Mathematical models of renal fluid and electrolyte transport: acknowledging our uncertainty

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Weinstein, Alan M. Mathematical models of renal fluid and electrolyte transport: acknowledging our uncertainty. Am J Physiol Renal Physiol 284: F871–F884, 2003; 10.1152/ajprenal.00330.2002.—Mathematical models of renal tubular function, with detail at the cellular level, have been developed for most nephron segments, and these have generally been successful at capturing the overall bookkeeping of solute and water transport. Nevertheless, considerable uncertainty remains about important transport events along the nephron. The examples presented include the role of proximal tubule tight junctions in water transport and in regulation of Na⁺ transport, the mechanism by which axial flow in proximal tubule modulates solute reabsorption, the effect of formate on proximal Cl⁻ transport, the assessment of potassium transport along collecting duct segments inaccessible to micropuncture, the assignment of pathways for peritubular Cl⁻ exit in outer medullary collecting duct, and the interaction of carbonic anhydrase-sensitive and -insensitive pathways for base exit from inner medullary collecting duct. Some of these uncertainties have had intense experimental interest well before they were cast as modeling problems. Indeed, many of the renal tubular models have been developed based on data acquired over two or three decades. Nevertheless, some uncertainties have been delineated as the result of model exploration and represent communications from the modelers back to the experimental community that certain issues should not be considered closed. With respect to model refinement, incorporating more biophysical detail about individual transporters will certainly enhance model reliability, but ultimate confidence in tubular models will still be contingent on experimental development of critical information at the tubular level.

proximal tubule; distal tubule; collecting duct; sodium; potassium; chloride; acid/base

IN THE PRESENTATION OF A PHYSIOLOGICAL model, declaration of success typically comes with the demonstration of faithful predictions of function in simulations of a number of experiments. Arguably, one may endorse a standard of model presentation in which the model builder shows not only what works but where the model fails, or where it makes novel predictions that have yet to be tested. In this regard, “model failure” may vary from small to large, where “large” implies an important observation that just cannot be captured by a reasonable model with realistic parameters. In some sense, this situation is the most interesting, because its resolution may provide new insights. In renal physiology, models of glomerular filtration have probably enjoyed the closest working relationship with experimental data, initially representing hemodynamics and subsequently examining issues of glomerular permselectivity. The successes and limitations of these models have recently been reviewed in this series (20). Perhaps the oldest and most intense renal modeling effort has been to represent the tubules and vasculature of the kidney medulla in antidiuresis and the formation of a concentrated urine. The serious difficulties encountered with representations of inner medullary function have been well documented (56, 101) and will not be taken up here. With respect to tubular models, the greatest attention has been given to the proximal tubule, initially with regard to forces and routes of water transport and subsequently focused on ion transport through the transcellular pathway. More recently, segments of the distal nephron have been modeled, and these simulations have been used to extrapolate in vitro transport observations to tubules in vivo. It is the aim of this review to survey models of renal tubular transport, specifically with regard to uncertainties encountered by the model builders. The points of interest will be model limitations and the
authors’ responses, either to question experimental data or to propose testable mechanisms that might render the models satisfactory.

**PROXIMAL TUBULE: WATER TRANSPORT**

Figure 1 is a schematic of the proximal tubule epithelium, in which the lateral intercellular space (LIS) is separated from luminal and peritubular solutions by the tight junction (TJ) and basement membrane (BM). All of the early models of proximal tubule function had focused on water transport, specifically to try to understand the forces responsible for isotonic water reabsorption and the pathways for transepithelial water flow (45, 79, 80, 91, 114). The last review of these issues here (116) was principally concerned with the paracellular pathway. Models of the proximal tubule had agreed that most of the transepithelial water flowed through the LIS and out across the BM. Points of disagreement among the models included 1) the magnitude of the flow that arrived in the LIS via the TJ and via a transcellular route across luminal and lateral cell membranes in series and 2) the magnitude of the solute permeability of the outlet BM (i.e., whether there was any significant resistance to solute flux across this barrier). A finite solute permeability of the BM would result in “middle compartment” behavior by the LIS. This refers to active solute transport into the LIS across the lateral cell membrane, creating a region of local hypertonicity, which acts to pull water from cell to LIS, and ultimately from lumen to cell. The net result is the possibility of transepithelial reabsorptive water flux in the absence of a transepithelial osmotic gradient (coupled water transport). Furthermore, a finite solute permeability for the LIS BM would result in solute polarization effects, namely, an overall epithelial water permeability less than that of the cell membranes in series and an overall epithelial solute reflection coefficient less than that of the TJ and cell in parallel (“pseudo-solvent drag”). A previous model had argued that large flows of isotonic water reabsorption could exist despite relatively low proximal tubule water permeability if there were substantial solute polarization within the LIS (114). In the review (116), it was estimated that coupled water transport was anywhere from 55–81% of isotonic water reabsorption, depending on which of the experimental measurements of overall epithelial water permeability one believed. Furthermore, it was estimated that proximal tubule could transport water against an adverse osmotic gradient (hypertonic lumen) of 8–23 mosmol/kgH2O. These predictions were subsequently tested experimentally by Green et al. (37) using in vivo microperfusion of rat proximal tubules and peritubular capillaries. It was found that coupled water transport was ~75% of isotonic transport and that the adverse osmotic gradient required to null volume reabsorption was between 13.2 and 29.4 mosmol/kgH2O, depending on the concentration of peritubular protein.

