit CNT, PTH increased intracellular Ca\(^{2+}\) by a mechanism that was dependent on extracellular Ca\(^{2+}\) (27). This suggested that PTH activates an apical Ca\(^{2+}\) entry process that is subsequently followed by extrusion of Ca\(^{2+}\) across the basolateral membrane.

2. Mechanisms of Ca\(^{2+}\) transport

The nephron segment localization of the Ca\(^{2+}\) transport-related proteins has been investigated using functional, immunological, and molecular methods (see Fig. 9). Discrepancies exist between studies, some of which may be due to differences in the species examined (7, 199) or the methods used (28, 30, 65, 66, 92, 242, 265, 273).

The effects of PTH on distal tubular Ca\(^{2+}\) transport are well known. Parathyroid hormone binds to its receptor in the basolateral membrane and increases transepithelial Ca\(^{2+}\) flux. Two recent studies employed molecular methods (isolated nephron segment RT-PCR and in situ hybridization) to localize the PTH receptor in rat kidney. Expression was noted in the glomerulus, proximal tubules, cortical thick ascending limb (cTAL), and the DCT, but not in the CCD (216). (165). The CNT was not addressed as a distinct segment in these studies.

Early radioligand binding studies suggested that 1,25-dihydroxyvitamin D\(_3\) receptors were expressed only by cells of the distal tubule (142). Kumar et al. (157) recently raised antibodies to the cloned 1,25-dihydroxyvitamin D\(_3\) receptor and confirmed that the highest levels of expression in human kidney were along the DCT, and CCD, with lower but significant expression in the proximal tubule (157).

Calbindins D28K and D9K are intracellular Ca\(^{2+}\) binding proteins that are thought to participate in shuttling Ca\(^{2+}\) from the apical to the basolateral membrane (115). In the rabbit and rat, calbindin D28K is expressed predominantly in the DCT and CNT (266). One study has also shown significant levels of expression in the CCD of rat (215). Calbindin D9K in rat is expressed in the cTAL, DCT, CNT, and CCD (18, 232). Interestingly, while calbindin D28K is expressed throughout the cytoplasm, calbindin D9K expression is concentrated in the basolateral aspect of the cell cytoplasm.

The recently discovered Ca\(^{2+}\) sensing receptor is another gene that is involved in regulated Ca\(^{2+}\) transport (217). Reverse transcription-PCR studies in rat kidney show that this receptor is expressed in the glomerulus, proximal convoluted tubule, proximal straight tubule, cTAL, DCT, and CCD (216). Immunofluorescence studies reveal that the Ca\(^{2+}\)-sensing receptor may be trafficked either to the apical or the basolateral membrane depending on the tubular segment. In proximal tubule and medullary collecting duct, it is expressed in the apical membrane, whereas in the loop of Henle, distal tubule, and CCD, expression was detected in the basolateral membrane (216). In the rat inner medullary collecting duct, the receptor colocalizes with aquaporin-2, indicating that it plays a role in vasopressin-mediated water permeability (221). In the thick limb of Henle, high external Ca\(^{2+}\) concentrations at the basolateral membrane activate the receptor. This results in inhibition of the apical K\(^+\) channel through the action of a cytochrome P-450 metabolite of arachidonic acid. Without apical K\(^+\) recycling, Na\(^+\)-K\(^+\)-...
2Cl- cotransport activity is decreased and the medullary countercurrent gradient is reduced, as is paracellular Ca2+-transport. This provides a mechanism for urinary Ca2+-mediated regulation of water permeability, which may aid in preventing the formation of Ca2+ stores.

The plasma membrane Ca2+-ATPases (PMCA) are a family of proteins first described by Schatzmann in 1966 (223). They are P-class ATPases that depend on the formation of an aspartyl phosphate intermediate and are inhibited by vanadate. Borke et al. (22) raised antibodies against the purified human erythrocyte PMCA and detected expression along the basolateral membrane of human DCT (22). Stauffer et al. (257) using Western analysis of whole kidney detected expression of PMCA-1 and -4 in human, but not PMCA-2 and -3 (257). In the rat, PMCA-1 was detected but not PMCA-2 and -3. Expression of PMCA-4 was not examined. Magocs et al. (178) employed nephron segment RT-PCR to examine the expression of PMCA-1,-2 and -3 in rat kidney (178). PMCA-2 was predominantly expressed in DCT and cTAL, whereas PMCA-1 was predominantly expressed in glomeruli. Expression of PMCA-3 has been detected principally in the thin descending limb of Henle (38).

3. Regulation of Ca2+ transport

Transepithelial Ca2+ reabsorption in the distal nephron involves the coordinated interplay of at least three transport pathways and two intracellular Ca2+ binding proteins (see Fig. 9). The transport rate is regulated by a 1,000-fold inward concentration gradient and the cell-negative membrane potential provides a large electrochemical driving force favoring the entry of Ca2+ across both the apical and basolateral membrane. Calcium entry across the apical membrane is believed to be mediated via Ca2+ channels driven by this gradient (32). Despite the high flux of Ca2+ in the distal nephron, only one laboratory has successfully patched Ca2+ channels from the apical membrane of microdissected tubules (264). These authors examined single-channel currents in microdissected CNT from rabbit. The patch-clamp data demonstrated a highly selective, 25-pS, cAMP-sensitive channel. Open probability peaked at 70 mV and declined with either hyperpolarization or depolarization. Spontaneous open probability is negligible, is stimulated to ~2% by agonists, and is reversibly inhibited by dihydropyridine antagonists. Calcium channels have also been patched from primary cultures of rabbit DCT (208) and an immortalized mouse distal tubular cell line (185). In rabbit primary cultures, an 8-pS channel with no voltage dependence that was blocked by lanthanum, verapamil, and nifedipine was identified. In the mouse, a 2.1-pS channel whose open probability was increased by hyperpolarizing voltages and inhibited by nifedipine but not verapamil was detected.

