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Ion transport by the cortical and outer medullary collecting tubule

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Anatomy and cell morphology. The rabbit collecting tubule originates near the surface of the kidney, descends through the cortex within the medullary rays and traverses the outer medulla as an unbranched structure. The cortical collecting tubule (CCT) receives the contents of an average of 6 nephrons [1]. The fusion of these nephrons generally occurs proximal to the origins of the collecting tubule. Thus, the collecting tubule is unbranched from the superficial portion of the cortex until it reaches the inner medulla where the collecting tubules begin to fuse to form larger and larger ducts eventually exiting through the papillary tip. Figure 1 schematically represents the collecting tubule as it courses through the cortex and medulla. The fact that the cortical and outer medullary collecting tubule (OMCT) in the rabbit are unbranched facilitates their examination using the technique of in vitro perfusion of isolated nephron segments.

It is generally agreed that the collecting tubule is comprised of two cell types, principal and intercalated cells. Several features characterize each cell type. The cytoplasm of the principal cell is generally less electron dense than is the cytoplasm of the intercalated cell (hence the designation of the intercalated cell as the "dark" cell). The principal cell also has fewer mitochondria and cellular organelles than does the intercalated cell. On transmission electron microscopy, the principal cell displays short microvillae while the intercalated cell has distinctly longer microvillae. A striking feature of the intercalated cell is the presence of vesicles near the apical membrane. Vesicles are rarely seen in principal cells. On scanning electron microscopy, the intercalated cells show prominent microplicae while the principal cells generally have short microvillae with a single central cilium. Histologically, intercalated cells stain with toluidine blue. In addition, these cells may contain carbonic anhydrase [2].

The distinction between the intercalated cell and the principal cell is not always clear. In addition, it is not certain that the principal cell and the intercalated cell represent functionally separate populations. There are gradations in the cytoplasmic staining patterns, variability in the number of mitochondria, and substantial variety in the configuration of the microplicae and microvillae of the apical membrane. Based on variations of the apical membrane, LeFurgey and Tisher [3] described four configurations of the intercalated cell and two configurations of the principal cell. Whether these cells have variable morphologic and/or functional characteristics or whether there are two functionally separable cells which have variable appearance is not clear. Stetson, Wade, and Giebisch [4] examined the intercalated cell of the rat collecting tubule using transmission electron microscopy and noted that the apical membrane contains small, regularly spaced, linear densities along the inner surface. These densities also appear in the membrane of the apical vesicles. These "studs" or "pegs" occur in the same cell population which, on freeze fracture, shows rod-shaped intramembraneous particles on the P face. It is likely that these particles have functional significance, although at the present time it is unclear as to which function they should be ascribed.

The principal cell outnumbers the intercalated cell in the cortical collecting tubule by about 2:1. In the outer medullary collecting tubule, the intercalated cells decrease to 18% [3] and are rarely seen in the inner medulla and the papilla. The principal cell also undergoes substantial morphologic changes from the cortex into the outer medulla and papilla. Its cell height increases from approximately 6 μm in the cortical collecting tubule to approximately 12 μm in the outer medullary collecting tubule to over 100 μm at the tip of the papilla. In the outer medullary collecting tubule the principal cell develops a more elaborate network of subapical microtubules and microfilaments. The "membranous labyrinth," a term used to describe the basilar infoldings, becomes greatly simplified as the collecting tubule descends into the medulla.

Although the principal cell of the cortical collecting tubule is sometimes thought to be analogous to the principal cell of the medullary collecting tubule and papillary collecting tubule, there is considerable evidence to indicate that such is not the case. Rosen [2] examined the carbonic anhydrase activity of the rabbit collecting tubule along its length and found that within the cortical portion, cells containing carbonic anhydrase appear irregularly. In contrast, collecting tubule cells from the medullary area contain cells with almost uniformly positive carbonic anhydrase activity. Whether it is the principal cell or the intercalated cell of the CCT which contains carbonic anhydrase is uncertain. However, from the studies of the urinary bladder of the fresh water turtle (an epithelium which has several functions analogous to the CCT of the rabbit), it is clear that the "mitochondria-rich cell" or "microplicated cell" contains carbonic anhydrase [5]. The similarities between the mitochondria-rich cell of the turtle bladder and the intercalated cell of the

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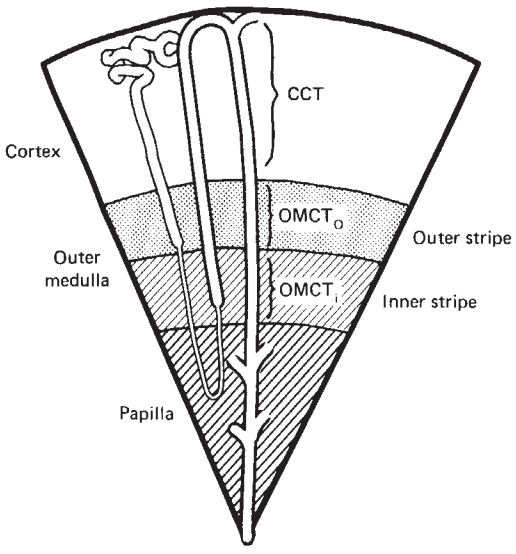

Fig. 1. *Schematic diagram of the cortical collecting tubule* (CCT) *and the outer medullary collecting tubule as it descends through the outer stripe* (OMCT$_o$) *and the inner stripe* (OMCT$_i$). (Reproduced from [24] with permission.)

rabbit CCT is striking, so one can deduce that the intercalated cell of the CCT contains carbonic anhydrase. From this deduction, it follows directly that the principal cells of the CCT are functionally different from the principal cells of the medullary and papillary collecting tubules, since within the medulla and papilla all cells contain carbonic anhydrase while the principal cells of the CCT do not. This conclusion is consistent with the previously mentioned morphologic differences in the principal cells along the collecting tubule. It is also consistent with the striking functional differences between the CCT and the outer medullary collecting tubule (OMCT) which will be described later.

Thus, the designation "principal cell" (and intercalated cell), while a morphologic convenience, must be considered within the specific region of the collecting tubule and cannot be assumed to represent a cellular counterpart to general functions such as sodium absorption, potassium secretion, or acidification.

Sodium transport

Cortical collecting tubule. There is now an extensive body of information which leads to the inevitable conclusion that sodium is transported actively by the CCT in a manner similar to that described for frog skin, toad bladder, and other "tight" epithelia. This model generally predicts that sodium moves across the luminal or apical membrane through specific channels into the cell "down" its electrochemical potential gradient. Sodium is extruded from the cell by an energy-consuming process through the Na-K pump (Na-K-ATPase). (For an extensive review, see [6].)

Early studies of the cortical collecting tubule noted that the spontaneous transepithelial voltage (V_T) was generally lumen-negative when perfusate and bath were solutions simulating an ultrafiltrate of plasma. Sometimes this voltage was lumen-positive although it is difficult to reproduce this situation consistently without inhibitors of sodium transport or removal of sodium from the bathing solutions. In general, the magnitude of this lumen-negative voltage correlates with the rate of sodium absorption. Thus, sodium is absorbed against its electrochemical potential gradient and the transport is, therefore, "active." Furthermore, it is the process of sodium absorption which generates the lumen-negative V_T.