The fraction of reabsorptive water flow that actually traverses the proximal tubule TJ is unknown. Arguments in favor of transjunctional water flow have included substantial solvent drag of ionic species (31, 44, 80); the appearance of streaming potentials with the application of an impermeant osmotic agent (30, 104); and ionic permeabilities roughly in proportion to their mobility in free solution (49). More direct evidence for TJ water flux in rabbit tubules came from Whittembury and associates (15, 34, 127), whose estimate of the water permeability of the peritubular cell membrane indicated a transcellular water permeability less than the overall epithelial tubular water permeability. In the rat, evidence for TJ water flow was the observation of convective entrainment of sucrose, despite relatively small diffusional flux (126). An important discrepant observation was made by Schnerrmann et al. (85), who found that mice, genetically defective for the proximal tubule cell membrane water channel aquaporin-1, had a reduction in proximal tubule epithelial water permeability of nearly 80% compared with control mice. These data are difficult to rationalize with significant TJ water permeability. However, before one can fully assess the implications of this observation, it must be acknowledged that there are no measurements of proximal tubule solute reflection coefficients in any strain.

![Fig. 1. Transport pathways across luminal and peritubular cell membranes of rat proximal convoluted tubule cell. All of the peritubular transport pathways shown on the basal surface also line the lateral cell membrane and communicate with the lateral intercellular space (LIS). There is a permeable tight junction. Adapted from Ref. 118.](https://www.ajprenal.org/content/284/5/F872/F872.large.jpg)
of mouse, so as yet we have no idea whether there is significant solute-water interaction that must be modeled in these tubules. Perhaps the most direct measure of TJ water flow was the work of Kovbasnjuk et al. (50), who were able to visualize standing concentration gradients of a fluorescent indicator trapped within the lateral interspaces of confluent Madin-Darby canine kidney cells. A sweeping away of marker from the neighborhood of the TJ would have indicated transepithelial water flux, but none was observed in this tight epithelium.

Consistent with early observations, the first proximal tubule models all included substantial TJ convective solute flux (45, 79, 80, 91, 114). Nevertheless, Rector and Berry (8, 74) resisted ascribing substantial water flux to the TJ based on pore-theoretic calculations that indicated that the junctions were not large enough to allow anything but a small fraction of transepithelial water flow. Preisig and Berry (72) measured the permeation of sucrose and mannitol across the rat proximal tubule. Applying the Renkin equations to their data, they computed the dimensions of the “sucrose pore” and indicated that it could be responsible for at most 2% of the tubular water permeability. These arguments provoked a quantitative examination of whether apparent convective epithelial solute flux could derive from solute polarization within the LIS (115). Specifically, could one construct a model of proximal tubule with just the right solute polarization to yield realistic reflection coefficients? In the reconsideration of rat data from Frümter et al. (31), all of the acceptable interspace models required substantial TJ convective Cl⁻ flux. An important contribution to this discussion came with the suggestion of Fraser and Baines (28) that the TJ might be represented as a fiber matrix, rather than as a collection of pores. The critical feature of the fiber matrix equations is that for a given solute permeability, the water permeability can be substantially greater than that predicted from the Renkin equations. This formulation was compatible with the permeabilities of rat proximal tubule, although it was a phenomenologic equation and not based on the fine structure of junctional strands. Most recently, Guo et al. (40) returned to this problem and examined representations of the TJ as a two-pore structure. It was found that an abundant small pore (consistent with interstices between claudin-2 molecules) could be used to represent small-solute permeability, and an infrequent large pore (such as 18 × 100-nm breaks in the TJ strands) could be used to represent sucrose permeability. This large pore could also be responsible for a substantial fraction of proximal tubule water flow, although it is not at all clear how such a pore could give rise to the differences in reflection coefficients that have been observed between Na⁺ and Cl⁻, or between Cl⁻ and HCO₃⁻. Ultimately, a true model of hindered transport, which incorporates electrical effects, will be required to represent TJ fluid and electrolyte fluxes.