On a molecular level, Yu et al. (304) employed isolated nephron segment RT-PCR to isolate a variety of Ca2+ channel α1-subunits from rat kidney (304). The predominant isoform detected in the distal nephron was α1A or CaCh4. The predominant β-subunit detected was β3 (304). The significance of this finding remains unclear given the α1A-isoform is insensitive to dihydropyridines. Antisense oligonucleotides directed against α and β Ca2+ channel subunits were employed to identify those isoforms that mediated Ca2+ entry into the mouse cell line discussed above. An antisense oligonucleotide complementary to the α1C-isoform sequence inhibited the rise of intracellular Ca2+ induced by chlorothiazide but not PTH, whereas an antisense oligonucleotide complimentary to the β3-sequence inhibited the rise of intracellular Ca2+ induced by chlorothiazide and PTH. These results suggest that the α1C- and β3-isoforms play a role in Ca2+ uptake in this cell line. However, one cannot be certain that these Ca2+ channel subunits mediate an apical Ca2+ entry process given that these cells are not polarized and that PTH may also activate Ca2+ channels in the basolateral membrane of the distal nephron (27).

Recently, Hoenderop et al. (119a) have expression cloned an apical membrane Ca2+ channel from a primary culture of rabbit kidney connecting tubule and collecting duct. This novel epithelial Ca2+ channel (ECaC) is a distant relative of the recently cloned capsaicin receptor and the transient receptor potential-related ion channel. Expression was detected predominantly in the proximal small intestine, distal nephron, and placenta. In kidney, the channel is expressed in the apical membrane of the distal nephron and colocalizes with calbindin D28. Lanthanum, cadmium, and manganese inhibit channel activity, whereas barium, magnesium, and strontium do not. L-type Ca2+ channel antagonists do not block the channel. These properties mimic those of transepithelial transport in the primary cultures. Interestingly, lowering the extracellular pH also inhibits the channel. Metabolic acidosis is a well known inhibitor of distal nephron transepithelial calcium transport. These authors subsequently showed that channel activation is hyperpolarization dependent, that conductance is regulated by Ca2+, and that the channel desensitizes during repetitive stimulation (1196).

Calcium exit across the basolateral membrane must be active and is carried out by at least two transporters, the Na+/Ca2+ exchanger and the PMCA. Two studies suggest that the Na+/Ca2+ exchanger, and not the Ca2+ ATPase, is responsible for the majority of basolateral Ca2+ exit (19, 242). Shimizu et al. (242) used in vitro microperfusion in rabbit to show that the Na+/Ca2+ exchanger plays a major role in the net flux of Ca2+ from distal tubular segments in the basal state and in response
to stimulation by PTH. When Na$^+$ was removed from the bathing fluid or 0.1 mM ouabain was added, net flux was dramatically reduced both in the baseline and PTH-stimulated states, suggesting that the basolateral exit of Ca$^{2+}$ was mediated, at least in part, by a Na$^+$-dependent secondary active transport process. Further proof that Na$^+$/Ca$^{2+}$ exchange plays a major role in basolateral Ca$^{2+}$ exit in distal nephron was also provided by Bindels et al. (19) in primary cultures of cells derived from both the CNT and the CCD of rabbit. Transcellular Ca$^{2+}$ transport was shown to depend on basolateral Na$^+$ and was inhibited by ouabain (0.1 mM) to the same extent as basolateral Na$^+$ removal. The authors concluded that ~70% of active Ca$^{2+}$ transport was driven by basolateral Na$^+$/Ca$^{2+}$ exchange and 30% by another mechanism, presumably a Ca$^{2+}$-ATPase.

On the basis of the sequence of the previously reported canine cardiac Na$^+$/Ca$^{2+}$ exchanger (196), a combination of RT-PCR and cDNA library screening was used to isolate two overlapping cDNA that spanned the entire coding region for a rabbit renal Na$^+$/Ca$^{2+}$ exchanger (213). A comparison of the deduced amino acid sequences of the exchangers revealed 95% amino acid identity. The majority of the amino acid differences occurred in two short regions. The first region, the initial 30 amino acids, was shown to represent a signal sequence (72); only 17 of these amino acids were conserved. The second region spanned amino acids 602–633; only 10 of these amino acids were conserved. The second region was shown to represent a signal sequence (72); only 17 of these amino acids were conserved.

Brunette and colleagues (26) recently examined the effects of calbindins on membrane Ca$^{2+}$ transport in distal nephron membrane vesicles. They showed that calbindin D9K increased ATP-dependent Ca$^{2+}$ transport by basolateral cell membranes, an effect that is similar to one reported previously in duodenal cells. In contrast, calbindin D28K stimulated Ca$^{2+}$ uptake across apical membrane vesicles. These studies suggest that expression of the Ca$^{2+}$-binding proteins by distal tubule cells may stimulate transepithelial transport both by increasing Ca$^{2+}$ diffusion and through direct interactions with transport proteins.

