Kidney International, Vol. 9 (1976) p. 88–102

Electrical phenomena in the nephron

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The epithelia lining the nephron form single cell layers that manifest distinctive physical properties such as transepithelial electrical potential differences and electrical conductances. The electrical behavior of epithelia is important to the understanding of ion movements across these structures since these are seen as electric current and ultimately depend on their conjugate force, the electrochemical potential difference and a membrane property, the ionic conductance.

In addition to earlier reviews [1, 2] we have recently surveyed electrical potential differences and resistances of renal tubules [3]. The majority of the presently available experimental data are derived from observations that treat the epithelium as a single diffusion barrier. However, transepithelial flows cannot be adequately understood from a description of the electrical properties of the full epithelial layer. First, morphologically renal epithelia constitute multicompartmental systems where several ion diffusion boundaries, in series or in parallel, exist rather than a single one. Moreover, electrolyte flows may appear macroscopically as electroneutral while, at the microscopic level of a single barrier, electroneutrality may be violated. Finally, electrochemical potential gradients across a complete epithelium may imply the active or passive nature of ion movements whereas entirely different inferences would follow from the driving forces that govern individual intraepithelial barriers.

The present study aims at an explanation of overall transepithelial electrical phenomena as a function of the discrete electrical characteristics of single barriers, more often single cell membranes. Clearly the cell membrane approach is only one level of analysis more advanced than the overall epithelial approach. Thin biological membranes are presently treated as black-boxes because of our lack of a molecular description of ion permeation channels within the membrane phase itself. Thus, the level of understanding of renal transport processes at which we aim in this paper remains essentially phenomenological in nature. In the following we will successively discuss electrical potential differences, electrical conductances and, finally, how these properties control ion flows through single barriers or through the full epithelial thickness. In each instance our focus will be on the single boundaries of tubule cells.

The only segments that have been investigated at the single membrane level are the proximal convoluted, distal and cortical collecting tubule [3]. As a rule intracellular impalements by means of microelectrodes are a prerequisite for information about individual cell membranes. Amphibian preparations such as *Necturus, Triturus* or *Amphiuma* are most useful because of the large size and less extensive basal infoldings of the tubule cells. Potentials in mammalian cells can be studied *in vivo* but only after extensive immobilization of the kidney [4, 5], in kidney slices [6] or in isolated tubules [7].

Proximal convoluted tubules

The open-circuit transepithelial potential difference across the proximal convoluted tubule is usually small, ranging from −6 to −15 mv in the amphibian [3] and from −6 to +2 mv in the mammalian kidney [3], where the sign indicates the polarity of the lumen as referred to a surface bath. Such potential differences have been measured in a variety of circumstances in kidneys *in vivo*, in perfused kidneys or in isolated tubules. The diverse techniques have been reviewed in detail [4].

The potentials of the individual membranes of cells have been reported, so far, only for preparations that exhibit zero or negative transepithelial potential. The two boundaries of the tubule cell exhibit sizeable potential differences opposite in sign and different in magnitude to the extent of the net transepithelial potential. The potential across the peritubular mem-

brane of the cell, i.e., the border facing the blood or the interstitium, is about −70 mv cell negative in most species studied: *Necturus* [2, 8–20] (unpublished data), newt [21, 22], rat [5, 23–25] and guinea pig [6, 26, 27]. The potential across the luminal cell membrane or brush border can be obtained as the difference between the potential across the peritubular membrane of the cell and the total transepithelial potential difference. Ideally the potential across the luminal membrane of the cell should be recorded from two microelectrodes, one impaling the cell and the other the tubule lumen, but this has been done only rarely [8] (unpublished data). The magnitude of the potential across the luminal membrane ranges from about +55 mv (lumen positive to cell cytoplasm) in *Necturus* [2, 8, 9, 12] (unpublished data) and *Triturus* kidneys [21, 22] to values equal to the potential across the peritubular membrane in mammalian kidneys [5].

Total transepithelial conductance in the proximal tubule of *Necturus* is invariably high, with specific resistance values ranging from 43 to 430 ohm × cm² [10, 13, 17]. Rat, dog and rabbit proximal convoluted tubules exhibit resistances at least one order of magnitude smaller, i.e., about 5 to 12 ohm × cm² [28–31]. If the transepithelial resistance were attributable solely to the resistances of the peritubular cell membrane and luminal cell membrane in series, much larger values would be expected. Indeed, direct estimates of the aggregate series resistance of the two plasma membranes in *Necturus* proximal tubule vary from 7900 to 9500 ohm × cm² [32, 33]. Approximate values for the single peritubular membrane resistance in *Triturus* and *Chironomus* renal tubules are 836 and 3000 ohm × cm², respectively [34, 35].

The discrepancy between total transepithelial conductance and that predicted from those of the single cell borders constitutes the major direct evidence for the existence of extracellular pathways of low resistance between cells [9]. Further circumstantial evidence in favor of a prevailing paracellular path has become available and lends strong support to the thesis of a major shunt conductance across the proximal epithelium [5, 9, 36] (unpublished data).

Several inferences can be made from the finding that tight junctions and lateral interspaces constitute a high conductance path for ion permeation. First, even the most abridged sketch plan of the proximal tubular epithelium should include three ion diffusion barriers: basolateral or peritubular plasma membrane, brush border or luminal plasma membrane, and tight junction or zonula occludens. Electrically, every barrier is endowed with its particular set of electromotive forces and ionic conductances. Second, since the diffusion boundaries are interconnected in a particular circuit, physiologically observed values of potential difference or resistance depend critically on the behavior of all barriers together. Third, a piecemeal study of the separate membranes is complicated by the interference of another membrane during the experiment. For example, externally imposed changes in ionic concentration differences across one membrane will, without exception, also affect the concentration difference across a second border. In addition, any change in potential difference recorded could have originated at either boundary or at both.

The model illustrated in Fig. 1 indicates the three barriers together with the observed potential differences V_1, V_2 and V_3 already mentioned. For each membrane the net equivalent of all ionic electromotive forces due to the diffusional pathways is represented by a single comprehensive emf E of either sign. Similarly, the symbol R represents the equivalent resistance of all ionic diffusion pathways within the ionic batteries, the series resistances and undefined leak resistances in parallel. More elaborate diagrams have been proposed for low resistance epithelia [37, 38] (unpublished data), but amount essentially to this elementary circuit model.