PROXIMAL TUBULE: REGULATION OF Na⁺ REABSORPTION

Once the TJ had been established as a route for solute flux, an hypothesis was advanced that the junction might be a key locus for the regulation of proximal Na⁺ reabsorption. Lewy and Windhager (57) demonstrated a correlation between single-nephron filtration fraction and proximal tubule Na⁺ reabsorption (57). Because a lower filtration fraction reduces protein oncotic pressure within peritubular capillaries, they surmised that this would lead to reduced capillary uptake of fluid from the renal interstitium and LIS, and, hence, elevated interspace pressure. In turn, this would produce a backflow of Na⁺ already transported into the interspace, that is, backflow across the TJ into the lumen. Before this proposal, it was known that proximal tubule Na⁺ reabsorption was depressed during extracellular volume expansion (22). In the intact dog, the ability to reverse this natriuresis with infusion of hyperoncotic albumin indicated that peritubular oncotic pressure could influence sodium reabsorption, and Earley et al. (24, 63) had proposed that renal interstitial pressure might be an intermediate variable. Micropuncture experiments in the rat reproduced the findings in the dog, namely, that depression of proximal sodium reabsorption that occurs with saline infusion could be reversed by perfusion of the efferent arteriole with a solution whose protein is at the control concentration (10, 90). Microperfusion of both proximal tubules and peritubular capillaries in the rat showed that peritubular oncotic force sharply increased isotonic Na⁺ reabsorption, well beyond a simple osmotic effect on water flux, thus suggesting a qualitative change in the epithelium (38). The precise mechanism by which LIS pressures modulate TJ sodium flux is uncertain. One possibility is that with increased interstitial pressure there is junctional widening and back-diffusion of sodium from interspace to lumen. Evidence from several sources has documented increased junctional permeability with volume expansion, both in Necturus (9) and in the rat (88). A second possibility is that backflow of sodium across the TJ occurs by convective flow. The TJs of leaky epithelia are sensitive to hydrostatic pressures applied from the contraluminal side, and volume expansion was found to decrease the proximal tubule NaCl reflection coefficient (7). In this regard, convective backflow across the tight junction of rat proximal tubule has been invoked by Ramsey et al. (73) to explain their observation that the luminal appearance of lanthanum deposited within the renal interstitium is enhanced during saline volume expansion.

To examine the backflux hypothesis quantitatively, a mathematical model of rat proximal nephron was developed, comprising tubular epithelium, glomerulus, peritubular capillary, and interstitium (117). In this model, the TJ was compliant in the sense that both junctional salt and water permeability increased and the salt reflection coefficient decreased in response to small pressure differences from lateral interspace to
tubular lumen. Although these compliance properties were empirical, they provided a model in which a decrease in peritubular protein concentration (which increased interspace hydrostatic pressure) could open the TJ and produce a secretory salt flux. This backflux was a combination of both diffusive and convective terms and did not specifically require either component to dominate. In this model of the TJ, once the interspace pressure fell below that of the lumen, the junction closed and junctional properties were fixed. The consequence of junctional closure is that beyond a certain value of peritubular protein, one may expect little influence of peritubular Starling forces on volume reabsorption. Figure 2 displays the results of calculations when afferent arteriolar tone was varied, changing both glomerular plasma flow and filtration fraction. What is shown in the log-log plot are the changes in proximal reabsorption (APR) as a function of filtration fraction (FF; Fig. 2A) or as a function of glomerular filtration rate (GFR; Fig. 2B). Within the region in which the TJ are “open” and influenced by pressure, the relative changes in APR and FF are identical. However, when related to changes in GFR, the fractional change in APR is only 41% (Fig. 2B). This derives from the fact that (within the model glomerulus) increases in renal plasma flow produce GFR increases even in the absence of changes in glomerular capillary pressure, i.e., without any change in filtration fraction. This model prediction was at odds with the nearly perfect glomerulotubular balance that has been observed in the rat kidney (86).

A prerequisite for the precise glomerulotubular balance that has been observed is that luminal fluid flow modulates epithelial Na⁺ reabsorption. This has been termed “perfusion-absorption balance” (128) and has been demonstrated in rat microperfusion studies (4, 41, 68, 78). One of the best illustrations of this phenomenon are the micropuncture data of Chan et al. (17), in which a three-fold increase in luminal perfusion rate (with trivial changes in luminal HCO₃⁻ concentration) produced a doubling of the rate of HCO₃⁻ reabsorption. It must be acknowledged that examination of rabbit tubules in vitro did not show flow-dependent reabsorption (13), but whether this is due to species difference or to the preparation of tubules for perfusion in vitro is not known. Although rat proximal tubules have been perfused in vitro (32), flow-dependent reabsorption has not been examined. In this regard, flow-dependent Na⁺ and HCO₃⁻ transport has been reported recently in mouse tubules both in vivo and in vitro (23). The underlying mechanism for flow-dependent changes in reabsorption has not been established. At one point, the proximal tubule brush border had been considered a possible unstirred layer. However, model calculations indicated that there was unlikely to be any appreciable convective stirring within this pile (5). More to the point, the diffusion barrier between the bulk luminal fluid and the cell membrane was not predicted to hinder Na⁺/H⁺ exchange (52). Two studies raised the possibility that increases in axial flow velocity recruit new transporters into the luminal membrane. Preisig (70) examined recovery of cellular pH from an acute acid load in vivo (ammonium pulse). With increases in luminal flow rate, the pH recovery mediated by Na⁺/H⁺ exchange was enhanced. Maddox et al. (59) subjected rats to acute changes in vascular volume to obtain hydropenic, euvoletic, and volume-expanded groups, with respective grouping according to decreased, normal, and increased GFR. When brush-border membrane vesicles were prepared from each of these groups and Na⁺/H⁺ kinetic parameters were assessed, it was found that the V_max determinations stratified in parallel with GFR.