Studies in cultured cell lines derived from the distal nephron have also contributed significantly to the understanding of how PTH and 1,25-dihydroxyvitamin D$_3$ regulate transepithelial Ca$^{2+}$ transport. Two groups, Bindels et al. (19) and Gesek and Friedman (101), have used immunodissected cell lines in rabbit and mouse, respectively, to investigate PTH-stimulated Ca$^{2+}$ transport. These studies verified that PTH increased transepithelial Ca$^{2+}$ transport in rabbit (19) and suggest that both protein kinase A and protein kinase C participate in this process (91).

The rabbit primary culture used by Bindels et al. (19) is immunodissected with a monoclonal antibody to an unknown “distal tubule antigen” and contains a mix of CNT and CCD cells. The murine cell line used by Pizzonia et al. (205) was immunodissected with a monoclonal antibody directed against the Tamm-Horsfall protein, a glycoprotein expressed in the cTAL and DCT. This cell line was subcloned and classified as originating from the DCT based on the expression of the thiazide-sensitive NaCl cotransporter (101).

Clearance studies in rats show that 1,25-dihydroxyvitamin D$_3$ increases renal Ca$^{2+}$ reabsorption across a wide range of serum Ca$^{2+}$ concentration (53). The molecular mechanisms responsible for these effects remain unknown. The effect of 1,25-dihydroxyvitamin D$_3$ on transepithelial Ca$^{2+}$ transport appears to vary depending on the species examined. It increases transepithelial Ca$^{2+}$ transport directly in the rabbit (19), whereas in the mouse it has no direct effect but potentiates PTH-induced Ca$^{2+}$ uptake (93).

V. THE DISTAL TUBULE IN HUMAN DISEASE

A. Gitelman’s Syndrome

Gitelman’s syndrome (Online Mendelian Inheritance in Man no. 263800) is an autosomal recessive disorder of hypokalemia, metabolic alkalosis, hypocalciuria, and hypomagnesemia. The triad of hyperaldosteronism, hypokalemia, and hyperplasia of the juxtaglomerular apparatus was described by Bartter and colleagues in 1960 (209) and
Gitelman's syndrome had tetanic episodes. It is characterized by hypokalemia, hypomagnesemia, and normal blood pressure (106). For many years, Gitelman's syndrome and Bartter's syndrome were considered to be one in the same. Bettinelli's careful analysis of phenotypic features, however, indicated clearly that Gitelman's syndrome is distinct from classical Bartter's syndrome (16). These workers differentiated the disorders on the basis of urinary Ca\(^{2+}\) excretion (Gitelman's patients had urine Ca\(^{2+}\)-to-creatinine ratios <0.20) and serum magnesium concentration (Gitelman's patients had plasma magnesium concentrations <0.75 mM). Bettinelli (16) also reported that patients with Bartter's syndrome were more often products of pregnancies complicated by polyhydramnios or preterm delivery. Many Bartter's patients also had short stature, polyuria, polydipsia, and a tendency to dehydration during infancy and childhood. In contrast, patients with Gitelman's syndrome had tetanic episodes.

Because the phenotype of Gitelman's syndrome resembles the consequences of thiazide diuretic therapy, the thiazide-sensitive NCC was examined as a candidate gene. Simon et al. (246) showed complete linkage of Gitelman's syndrome to the locus encoding the human thiazide-sensitive NCC. Seventeen different molecular variants were identified that were predicted to alter the structure of the encoded protein (246). Other groups have subsequently reported mutations in the same gene that were linked to this disorder (138, 167, 183, 207, 263). In a preliminary report, Simon et al. (245) collected a total of 82 distinct mutations that were associated with Gitelman's syndrome. Nearly all of these mutations are non-conservative and affect amino acids that have been conserved throughout evolution. To date, mutations in the thiazide-sensitive NCC have been detected in most cases of Gitelman's syndrome that have been screened; mutations in other genes have not been shown to cause the Gitelman's phenotype. Thus the syndrome appears to be caused by mutations in a single gene.

Identification of the gene defect responsible for Gitelman's syndrome has permitted the phenotype to be defined more clearly. In general, Gitelman's syndrome is a mild disease, but recent case reports indicate that adverse outcomes, such as hypokalemic rhabdomyolysis, can occur (197). Simon et al. (245) showed that affected patients were diagnosed at a mean age of 22.5 yr (range 0.75–65 yr). The mean serum K\(^+\) concentration was 2.6 mM (range 1.0–3.5 mM), the serum bicarbonate was 30.7 mM (range 22.0–37.4 mM), the serum Mg\(^+\) was 1.2 mM (range 0.3–1.7 mM), and the urine Ca\(^{2+}\)-to-creatinine ratio was 0.088 (range 0.001–0.36). In addition, 83% of patients had diastolic blood pressures below the 50th percentile for age and sex. Fifty-one percent of patients had diastolic blood pressures below the 50th percentile (P < 0.001). These findings emphasize that Gitelman's syndrome can present at any age but that hypokalemia, hypocalciuria, and hypomagnesemia are uniform features of the disease. Furthermore, they suggest that patients with Gitelman's syndrome have lower mean arterial pressures than normal controls.