The evidence which indicates that the absorption of sodium generates the lumen-negative V_T is derived mainly from the comparison with more extensively studied epithelia such as toad bladder and frog skin [6]. Removal of sodium from the perfusate (only) reduces the magnitude of the V_T toward zero, sometimes causing the V_T to go positive [7, 8]. Amiloride is an agent that blocks the entry of sodium across the apical membrane [9] and in the CCT also causing the V_T to become positive [10–16]. Furthermore, amiloride (0.1 mM) abolishes the net absorption of sodium [10, 11].

Inhibition of Na-K-ATPase also reduces the magnitude of the lumen-negative V_T. Ouabain applied to the peritubular surface causes the V_T to become positive [7, 16–18]. Simultaneously, ouabain reduces the rate of sodium absorption to near zero [7, 16]. Inhibition of Na-K-ATPase by removal of bath potassium also reduces the magnitude of V_T to near zero and abolishes net sodium absorption [7].

Recently, Koeppen, Biagi, and Giebisch [19] and Koeppen and Giebisch [20] conducted intracellular voltage measurements under several conditions. The basolateral membrane voltage was approximately 96 mV (intracellular negative). Amiloride reduced the basolateral membrane voltage by 11 mV and ouabain depolarized it by 34 mV. Amiloride also increased the transepithelial resistance [12] and increased the ratio of the resistance of the apical membrane to that of the basolateral membrane [20].

Taken together, these findings indicate that the process of sodium absorption occurs in a fashion remarkably similar to that which occurs in toad bladder and frog skin. Although the process of sodium absorption appears to be the same, there are other important differences, the most notable of which is the ability of the cortical collecting tubule to secrete potassium.

The electrogenicity of sodium absorption is supported by the observations that most maneuvers which reduce the magnitude of the lumen-negative V_T also reduce net sodium absorption. However, there are two exceptions: Boudry, Stoner, and Burg [13] and Shareghi and Stoner [21] noted that acidification of the perfusion solution acutely increases the magnitude of V_T but has no effect on sodium transport (acidification of the bath solution produces a biphasic effect on V_T [22]). Iino, Troy, and Brenner [18] reported that isoproterenol reduces the magnitude of V_T without influencing sodium absorption. Thus, it is clear that the process of sodium absorption is not the sole determinant of the magnitude of V_T but that other processes, such as acidification and potassium secretion probably contribute to V_T.

Outer medullary collecting tubule. The morphological difference between the CCT and OMCT suggests that there is a functional alteration as the collecting tubule descends into the medulla. One of the first indications that this morphological change corresponded to a change in the nature of ion transport was the finding of a spontaneously lumen-positive voltage in medullary collecting tubules dissected from rabbits having

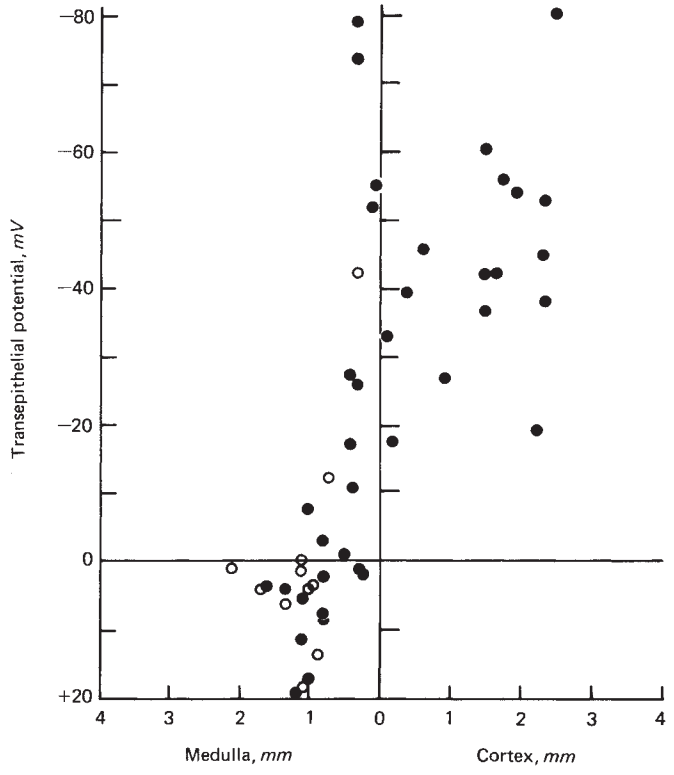

Fig. 2. *Transepithelial electrical potential of rabbit collecting tubule as a function of position within the kidney.* All tubules were dissected from DOCA-treated rabbits. Each *point* represents a single tubule perfused antegrade. Proceeding from cortex to medulla (right to left) effectively amputates the active sodium adsorption process which is present in the cortical collecting tubule and diminishes rapidly within the outer stripe of the outer medulla (~1 mm). *Closed circles* represent tubules perfused and bathed with an artificial isotonic solution. *Open circles* represent tubules perfused and bathed with an artificial solution designed to simulate the more hypertonic outer medullary interstitium. (Reproduced from [23] with permission.)

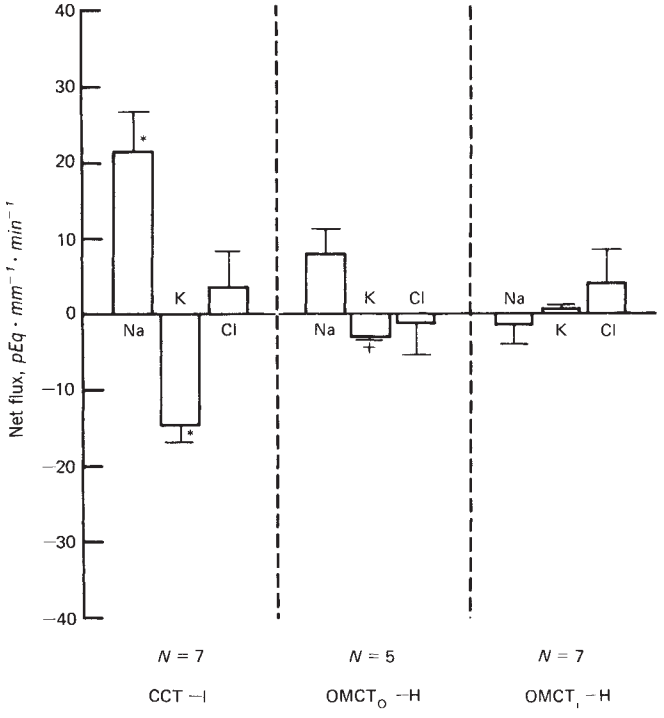

Fig. 3. *Net flux of sodium, potassium, and chloride across segments of the cortical and outer medullary collection tubule.* All tubules were dissected from normal rabbits. Negative values indicate secretion. Abbreviations: CCT, cortical collecting tubule; $OMCT_o$, outer medullary collecting tubule dissected from the outer stripe; $OMCT_i$, outer medullary collecting tubule dissected from the inner stripe; I, isotonic solution used in perfusate and bath; H, hypertonic solution used in perfusate and bath; * $P < 0.01$; † $P < 0.05$ (compared to 0). (Reproduced from [24] with permission.)