The potential differences for a given barrier are a function of all emf's and all resistances according to the following equations

$$V_1 = \frac{E_1(R_2 + R_3) + R_1(E_3 - E_2)}{R_1 + R_2 + R_3} \quad (1)$$

$$V_2 = \frac{E_2(R_1 + R_3) + R_2(E_3 - E_1)}{R_1 + R_2 + R_3} \quad (2)$$

$$V_3 = \frac{(E_1 + E_2)R_3 + E_3(R_1 + R_2)}{R_1 + R_2 + R_3}. \quad (3)$$

The overall transepithelial resistance R_{te} is a function of all barriers according to the following:

$$R_{te} = \frac{(R_1 + R_2)R_3}{R_1 + R_2 + R_3} \quad (4)$$

In order to estimate quantitatively the permeability properties of membrane 1 (peritubular cell membrane), ionic substitution methods have been used [8, 39] (unpublished data) and the induced changes in V or R recorded. With respect to the use of e.g. observed changes in V_1 or ΔV_1, equation (1) shows clearly that they fail to estimate E_1 or ΔE_1 without an inclusive determination of all parameters. Unlike the observed potential difference, the emf relates directly to the chemical potential differences across the membrane m:

$$E_m = -\frac{1}{F}\sum_j \frac{t_j}{Z_j}\Delta\mu_j \quad (5)$$

where F is the Faraday constant; t_j, the transference number; Z, the valence; and $\Delta\mu$, the transmembrane chemical potential difference for the jth ion. Instead of changes in V_1, knowledge of actual changes in F_1 is required in order in determine permeability properties from electrophysiological experiments. Similarly, changes in R of the membrane m may be related to any of the ionic conductances G_j according to

$$\frac{1}{R_m} = G_m = \sum_j G_j \qquad (6)$$

where G_m is the total membrane conductance and G_j is the partial ionic conductance of the jth ion and equal to $t_j G_m$. Permeability coefficients can be derived from partial ionic conductances. Obviously, determinations of the resistances of membranes are an essential prerequisite.

The nature of the arrangement of the individual barriers of the epithelium precludes separate experimental handling of a single membrane. Substitution of an ion or salt in either the lumen alone or the plasma alone affects the $\Delta\mu$ for one of the two cell membranes together with that of the paracellular shunt. Ion substitutions on both surfaces would lead to a $\Delta\mu$ for both cellular barriers 1 and 2 and leave barrier 3 possibly unaltered. In order to circumvent this ambiguity, we have recently resorted to a combination of potential recordings during threefold applications of the same concentrations change: first, during ion substitution on the peritubular surface alone; second, during similar ion substitution in the lumen alone; and, finally, during bilateral or symmetrical ion substitution in both capillaries and tubule lumen [39].

To sum up the above-mentioned considerations, electrophysiological studies of the proximal tubular epithelium should center on the determination of E_1, E_2, E_3 and the respective ions contributing to each emf, in addition on the evaluation of R_1, R_2, R_3 and the respective ionic channels responsible for each resistance.

The peritubular cell membrane

The nature of E_1, the emf of the peritubular membrane was implied, at least qualitatively, by the observations of Giebisch on perfused *Necturus* kidneys [12]. From determinations of V_1 during simultaneous changes of potassium and chloride concentrations, the peritubular membrane was thought to behave approximately as a potassium or chloride battery with only a small sodium permeability. By means of single ion substitutions bilaterally in both capillary circulation and lumen, we have confirmed these observations and added some quantitative estimates of absolute and relative permeability [8] (unpublished data). Instantaneous potential changes were obtained immediately after single ion substitution of potassium and chloride starting from a steady-state condition. From the relationship between instantaneous changes in potential difference and the concentration of external K or Cl, it was possible to estimate individual transference numbers for potassium and chloride of, respectively, $t_K = 0.49$ and $t_{Cl} = 0.20$ [8] (unpublished data). Relative permeability coefficients were computed from observed potential difference displacements and the following ranking obtains $P_K = 1.0 > P_{Cl} = 0.4$ to $0.15 > P_{Na} = 0.04$ (unpublished data). Determinations of the intracellular activity of K^+ together with corresponding measurements of potential difference indicate an equilibrium distribution of potassium across the peritubular cell boundary of *Necturus* maculosus [15].

Peritubular membranes of mammalian proximal tubules also display a marked dependence on the external potassium concentration [6, 25] with transference numbers for potassium of $t_K = 0.3$ but low chloride permeability $t_{Cl} = 0.07$ [5]. However, the sum of all transference numbers for the rat proximal tubule is markedly short of unity, indicating a technical inadequacy of superfusion alone in effecting truly instantaneous replacement of the ions in contact with the basolateral membranes of the cells. During studies involving peritubular perfusion, the sum of trans-

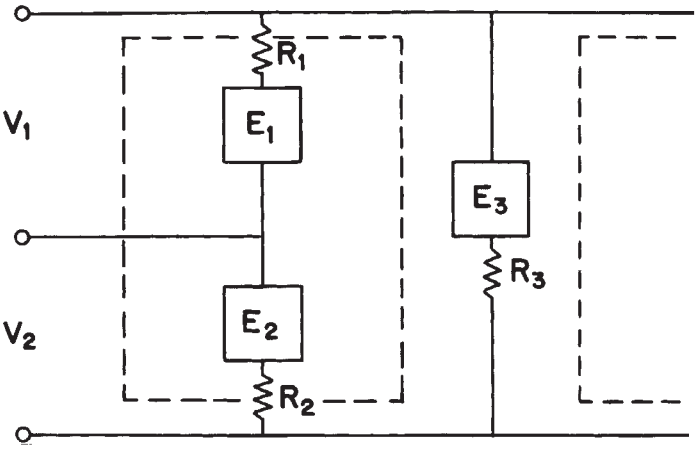

Fig. 1. *Model of the equivalent electrical circuit for the proximal tubule cell and overall epithelium.* The *dashed lines* indicate the cell borders. V_1 = potential difference across the peritubular membrane of the cell; E_1 = electromotive force of the peritubular membrane which may be of either polarity and is equivalent to the combined electromotive forces of all diffusional pathways; R_1 = resistance of the peritubular (and lateral) membrane of the diffusional pathway. The same elements are represented for the luminal membrane with subscript 2. V_3 = transepithelial potential difference. E_3 = paracellular electromotive force (of either polarity) resulting from dissipative leaks; R_3 = paracellular resistance.

ference numbers approached unity with $t_K = 0.45$ and $t_{Cl} = 0.05$ [40]. The ratio of potassium to sodium permeability for rat tubule cells was 1:0.05 [5] with an apparent t_{Na} of 0.05 [40]. Again, joint estimates of intracellular K activity and potential differences satisfy equilibrium conditions across this border of rat proximal tubule cells [23].

The removal of external bicarbonate depolarizes the peritubular membrane potential of amphibian proximal tubules to an extent that would indicate an extremely high HCO_3^- permeability if the effect were due to a bicarbonate diffusion potential (unpublished data). Since modifications in intracellular bicarbonate concentrations may occur during these relatively long-term ion substitutions, a calculation of the relative bicarbonate permeability is not strictly valid. On the other hand, Khuri et al found an intracellular bicarbonate activity of 11.1 mM, a value far in excess of that expected from equilibrium distribution across the peritubular membrane [14]. This also precludes a permeability calculation from instantaneous potential changes after sudden omission of external bicarbonate.