Based on the autosomal recessive inheritance and the phenotypic features of the syndrome, Simon et al. (246) postulated that Gitelman's syndrome results from defective Na\(^+\) and Cl\(^-\) transport by the mutant gene product. In partial confirmation of this hypothesis, Schulteis et al. (233) detected several phenotypic features of Gitelman's syndrome in an NCC-knockout mouse. Null mice exhibited lower rates of urinary Ca\(^{2+}\) excretion and lower serum magnesium concentrations than did wild-type mice. In addition, the mean arterial pressure of the null mice was lower than the pressure of wild-type mice during dietary Na\(^+\) depletion. The size and number of DCT cells was reduced in null mice, and the number of mitochondria in DCT cells appeared to be reduced. Surprisingly, however, the arterial pH and P\(_{\text{CO}_2}\), and the serum bicarbonate and K\(^+\) concentrations did not differ between null mice and controls. Furthermore, NCC knockout did not affect levels of renin mRNA or serum aldosterone. The authors suggest that the mild phenotype observed in the knockout animals is similar to the phenotype in humans. The data of Simon et al. (245), discussed above, however, suggest that there are important differences in phenotype between the knockout mouse and human patients who suffer from Gitelman's syndrome. Most importantly, hypokalemia is consistently observed in humans, and the mean arterial pressure is usually reduced, even when patients consume a normal-salt diet (245).

Mutations in many regions of the NCC gene have been reported to cause the Gitelman's phenotype. Kunchaparty et al. (160) compared the properties of wild-type and mutant NCC clones, expressed in Xenopus oocytes. In contrast to the wild-type clone, which imparted thiazide-sensitive \(^{22}\)Na uptake, a clone containing a Gitelman's mutation that deletes the carboxy-terminal 54 amino acids (R948X) did not increase Na\(^+\) uptake above levels achieved in water-injected oocytes. Thus this Gitelman's mutation completely abrogates the ability of the protein to transport salt. The mechanism by which Gitelman's mutations impair transport was investigated using the oocyte expression system. The NCC is normally glycosylated at two asparagine residues, located on the fourth extracellular domain. The NCC R948X was found to be translated normally by the oocytes, but it was not glycosylated. Furthermore, the mutant protein was not delivered to the plasma membrane. These data suggest that at least one Gitelman's mutation leads to misfolding of the protein, activating the “quality control” system of the endoplasmic reticulum. The quality control system is able to detect misfolded proteins, targeting them for deg-
radation, often via an ubiquitin-dependent interaction with the proteasome. In preliminary experiments, at least 10 other Gitelman’s mutations were shown to disrupt function of the protein by causing misprocessing (Ellison, unpublished data). Thus most cases of Gitelman’s syndrome appear to result because misfolding of the protein activates the quality control system of the endoplasmic reticulum. This leads to a failure of delivery to the plasma membrane.

Identification of a single gene defect that is responsible for most or all cases of Gitelman’s syndrome implies that the phenotypic features of the disease all result from dysfunction of this protein. The observation that the gene for the thiazide-sensitive NCC is expressed predominantly by renal tissue and not elsewhere (184; Ellison, unpublished data) implies that all of the phenotypic features of Gitelman’s syndrome result from abnormalities of renal function. Hypokalemia and alkalosis probably develop because extracellular fluid volume contraction results from loss of Na⁺ and Cl⁻. It is further exacerbated because Na⁺ delivery into the collecting duct is increased (owing to the absence of Na⁺-Cl⁻ cotransport by DCT cells, see Fig. 10). Patients with Gitelman’s syndrome generally have high levels of plasma renin activity (16) that enhance aldosterone secretion and favor K⁺ and H⁺ secretion along the distal tubule and collecting duct. Mild contraction of the extracellular fluid volume also reduces the blood pressure, as observed by Simon et al. (245). An early observation was that patients with Bartter’s and Gitelman’s syndromes are resistant to the effects of angiotensin II. This resistance probably results from chronically elevated levels of plasma renin and angiotensin II leading to a desensitization phenomenon. It should be noted that patients with Gitelman’s syndrome exhibit levels of plasma renin activity that are not as high as the levels observed in patients with classical Bartter’s syndrome. This is likely to reflect the fact that Bartter’s syndrome results from dysfunction of TAL cells (via disruption of either the Na⁺-K⁺-2Cl⁻ cotransporter, the apical K⁺ channel, or the basolateral Cl⁻ channel). Macula densa cells are specialized TAL cells that suppress renin secretion when transcellular NaCl transport occurs (31). Thus plasma renin activity is stimulated in Bartter’s syndrome both because extracellular fluid volume is contracted and because the macula densa signal is blocked.

The pathogenesis of the hypocalciuria and hypomagnesemia remains less fully understood. As discussed above, blocking Na⁺ entry across DCT cells leads to increases in Ca²⁺ reabsorption, probably by increasing Ca²⁺ entry, via a voltage-activated luminal Ca²⁺ channel (see Fig. 10) and secondarily by stimulating basolateral Na⁺/Ca²⁺ exchange. These effects are believed to occur because the intracellular Cl⁻ concentration declines, owing to the absence of an apical Cl⁻ entry pathway. The
lower intracellular Cl⁻ hyperpolarizes the cell, enhancing Ca²⁺ entry via the voltage-activated channel. The hyperpolarization also stimulates the basolateral Na⁺/Ca²⁺ exchanger, because this transporter is electrogenic. Finally, lower intracellular Na⁺ concentrations stimulate the Na⁺/Ca²⁺ exchanger by increasing the Na⁺ gradient across the basolateral membrane. All of these factors probably participate in the increased Ca²⁺ transport.