received desoxycorticosterone acetate (DOCA). In rabbits treated with DOCA, the spontaneous V_T across the CCT (perfused and bathed with Ringer's solution) is always lumen-negative. The results of experiments designed to determine whether or not this voltage was a function of position within the outer medulla are displayed in Figure 2 [23]. Each point represents the spontaneous V_T from collecting tubules dissected from the indicated position within the cortex or outer medulla and perfused antegrade. Thus, as one moves from the surface of the kidney into the outer medulla, one sequentially amputates the active sodium absorption mechanism responsible for generating the lumen-negative voltage. It is clear that the V_T of the collecting tubules dissected from the outer stripe of the outer medulla (which is located in the outer millimeter of the outer medulla) maintained their lumen-negative voltage but at a substantially decreased magnitude. Tubules dissected from the inner stripe of the outer medulla (greater than 1 mm into the medulla) displayed a uniformly lumen-positive voltage. This functional heterogeneity must be due to a change in the nature of active ion transport since all studies were conducted using identical solutions in the perfusate in the bath. The morphological change, examined in these same tubules, was equally clear. In tubules which had a spontaneously negative voltage the population of intercalated cells (dark cells) was approximately 20 to 30%. In tubules dissected from the inner stripe of the outer medulla which displayed a spontaneously positive voltage the dark cell population was reduced to less than 10%. As mentioned above, the designation of principal cell and intercalated cell is somewhat arbitrary since the morphology of the principal cell of the outer medulla is different from that of the CCT.

Additional functional aspects of this axial heterogeneity have been examined recently [24]. Figure 3 displays the net flux of sodium, potassium, and chloride of the CCT and from the outer and inner stripes of the outer medullary collecting tubule. In contrast to the CCT which absorbed sodium, secreted potassium, and had a lumen-negtive V_T, collecting tubules dissected from the inner stripe of the outer medulla had no significant net sodium or potassium transport and had a consistently lumen-positive V_T. The collecting tubule dissected from the outer stripe of the outer medulla displayed an intermediate function and appears to be a transitional segment. The mechanism of sodium transfer across the inner stripe of the outer medullary collecting tubule has been examined further recently [16]. Figure 4 displays the effect of 0.5 mM ouabain applied from the bath surface on sodium efflux across the cortical collecting tubule and the OMCT dissected from the inner stripe. In

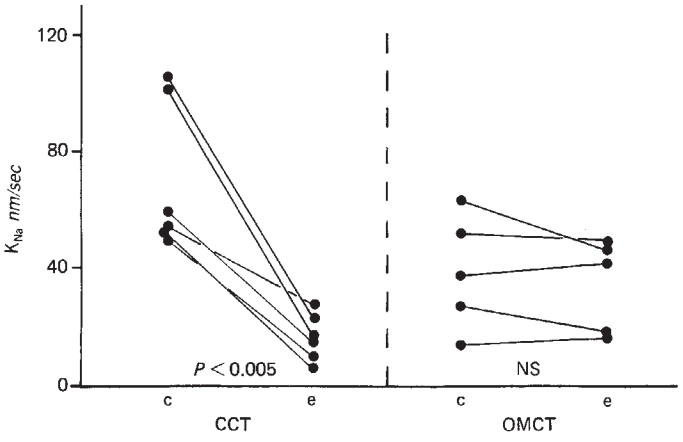

Fig. 4. *Effect of ouabain (0.5 mM) on the sodium efflux rate coefficient (K_{Na}) across the CCT and OMCT.* All tubules were dissected from normal rabbits. Tubules designated OMCT were dissected from the inner stripe of the outer medulla and all had lumen-positive V_T. (Figure was made from data reported in [16].)

contrast to the CCT, ouabain had no effect on either V_T or sodium efflux across OMCT. These results indicate that sodium efflux across OMCT has no component analogous to the cellular pathway of the CCT. Sodium transfer cross the OMCT is not carrier-mediated since recent experiments demonstrated that the tracer efflux rate coefficient, which is the tracer flux divided by the tracer concentration, was independent of the sodium concentration. This behavior is predictably different from the sodium concentration effects on the tracer rate coefficient across the CCT [16]. In this segment, increasing the sodium concentration reduces the tracer rate coefficient as though sodium absorption occurred by a carrier-mediated process. Taken together, this evidence strongly suggests that sodium transfer across the inner stripe of the OMCT occurs by simple ionic diffusion. Thus, the active absorptive process disappears as the collecting tubule descends into the medulla.

All experimental evidence to date indicates that sodium transport across the OMCT (dissected from the inner stripe) occurs by simple ionic diffusion [16, 24]. The permeability coefficient for this segment ranges from 10 to 100 nm/sec. The CCT has a sodium permeability coefficient which is generally lower (8 to 25 nm/sec), but this value and the technique used to measure it vary greatly by investigator [8, 10, 13, 16, 25–27]. Despite these modest differences in the absolute value of the sodium permeability coefficient, the permeabilities of both segments are relatively low, an observation consistent with the fact that the urine sodium concentration can be low. Thus, the collecting tubule is able to maintain steep chemical gradients for sodium.

The "disappearance" of active sodium absorption in OMCT raises several questions regarding its functional significance: First, if sodium transport occurs by diffusion, will not the steep chemical gradients generated in the CCT tend to be dissipated? An affirmative answer appears inescapable. Although this issue has not been examined in the rabbit in vivo, Sonnenberg [28] described net sodium entry into the medullary collecting tubule of the rat during volume expansion. Extrapolation of results from rat to rabbit should be done cautiously, but the data from each species are consistent with the interpretation that sodium can be "secreted" by the medullary collecting tubule (consistent with its chemical gradient), at least under the appropriate conditions.

A second question is a corollary to the first: Can the permeability of the OMCT be altered? At present there is little information on which to base a firm answer. However, the in vivo observation in the rat indicating that measurable sodium addition occurs only under certain circumstances suggests that permeability might be regulated. In addition, the wide range of rate coefficients for the OMCT perfused in vitro also suggests a possible regulation of OMCT permeability. However, one likely candidate, mineralocorticoid hormone, does not appear to affect sodium permeability since DOCA pretreatment failed to induce a change in either V_T or the sodium efflux rate coefficient [24].

A third question is teleological: What is the significance of this axial change in sodium transport to the kidney? The answer(s) to this question is, of course, speculative and probably incomplete, but some preliminary thoughts should be mentioned. Possibly this difference plays a role in the natriuresis of volume expansion. The in vivo experiments in the rat address this issue explicitly [28]. Although the mechanism of sodium transport in the papillary collecting duct of the rabbit is unknown, in vivo experiments on the rat indicate that net sodium absorption can be accomplished [29, 30], and if a similar situation exists for the rabbit, net reabsorption of Na(Cl) in the papilla may be the final regulator of sodium excretion. Without such a mechanism it is difficult to envision how the urine could be rendered sodium-free.