Ultimately, perfusion-absorption balance must derive from an afferent sensor of fluid flow rate in series with a cascade of effector steps that activate luminal transporters or insert new membrane transporters. Model calculations (39) have indicated that the proximal tubule microvilli are physically suitable to func-
tion as such a sensor. A striking feature of proximal tubule epithelium is the observation that the microvilli are remarkably uniform in height and form a highly organized hexagonal array (64). Although the main function that has been attributed to the brush border has been luminal membrane area amplification, such regular organization is not necessary to accommodate more transporters. However, the model in Guo et al. (39) shows that such regularity in height and spacing is highly advantageous if the microvilli are to function as a flow sensor, because the bending deformation of the microvilli would be both small and uniform. The critical component of this system may well be the actin cytoskeleton, which is abundant within and beneath the brush border (64). The model in Guo et al. (39) describes how the actin filament bundle that is the central core of the microvillus deforms under hydrodynamic loading. The proposed role for the microvilli is that they can not only sense fluid drag forces but are also capable of greatly amplifying these stresses as the forces are transferred to the intracellular cytoskeleton. This is due to the hydrodynamic torque exerted on the terminal web, where the actin filament bundle within the microvillus attaches at its roots to the main cell body. To serve the hypothesized function, the microvilli should be relatively stiff structures that are able to transmit, without significant bending, the torque due to the hydrodynamic drag acting on the microvilli tips (113). In this scheme of signal transduction, specific interaction between the proximal tubule cytoskeleton and the apical cell membrane Na+/H+ exchanger is a critical feature. In this regard, Lamprecht et al. (55) have shown that the Na+/H+ exchanger in brush-border microvilli is linked via ezrin, a kinase anchoring protein, to the actin cytoskeleton. Finally, implication of the cytoskeleton in the flow-dependent modulation of luminal Na+ entry invites an immediate means for coordinating peritubular solute exit in response to changing throughput. In sum, model failure to represent a fundamental aspect of glomerulotubular balance has been the impetus to formulate testable hypotheses for regulation of proximal tubule Na+ transport.

PROXIMAL TUBULE: Cl− REABSORPTION

Proximal tubule Cl− reabsorption proceeds via both paracellular and transcellular pathways (3). Evidence for a transcellular component to Cl− flux includes observations that in the absence of transepithelial electrochemical driving force, there is still substantial Cl− reabsorption (1) and that a substantial component of Cl− reabsorption can be blocked by specific inhibitors of membrane transporters (84, 110). The principal candidates for luminal membrane Cl− uptake are all Cl−/base exchangers, in which the base may be HCO3−, hydroxyl, formate, or oxalate (Fig. 1). A major advance in this inquiry came with the finding by Karniski and Aronson (46) that formate could catalyze Cl− uptake into proximal tubule brush-border membrane vesicles, and, in the presence of a vesicle-to-medium formate gradient, vesicle Cl− concentration could be driven above that of the ambient medium. In rabbit proximal tubules perfused in vitro with a high-Cl−, low-HCO3− solution, addition of luminal formate (0.5 mM) increased volume reabsorption by 60% (84) and was associated with a small increase in cell volume (82). In rat tubules perfused in vivo with the same high-Cl− solution, luminal formate increased volume reabsorption by 45% (110). This increase in volume and Cl− reabsorption could be blocked, not only by an inhibitor of the Cl−/HCO3− transporter but also by an inhibitor of the luminal membrane Na+/H+ exchanger (109). Under scoring the importance of the Na+/H+ exchanger in this observation, the effect of formate to enhance proximal Cl− reabsorption (present in normal mice) was absent in Na+/H+ exchanger 3 (NHE3)-deficient mice studied with in vivo microperfusion (111). For formate-enhanced Cl− transport to be significant, submillimolar concentrations of formate (and micromolar concentrations of formic acid) must mediate reabsorption of a substantial portion of the filtered Cl− load. The scheme that has emerged is one in which cellular formate exchanges for luminal Cl−, the formate is protonated to formic acid within the tubular lumen, and formic acid recycles back into the cell. At minimum, the luminal membrane Na+/H+ exchanger is a proton source, but it may be more tightly coupled to the formate flux pathways (3).