Gitelman's syndrome is associated with Mg²⁺ wasting. This suggests that the cellular events that enhance Ca²⁺ absorption inhibit Mg²⁺ absorption. Surprisingly, if immortalized DCT cells are hyperpolarized, as postulated to occur in Gitelman's patients, Mg²⁺ uptake increases rather than decreases (60, 210). Recently, a paracellular magnesium transport protein, named paracellin, has been cloned (245a). As an alternative hypothesis, therefore, Figure 10 postulates that Mg²⁺ wasting in Gitelman's syndrome occurs via alterations in paracellular Mg²⁺ movement. According to this model, Gitelman's syndrome converts some DCT cells from predominantly electroneutral cells to cells that reabsorb Na⁺ in an electrogenic manner. As discussed in section 1B3A, these cells are also responsive to the actions of aldosterone. This combination of the dominance of electrogenic ion transport pathways, the stimulation by aldosterone, and the increased luminal Na⁺ concentrations all favor electrogenic Na⁺ reabsorption. This greatly increases the magnitude of the transepithelial voltage. The high transepithelial voltage would favor Mg²⁺ secretion, via the paracellular pathway. Until mechanisms of Mg²⁺ transport by kidney cells are better understood, however, the pathogenesis of Mg²⁺ wasting in patients with Gitelman's syndrome will remain speculative.

**B. Distal Disorders of Urinary Acidification**

The secretion of H⁺ by tubular epithelial cells serves at least two purposes: 1) the reabsorption of filtered bicarbonate and 2) the excretion of the dietary proton load. The distal tubule is largely responsible for the latter, via the acid-excreting α-intercalating cell shown in Figure 11. Aldosterone stimulates Na⁺ reabsorption in the distal nephron by increasing apical Na⁺ entry via the amiloridesensitive epithelial Na⁺ channel and Na⁺ exit via the basolateral Na⁺-K⁺-ATPase. This promotes H⁺ secretion by establishing a more favorable electrochemical gradient. In addition, aldosterone stimulates H⁺ secretion directly (261).

Defects in any of the α-intercalated cell transport proteins shown in Figure 11, the epithelial Na⁺ channel, or the mineralocorticoid receptor could result in a metabolic acidosis. To date, distal acidification defects as a result of either acquired or inherited defects in the H⁺-ATPase, the Cl⁻/HCO₃⁻ exchanger, the epithelial Na⁺ channel, and the mineralocorticoid receptor have been described. Autosomal dominant familial distal renal tubular acidosis is caused by mutations in the renal Cl⁻/HCO₃⁻ exchanger (33) (137). Mutations in the amiloride-sensitive epithelial Na⁺ channel cause autosomal recessive pseudohypoaldosteronism type 1 (PHA-1) (42), whereas mutations in the mineralocorticoid receptor result in autosomal dominant PHA-1 (98).

Several groups have reported that immunohistochemical staining of kidney in patients with distal renal tubular acidosis and Sjogren's syndrome failed to detect staining of H⁺-ATPase in intercalated cells (11, 49). Intercalated cells were present in these patients, and antibody
to the H\(^+\)-ATPase was not detected in serum. In addition, in two patients, the Cl\(^-/HCO_3\)\(^-\) exchanger was also not detected in the basolateral membrane by immunofluorescence. T cell-mediated damage to intercalated cells has been postulated.

C. Adapation to Diuretic Drugs

Diuretic drugs are effective therapy for edematous disorders and hypertension. However, several adaptations to diuretic treatment may affect the ability of these drugs to lead to natriuresis. When NaCl reabsorption along the TAL is inhibited by loop diuretics, the NaCl concentration in fluid that enters the distal tubule is greatly increased. In one study, the Na\(^+\) concentration in fluid entering the distal tubule of rats rose from 42 to 140 mM during acute loop diuretic infusion (122). The increased luminal NaCl concentration drives increased Na\(^+\) absorption along the distal tubule (from 148 to 361 pmol/min) (122) because NaCl transport by the distal tubule is load dependent (as discussed above). This increase in solute reabsorption by segments that lie distal to the site of diuretic action is the first form of diuretic adaptation and limits the intrinsic potency of the diuretic drug. The net effect of acute diuretic administration on urinary Na\(^+\) and Cl\(^-\) excretion, therefore, reflects the sum of effects in the diuretic-sensitive segment (inhibition of NaCl reabsorption) and in diuretic-insensitive segments (secondary stimulation of NaCl reabsorption).