Potassium transport

Cortical collecting tubule. Potassium secretion by the isolated, perfused CCT was first reported by Grantham, Burg, and Orloff [7]. These studies provided clear evidence for active potassium secretion and formed the foundation for further experiments on its mechanism. One of the striking observations these investigators made was that the concentration of potassium in the luminal fluid could be raised to over 100 mM if the contact time was greater than 600 sec. This observation indicates that potassium secretion is "active" since the measured V_T could not explain such a concentration gradient. The relationship between the measured V_T and the observed and predicted concentration gradients can be evaluated by the Nernst equation

$$V_T = \frac{RT}{zF} \ln \frac{[K]_b}{[K]_l} \qquad (1)$$

where the transepithelial voltage (V_T) is oriented with the bath as ground, $[K]_b$ and $[K]_l$ are the potassium concentrations of the bath and the collected fluid, respectively, and z, R, T, and F have their usual meanings. If the calculated V_T exceeds the measured value, the inescapable conclusion is that potassium secretion cannot be due to simple ionic diffusion across a single membrane. The physical interpretation of this statement is that potassium secretion cannot be due entirely to a paracellular flow through the limiting junctions but that a substantial fraction occurs via a cellular pathway and consumes energy (directly or indirectly). Experiments subsequently conducted in this

laboratory [8] at physiologic flows (4 to 5 nl/min) have shown that in half the tubules examined $[K]_l$ exceeds that predicted by the Nernst equation despite the fact that contact time was less than 15 sec and that equilibrium had not yet been achieved.

Stoner, Burg, and Orloff [10] used a second method to confirm that potassium secretion is greater than that predicted by simple ionic diffusion. These investigators examined the sequential unidirectional potassium fluxes using the Ussing flux ratio equation

$$\frac{J_K^{lb}}{J_K^{bl}} = \frac{C_b}{C_l} \exp\left(\frac{-zF\,V_T}{RT}\right) \qquad (2)$$

where J_K^{lb} and J_K^{bl} are the unidirectional fluxes of potassium from lumen-to-bath and from bath-to-lumen, respectively, and C_b and C_l are the concentrations of potassium in the bath and lumen, respectively. By this analysis, the V_T required to account for the observed flux ratio was −50 mV while the measured V_T was only −35 mV. Thus, by this evaluation K secretion is "active."

A third method of evaluating the contribution of diffusional potassium secretion is to determine the permeability coefficient (P) and apply the Goldman-Hodgkin-Katz equation. The upper estimate of the permeability coefficient is the lumen-to-bath tracer rate coefficient. This upper limit designation applies because the unidirectional flux includes not only the diffusional components but also any carrier-mediated component. This value, corrected to a V_T of zero, determined both by Stoner, Burg, and Orloff [10] and Stokes [8], is approximately 100 nm/sec. The magnitude of this value reinforces the notion that it is an upper limit, for it is several times larger than the permeability coefficient for sodium [8, 10, 13, 16, 25–27]. The voltage-dependent component of potassium secretion can be determined using

$$J_K^{net} = P\,\xi\,\frac{C_b - C_l\,e^{-\xi}}{\xi - 1} \qquad (3)$$

where $\xi = zFV_T/RT$. J_K^{net}, the net potassium secretory rate, calculated assuming a constant absorptive flux (a worst-case analysis), was only 10% of the observed flux [8]. Thus, by these three independent approaches, potassium secretion must be a cellular process and cannot be explained by paracellular diffusion.

There is extensive literature on the dependency of sodium absorption for urinary potassium excretion. The nature of this dependency for potassium secretion by the CCT is now clear. Grantham, Burg, and Orloff [7] demonstrated that perfusion with a sodium-free solution caused a marked reduction in potassium secretion. Stoner, Burg, and Orloff [10] and O'Neil and Helman [11] demonstrated that amiloride, an agent which inhibits the cellular entry of sodium across the apical membrane, also abolishes potassium secretion. Grantham, Burg, and Orloff [7] also showed that the administration of ouabain to the peritubular surface to inhibit Na-K-ATPase also abolished sodium absorption and potassium secretion.

Recently the dependency of potassium secretion on sodium absorption has been examined in this laboratory [8]. The magnitude of potassium secretion was examined over a wide range of rates of sodium absorption, a range extended beyond the normal variability by pretreating some rabbits with DOCA to increase sodium absorption. The results of these experiments, depicted in Figure 5, demonstrate three important features: First, potassium secretion rates and sodium absorption rates are related linearly. Second, extrapolation of sodium absorption to zero predicts that the potassium secretion rate would likewise be zero, a result consistent with the results using amiloride, ouabain, and a sodium-free perfusate. Thus, it is apparent that sodium absorption is required for potassium secretion. Finally, the ratio of sodium absorption to potassium secretion (the reciprocal slope of the regression line in Fig. 5) is ~ 1.35. This ratio is close to the stoichiometry of the Na-K pump (3:2) as determined in the red cell [31], frog skin [32], and turtle colon [33]. A similar value can be obtained by calculating the ratio of the change in sodium absorption and potassium secretion induced by DOCA treatment as determined by Schwartz and Burg [26] in grouped experiments. Their results yield a ratio of 1.53, a value reasonably consistent with the value determined using individual experiments (Fig. 5).

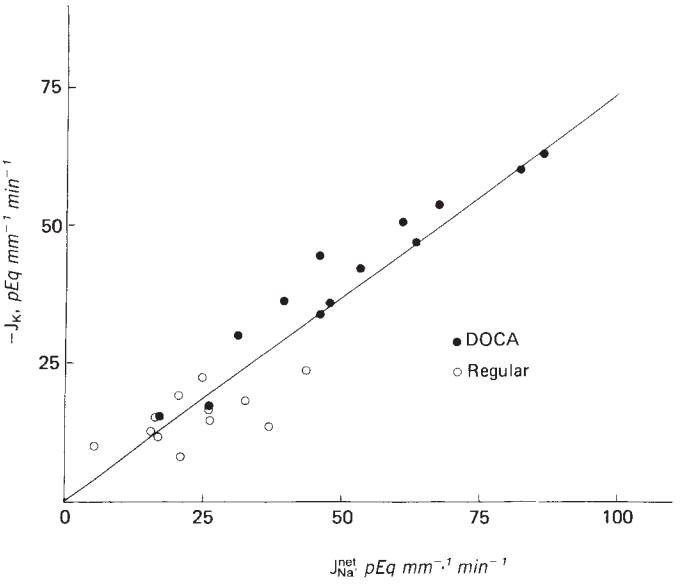

Fig. 5. *Relationship between net potassium secretion ($-J_K$) and net sodium absorption (J_{Na}^{net}) in CCT. Open circles* represent tubules dissected from normal rabbits; *closed circles* represent tubules dissected from rabbits treated with DOCA for 1 to 7 days prior to sacrifice. Linear regression equation is $y = 0.745\times + 0.34$, $r = 0.945$. The sodium:potassium flux ratio (the reciprocal slope, $\Delta J_{Na}^{net}/-\Delta J_K$) is 1.34. (Reproduced from [8] with permission.)