Luminal membrane Cl−/HCO3− exchange has been problematic for the modeling of proximal tubule. In model simulations with luminal membrane Cl−/HCO3− exchanger, the addition of formate produced cell swelling, and increased cytosolic Cl− concentration, (118). Unfortunately, variation of the density of the Cl−/HCO3− exchanger had virtually no effect on overall NaCl reabsorption along the tubular segment, whereas in similar simulations, the density of the Na+/H+ exchanger had a powerful impact on NaCl reabsorption; i.e., the rate of Na+ reabsorption was clearly rate limiting (Fig. 3). In this model, although transcellular Cl− flux was substantial, it was only about one-half the estimate of paracellular Cl− flux. When the transcellular pathway was diminished, the forces favoring paracellular flux were augmented. In these model calculations, the luminal membrane permeability to formic acid was about one-third that of a lipid bilayer to CO2. Unfortunately, the only measurement of the formic acid permeability of the proximal tubule cell membrane is ~5% of the value selected for the model parameter (71), and using the measured value, the recycling scheme fails. This prompted a modeling investigation into the possibility that the microvillous configuration of the proximal tubule brush border could provide a diffusion barrier, so that the experimental assessment of membrane formic acid permeability might have been artificially low. The result of these calculations was that the brush border only depressed formic acid permeability measurement by ~10%; even if the formic acid diffusion coefficient within the brush border were one-tenth that in free solution, the permeability assessment would only be off by 25% (51). Additional calculations, which included
brane conductance. In rabbit proximal convoluted tubule, cell volume was unaffected by changes in peritubular Cl− concentration. However, prior hypotonic cell swelling rendered the cell volume sensitive to peritubular Cl− concentration, and this effect was eliminated by application of a Cl− channel blocker (83). Electrophysiological study of rabbit convoluted tubule during osmotic shock estimated the fractional Cl− conductance to increase from 3% to a maximum of 16%, with relaxation to 8%. During these same experiments, the fractional conductance of the Na+−HCO3− pathway declined from 41% to 16%, with little change in the absolute conductance through this pathway (125). The data from this type of experiment give some guidance for estimating the reabsorptive flux of Cl− through peritubular channels in rat experiments. If one assumes that the peritubular membrane conductance under control conditions is 10 mS/cm² (29), then the conductance of the Na+−HCO3− pathway is ~4 mS/cm², and in the relaxation phase after cell swelling, the steady-state Cl− conductance increased to 2 mS/cm². For a peritubular membrane electrical potential of ~75 mV, and cytosolic and peritubular Cl− concentrations of 18 and 118 mM (16), respectively, the cytosolic Cl− potential is ~25 mV. Multiplication by the steady-state Cl− conductance of the swollen cell, 2 mS/cm², yields a Cl− current of 50 μA/cm², or 0.5 nmol·s⁻¹·cm⁻², or 25 pmol·min⁻¹·mm⁻¹ for a tubule with a 25-μm diameter. If in the conditions of these experiments, cytosolic Cl− had been doubled to 36 mM, then the Cl− potential might have been as high as 45 mV, and the conductive flux 45 pmol·min⁻¹·mm⁻¹. These estimates must be considered in light of the control volume fluxes of 2.5 nl·min⁻¹·mm⁻¹ (110), which for isotonic transport corresponds to Cl− reabsorption of 350 pmol·min⁻¹·mm⁻¹. Several-fold higher than the conductive maximum. In the mathematical model, the formate-induced increases in luminal Cl− entry exited largely via the potent Na+−2HCO3−/Cl− exchanger within the peritubular membrane (118).

**DISTAL NEPHRON: K⁺ SECRETION**

Micropuncture study of K⁺ handling by the rat kidney has identified the accessible portion of the distal convoluted tubule (DCT) as the principal site for K⁺ secretion (61, 62). Further along the nephron, there is little change in K⁺ flow, at least from a comparison of K⁺ delivery to the collecting duct (CD) with its appearance in the final urine. In rats on a low-Na⁺ diet and treated with mineralocorticoids, maneuvers designed to enhance renal K⁺ excretion, determinations of late distal fluid-to-plasma K⁺ concentration yielded a ratio of 3.8 (62). In a microperfusion study, the limiting K⁺ concentration of rat DCT has been estimated to be 13–15 mM by extrapolating between secretory (10

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**Fig. 3. Proximal reabsorption of Na⁺, Cl−, and HCO3⁻ predicted by an electrolyte model of proximal tubule (Fig. 1).** Perfusion rate is 30 nl/min into a 5-mm tubule segment. In the top panel, electrolyte reabsorption is computed over a range of values for the activity of the luminal NH₄⁺/H⁺ exchanger. The arrow indicates the reference value for this parameter. In the bottom panel, the independent variable is the activity of luminal Cl⁻/HCO3⁻ exchange. Adapted from Ref. 118.

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With respect to peritubular exit of Cl−, the early electrophysiological data indicated only a small role for a conductive pathway (6, 12, 14). Welling and O’Neil (124) found that in rabbit proximal straight tubule the conductive Cl− pathway in peritubular membrane could account for ~6% of the total membrane conductance. However, after cell swelling induced by a 150 mosmol/kgH₂O hypotonic osmotic shock, Cl− conductance increased to 20% of the total peritubular mem-
mM) and reabsorptive (25 mM) luminal K⁺ concentrations (35). Perhaps the most direct determination of the limiting K⁺ concentration was the “stationary K⁺ concentration” observed in a split drop, 30.6 mM under control conditions (2). These values are about an order of magnitude lower than urinary K⁺ concentrations of antidiuretic rats on a control diet (61) and reflect the fact that K⁺ secretion in the distal tubule precedes final water abstraction from CD fluid. The idea of the CD as a K⁺-passive conduit to the final urine stands in contrast to observations of cortical CD (CCD) function in vitro, revealing that in rabbit (66, 87, 97, 95) and rat tubules (81, 102), the CCD is a site of Na⁺ reabsorption and K⁺ secretion, with transport enhanced by aldosterone and antidiuretic hormone. Thus one issue is understanding the observed DCT K⁺ concentrations in terms of the measured fluxes and permeabilities of this segment, and a second issue is rationalizing the CCD K⁺ fluxes in vitro with the negligible K⁺ secretion in vivo.