As the acute effect of a diuretic declines, a period of NaCl retention usually commences. This phenomenon, often termed postdiuretic NaCl retention, has been reviewed recently (75). One mechanism by which diuretic drugs may increase the tendency for NaCl retention directly, without participation of changes in extracellular fluid volume, involves diuretic-induced activation of ion transporters within the diuretic-sensitive nephron segment. Within 60 min of thiazide administration, the number of thiazide-sensitive Na\(^+\)-Cl\(^-\) cotransporter (NCC) mRNA (measured as the number of \([\text{H}]\)metolazone binding sites) increases substantially (45). The techniques used to estimate the number of transporters in these experiments do not permit one to determine whether the increased number reflects insertion of preexisting transporters from a subapical storage pool or activation of transporters that are present but inactive in the apical membrane. Recent immunocytochemical studies show that a subapical compartment contains thiazide-sensitive NCC in DCT cells of rats (206), suggesting that a shuttle system may regulate functional activity of the thiazide-sensitive NCC in the distal tubule. An increase in the number of activated ion transporters at the apical membrane would be expected to increase the transport capacity so that when diuretic concentrations decline, increased Na\(^+\) and Cl\(^-\) transport would result.

Another mechanism by which diuretic drugs may enhance the tendency to NaCl retention directly involves stimulation of transport pathways in nephron segments that lie distal to the target of diuretic action (segments that are insensitive to the diuretic drug). For example, the number of thiazide-sensitive Na\(^+\)-Cl\(^-\) transporters in the kidney (and presumably in the DCT) increases within 60 min after a loop diuretic has been administered (45). Because thiazide-sensitive transporters are expressed only by nephron segments that do not express loop diuretic-sensitive pathways, the increased number of thiazide-sensitive NCC is believed to result from increases in salt and water delivery to DCT cells.

Another factor that can enhance the reabsorptive capacity of the distal tubule is adaptive changes in the
nephron itself. When solute delivery to the distal tubule is increased chronically, distal cells undergo both hypertrophy and hyperplasia. Infusion of furosemide into rats continuously for 7 days increased the percentage of renal cortical volume occupied by DCT cells by nearly 100% (79, 132, 135). Biochemical and functional correlates of these structural changes are shown in Figure 12. Chronic loop diuretic administration increases the Na\(^+-\)K\(^+-\)ATPase activity in the distal convoluted and CCT (226, 288) and increases the number of thiazide-sensitive NCC, measured as the maximal number of binding sites for \[^{3}H\]metolazone (45, 199). In one study, chronic furosemide treatment increased expression of mRNA encoding the thiazide-sensitive NCC, as detected by in situ hybridization (see Fig. 12) (199). In another study, however, mRNA expression of the thiazide-sensitive NCC as well as the ouabain-sensitive Na\(^+-\)K\(^+-\)ATPase was not affected by chronic furosemide infusion, when detected by Northern analysis (188). Distal tubule cells that express high levels of transport proteins and are hypertrophic have a higher Na\(^+\) and Cl\(^-\) transport capacity than normal tubules; compared with tubules from normal animals, tubules of animals treated chronically with loop diuretics can absorb Na\(^+\) and Cl\(^-\) up to three times more rapidly than control animals. Even salt and water delivery is fixed by microperfusion (Fig. 12); when distal tubules are presented with high NaCl loads, as occurs during loop diuretic administration in vivo, Na\(^+\) and Cl\(^-\) absorption rates approach those commonly observed only in the proximal tubule (79). Recently, it has been observed that chronic treatment of rats with loop diuretics also results in significant hyperplasia of cells along the distal nephron. Whereas mitoses of renal tubule epithelial cells are infrequent in adult kidneys, distal tubules from animals treated with furosemide chronically demonstrate prominent mitoses; increased synthesis of DNA in these cells was confirmed by showing increases in labeling of DCT cells with bromodeoxyuridine and proliferating cell nuclear antigen (171).

The diuretic-induced signals that initiate changes in distal nephron structure and function are poorly understood. Several factors, acting in concert, may contribute to these changes; these include diuretic-induced increases in Na\(^+\) and Cl\(^-\) delivery to distal segments, effects of extracellular fluid volume depletion on systemic hormone secretion and renal nerve activity, and local effects of diuretics on autocrine and paracrine secretion. Increased production of angiotensin II or increased secretion of aldosterone resulting from increases in renin activity may contribute to hypertrophy and hyperplasia. Angiotensin II is a potent mitogen; angiotensin II receptors have not been localized definitively to DCT cells, but recent functional studies do suggest that DCT cells express angiotensin II receptors. Aldosterone also promotes growth of responsive tissues under some circumstances (136); when salt delivery to the collecting duct is increased in the presence of high levels of circulating aldosterone, principal cell hypertrophy develops; when salt delivery is high in the absence of aldosterone secretion, hypertrophy is absent. This indicates that aldosterone plays a permissive role in the development of cellular hypertrophy in this aldosterone-responsive renal epithelium. Although recent experiments suggest that aldosterone does affect ion transport by cells of the DCT, and aldosterone almost certainly contributes to adaptations along the CCT, hypertrophy of DCT cells has been shown to occur during chronic loop diuretic infusion even when changes in circulating mineralocorticoid, glucocorticoid, and vasopressin levels are prevented (135).