The dependence of potassium secretion on sodium absorption was further examined by measuring the rate of potassium secretion as a function of perfused sodium concentration [8]. The results are in agreement with those of Grantham, Burg, and Orloff who [7] demonstrated that when the lumen sodium concentration is 30 mM or greater, potassium secretion is little affected. However, when mean sodium concentration in the lumen approached zero, potassium secretion also approached zero. Half maximal potassium secretion was observed when mean lumen sodium concentration was ~ 8 mM. This concentration of sodium is also that at which half-maximal sodium transport occurs in other "tight" epithelia such as the frog skin

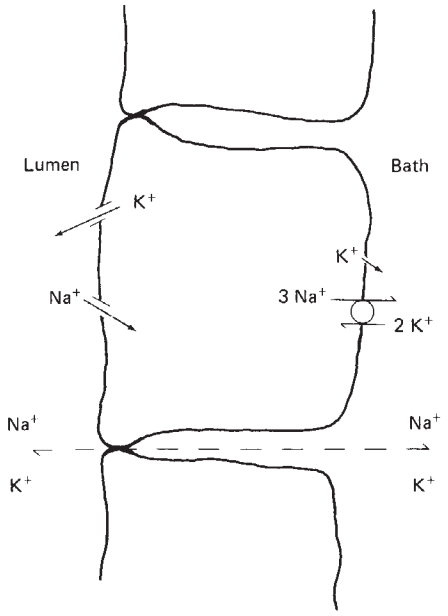

Fig. 6. *Proposed model of sodium and potassium transport across CCT.* Sodium enters the cell across the apical membrane "down" a favorable electrochemical potential gradient. Extrusion across the basolateral membrane occurs via the Na:K pump where three sodium ions are exchanged for two potassium ions. Potassium accumulates in the cell and exits down its electrochemical potential gradient. Under conditions where potassium secretion is maximal, the apical membrane permeability appears to be larger than the basolateral permeability. A similar model has been proposed by O'Neil [39]. (Reproduced from [8] with permission.)

[34] and turtle colon [35]. The results of these experiments indicate that potassium secretion is critically dependent on sodium absorption. The similarities of the kinetics of sodium absorption and potassium secretion together with the observed flux ratios suggest that in the CCT almost all secreted potassium traverses the cell via the Na-K pump on the basolateral membrane and exits the cell down its electrochemical potential gradient across the apical membrane. This model is diagramatically depicted in Figure 6. The two rate-determining processes would thus be: (*1*) the rate of sodium absorption, and (*2*) the relative permeabilities of the apical and basolateral membranes to potassium.

This model for potassium secretion would have potassium exit the cell either across the apical membrane or across the basolateral membrane. Although under normal conditions the respective electrochemical potential gradients would favor potassium exit across the apical membrane, a reduction in apical membrane permeability and/or an increase in basolateral membrane permeability would result in less net secretion and more potassium "recycling" across the basolateral membrane. In this way, sodium absorption could be separated from potassium secretion.[1]

[1]Helman and O'Neil [36], using equivalent circuit analysis, proposed two separable EMF's for sodium and potassium transport across the CCT. Their analysis does not preclude a high degree of coupling between the two flows.

Table 1 lists all experiments where sodium absorption and potassium secretion have been examined in the same tubule. Three observations seem noteworthy: First, sodium absorption always exceeded potassium secretion with the lowest ratio (J_{Na}/J_K) being approximately 1.3. This ratio is close to the stoichiometry of the Na-K pump so that these data are consistent with the idea that most potassium secretion occurs by a transcellular process and depends on the integrity of the pump. Ratios of less than 1.5 might be explained, at least partially, by voltage-dependent potassium secretion which, as noted above, could account for 10% of the observed net secretion [8]. Voltage-dependent sodium backflux might also account for a flux ratio less than 1.5.

Second, in the three studies where sodium absorption and potassium secretion have been examined by the same investigators in both control and DOCA-pretreated rabbits, the J_{Na}/J_K ratio is lower in tubules from DOCA-pretreated animals. This decrease is owing to a relatively greater increase in potassium secretion than in sodium absorption. The tendency for J_{Na}/J_K ratios to be higher than 1.5 in CCT from normal rabbits is also evident when the studies are considered in the aggregate.

Third, it is evident that acidification of the perfusate correlates with a higher J_{Na}/J_K ratio. The first explicit examination of the effect of luminal acidification on ion transport in CCT was conducted by Boudry, Stoner, and Burg [13]. These investigators demonstrated that acidification of the lumen reduced potassium secretion by approximately 50% without affecting sodium absorption significantly. Subsequently, Shareghi and Stoner [21] demonstrated that the removal of bicarbonate from the perfusate caused a 50% reduction in potassium secretion without affecting sodium absorption, an effect also likely due to acidification of the lumen contents by the entry of carbon dioxide [37]. In these studies, the ratio of sodium absorption to potassium secretion increased from 1.5 to 3.0. Thus, it appears that for maximum potassium secretion to occur the pH of the perfusate should be close to 7.4 although the precise relationship to lumen pH and potassium secretion has not yet been determined. The data in Table 1 support these experimental results since, in studies where bicarbonate was eliminated from the perfusate, the J_{Na}/J_K ratio was generally higher than when bicarbonate was present.

The thesis that the apical membrane has a relatively high potassium permeability has been examined by several investigators using electrophysiologic techniques. Increasing potassium concentration in the lumen causes a deflection in V_T consistent with a high selectivity for potassium [11, 12, 16]. Replacing lumen sodium with potassium also increased transepithelial conductance [12]. Recently, Koeppen, Biagi, and Giebisch [19] and Koeppen and Giebisch [20] measured intracellular voltage of cells from the isolated, perfused CCT. They found that raising lumen [K] depolarized the apical membrane, a result consistent with a large potassium conductance. Furthermore, they found that barium, an agent which blocks potassium channels [38], also depolarized the apical membrane and mitigated the depolarizing action of high luminal potassium.

The results of the experiments measuring fluxes when considered with the electrophysiologic data provide strong evidence that potassium secretion occurs by a process depicted in Figure 6. This model has been proposed independently by two groups of investigators [8, 39] and satisfies all of the data accumulated

Table 1. Comparison of simultaneous net sodium absorption and potassium secretion in rabbit CCT

Reference	Temp °C	V_T mV	J_{Na}^{net} pEq/cm · sec	J_K^{net} pEq/cm · sec	J_{Na}/J_K	Comments
Boudry, Stoner, and Burg [13]	37	−32	7.4	1.7	4.3	[a]
Holt and Lechene [63]	37	−10	5.3	1.7	3.1	
Iino and Imai [69]	37	−23	8.6	4.9	1.7	DOCA
O'Neil and Helman [11]	24	−8	2.7	0.5	5.4	[a]
		−58	6.3	3.1	2.0	[a] DOCA 11 to 18 days
		−29	4.9	3.9	1.3	[a] DOCA 23 to 31 days
Schwartz and Burg [26]	37	−16	5.7	2.3	2.5	
		−46	15.4	8.8	1.7	DOCA 10 to 15 days
Shareghi and Stoner [21]	37	−21	5.1	3.4	1.5	
			5.1	1.6	3.2	[a]
Stokes et al [24]	37	−21	3.8	2.4	1.6	
		−42	9.0	7.2	1.3	DOCA 2 to 6 days
Stoner, Burg, and Orloff [10]	37	−38	7.2	3.0	2.4	[a]

[a] Perfusate contained no HCO_3^-.

thus far regarding sodium and potassium transport in the CCT. There are at least two attractive features to this model: (*1*) It is efficient, and (*2*) in principal, the relationship between sodium absorption and potassium secretion can be varied over a wide range. Whether this model reflects all of the mechanisms responsible for sodium and potassium transport remains to be determined.