The only mathematical model of rat DCT that has been developed was done by Chang and Fujita (18, 19) and includes K⁺ fluxes and acid-base transport in this segment. The late DCT principal cell, which is responsible for the K⁺ secretion of this segment, is depicted in Fig. 4. Chang and Fujita applied a novel method to obtain model parameters by devising a penalty function that examined the results of simulations of several different experiments, and notably none of the reference experiments included measurements of the limiting DCT K⁺ concentration. What their results show is that K⁺ secretion occurs predominantly in the first half of the late DCT and then goes to zero by the end of this segment, when luminal K⁺ concentration reaches a limiting value of 15 mM (18). This represented an approximately sixfold increase from the entering K⁺ concentration and reflects an approximate doubling of axial K⁺ flow in conjunction with reabsorption of two-thirds of the delivered water. Their model prediction for the limiting concentration was derived from data obtained under control conditions and thus appears realistic. A model of rat CCD by Weinstein (122) was developed with parameters designed to yield fluxes and permeabilities characteristic of tubules exposed to both aldosterone and antidiuretic hormone stimulation. With these parameters, the limiting K⁺ concentration for CCD was 23 mM. When simulations were run for a 2-mm tubule in which entering fluid was hypotonic with 12 mM K⁺, there was rapid water reabsorption to isotonicity, a prompt doubling of luminal K⁺, and virtually no change in the axial flow of K⁺. When entering K⁺ was 24 mM, the luminal concentration was driven above 40 mM, and 23% of delivered K⁺ was reabsorbed. This model was subsequently extended to a model of the whole CD, by appending outer medullary (OMCD) and inner medullary (IMCD) segments (123). Under antidiuretic conditions, the model predicted that ~80% of the K⁺ delivered to the CD would be reabsorbed; most of this flux occurred within the OMCD and was paracellular (Fig. 5). In these CD segments, the model K⁺ permeabilities had been guided by experimental measurements of rat tubules perfused in vitro: NH₄⁺ permeability in OMCD (25) and K⁺ permeability in IMCD (76). The most immediate rationalization of the predicted CD K⁺ reabsorption with the micropuncture data is that the tubules in vivo either have a greater K⁺ secretory capacity or a lower K⁺ permeability, or both.

Plasma HCO₃⁻ concentration has a profound effect on renal K⁺ handling, with metabolic alkalosis increasing excretion (27, 75) and acidosis decreasing excretion (103). Distal micropuncture by Malnic et al. (60) localized much of this effect to the accessible DCT. Microperfusion of this segment by Stanton and Giebisch (93) established that the effect of HCO₃⁻ concentration to modulate DCT K⁺ secretion derived from peritubular events and not the luminal concentration. These workers found a 65% increase in K⁺ secretion with alkalosis and a 50% decrease with acidosis, whereas the effects on Na⁺ transport were substantially less. Examination of rabbit CCD in vitro confirmed that reduction in peritubular HCO₃⁻ decreased K⁺ secretion, with little effect on Na⁺ reabsorption, but with an increase in apical membrane resistance (100). With respect to underlying mechanisms, pH dependence of cation channels is a prime consideration, and in this regard, Palmer and Frindt (67) patch clamped rabbit CCD and demonstrated that alkalosis opened and acidosis closed the Na⁺ channel of the luminal membrane.
Wang et al. (112) observed a comparable pH effect on the small-conductance luminal K+ channel. For these channels, there was an ~60% reduction in open probability as cytosolic pH was reduced from 7.4 to 7.0. Strieter et al. (98, 99) used a mathematical model of rabbit CCD to try to assess the relevance of the channel kinetics to the pH effect on overall tubular K+ transport, and a summary of their observations is displayed in Table 1. Each section of the table shows the ion fluxes and epithelial PD for three values of the peritubular HCO3- concentration, C\textsubscript{S}(HCO\textsubscript{3}-). When all permeabilities are fixed or when luminal membrane K+ permeability (P\textsubscript{K\textsuperscript{\text{mp}}}) is the only pH-dependent permeability, peritubular HCO\textsubscript{3}- engenders only trivial changes in Na\textsuperscript{+} and K+ fluxes. When luminal Na\textsuperscript{+} permeability (P\textsubscript{Na\textsuperscript{\text{mp}}}) is pH dependent, alone or in combination with K+ permeability, the modulation of K+ flux is substantial, but this comes with an increase in Na\textsuperscript{+} reabsorption that appears to be too high to be compatible with observation. Strieter et al. (99) considered the possibility that, as in gallbladder (130), tight junctional Cl\textsuperscript{-} permeability (P\textsubscript{Cl\textsuperscript{\text{mp}}}), might decrease with alkaline pH. This would act to hyperpolarize the epithelium, increasing K+ secretion while decreasing Na+ reabsorption, so that when all three permeabilities are pH dependent, there is a doubling of K+ secretion in going from acidosis to alkalosis, with virtually no change in Na+ flux. Whether there is such pH dependence of CCD TJ permeabilities remains to be examined.