One intriguing hypothesis is that cellular ion concentrations regulate epithelial cell growth directly (256). Increases in Na\(^+\) uptake across the apical plasma membrane precede cell growth in the TAL during treatment with antidiuretic hormone (24), in principal cells of the CCT during treatment with mineralocorticoid hormones (131, 204), and in the DCT during treatment with loop diuretics (79, 135). Although the cause of the increased Na\(^+\) uptake varies, changes in the intracellular Na\(^+\) concentration appear to precede growth in each example (13). This hypothesis predicts that blockade of apical Na\(^+\) entry would lead to atrophy of epithelial cells. Chronic treatment of rats with DCT diuretics reduces activity of Na\(^+-\)K\(^+-\)ATPase and Na\(^+\) transport capacity of DCT segments (97, 193), but these experiments are complicated by other structural effects of chronic DCT diuretic treatment, discussed below. Regardless of the proximate stimulus for DCT cell growth, recent experiments have shown that immunoreactivity for IGF-I and for an IGFBP (IGFBP-I) increases during chronic treatment of rats with loop diuretics (150). The changes in IGF-I expression appeared not to result from changes in IGF-I mRNA expression, but rather appeared to reflect posttranscriptional events. IGFBP-I mRNA was increased by threefold 18 h after loop diuretic treatment was initiated. IGF-I has been shown to participate in regeneration of injured or ischemic renal tissue and promotes cell proliferation and differentiation in vitro. Whether these changes in IGF expression mediate the effects of diuretics on distal nephron structure remains to be established.

When DCT diuretics are administered chronically, Na\(^+-\)K\(^+-\)ATPase activity in the DCT is reduced (97), and the capacity of DCT cells to reabsorb Na\(^+\) and Cl\(^-\) declines (193). However, chronic administration of DCT diuretics to rats leads to profound changes in cellular morphology; DCT cells undergo apoptosis and necrosis with resulting interstitial fibrosis. Chronic treatment also leads to the disappearance of normal polarization of thiazide-sensitive NCC proteins. Under normal conditions, immunoreactivity for the thiazide-sensitive NCC is restricted to the apical membrane and to a small subapical
pool of vesicles. During chronic treatment with DCT diuretics, the protein is distributed uniformly throughout the cell. Surprisingly, based on the severe morphological degenerative changes in tubular morphology, chronic thiazide administration results in an increase in the density of [3H]metolazone binding sites (functional thiazide-sensitive transporters) in kidney cortex (97) despite a decline in mRNA expression for the transporter (171); this may reflect an increased ability of diuretic to bind to degenerating receptors that have been internalized into cells during the diuretic treatment.

Although experimental data concerning structural and functional responses of the distal nephron to chronic treatment with diuretic drugs come predominantly from studies employing experimental animals, Loon et al. (175) reported that chronic treatment with loop diuretics in humans enhanced ion transport rates in the distal tubule. They estimated the transport capacity of the DCT as the portion of Na\(^+\) and Cl\(^-\) reabsorption that could be inhibited by thiazide diuretics. When furosemide was administered to volunteers for 1 mo, the enhancement in Na\(^+\) excretion that occurred resulted from a dose of thiazide diuretic that was significantly larger. Although these data are necessarily indirect, they are entirely consistent with the data derived from experimental animals given loop diuretics chronically. The extracellular fluid volume-independent component of NaCl retention that occurs after loop diuretic administration (5) may also reflect changes in distal nephron structure and function.

D. Nephrolithiasis

Nephrolithiasis accounts for ~7–10 of every 1,000 hospital admissions in the United States and is a cause of considerable morbidity (1). It has been estimated that there is a genetic component involved in up to 45% of cases, and hypercalciuria is a major risk factor.

Recently, a common molecular etiology for three rare X-linked hypercalciuric syndromes characterized by nephrolithiasis (Dent’s disease, X-linked recessive nephrolithiasis, and X-linked recessive hypophosphatemic rickets) was identified using a positional cloning approach (169). Although these syndromes do differ slightly in their clinical presentation, they are all characterized by proximal tubular dysfunction, nephrocalcinosis, nephrolithiasis, and progressive renal failure. The defect is in the CLC-5 gene, which is a member of the CLC family of
There are at least nine known members of the CLC gene family, the first of which, CLC-I, was expression cloned from *Torpedo marmorata* by Jentsch et al. (128). Other members of the family include CLC2–7, CLC-Ka, and CLC-Kb. Defects in the expression of at least two additional members of the CLC gene family are associated with human disease. CLC-I is mutated in both the dominant and recessive forms of congenital myotonia (151), and mutations in CLC-Kb were reported to cause some cases of Bartter's syndrome (244).

CLC-5 has a high selectivity for Cl\(^-\), is strongly outwardly rectifying, and is activated at inside-positive membrane voltages exceeding 10–20 mV. It is expressed predominantly in the kidney in humans (90); in the rat, the transcript is also expressed in brain, liver, and lung (258).

To date, 19 mutations have been reported in 22 different families (224). The majority of these have been expressed in *Xenopus laevis* oocytes by the Jentsch group and have been found to have either no or marked reduced Cl\(^-\) channel activity (see Fig. 13). Interestingly, there appears to be little correlation between the phenotype and the nature of the severity of the Cl\(^-\) channel defect. Even within families, individuals sharing the same mutation can be either mildly or severely affected.

It is clear that this group of disorders is caused by a lack of expression of CLC-5. How this Cl\(^-\) channel defect results in low-molecular-weight proteinuria, moderate hypercalciuria, an exaggerated calcircetric response to an oral Ca\(^{2+}\) load, defective urinary concentrating ability, an acidification defect with acid loading, nephrocalcinosis, and ultimately renal failure remains unclear. The best arguments that these abnormalities are due to a primary renal abnormality as opposed to nonspecific renal injury from an increased filtered load of Ca\(^{2+}\) are as follows: 1) in humans the major site of expression of CLC-5 is the kidney, and 2) of patients that have undergone renal transplantation for these disorders, there has been no evidence of recurrent disease in the allograft.