The potential across the peritubular membrane in the rat also shifts in proportion to the logarithm of the bicarbonate concentration [5, 25]. Based on earlier studies a transference number, $t_{HCO_3} = 0.07$, had been advanced [5]. Recently, transient peak depolarizations upon removal of bicarbonate were described with a mirror-like hyperpolarization or temporary overshoot occurring after return to a normal bicarbonate concentration [24, 40, 41]. The transference number t_{HCO_3} was estimated in these studies to be 0.45 to 0.27 [24, 40]. It is noteworthy that similar transients and rebounds of peritubular membrane potential could also be elicited by sudden changes in concentrations of other lipid soluble buffers such as glycodiazine or butyrate. We believe that the time course of the drift of membrane potential is not a direct measure of the permeability to bicarbonate or buffer. Instead, if such potential changes are associated with changes in the intracellular concentration of the buffer ion, permeation of the undissociated buffer and rates of H^+ or OH^- ion movements will determine the time course of redistribution.

An increase or a decrease of pH in the *Necturus* peritubular circulation, at constant buffer concentration, respectively, hyperpolarizes or depolarizes the peritubular membrane [42] (Steels and Boulpaep, unpublished data). Determinations have also been made of the relative permeability of the peritubular membrane of *Necturus* proximal tubule to other ions such as choline, Mg^{++}, isethionate, nitrate and sulfate when these were substituted for Na^+ or Cl^- ions. In all cases depolarizations were noted implying a higher permeability to the test cations than to Na^+ and a lower permeability to the test anions than to Cl^- [9] (unpublished data). Observations at variance with the above have been described for acetylglycinate and benzene sulphonate [43]. However, comparison of the results would require knowledge of actual changes in E_1 rather than V_1 since a different (unilateral vs. bilateral) substitution method was used. In agreement with the findings on amphibian tubules, in rat proximal tubules, substitutions of choline and tetraethylammonium for Na^+ and substitutions of nitrate, sulfate, ferrocyanide and cyclamate for Cl^- all lead to depolarization of the peritubular membrane [5]. Only bicarbonate or acetate ions substituting for chloride bring about hyperpolarization [5].

In a study designed to calculate the actual changes of E from a set of observations of V during three forms of identical concentration changes, unilaterally peritubular, unilaterally luminal and finally bilaterally, we have recently estimated the actual change in peritubular emf during single salt dilutions [39]. A tenfold change in $\Delta\mu$ jointly for both Na^+ and Cl^- ions across the peritubular membrane caused a ΔE_1 of +21 mv [39], which would point to a chloride transference number larger than that of sodium as was previously [9] (unpublished data) inferred from observed ΔV_1 values during single ion replacements.

The peritubular membrane potential V_1 is altered during various experimental manipulations when the actual electrochemical potential difference $\Delta\mu$ across the peritubular membrane is unchanged for all ions, i.e., when intracellular concentration alterations are unlikely and the electrolyte composition of the external medium is unmodified. Thus, a change of composition in the lumen alone elicits frequently a peritubular response. Such coupling was first described for *Necturus* proximal tubule [8, 44] and supported strongly the concept of a low resistance paracellular shunt and a circuit diagram similar to Fig. 1. Equation 1 clearly explains how, for specified changes in E_2 or E_3, elicited by unilateral luminal substitutions, a change in V_1 can be foretold. Such electrical coupling data yield no information about peritubular permeability characteristics but define the relative importance of the resistor R_1.

Another instance of potential changes V_1 not related to E_1 is that evoked by intraluminal injection of transported sugars or amino acids. Studies on the newt kidney first demonstrated depolarization of the peritubular membrane during perfusion of the lumen with glucose; such depolarization was not found during perfusion with mannitol [21]. The depolarization is inhibited by phlorizin, depends on luminal Na^+

concentration, is saturable and follows Michaelis–Menten kinetics. The response of the peritubular membrane to glucose resembles that noted for the basal membrane of the small intestine [38, 45]. Since the change of the luminal potential (ΔV_2) exceeds ΔV_1, these results are not likely to be due to a direct effect of transported sugars on E_1. A similar observation has been reported after perfusion of the lumen with alanine in the newt kidney [46] and in *Necturus* kidney (Steels and Boulpaep: unpublished observations). In rat proximal tubules *in vivo* it was also shown that several transported sugars are able to evoke a potential response while the nontransported sugars lack this influence [47]. Furthermore, it was demonstrated in rat proximal tubules that both neutral, di-amino and di-carboxylic amino acids are able to alter the cell membrane potential [48]. We will discuss below the nature of the primary electrical events occurring at the luminal membrane, but it appears probable that cotransport of Na with sugars or amino acids across the luminal membrane is not electroneutral.

Only scant information is available regarding the influence of pharmacologic agents on the ion permeability of the peritubular membrane. Ouabain in high concentrations [12], mercurial diuretics [12, 22] and amphotericin B [49] diminish the potential difference but it is not established whether these are direct effects on cell membrane parameters. Acetazolamide appears to reduce the transference number for bicarbonate but to enhance that for potassium and chloride in rat proximal tubular cells [41]. Finally, isotonic volume expansion leaves V_1 unchanged [10]. Spring reported V_1 changes during current imposed transepithelial voltage transients that lead to volume flows [16].

Evaluation of the absolute electrical resistance of the peritubular membrane necessitates extensive two-dimensional cable analysis for a double cylindrical model of the tubule. In this model the peritubular membrane is assumed to occupy a continuous outer cylindrical shell and the luminal membrane constitutes a uniform inner shell [33]. As mentioned above, R_1 markedly exceeds overall transepithelial resistance (R_{te}). Relative estimates of cell membrane resistance were calculated from the input resistance of cells measured either with double-barreled microelectrodes or with single-barreled electrodes in combination with a bridge arrangement (unpublished data). In order to determine whether the plasma membrane resistance is a function of the electric field or the current applied, current-voltage relationships were determined for single cells of *Necturus* proximal tubule. Nonlinearity was very striking at all extracellular K concentrations, and persisted in the absence of any other permeating ions [50] (unpublished data). The rectifying properties of the cell membrane resistance can be traced to nonlinear characteristics of the potassium conductance of a type not predicted by the constant field equations. Moreover, it was found that $\Delta\mu_K$ was a strong determinant of cell membrane resistance. From these studies a permeability coefficient for potassium emerges that is not constant but a monotonic decreasing function of $V_1 - E_K$ and a monotonic decreasing function of V_1 (unpublished data).

During subsitutions of Na and Cl, changes in input resistance may be used for indirect estimates of the partial conductance for these ions. t_{Cl} from these measurements varies from 0.35 to 0.51 [8] (unpublished data). Finally, determinations of R_1 during single ion substitutions in *Necturus* when compared to control R_1 lead to the following ranking of the partial conductance for cations $t_{choline^+} > t_{Na^+} > t_{Mg^{++}}$, for the anions $t_{Cl^-} > t_{NO_3^-} > t_{isethionate^-} > t_{SO_4}$ (unpublished data). No changes in cell membrane resistance have been detected for *Necturus* proximal tubule during isotonic volume expansion [10]. The resistance of rat proximal tubular cells rises after acetazolamide treatment [41].