Outer medullary collecting tubule. In contrast to the cortical collecting tubule, potassium secretion in the inner stripe of the outer medullary collecting tubule does not occur spontaneously (Fig. 3) [24]. As is the case with sodium absorption and V_T, potassium transport in the outer stripe of the outer medullary collecting tubule appears to be transitional. Within the inner stripe of the outer medulla, there is now substantial evidence to indicate that potassium transport as well as sodium transport is diffusional. Support for this conclusion is based on four observations: First, there is no spontaneous net potassium transfer in the isolated perfused outer medullary collecting tubule dissected from the inner stripe whether or not the rabbit has received DOCA previously [24]. Second, in contrast to that found in the cortical collecting tubule, there is no potassium selectivity as evidenced by biionic diffusion potentials when potassium in the lumen is raised to 50 mM. The diffusion potential is equal (and opposite in sign) to the liquid junction potential, indicating that the ratio of the conductivities of sodium and potassium across the outer medullary collecting tubule is not different than the mobilities of those ions in water. Third, the tracer efflux rate coefficients for potassium and sodium measured simultaneously conform to the ratio of their mobilities in water (1.43), thus indicating that both sodium and potassium likely traverse nonselective aqueous pathways across the outer medullary collecting tubule. Finally, raising lumen potassium concentration at the expense of sodium (as would likely be the situation in vivo) produces net absorption of potassium and net sodium secretion, the magnitude of which is consistent with the rate coefficients determined isotopically. Together with the independence of the tracer rate coefficient on the abundant concentration of sodium mentioned previously, there is no evidence for any carrier-mediated transfer process for either sodium or potassium across the inner stripe of the outer medullary collecting tubule of the rabbit, at least as it is perfused and bathed in vitro [16].

The disappearance of the active transport processes for sodium and potassium within the outer medulla raises the reasonable question of its physiologic significance. A possible explanation for the elimination of these active transport processes in this segment of the collecting tubule likely relates to the phenomenon of potassium recycling recently described by Battilana et al [40], Dobyan, Lacy, and Jamison [41], and Arrascue, Dobyan, and Jamison [42]. As it is currently understood, potassium recycling to the renal medulla is critically dependent on the magnitude of potassium secretion and the concentration of potassium in the urine. The elimination of active transport in this segment allows a small but (probably) significant amount of potassium to diffuse back to the renal medulla. The resulting accumulation in the medulla would provide the chemical gradient required for potassium secretion into the pars recta and/or descending limb of Henle's loop. Preliminary experiments from this laboratory indicate that this accumulation might affect ion transport across the medullary thick ascending limb [43].

It is unlikely that the diffusional nature of sodium and potassium transfer in the outer medullary collecting tubule continues along the papillary collecting tubule. Nevertheless, the differences between the cortical and outer medullary collecting tubules with respect to sodium and potassium transport are striking. The complete significance of this arrangement is yet to be determined.

Chloride transport

Cortical collecting tubule. The nature of chloride transport across the cortical collecting tubule has been studied by several investigators and appears to be more complex and less well understood than in sodium or potassium transport. There is general agreement that a major fraction of the tracer chloride flux can be ascribed to an electroneutral process which appears to be a chloride-chloride exchange. Evidence in support of this process comes from two groups of investigators. Stoner, Burg, and Orloff [10] found that the rate coefficient for chloride transfer was 470 nm/sec, a value 50 times larger than the apparent permeability coefficient for sodium. Since the calculated partial ionic conductance for chloride was 20.2 mS/cm^2, and the total electrical conductance of the tissue was only 3.78

mS/cm^2, these investigators concluded that much of the chloride tracer flux must be electrically silent. These investigators also concluded that approximately 85% of this flux could be due to chloride-chloride exchange. Hanley and Kokko [44] supported this notion with experiments demonstrating that the lumen-to-bath tracer efflux was unaffected by transepithelial voltage of −35 to +28 mV. In addition, the efflux rate coefficient fell from 241 to 69 nm/sec when the chloride concentration in the bath solution was removed. Taken together, these data provide strong evidence for an element of chloride-chloride exchange which comprises at least 70% of the tracer efflux.

The presence of this chloride-chloride exchange process complicated the evaluation of the nature net chloride absorption. Net absorption of chloride was inferred from the experiments of Stoner, Burg, and Orloff [10] and O'Neil and Helman [11] by calculating the difference between sodium absorption and potassium secretion. Net chloride absorption was measured directly by Hanley and Kokko [44] and was found to vary with the mineralocorticoid hormone status of the rabbit prior to study of the collecting tubule. These investigators found that net chloride absorption in collecting tubules from normal rabbits was small with a difference in chloride concentration of only 2.3 mM. In contrast, chloride absorption increased substantially in collecting tubules taken from rabbits pretreated with DOCA. This observation regarding the net absorption of the chloride increasing in response to DOCA pretreatment was confirmed by Stokes et al [24]. In collecting tubules from normal rabbits, chloride absorption was approximately 0.5 pEq/cm·sec. Chloride absorption in DOCA-treated rabbits increased to over 3 pEq/cm · sec, a value significantly greater than normals. In these experiments the rate of chloride absorption was not significantly different from the difference between the rates of sodium and potassium transport, and thus net chloride absorption might be explained completely on the basis of a passive mechanism. However, it should be emphasized that small amounts of bicarbonate absorption or secretion could have been missed by these techniques and a component of carrier-mediated net chloride absorption cannot be eliminated.

The possibility that chloride could be absorbed by an "active" process has been considered by several groups of investigators. The unambiguous demonstration of net chloride absorption against an electrochemical gradient such as occurs in the thick ascending limb of Henle's loop would constitute sufficient evidence for the consideration of an "active" process. The demonstration of a lumen-positive V_T in the cortical collecting tubule has provided such an opportunity to examine chloride transport under the appropriate electrical gradient. This lumen-positive V_T can be observed spontaneously in some rabbits treated with a high sodium diet. However, most investigators have found that high sodium diets do not consistently produce a CCT with lumen-positive V_T, and thus, it is necessary to utilize pharmacologic techniques to obtain tubules with such voltages consistently. A positive V_T can be produced by either adding amiloride to the perfusate (to inhibit sodium absorption), eliminating sodium from the perfusate, or by treating the tubule with ouabain. The lumen positive V_T produced by these maneuvers can thus be examined to determine its nature.

The magnitude of the lumen positive V_T is reduced by treatment of the tubule with acetazolamide [10, 45, 46], elimination of carbon dioxide and bicarbonate from the perfusate and bath [10], and by treatment of the tubule with SITS [46]. Additional experiments have demonstrated that this positive V_T, when induced by a sodium-free perfusate, is not inhibited by ouabain [45] nor is it inhibited by removal of potassium from the bathing solutions [46]. Furosemide in the perfusate also has no effect on the magnitude of this positive V_T [45]. Thus, if this V_T represents some degree of electrogenic chloride absorption, it is different than the chloride absorption process in the thick ascending limb of Henle's loop where the positive voltage is sensitive to both ouabain and furosemide. In contrast, the effects of these drugs are consistent with the V_T being due to acidification.

The evidence for electrogenic, active chloride absorption rests with two observations made by Hanley et al [45]. First, when the perfused concentration was 8 to 10 mM, net chloride was absorbed (against its chemical gradient) and the collected concentration was well below the value predicted by the Nernst equation (equation *1*). While this information by itself does not indicate an electrogenic absorptive process, the authors conclude that it is consistent with such. It is also consistent with a neutral process. The second observation they made was that removal of chloride from all solutions (by substituting methyl sulfate) caused the spontaneously positive V_T to go to zero or a slightly negative value. They thus concluded that the positive V_T was due to active electrogenic chloride transport.