### CD: H+ SECRETION

Net acid excretion by the kidney is determined within the CD as a consequence of luminal proton secretion and buffer availability. Our information about this derives from studies of CCD and OMCD segments in vitro (albeit with significant uncertainty over extrapolation to conditions in vivo), and to a limited number of micropuncture and microcatheterization studies and far fewer perfusions of rat IMCD. Recently, component models of rat CD segments have been concatenated to yield a simulation of the complete structure, from cortex to papilla (123). In this model, the OMCD emerges as the most important site for CD acidification, and the α-intercalated cell of this segment is shown in Fig. 6. In the rat, when luminal HCO\textsubscript{3}- is 24 mM, luminal membrane proton secretion is apportioned 5:2 between the H-K-ATPase and H+-

### Table 1. Tubular fluxes and luminal potential difference during acidosis, control, and alkalosis

<table>
<thead>
<tr>
<th>Permeability</th>
<th>C\textsubscript{S}(HCO\textsubscript{3}-), mM</th>
<th>J\textsubscript{Na\textsuperscript{\text{na}}}, pmol-cm\textsuperscript{-2}-s\textsuperscript{-1}</th>
<th>J\textsubscript{K\textsuperscript{\text{mp}}}, pmol-cm\textsuperscript{-2}-s\textsuperscript{-1}</th>
<th>J\textsubscript{Cl\textsuperscript{\text{cl}}}, pmol-cm\textsuperscript{-2}-s\textsuperscript{-1}</th>
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<td>10</td>
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C\textsubscript{S}(HCO\textsubscript{3}-), peritubular HCO\textsubscript{3}- concentration; J\textsubscript{Na\textsuperscript{\text{na}}}, J\textsubscript{K\textsuperscript{\text{mp}}}, and J\textsubscript{Cl\textsuperscript{\text{cl}}}, Na\textsuperscript{+}, K+ and Cl\textsuperscript{-} flux, respectively; \(\Psi\), electrical potential; P\textsubscript{mp} and P\textsubscript{Na\textsuperscript{\text{mp}}}, luminal membrane K+ and Na+ permeability, respectively; P\textsubscript{Cl\textsuperscript{\text{mp}}}, tight junctional Cl\textsuperscript{-} permeability. Data are from Ref. 99.
ATPase transporters (33). Although cytosolic carbonic anhydrase (CA) is present (58), there is no membrane-bound luminal CA (11, 26). Luminal proton secretion is balanced by peritubular base exit, which for OMCD is almost exclusively HCO$_3^-$, and it is believed that exit is almost exclusively via AE1, the peritubular Cl$^-$/HCO$_3^-$ exchanger. The first evidence for this was the finding that rabbit OMCD proton secretion is eliminated by removal of peritubular Cl$^-$/HCO$_3^-$ or by application of a stilbene inhibitor of AE1 (96). Confirming the importance of Cl$^-$/HCO$_3^-$ exchange are experiments in which OMCD cell pH was monitored and in which removal of ambient Cl$^-$/HCO$_3^-$ reduced peritubular HCO$_3^-$ permeability by 90% (42). Cl$^-$ that enters via AE1 can exit the cell via peritubular Cl$^-$ channels. Although the absolute conductance of the membrane is not known, it has been established that the major conductive pathway is Cl$^-$-selective (47, 65). In the model OMCD, assignment of all Cl$^-$ exits to a peritubular conductive pathway did yield an estimate for the absolute conductance of this pathway (~22 mS/cm$^2$) but also provided a dilemma (121). The model attempted to accommodate the fact that Cl$^-$ channels typically have a HCO$_3^-$ permeability (54, 69) and used a conservative estimate of 1:8 for the HCO$_3^-$-to-Cl$^-$ permeability ratio. This provided a peritubular exit pathway for HCO$_3^-$ that carried about one-half of the generated HCO$_3^-$.

Thus in this model, even when peritubular AE1 activity was reduced to near zero, model proton secretion decreased by only one-third. A number of explanations could be invoked to rationalize this important discrepancy, but one attractive hypothesis is that a substantial portion of peritubular Cl$^-$ exits occurs via electroneutral K-Cl cotransport and not all via Cl$^-$ channels. Koeppen (48) had suggested the existence of this pathway to rationalize the slow hyperpolarization of SITS-inhibited OMCD cells in terms of loss of cell Cl$^-$ via an electroneutral pathway. One additional appeal of this hypothesis is that it may also provide a possible mechanism for blunting cell volume perturbations that are predicted to accompany any changes in flux through the luminal H-K-ATPase (121).