The experiments reported so far have all used extracellular salt or ion substitutions to gauge the respective part contributed by each ion to E_1 or to R_1. Alternatively, intracellular concentration changes can also be reproducibly induced. A variety of different techniques is available. First, presoaking of kidney cortex slices in the cold or in low potassium media, followed by reimmersion in warm media containing variable Na and K concentrations, provides a means for studying the relationship between cell membrane potential difference (V_1) and the chemical potential difference, $\Delta\mu$ [6]. In studies on guinea pig kidney, Na extrusion was found to occur against an electrochemical potential gradient, while K uptake follows Na extrusion. At low extracellular K concentrations, however, active potassium uptake became apparent [6]. Of particular interest is the evolution of V_1 during rewarming in K-free media. A net efflux of Na with Cl is accompanied by hyperpolarization of V_1, while no change in $\Delta\mu_K$ occurred, thus suggesting the activity of a rheogenic Na pump [26, 51].

Microelectrophoretic injection of specific ions is another technique to modify intracellular ionic activities in proximal tubules of *Necturus in vivo*. Although quantitative estimates of the actual change in $\Delta\mu_j$ caused by the injection remain approximate, it has been possible to inject repeatedly a defined amount of ions into single *Necturus* cells over a specified time

interval and to record the corresponding changes of membrane potential [52]. In particular we succeeded in monitoring the time course of recovery of V_1 following a standard depolarization induced by the intracellular injection of Na ions. The analysis of such transients of potential reveals a component directly related to the rate of coupled Na-K exchange. Intracellular electrodeposition of Na in normal or high potassium media invariably depolarized V_1. However, after prolonged exposure to zero K concentrations hyperpolarizations can be seen, thus unmasking the existence of rheogenic sodium extrusion [52].

In conclusion, the equivalent peritubular emf, E_1, is composed of a set of parallel diffusion cells for K^+, Cl^-, Na^+ and probably HCO_3^-. With respect to the absolute value of V_1, and assuming that no paracellular shunt existed or $V_1 = E_1$, it would be safe to assume so far that the peritubular membrane potential may be entirely due to diffusion potentials. Indeed, E_K or the electrochemical potential for potassium nearly always exceeds V_1. We have already mentioned noteworthy exceptions to this observation. After examination of the properties of the cell membrane and the paracellular membrane, we will show the need for amending the concept that the peritubular membrane arises solely from ion diffusion potentials.

The luminal cell membrane

Previous investigations had suggested that the emf, E_2, of the luminal membrane is composed of a potassium diffusion potential in parallel with a more powerful Na battery than that residing within the peritubular membrane [12]. The higher sodium permeability would cause the luminal cell membrane potential to deviate more from the theoretical potassium equilibrium potential than would be the case for the peritubular membrane. We have more recently reassessed the contribution of potassium ions to the potential of the luminal membrane and have tested the possible role of other ions in *Necturus* proximal tubules [8, 9] (unpublished data). Most observations were made during ion substitutions in the lumen only. If E_2 were related solely to V_2, instantaneous potential changes after single ion substitutions of potassium and chloride would indicate that the combined share of K and Cl in the membrane current is limited, $t_K + t_{Cl} \simeq 0.2$ [8, 9] (unpublished data). These studies have indicated a substantial contribution of sodium ions to the membrane current, while bicarbonate ions do not appear to participate appreciably. Such findings greatly compromise the possibility that V_2 is mainly a function of E_2. Even assuming a large sodium transference number of up to 0.8, only values of E_2 lumen negative to cell could be expected. The actual sign of V_2, however, is the opposite (lumen positive with respect to cell).

Measurements of intracellular K activities in *Necturus* proximal tubule have indicated passive distribution of this ion species across the luminal membrane in accord with V_2 [15]. Bicarbonate ion distribution, in contrast, does not satisfy equilibrium conditions across the same membrane [14].

Our information on the electrical properties of the luminal membrane of mammalian renal tubules is quite restricted, due in part to the requirement of more intricate techniques. Moreover, separate studies of the luminal potential difference in the mammalian kidney seem less important in view of the small overall transepithelial polarization. Thus, it was assumed that studies of the peritubular potential assess the electrophysiological properties of the cell membranes in full. Potassium distribution, as determined from activity ratios, agrees well with the potential across the luminal membrane in rat proximal tubules [23]. However, in view of our studies on *Necturus*, there is no reason to believe that the electrical behavior of luminal membranes resembles that of peritubular membranes. Single ion substitutions in the lumen of mammalian kidneys have been performed nearly exclusively in association with transepithelial measurements. Occasionally changes in the potentials across the peritubular membrane have been reported during luminal perfusion, e.g., hyperpolarization of V_1 during perfusion with bicarbonate solutions [24], but no simultaneous records of V_1 and V_3 are available for the mammalian kidney. As pointed out previously any direct study of E_2 is hampered by the possibility of external current flow and such current flow is more likely in mammalian tubules because of their lower transepithelial resistance.

Our recent study of *Necturus* proximal tubules, using a protocol of composition changes in the peritubular capillaries, the lumen or both, allows a correct estimate of actual changes in E_2 from observed changes in V_2 [39]. Thus, a tenfold reduction of both the Na and Cl concentration ratios across the luminal membrane leads to an observed ΔV_2 of -35 mv during microperfusion of the lumen alone. However, assuming that the change of the potential difference across the luminal membrane had not been shunted by current flow in the extracellular path, the computation of actual ΔE_2 yields a value of -65 mv (unpublished data). This value exceeds the theoretical value of 58 mv for the behavior of a chloride electrode. More information is required from similar single ion substitutions to establish the role played by sodium ions across the luminal cell membrane.

As mentioned for the peritubular membrane, the luminal membrane potential was similarly found to be electrically coupled to events at the peritubular cell border [8] (unpublished data). For instance, a change in $\Delta\mu_K$ across the peritubular membrane altered V_2 while no $\Delta\mu$ occurred over the luminal boundary. Equation 2 accounts for the observation that V_2 can be modified by interfering with E_1.

Of special interest are the shifts in potential difference across the luminal membrane elicited by nonelectrolyte transport; these have already been mentioned in the section on the peritubular membrane. Perfusion of the lumen with glucose depolarizes the luminal boundary of the cell to a greater extent than the effects seen on the peritubular cell membrane [21, 47]. Lowering the sodium concentration in the lumen decreases markedly the electrical response to glucose, supporting the view that cotransport of carbohydrates with Na^+ is rheogenic [21]. Nevertheless, such rheogenic movements need not be active since Na can move passively along a favorable electrochemical potential gradient from lumen to cell. Similar depolarization of the luminal membrane was also found in newt, rat and *Necturus* proximal tubules when they were microperfused with alanine [46, 48] (Steels and Boulpaep, unpublished observations). The presence of sodium in the lumen is also a requisite for amino acids to depolarize the luminal membrane [46, 48]. It was concluded that amino acids can traverse the luminal membrane with more than one Na ion per molecule, and that the change in V_2 is created by net current flow.

With respect to interventions with pharmacologic agents, amphotericin B decreased the potential of the luminal membrane in the proximal tubule, apparently through an increase in sodium permeability [49].