The dependency of the positive V_T on the presence of chloride contradicts other reports. Stoner, Burg, and Orloff [10] and Koeppen and Helman [46] reported that substitution of sulfate for chloride does not affect the magnitude of this V_T. Although the difference between substituting sulfate or methyl sulfate for chloride has not been scrutinized carefully, the discrepancies may be due to the nature of the ion substitution. Recently, however, experiments by Fischer, Husted, and Steinmetz [47] in the turtle urinary bladder have suggested a much more likely explanation for the apparent dependence of chloride on the lumen positive voltage. In the turtle's urinary bladder, the electrogenic hydrogen ion secretory mechanism is located on the apical membrane. Intracellular pH is maintained by bicarbonate exit across the basolateral membrane. Fischer's experiments indicate that this exit step is a chloride-bicarbonate exchanger, and thus the process of acidification is dependent on the presence of chloride in the solution bathing the basolateral membrane. The affinity of this exchanger for chloride is extremely high with a K_m of ~ 0.1 mM. Thus, seemingly trivial amounts of chloride in the serosal solution can produce maximal amounts of hydrogen ion secretion. Based on the similarities of the positive lumen voltage in the CCT with that of the turtle urinary bladder, it is quite likely that the discrepancies regarding the dependence of this positive voltage on the presence of chloride are not due merely to the nature of the anion substitution but rather the completeness with which chloride was eliminated from the solutions. The chloride-sensitive (positive) V_T could in this way be due to electrogenic hydrogen ion secretion. Nevertheless, there appears to be a carrier-mediated process for chloride absorption based on the reduction of luminal chloride concentration which is unexplained by diffusion. Whether or not this absorptive process is electrogenic will require further experimentation.

Outer medullary collecting tubule. The net flux of chloride across the inner stripe of the outer medullary collecting tubule

is, as for sodium and potassium, not significantly different from zero. This finding appears to be true whether or not the OMCT is dissected from normal or DOCA-treated rabbits or whether the segment is perfused in hypertonic or isotonic solutions [24]. It is not known whether there is chloride exchange diffusion in this segment. There is, however, a consistently positive V_T which exhibits many of the same characteristics of the positive V_T in the CCT [48]. In contrast to the CCT, however, the OMCT absorbs bicarbonate at a substantially greater rate [48]. This observation together with the lack of substantial chloride flux suggests that this (positive) V_T is due predominantly if not exclusively to hydrogen ion secretion. The elucidation of the nature of chloride transfer in this segment must await further experimentation.

Human cortical collecting tubule. Two studies have been made on the voltage characteristics of the human CCT. Jacobson et al [49] found that the spontaneous V_T was consistently lumen positive and inhibited by either furosemide or ouabain. Acetazolamide had no effect and the elimination of chloride from the solutions reduced the voltage to near zero. Yanagawa et al [50] likewise found that ouabain inhibited the lumen positive V_T and, in addition, found two tubules where a spontaneous lumen negative V_T occurred. Ouabain reduced the magnitude of both the spontaneously positive and the spontaneously negative V_T. Although the nature of this voltage in the human collecting tubule is uncertain, the unambiguous differences between the positive voltage found in the human compared to those in the rabbit indicate that the nature of the transport processes are completely different. Based on these pharmacologic results, the human CCT behaves more like the thick ascending limb of Henle's loop while the lumen-positive V_T of the rabbit CCT is more likely owing to electrogenic hydrogen ion secretion.

Hormonal regulation of ion transport

Mineralocorticoid hormone. The cortical collecting tubule is the principal site of action for aldosterone, the major mineralocorticoid hormone. The localization to the CCT is consistent among the studies of Gross, Imai, and Kokko [15], Imai [51], and Stokes et al [24] who showed clearly that the cortical collecting tubule alone responded to mineralocorticoid hormone while the distal convoluted tubule, the connecting tubule, and the outer medullary collecting tubule did not respond in a similar fashion. The major physiologic effect of mineralocorticoid hormones is to increase sodium absorption and potassium secretion [11, 24, 26]. The data regarding the comparative effects of DOCA-pretreatment on net sodium absorption and potassium secretion are included in Table 1. In addition to the mineralocorticoid effect on the magnitude of net sodium absorption and potassium secretion, the J_{Na}/J_K ratio tends to fall. As previously noted, this change in ratio is owing predominantly to a relatively greater increase in potassium secretion. Although it is clear that the magnitude of potassium secretion can be modulated by variations in circulating mineralocorticoid hormone concentrations, recent experiments of Wingo, Kokko, and Jacobson [52] demonstrated that potassium secretion can be modulated in adrenalectomized rabbits and that variations in dietary potassium can alter the magnitude of potassium secretion in vitro. The mechanism whereby potassium secretion is altered in this setting is not clear.

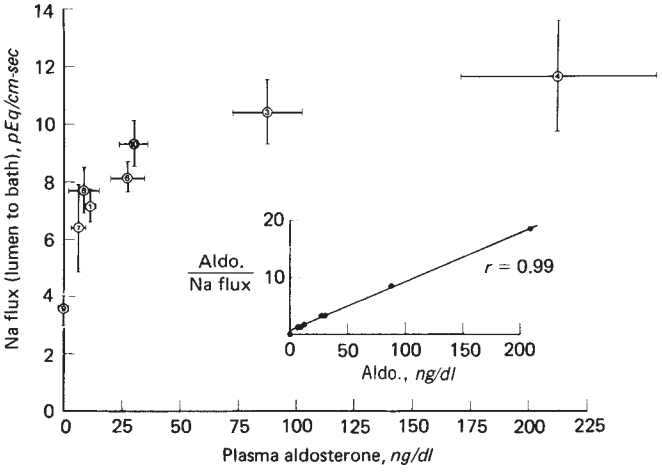

Fig. 7. *Relation between plasma aldosterone concentration at the time of sacrifice to the subsequently determined (in vitro) rate of sodium absorption across the CCT.* Numbers within *circles* refer to groups of rabbits which had alterations in diet to produce changes in endogenous aldosterone production. Insert is a Hanes plot demonstrating the $K_{1/2} \approx$ 7 ng/dl and $V_{max} \sim$ 12 pEq/cm · sec. (Reproduced with permission from [26].)

The relation between the concentration of circulating aldosterone and the magnitude of sodium absorption and potassium secretion was examined by Schwartz and Burg [26]. Studying an extensive number of tubules, these investigators demonstrated a classic dose-response relationship between the plasma aldosterone concentration at the time of sacrifice and the in vitro rates of sodium and potassium transport. Figure 7 displays their dose-response curve for sodium absorption and plasma aldosterone concentration. They obtained similar curves for potassium secretion and V_T. The apparent $K_{1/2}$ for these experiments was 0.2 to 0.5 nM. The K_d of the high affinity aldosterone binding site determined by Marver [53] using rabbit renal cortex was 3 to 3.7 nM. Although these values appear somewhat disparate, they are in reasonable agreement considering the methodologic differences in their determination.