Obermüller et al. (200) employed in situ hybridization to show that CLC-5 is expressed by type A intercalated cells in the CNT and collecting duct (200). This would indicate that the highest level of expression of the CLC-5 transcript is in the intercalated cell in CNT and CCD. However, on the basis of the clinical presentation, it would appear that the CLC-5 gene product is likely expressed more widely than distal nephron. The low-molecular-weight proteinuria is suggestive of proximal tubular expression that was below the level of detection of the in situ hybridization technique that we employed. More recently, two laboratories used polyclonal antibodies to immunolocalize CLC-5. Luyckx et al. (176) showed that CLC-5 is expressed in the S3 segment of the proximal tubule and in the medullary TAL (176). Devuyst et al. (62) immunolocalized CLC-5 to the proximal tubule, TAL, and CCD intercalated cells (62). In both studies, CLC-5 was shown to be associated with endosomes of the receptor-mediated endocytic pathway. An inward Cl\(^-\) flux is required in these vesicles to dissipate the positive charge that results from the secretion of protons into the interior of the vacuole by a H\(^+\)-ATPase. It is likely that defects in protein trafficking due to dysfunction of CLC-5 are responsible for the phenotype of these disorders.

E. Treatment of Nephrolithiasis With Distal Tubular Diuretics

The treatment of choice in patients with Ca\(^{2+}\)-containing stones and hypercalciuria is thiazide diuretics. Two randomized placebo-controlled trials have shown that thiazides reduce the risk of Ca\(^{2+}\) lithiasis by 54 and 58% (81, 161). It is thought that thiazides reduce the risk of stone formation by increasing distal tubular reabsorption of Ca\(^{2+}\). Interestingly, the majority of patients in these studies were not hypercalciuric. Therefore, it may be possible that thiazides not only reduce the risk of recurrent nephrolithiasis by lowering urinary Ca\(^{2+}\) but may also act via other mechanisms as well.

At least two different mechanisms have been postulated to explain the effect of thiazides to increase distal tubular Ca\(^{2+}\) transport. Brunette et al. (34, 35) have identified two kinetically distinct Ca\(^{2+}\) uptake pathways in the luminal membrane of distal tubules. The first is a high-affinity, low-capacity component. The maximum velocity of this component is increased by preincubation with PTH. The second is a low-affinity, high-capacity component whose maximum velocity is enhanced by hydrochlorothiazide (102). Hydrochlorothiazide and PTH produced an additive stimulation of Ca\(^{2+}\) uptake. Calcium channel blockers alone did not affect Ca\(^{2+}\) transport; however, when added with PTH, they completely abolish the PTH-induced stimulation of Ca\(^{2+}\) uptake.

Hydrochlorothiazide has no effect on basolateral Ca\(^{2+}\) transport mediated by either the Na\(^+\)/Ca\(^{2+}\) exchanger or the Ca\(^{2+}\)-ATPase (163). Only when hydrochlorothiazide was incubated with apical membrane vesicles of the distal tubule was an increase in Ca\(^{2+}\) uptake noted. Sodium was required for the effect. Sodium decreased Ca\(^{2+}\) uptake in a dose-dependent manner, and hydrochlorothiazide partially reestablished Ca\(^{2+}\) uptake to those observed in Na\(^-\)-free media. It was concluded that hydrochlorothiazide decreased the inhibitory effect of Na\(^+\) on Ca\(^{2+}\) uptake in the luminal membrane of the distal tubule.

Gesek and Friedman (100) also examined the mechanism whereby hydrochlorothiazide increases distal
nephron Ca\(^{2+}\) transport in an immortalized distal convoluted cell line. In this cell line, chlorothiazide hyperpolarized membrane voltage and stimulated Ca\(^{2+}\) entry. They went on to show, in inside-out patch-clamp experiments in these cells, the presence of a Ca\(^{2+}\) channel whose open probability was increased by hyperpolarization (101).

The diuretic drug amiloride inhibits the epithelial Na\(^{+}\) channel in nanomolar concentration and has also been used in the treatment of Ca\(^{2+}\) nephrolithiasis. Its hypocalciuric effects are additive with thiazide diuretics, suggesting that it acts in a different segment than that which expresses the NaCl cotransporter (54). In vivo microperfusion experiments in rats confirmed that the addition of 10\(^{-5}\) M amiloride to the luminal perfusate enhanced Ca\(^{2+}\) reabsorption in the late segments of the distal nephron. The drug had no effect on early distal function (51).

VI. SUMMARY

The distal tubule is a short nephron segment between the region of the macula densa and the confluence to form the collecting duct. Its importance in sodium, chloride, potassium, calcium, and magnesium balance is emphasized by human diseases that result from ion transport defects along its length. During the past 10 years, nearly all of the dominant ion transport proteins that control its function have been identified and cloned. This has permitted earlier physiological information to be reassessed based on molecular information. It has also permitted specific transport functions to be assigned to specific cell types within this extremely heterogeneous tubule segment.

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