Direct absolute measurements of the resistance of the luminal membrane are not available, but can be calculated from the sum of the two cell membrane resistances in series and peritubular membrane resistance. Values of relative resistance, luminal membrane to peritubular membrane, can be determined from records of the electrotonic voltage deflections across, respectively, the luminal membrane and the peritubular membrane of a given cell. Despite the fact that actual current flows across the cellular versus paracellular pathways are unknown, relative resistance values of R_2 and R_1 have been obtained from estimates of ΔV_2 vs. ΔV_1 treating the two cell membranes as a simple voltage divider of total transepithelial deflections. The ratio R_2/R_1 in *in vivo* kidneys is 2.5 for *Necturus* proximal tubule (unpublished data). Neglecting the expanded area that may result from infoldings, luminal membranes thus have a higher apparent resistance than basolateral membranes. Concerning the partial ionic conductances of the luminal membrane, no data are available from measurements of membrane conductance during ion substitutions.

An exhaustive analysis of the maximal possible value of the luminal equivalent electromotive force, E_2, predictable from all possible ion diffusion potentials, indicated that E_2 could not exceed about +7 mv, as opposed to a measured value of V_2 of 55 mv [3, 8, 9]. In other words, even if V_2 were equal to E_2, the major fraction of V_2 remains unaccounted for. Clearly such calculation assumes no external current flow or source of current across the luminal membrane. Two different possibilities exist to account for the fact that the luminal membrane potential exceeds E_2:

a) Rheogenic active ion transport of either cations from cell to lumen or of anions from lumen to cell may occur. In order for this transport to contribute an additional source for the genesis of the potential of the luminal membrane, such rheogenic ion flows cannot be due to passive dissipation of existing diffusion potentials. The sum of all diffusion potentials is included in the symbol E_2 of Fig. 1. For instance, the rheogenic entry of Na stimulated by sugars or amino acids would deliver a depolarizing transmembrane current but may be the result of movement down its electrochemical gradient initiated by the opening of a sodium permeation channel. Maruyama and Hoshi [21] have added another emf at the luminal membrane in order to represent this sodium element, but in our representation of Fig. 1, such a component is, for all practical purposes, incorporated in the total equivalent emf E_2. What is needed is a current source opposite to that of passive Na influx into the cell. It can be represented by the additional symbol for a constant current source added in Fig. 2. At present, the ion species involved are unknown.

b) Another possibility is that there may be current flow across the luminal membrane created by an extraneous source. Clearly, no electrical field is imposed externally across the epithelium. However, the existence of a low resistance paracellular shunt makes current flow in a closed loop quite likely in the presence of any imbalance among the 3 emf's, E_1, E_2 and E_3, or if other current sources exist at other barriers. Basically, the two possibilities mentioned differ only in the origin of the current flow. At present no evidence is available to exclude one of the alternatives. We conclude that intraepithelial current flow is responsible for the wide disparity between V_2 and apparent E_2.

The paracellular pathway

The existence of an appreciable intercellular emf, E_3, is debatable on theoretical grounds. First, chemical potential differences across the paracellular shunt, $\Delta\mu_j$, are small for most ions, when taken as overall $\Delta\mu$ between lumen and capillaries. Second, if the tight junctions offer the major barrier to solute movement [10, 13, 53] and are water-filled channels, little discrimination among ions is expected. Third, the observed values of V_3 in the proximal tubule are modest. The sign and amplitude of transepithelial potential differences have recently been reviewed in detail [3]. No direct determinations of E_3 have been reported for the proximal tubule. Since E_3 is by definition caused by ionic diffusion only, expected values can be calculated according to equation 5 providing t_j and $\Delta\mu_j$ are known.

The total transepithelial $\Delta\mu$ for both chloride and bicarbonate is known for late proximal tubules of mammalian kidneys in free flow. Since t_{Cl} exceeds t_{HCO_3} [5, 28], a positive value of E_3 is expected, and is in agreement with a positive late proximal potential difference V_3 [3, 54, 55, 56]. Although there are no sizeable values of $\Delta\mu$ for any ion in the early proximal tubule of amphibians or mammals, where sugars and amino acids are present, the values of V_3 are significantly negative. It may be speculated that, in reality, concentration differences exist across the tight junction which are not detected in the peritubular space. If solute is transported from the cells to the interspaces, a salt concentration difference between interspace and lumen could arise. From diffusion potentials occurring during active transport, Machen and Diamond have estimated the salt concentration in the lateral intercellular spaces of the rabbit gall bladder [57]. Using a variety of experimental conditions and two different "hypertonic interspace" models for the coupling of salt and water, we have recently computed the salt concentrations expected within the intercellular channel of *Necturus* proximal tubules [58]. A 10% hypertonicity as compared to the lumen is probably a generous estimate where NaCl is assumed to be the solute [58].

It remains for us to explore the partial ionic conductances of the barrier across which E_3 could be generated. The ionic permeability properties of the proximal tubular epithelium have been investigated in *Necturus*, dog and rat kidney by means of ionic or salt substitutions [5, 9, 28] (unpublished data) and were recently reviewed [3]. In each instance, changes in V_3 following a change of ion composition were employed for the calculation of paracellular transference numbers or permeability ratios. As mentioned earlier ion substitutions never uniquely affect a single emf (see Fig. 1) and interactions from changes of cell emf are possible. *A priori*, the high resistance of the cell membrane relative to that of the paracellular shunt appears as a redeeming feature: its presence avoids the short-circuiting of E_3 so that changes in E_3 may be reasonably approximated by readings of V_3. The view that changes of cellular emf interfere little with the measurements of V_3 during transepithelial concentration changes was supported by the wide discrepancy between the pattern of transepithelial permeability ratios obtained in this manner and those estimated for the cell membranes [9]. Symmetry in the response of V_3 to identical salt gradients of opposite sign constitutes additional evidence that transepithelial ion selectivity patterns simply reflect the permeability of the paracellular pathway [5, 39]. We have recently used a quantitative approach evaluating the changes in E_3 that occur in proximal tubules of *Necturus* exposed to multiple salt dilutions in either luminal or peritubular media or both. Thus, in *Necturus* proximal tubule, a single-sided tenfold NaCl salt dilution modified V_3 by 24 mv at a time when the shift in emf E_3 also amounted to 24 mv [39]. Hence, estimates of transepithelial transference number serve as a measure of paracellular, presumably junctional, partial ionic conductance. For the mammalian kidney, t_{Na}/t_{Cl} ratios of the paracellular path vary from 1.4 to 1.5 [5, 28], suggesting that the permeability to anions is restricted compared to that to cations. On the other hand, amphibian proximal tubules in diverse experimental conditions invariably discriminate against cation diffusion in such a way that t_{Cl}/t_{Na} ranges from 3.6 to 1.5 [39] (unpublished data).

Using the above-mentioned data, what is the maximal emf E_3, that would result from a hypothetical hypertonic lateral interspace and a selective tight junction? Mammalian proximal tubules would yield a positive E_3 while V_3 has the opposite sign with symmetrical outside solutions containing transported nonelectrolytes [3]. From $t_{Cl} = 0.78$ and $t_{Na} = 0.22$ [39] and a concentration ratio of 1.1 across the tight junction, amphibian tubules are predicted to produce a -1.3 mv salt diffusion potential, an order of magnitude smaller that the free-flow potential difference for *Necturus*. We infer that mechanisms other than paracellular diffusion potentials are responsible for the generation of the transepithelial potential difference [3, 8, 59].