The IMCD cell, like that of the proximal tubule or thick ascending limb of Henle, must coordinate both Na$^+$ reabsorption and H$^+$ secretion within a single cell type. A schematic is shown in Fig. 7 (119). The Na$^+$ fluxes are variable but can be perhaps nearly as large as those of the proximal tubule (21, 89) and occur without generating a significant transepithelial PD (36, 43). In view of the reports of thiazide inhibition of IMCD Na$^+$ reabsorption (77, 129), a luminal Na-Cl cotransporter appears to be the dominant pathway. To accommodate this large transcellular Cl$^-$ flux, along
with the observation that the peritubular membrane conductance is predominantly K⁺ (92), a peritubular K-Cl cotransporter has been included in the model cell. Luminal membrane H-K-ATPase has been identified as the major proton transporter (108). As in rat OMCD, cytosolic CA is present (58) but not within the luminal membrane (11, 106). Peritubular base exit may occur as HCO₃⁻, via a Cl⁻/HCO₃⁻ exchanger and is thus susceptible to inhibition with CA inhibition (53, 94). What has been noteworthy about IMCD is a second mechanism for peritubular base exit, which has been identified by Wall (105), and involves ammonia recycling. In this scheme, peritubular NH₄⁺ enters on the Na-K-ATPase in competition for K⁺ (107), elevates cytosolic ammonia, and thus promotes diffusive exit of NH₃. Predictions from this mechanism are that base exit and thus luminal acid secretion 1) should have a CA-insensitive component that would vary directly with peritubular NH₄⁺ concentration; 2) should vary inversely with peritubular K⁺ concentration; and 3) should vary directly with the rate of IMCD Na⁺ reabsorption (i.e., Na⁺ flux through the Na-K-ATPase). In the model IMCD, this scheme was represented with competition of K⁺ and NH₄⁺ on the Na-K-ATPase, and the first prediction was realized (120). Inhibition of proton secretion by peritubular K⁺ was stronger than expected, because increasing K⁺ also produced an increase in cell Cl⁻ (via K-Cl cotransport) and thus also inhibited the CA-sensitive component of base exit. The predicted effect of Na⁺ reabsorption on proton secretion turned out to be false (at least in the model) and the results of that calculation are shown in Fig. 8 (120).

In this simulation, the abcissa for all panels is luminal NaCl concentration, from 2 to 110 mM. Figure 8A indicates cytosolic conditions, and Fig. 8C shows the rate of luminal H⁺ secretion via the H-K-ATPase. Figure 8B, left, displays the peritubular membrane fluxes of NH₄⁺ via the Na-K-ATPase and K⁺ channels (G_{NH₄}), and in Fig. 8B, right, are peritubular HCO₃⁻ fluxes (G_{HCO₃}) via Cl⁻/HCO₃⁻ exchange and Cl⁻ channels. Over the range of luminal NaCl concentrations, the peritubular pump rate for Na⁺ varied from 0.9 to 6.1 nmol·s⁻¹·cm⁻². In these calculations, however, there was virtually no change in luminal membrane H⁺ secretion (Fig. 8C) or in cell pH (Fig. 8A, left). Reference to Fig. 8B, left and right shows that the changes in Na⁺ transport led to opposing effects on NH₄⁺ cycling and HCO₃⁻ exit. As luminal entry of NaCl decreased, there was a decrease in cytosolic Cl⁻ (Fig. 8A, right) and thus increased peritubular HCO₃⁻ exit via the Cl⁻/HCO₃⁻ exchanger (Fig. 8B, right). Thus this model predicted that with these two base exit mechanisms operating in parallel, acid secretion would be stable over a wide range of IMCD Na⁺ transport. At present, dissected and perfused IMCD do not transport Na⁺ well, if at all, and in situ peritubular conditions are not easily assessed, so that the prospects for subjecting these model predictions to testing appear distant.

CONCLUSION

It should be clear that there remains considerable uncertainty as to our ability to model renal tubular function. The issues presented here can be formulated as specific questions: How much proximal tubule water flux traverses the Tj? What is the role of proximal TJs in the regulation of Na⁺ reabsorption? Which luminal cell membrane transporters of proximal tubule have flow-dependent changes in density, and what is the signaling pathway? What are the important transcellular pathways for proximal tubule Cl⁻ flux, and which transporters are modulated by formate? Do CD K⁺ fluxes and tubular permeabilities measured in vitro correspond to those in vivo? What are the Cl⁻ exit pathways in OMCD, and in particular, is a K-Cl co-transporter important? Does variation in H-K-ATPase activity in OMCD produce derangements in cell volume, or is there coordinate activation of other path-

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ways? Does the IMCD shift base exit between CA-dependent and CA-independent mechanisms to maintain stable proton secretion in the face of variable Na+ fluxes? This list of questions hardly exhausts the uncertainties that have come to the fore in the published model investigations. Some of the issues have had intense experimental interest well before they were cast as modeling problems, but some truly did arise out of model exploration. What should also be clear is that some of these questions are quantitatively very important; i.e., the essence of some phenomena have not been captured, and this is not just an effort to fine-tune a nearly completed picture. What must also be acknowledged is that, for all its obvious value to understanding the kidney, structural information alone will not suffice to answer many of our most important questions, and there is no escape from the conclusion that additional functional data are required. It may be legitimate to question whether existing experimental technology is up to the task, or whether new techniques are necessary. However, at the level of detail considered here, the available numerical methods and computing power are certainly up to the task of simulating renal tubular transport. With respect to model refinement, incorporating more biophysical detail about individual transporters will no doubt enhance model reliability, but ultimate confidence in these tubular models will still be contingent on critical experimental information to be developed at the tubular level.

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REFERENCES