The in vitro response of the CCT to aldosterone was not as clearly demonstrated as was the effect of increasing plasma concentration prior to sacrifice. Gross and Kokko [54] showed an increase in the magnitude of the negative V_T after 40 to 50 min of in vitro exposure to aldosterone. The concentration of aldosterone required to produce an effect was 0.2 mM, six orders of magnitude larger than the apparent $K_{1/2}$ determined for antemortum plasma aldosterone effect. These investigators reported the effect only from the luminal surface. This voltage-response to in vitro aldosterone has proven difficult to reproduce. Schwartz and Burg [26] could not reproduce these results. Preliminary results reported by Wingo, Kokko, and Jacobson [55] confirm the lack of an effect of in vitro aldosterone on V_T. However, they noted that net sodium absorption increased. Since these tubules were harvested from previously adrenalectomized rabbits, presumably the results are independent of previous aldosterone exposure. The mechanism whereby sodium absorption increases without a change in V_T is unclear.

Another enzyme of crucial importance to sodium and potassium transport is Na-K-ATPase. The effects of mineralocorticoid

hormone on this enzyme has been examined by several investigators. Garg, Knepper, and Burg [56] reported that chronic stimulation by either a low sodium diet or DOCA increased CCT Na-K-ATPase activity over normal controls. They also found that Na-K-ATPase activity in the connecting tubule was increased by DOCA but not by low sodium diet. Whether this stimulation reflects "contamination" by functional CCT or whether the connecting tubule responds to DOCA along its entire length is uncertain. Horster, Schmid, and Schmidt [57] demonstrated that in vitro exposure of the CCT to aldosterone stimulates Na-K-ATPase. The nature of this stimulation following adrenalectomy was examined recently by Petty, Kokko, and Marver [58]. These investigators found that *(1)* aldosterone stimulated Na-K-ATPase at 3 hr but not at 1.5 hr after administration while aldosterone stimulated citrate synthase at 1.5 hr [59], *(2)* there was no stimulation with dexamethasone, *(3)* the stimulation was blocked by prior administration of spironolactone, and *(4)* the stimulation was also blocked by prior (in vivo) treatment with amiloride. These experiments provide evidence that the mineralocorticoid-induced increase in Na-K-ATPase is secondary to an increased availability of sodium to the pump and not a primary effect of the hormone.

There is morphologic and autoradiographic evidence which supports the fact that the CCT is the target for aldosterone. Wade et al [60] measured the basolateral membrane surface area of CCT from DOCA-treated rabbits and found a two- to threefold increase over the normal controls. This increase was localized to the principal cell. Apical membrane area was not increased. Vandewalle et al [61] reported that, using autoradiography, aldosterone binding was localized to the distal convoluted tubule and CCT. Thus, based on functional, biochemical, and morphological data, the CCT is a major, if not the only, site where mineralocorticoid produces its classic effects: sodium retention and kaliuresis.

Vasopressin and prostaglandins. Several investigators [15, 17, 27, 62, 63] reported that exposure of the peritubular surface of the CCT to vasopressin causes an increase in the magnitude of (the lumen-negative) V_T. This effect is transient, and V_T is reduced ultimately below control values [15, 51, 63]. This transient lasts only 5 to 15 min at 37°C. The changes in V_T parallel those made in net sodium absorption [27, 63]. The stimulation of sodium absorption is similar to its effect on the toad bladder [6], but the subsequent reduction may represent a different action. The cellular responses to vasopressin vis-à-vis ion transport were not as thoroughly investigated in the CCT as were its effects on water transport. The reduction in sodium absorption is not accompanied by a change in transepithelial resistance [62] and is not accompanied by a reduction in potassium secretion [63].

There is accumulating evidence that vasopressin stimulates production of prostaglandins by the cells of the collecting tubule. The isolated medullary collecting tubule of the rat can synthesize PGE_2 and $PGF_{2\alpha}$ [64] and the rabbit CCT also contains cyclooxygenase [65] so that the CCT should also synthesize prostaglandins. Preliminary data from Kirschenbaum et al [66] indicate that such synthesis can occur. Since vasopressin can stimulate prostaglandin synthesis [67, 68] and prostaglandins can inhibit sodium absorption [25, 69], Holt and Lechene [63] examined the effect of cyclooxygenase inhibitors on the vasopressin-induced reduction in sodium absorption. These investigators found that the (subsequent) reduction, but not the magnitude or the duration of the transient increase, in sodium transport was prevented by cyclooxygenase inhibitors. Furthermore, the vasopressin-induced reduction in V_T and sodium transport could be returned to control values if cyclooxygenase inhibitors were added to the bath even in the presence of vasopressin. It appears that the action of vasopressin may be, at least in part, influenced by endogenously-produced prostaglandins.

There are some differences between vasopressin and prostaglandin effects on transport. Vasopressin usually causes a transient stimulation of V_T whereas PGE_2 (the major prostaglandin of the collecting tubule) seldom does [25, 69]. In addition, PGE_2 inhibits potassium secretion [63, 69] while vasopressin does not. Thus, there are some apparent differences between the vasopressin and prostaglandin effects on ion transport.

Although there is qualitative unanimity regarding the effects of vasopressin on sodium transport, there is not such unanimity regarding the effects of the prostaglandins. Fine and Trizna [70] reported no effect of PGE_2, $PGF_{2\alpha}$, or PGA_2 on sodium transport or V_T of medullary collecting tubules. This discrepancy is not explained by regional heterogeneity of the collecting tubule. PGE_2 also inhibits the V_T of medullary collecting tubules with a lumen-negative V_T [25, 69]. Neither are the differences due to the mineralocorticoid hormone status of the rabbit since prostaglandins inhibit V_T in CCT from normal or DOCA-pretreated rabbits [25, 63, 69]. The differences probably associate with the method of preparation of the prostaglandins.

Catecholamines. The effects of catecholamines on the cortical collecting tubule were examined. Imai [51] found that the CCT was considerably less sensitive than the connecting tubule, at least with respect to the alterations in V_T. Iino, Troy, and Brenner [18] reported a decrease in the magnitude of lumen negative V_T with isoproterenol and an increase in the magnitude of the lumen positive voltage after treatment with ouabain or removal of sodium from both perfusate and bath. Pretreatment with acetazolamide prevented this effect on V_T. There was no effect on unidirectional sodium absorption. These results suggest that isoproterenol stimulates electrogenic acidification. Although bicarbonate absorption was not measured, and thus this explanation was not examined directly, net chloride transport was measured. Net chloride absorption increased after isoproterenol. Removal of chloride from perfusate and bath also abolished this voltage response. These observations suggest that isoproterenol induces chloride absorption. The mechanism of this increase in chloride absorption is unknown.

Summary. The cortical and outer medullary collecting tubule can play a major role in the regulation of sodium, potassium, chloride and hydrogen ion balance. The rabbit collecting tubule displays striking heterogeneity in its structure and function as it descends from the cortex into the outer medulla. The CCT has transport systems for each of these ions. It absorbs sodium and chloride, secretes potassium, and can acidify the contents of the lumen. Furthermore, at least some of these transport processes can be modified by hormones. In contrast, the collecting tubule from the inner stripe of the outer medulla has no carrier-mediated system for sodium or potassium transport and has a more active system of acidification. These differences probably play a role in the intratubular ion transfer.

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