From the previous considerations it is quite obvious that the paracellular resistance R_3 will deviate only minimally from the measured transepithelial resistance R_{te}. Hence, changes in transepithelial resist-

ance approximate fairly well changes in paracellular resistance. Factors which affect transepithelial conductance have been summarized recently [3]. Of particular interest is the agreement of estimates of partial ionic conductance of the paracellular path calculated from the modification of transepithelial conductance induced by the absence of, e.g., chloride with estimates of t_{Cl} from measurements of potential difference [28]. In conclusion, the composition of the equivalent paracellular emf E_3 is not fully solved, but enough information is available to postulate that current flow across R_3 needs to contribute the major part of V_3. The following section examines whether the combined elements of the equivalent circuit can generate enough current to account for the transepithelial potential difference.

Intraepithelial current loops

In our attempts to explain the potential differences across the individual membranes on the basis of the diffusion potentials associated with each particular membrane, only the peritubular membrane emf was found possibly to meet the requirements. As outlined above, additional current flow over the luminal membrane and the paracellular shunt is required to create an important fraction of the actually observed potential differences. Whereas the need for and the feasibility of intraepithelial current loops is beyond dispute considering the low shunt resistance, the nature and site of origin of the current is not obvious. Which membrane is the primary site of current generation even though no net current runs from lumen to capillaries or in the reverse direction? What ions carry this current across each membrane? Finally, what provides the free enthalpy change necessary for this circular current?

With respect to the last of these three questions, two alternatives exist. Either ionic currents have their source in passive ion permeation down an electrochemical potential gradient or, alternatively, they are directly generated by active ion movement against an energy barrier. Note that in both cases free enthalpy is dissipated. The distinction depends solely on the final link between ion current and energy.

Diffusion potentials and current flow

The possibility that the necessary ion flows are driven by preexisting differences of transmembrane concentration and potential can best be analyzed on the basis of the configuration of Fig. 1. Net current flow across each of the three barriers in the equivalent circuit is implicit and arises whenever $E_1 + E_2 - E_3 \neq 0$. The closed loop current I defined as positive when flowing in a clockwise direction in the loop is given by the following:

$$I = -\frac{E_1 + E_2 - E_3}{R_1 + R_2 + R_3} \cdot \qquad (7)$$

Kirchhoff's laws require the total net current across each membrane to be the same. Thus, $I = I_1 = I_2 = I_3$ where I_1, I_2, I_3 are currents traversing the peritubular, luminal and paracellular membrane, respectively. Since

$$V_1 = E_1 + I_1R_1 \qquad (8)$$

$$V_2 = E_2 + I_2R_2 \qquad (9)$$

$$V_3 = E_3 - I_3R_3 \qquad (10)$$

it is possible to establish from the first term which fraction of each potential is produced by its emf E, and from the second term IR which part is the consequence of passive current flow.

In order to maximize the current, one could, for the sake of the argument, postulate that E_1 is an ideal K electrode and E_2 an ideal Na electrode, much alike the Koefoed-Johnsen and Ussing model for the frog skin [60]. Using maximal concentration ratios of 40 for potassium across the peritubular membrane and 4 for sodium across the luminal membrane in *Necturus* proximal tubules, E_1 would reach −93 mv, and E_2, −35 mv. We suggested above an upper limit for E_3 of −1.3 mv. The total of all emfs, therefore, would be less than −130 mv. Since the sum of all resistances of Fig. 1 in series amounts to at least 8,000 ohm·cm² [10, 33], the maximum value for the circular current is 16 $\mu A \cdot cm^{-2}$. Is V_3 or V_2 explained in this way? For the paracellular shunt path, I_3R_3 would be 16 $\mu A \cdot cm^{-2} \cdot 70$ ohm·cm² or +1.1 mv. Thus, total V_3 according to equation 10 would be at best −2.4 mv, a value far below the observed value in *Necturus* proximal tubules [3, 59]. A similar calculation for the mammalian paracellular shunt of lower resistance would be even less favorable. Regarding the luminal membrane, I_2R_2 may be about +95 mv which combined with a value of E_2 of −35 mv, according to equation 9, may generate V_2 of +60 mv. Finally, the peritubular I_1R_1 could be about +32 mv if R_1 were 2000 ohm·cm². Hence, the resulting V_1 from equation 8 is −61 mv. We conclude that, given the approximations for the cellular parameters used in this calculation, the membrane potentials are reasonably well-matched by the theoretical predictions, thus indicating that the cell properties of a leaky epithelium, such as the proximal tubule, might not differ essentially from the classical picture proposed for tight epithelia such as frog skin [60]. It should be noted

that the actual emf across the luminal membrane can, thus, very well be of a sign opposite to the actual potential difference recorded. In other words, despite a fundamental similarity, a leaky epithelium such as the proximal tubule fails to demonstrate a two-step potential profile only because circulation of current imposes a well-type electrical potential profile instead. However, the discrepancy between predicted and observed V_3 demands the exploration of additional sources of current flow.

Active rheogenic pumps and intraepithelial current flow

The second alternative suggests that ion flows are the immediate result of active ion transport across any of the three barriers. In Fig. 2 we represent this proposal by the introduction of two additional symbols for the peritubular and luminal membrane, respectively. Rheogenic active transport systems (electrogenic pump or lumped pumps) are illustrated by two open circles. This sign is preferred over that of a battery or emf because we assign to it the property of generating a constant current independent of changes of the load, i.e., changes in R_1, R_2 or R_3. Electrogenic Na pumps have often been characterized by an emf, E_{Na} [61]. Actually, real generators neither behave as ideal constant voltage sources with zero internal resistance, nor as ideal constant current sources with infinite internal resistance. Nevertheless, we presume that rheogenic pumps, at a given metabolic level of substrate availability, pool size and number of transport sites, are able to maintain a constant ion flux across a single barrier independent of the resistive load imposed by the other barriers. At constant coupling of the transport system proper with other ions, such pumps would generate a constant electric current. In addition, electrophysioligical measurements of membrane conductance generally are assumed to gauge only the passive channels for ion permeation through the membrane, neglecting the active pump sites. Such an assumption implies an infinite internal resistance of the active pump pathway in agreement with the behavior of a constant current source. In contrast, an ideal constant voltage source should have zero internal resistance. Finally, a constant voltage source E should, under short-circuited conditions, generate an infinite current, a situation definitely ruled out for biological membranes. In spite of our ignorance with respect to the intricate intramembrane mechanisms of charge separation and thus of pump current flows, we feel more justified in approximating rheogenic pumps by means of an ideal constant current source, keeping in mind that neither ideal configuration of generator exists.

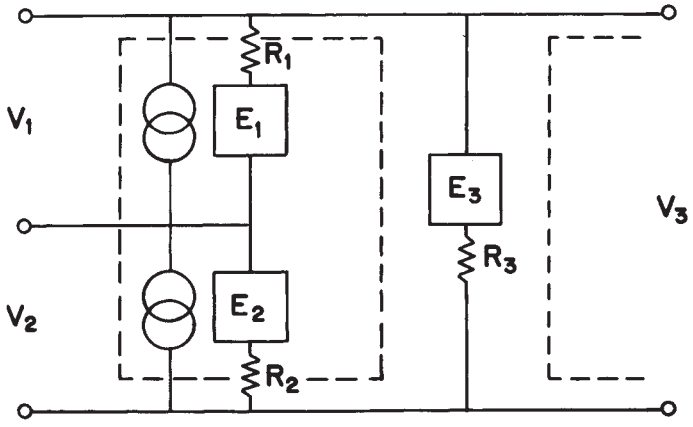

Fig. 2. *Equivalent electrical circuit model similar to that of Fig. 1 with the addition of two* open circles *at both the peritubular and luminal cell border.* The *upper open circles* i_1 represent a constant current source of either polarity designating the ion current driven by a rheogenic pump or pumps. The *lower open circles* i_2 represent a similar but not necessarily identical constant current source driven by a rheogenic pump or pumps. (Reprinted with permission from [59].)

Theoretically all three barriers could be candidates for rheogenic pumps. No such mechanism is shown for the paracellular path since it is doubtful that there is membrane material endowed with the proper transport sites across the zonulae occludentes. *A priori* it is not necessary to have a rheogenic mechanism at each plasma membrane of the cell. If i_1 were the current generated by a peritubular active ion pump only, two conditions have to be satisfied in the steady state; electroneutrality and mass balance.

For a peritubular active current flow i_1, due to Na extrusion, electroneutrality can be respected in two ways: *a*) The first is the counterflow of a cation or parallel flow of anions across the same peritubular membrane, utilizing one or more passive diffusional pathways within E_1 and R_1. The potential difference V_1 thus developed will provide the electrochemical potential just necessary to match the transmembrane flow of the other ion with that of the actively transported species, such that total peritubular membrane current flow $I_1 = 0$. Note that this mechanism represents the single membrane as if it were not interconnected with the others. *b*) The second is the flow of current across the luminal membrane, I_2, that is exactly identical to I_1. Again, the current I_2 can be composed entirely of ion flows through the diffusional pathways represented in bulk by R_2 and E_2. In turn, rheogenic pumps, i_2, at the luminal membrane may entirely or in part balance i_1 in order to reach electroneutrality. Since it is difficult to visualize how two rheogenic pumps in the two membranes in series would perform electrical work at exactly identi-

cal rates while faced with different transport pools, it is most likely that rheogenic pump current is to some extent counterbalanced electrically by simple passive electrodiffusion of ions across the same or the opposite membrane.

Mass balance furthermore requires that in the steady state the net flux of one species across, e.g., the peritubular membrane is exactly offset by an equal flux across the luminal membrane. Most explicitly, to the extent that $I_1 \neq 0$ and that the net current I_1 is carried by sodium ions, I_2 will be equal to I_1 and, if net current persists in the steady state, I_2 will also be carried by sodium ions across the luminal border. Obviously two restrictions to this principle should be kept in mind. First, in the steady state I_1 and I_2 can each be exactly zero despite the presence of powerful rheogenic pumps. Second, displacements from steady state can engender currents in the closed loop which, at least for a given time, rely on the net movement of ions of a different species across the two cell membranes until intracellular composition is altered and a new steady state is realized.

From the point of view of the paracellular shunt, the total current, I_3, can be partitioned into a component that stems from the diffusional path across the cell membranes and one that originates from rheogenic pumping sites. Indeed if, for the sake of simplicity, we assume that $i_1 = i_2$ or that the two cell membranes have exactly identical rheogenic pumps oriented in the same direction, then the total current across the shunt I_3 would, in this particular case, be given by the following:

$$I_3 = -\frac{E_1 + E_2 - E_3}{R_1 + R_2 + R_3} + \frac{R_1 + R_1}{R_1 + R_2 + R_3}\, i_{pump} \qquad (11)$$

where i_{pump} is assumed to be $i_1 = i_2$. As can be judged from equation 11, the first term is simply the equivalent of the current due to a simple diffusional configuration as in equation 7, whereas the second term reflects the contribution of the rheogenic active pump. Since $R_1 + R_2/R_1 + R_2 + R_3$ is close to unity, the impact of pump current i_1 or i_2 on paracellular current flow, I_3, can be appreciable, and thus on V_3 according to equation 10. The equations for I_3 are more involved if $i_1 \neq i_2$ and part of the electroneutrality is effected via diffusional pathways. Essentially, the first term of equation 11 would remain but two additional terms, one in i_1 and another in i_2, would appear.

An important feature of the equivalent circuit of Fig. 2 that sets it markedly apart from the circuit of Fig. 1 is the effect of intraepithelial current flow on the membrane potential differences. In the case of two constant current sources in series at both cell borders, current flow essentially bypasses the passive diffusional pathways of the cell membranes. Only a fraction of the pump current, i_{pump}, passes over the cellular passive ionic channels due to their larger resistance as compared to the paracellular path. Practically, it means that a paracellular current of, e.g., 200 $\mu A \cdot cm^{-2}$ over R_3 generates a voltage drop of -14 mv, without undesirable current flow across the resistances R_1 and R_2. The extent of transcellular current flow across the diffusional paths of the cell depends on the agreement between i_1 and i_2 for obvious reasons of electroneutrality. On the contrary, as shown in the previous section, the diagram of Fig. 1 shows only a single closed loop and current flow, if any, would have to be the same over all three barriers, in the absence of current over the whole wall of the tubule. The low loop current of 16 $\mu A \cdot cm^{-2}$ in the diffusional model was insufficient for the transepithelial potential difference but created a luminal voltage drop of +95 mv. Any additional current necessary to give rise to a realistic value V_3 would, in the case of a single loop circuit, have influenced the cellular potential differences excessively.

Experimental evidence for net ion currents

As pointed out above, the need for net ion currents can be inferred from the magnitude of the transepithelial potential differences across a low resistance shunt. From a quantitative comparison, the necessity for a rheogenic pump or pumps was postulated. It can be speculated that in the proximal tubule Na ions carry such current at the peritubular membrane since they are transported at that site against their electrochemical gradient. However, contrary to simplifying statements postulating the presence of "active transport potentials" in the rat proximal tubule in certain circumstances [62, 63] a direct link between active rheogenic transport and transepithelial potential difference has not yet been proven. The finding that a potential difference remains across the rat proximal tubule in the absence of concentration differences for water and solutes as well as in the absence of hydrostatic pressure or external current flow has been defined to indicate the presence of "active transport potentials" as opposed to diffusion potentials and streaming potentials [62]. However, from the above-mentioned demonstration of current flows, possibly driven only by diffusion potentials across each membrane barrier, it is clear than an "active transport potential" cannot be construed to indicate active rheogenic ion transport that generates potentials directly. In the last analysis active transport or cellular metabolism will of course be

necessary to rebuild the free enthalpy stored in transmembrane concentration gradients; this is not the real issue but that of direct linkage of current generation and energy. Clearly, passive transmembrane transport will ultimately also rely on metabolic resupply of its stores.

Likewise, inhibition of a transepithelial potential difference by metabolic inhibitors or cardiac glycosides has led to the mistaken belief that this establishes the existence of rheogenic mechanisms [63–65]. In order to establish the existence of rheogenic pumping, only demonstrations, at the single cell boundary, of ion current flow in excess of or against that predicted by the transmembrane driving forces across a single boundary constitute valid proof.

An excellent example of net current flow without active transport is the electrical manifestations of presumed cotransport of Na with sugars and amino acids across the luminal membrane [21, 46–48]. Until evidence to the contrary is developed, there is, on the basis of the reported experiments, no reason to conclude that *active* rheogenic transport is involved. Thus, a sudden depolarization can be brought about by a sudden change in effective Na conductance induced by the transport of sugars or amino acids. Such a process would lead to a sudden influx of sodium ions, much like the sudden rise in Na conductance that accompanies the generation of an action potential in excitable tissues. As in nerve or muscle, Na ions do not enter the tubule cell in an electrically "silent" fashion but such sodium movement constitutes inadequate evidence for rheogenic active transport. Instead, sodium current might flow through the diffusional pathways of the luminal membrane and the same moiety then traverses the peritubular membrane, perhaps by means of a truly rheogenic pump.

Ion and water flows across single boundaries of proximal tubule

Knowledge of the electrical characteristics of tubular structures allows a prediction of ion fluxes across individual barriers. Such ion fluxes have been extensively reviewed [1]. We have recently summarized how ion or fluid transport is influenced by electrical parameters [59]. Different approaches have been used in the past. First, net passive ion flux has been simply related to the electrochemical potential difference and the partial conductance [28]. Alternately, using the formalism of the constant field equations, undirectional fluxes have been related to the driving forces and the permeability coefficient of the particular ion. In such a manner passive and active components were calculated along the intercellular spaces of *Necturus* proximal tubule [10, 13]. A third approach, still at the bulk transepithelial level, was used by Frömter, Rumrich and Ullrich in which the contribution of solvent-solute interaction was included in the evaluation of transepithelial fluxes [66].

None of the above approaches is, strictly speaking, correct if the proximal tubule is composed of several compartments and barriers. We have recently proposed a multicompartmental analysis as shown in Fig. 3 that introduces five separate compartments: *1*) lumen, *2*) cell, *3*) interspace, *4*), peritubular space and *5*) capillary. Five specific barriers are α) the tight junction, β) the luminal cell membrane, γ) the peritubular or basolateral membrane, δ) the basement membrane, and ϵ) the capillary wall [58]. Each barrier is endowed with its own set of membrane parameters. Using the formalism of irreversible thermodynamics introduced by Kedem and Katchalsky [67]. we have computed the ion and water fluxes across each membrane as a function of hydrostatic, colloid osmotic, chemical and electrical driving forces. The comprehensive approach employed allowed us to verify earlier predictions made with respect to the magnitude of intercellular backleak of Na ions through the tight junctions, and its modifications by alterations in R_3 and V_3 [10, 13].

Distal convoluted tubules

The open circuit potential difference across distal convoluted tubules ranges from +12 to −60 mv [3].

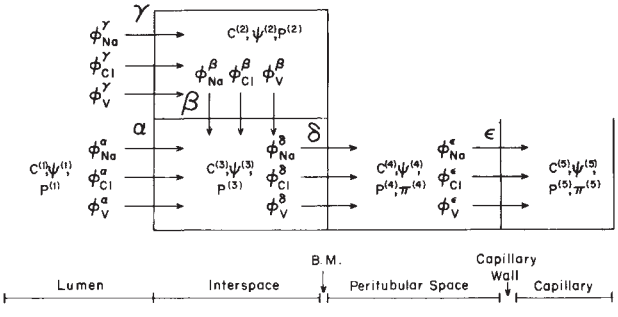

Fig. 3. *A five-compartment model for Na^+, Cl^-, and water.* α denotes the tight junction; β, the lateral membrane of the cell; γ, the luminal membrane; δ, the open end of the interspace and basement membrane; ϵ, the capillary endothelium. C_j denotes concentration of ion j; P denotes hydrostatic pressure; Ψ denotes electrical potential; π denotes colloid osmotic pressure. The superscripts indicate the particular compartments of each of the above parameters where 1 denotes the tubular lumen; 2, the cell; 3, the lateral interspace; 4, the peritubular space; and 5, the capillary lumen. The direction of the *arrows* indicates the convention chosen for positive fluxes with subscript Na, Cl or V indicating sodium, chloride and volume fluxes. (Modified from [58].)

The potentials of individual cell membranes have been measured most reliably on distal tubular cells of *Amphiuma* [68–70]. Fewer determinations are available for *Necturus* and rat kidney [3].

The potential across the basal border of the distal cell of *Amphiuma* ranges from −70 to −79 mv [67, 70] cell-negative to the capillaries. The potential of the luminal membrane in free flow is about +30 mv, lumen-positive to cytoplasm [69]. Determinations of the conductance of single cell membranes have not been reported.

The configuration of an equivalent circuit as in Fig. 1 may serve as a useful basis for the exploration of the properties of distal cells. In view of the higher transepithelial resistance of mammalian distal tubules, ranging from 400 to 600 ohm·cm² [28, 71], it has generally been held that R_3 has an infinite resistance. However, no unequivocal evidence exists in favor of this assumption.

Since both the peritubular and luminal membranes depolarize in high K media, both E_1 and E_2 may be considered to include a potassium emf [69]. A transport number for potassium t_K of 0.6 was calculated for the luminal membrane [70]. Since replacement of Na by choline in the lumen hyperpolarizes V_2, the luminal E_2 should also include a sizeable contribution from a sodium battery [69].

Hyperpolarization of the peritubular membrane of *Amphiuma* distal tubule cells occurs whenever the sodium concentration at the contralateral membrane, i.e., in the lumen, is elevated [70, 72]. In the presence of chloride in the lumen, the same Na-induced peritubular hyperpolarization is inhibited by amiloride, acetazolamide or ouabain [72]. Therefore, it was postulated that an active rheogenic sodium pump, generating a current i_1 of the type shown in Fig. 2, may contribute to the peritubular membrane potential [72]. Additional studies are desirable to establish this hypothesis.

Cortical collecting tubules

Studies of single cells were performed on isolated perfused rabbit cortical collecting tubules [7]. The peritubular membrane potential V_1 is increased by high K diets and DOCA administration, whereas ouabain depresses the potential difference [7]. Helman further reported that the luminal membrane resistance, R_2, exceeds peritubular membrane resistance, R_1, but that the sum of $R_1 + R_2$ exceeds appreciably the transepithelial resistance R_{te} [7, 73]. We infer that an equivalent circuit similar to that of Fig. 1 or 2 may be equally valid for epithelial cells of the collecting tubules.

Acknowledgments

This investigation was supported by Public Health Service research grant 5-RO1-AM-13844 and program project grant AM-17433 from the National Institute of Arthritis, Metabolic and Digestive Diseases.

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