Role of the Paracellular Pathway in Isotonic Fluid Movement Across the Renal Tubule

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Evidence for a highly permeable paracellular shunt in the proximal tubule is reviewed. The paracellular pathway is described as a crucial site for the regulation of net absorption and for solute-solvent interaction. Available models for the coupling of salt and water transport are assessed with respect to the problem of isotonic water movement. Two new models are proposed taking into account that the tight junctions are permeable to salt and water and that active transport sites for sodium are distributed uniformly along the lateral cell membrane. The first model (continuous model) is a modification of Diamond and Bossert’s proposal using different assumptions and boundary conditions. No appreciable standing gradients are predicted by this model. The second model (compartmental model) is an expansion of Curran’s double membrane model by including additional compartments and driving forces. Both models predict a reabsorbate which is not isotonic. For the particular case of the proximal tubule it is shown that in the presence of a leaky epithelium these deviations from isotonicity might have escaped experimental observation.

Considerable interest and effort have been directed toward a resolution of the intraepithelial events responsible for salt and water movement in the kidney. Glomerular ultrafiltrate which does not deviate appreciably in solute composition from blood is reabsorbed in isosmotic fashion by the proximal tubule. As in the case of small intestine and gallbladder, solute and water transport in the proximal tubule poses two problems. First, how does water move across a barrier separating two solutions of equal osmolarity? Second, how do salt and water move in what appears to be an isotonic ratio?

With respect to the first question, water transport in the proximal tubule has been proven to be a passive process depending on active solute transport [1,2]. An important step in explaining salt and water coupling was Curran’s development of a three-compartment double membrane model for intestinal water absorption [1]. As shown in Fig. 1, two membranes (a and b) of different permeabilities separate three compartments: 1, 3 and 4. Active transport of solute across membrane “a” elevates the solute composition in the middle compartment. If the reflection coefficient at “a” is larger than at “b,” the osmotic driving force from 1 to 3 exceeds that from 4 to 3. For an inelastic middle compartment, the net influx of water will cause an increase in hydrostatic pressure. If \( L_a^p \) is less than \( L_b^p \), the pressure driving force from 3 to 4 exceeds that from 3 to 1. This will lead to a net water flux from 1 to 4 in the absence of any external driving forces across the composite epithelium. By providing a suitable site for intraepithelial solute accumulation, Curran’s model resolves the first problem of how water moves without an apparent gradient.

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FIG. 1. Top: Three compartmental model of Curran. Active solute transport occurs into the middle compartment 3 with boundaries a and b. Bottom: Representation of leaky epithelium such as renal proximal tubule with various compartments: 1. tubular lumen or mucosal bath, 2. cell cytoplasm, 3. basolateral intercellular space, 4. peritubular or serosal interstitium.

THE PARACELLULAR PATHWAY: A POSSIBLE SITE FOR SOLUTE-SOLVENT INTERACTION

The site of coupling between volume flow and salt flow was initially poorly defined. Intracellular or extracellular subcompartments could offer a locus for intraepithelial osmosis providing certain conditions are fulfilled. The barriers separating such compartments should show asymmetrical properties with respect to hydraulic conductivity and reflection coefficient for the transported solute. Moreover active solute transport should be directed into that compartment.

A striking feature of renal and other epithelia is the existence of intercellular and basal infoldings of the plasma membrane. Extracellular spaces between the cell and the basement membrane, the lateral intercellular space, and specialized structures within the cell membrane were all suggested as possible sites of solute accumulation [3,4,5,6]. Whitlock and Wheeler first interpreted their findings on volume flow in gallbladder by identifying the lateral intercellular spaces as the special structures involved in local osmosis [7]. Extensive morphological studies correlating structural and functional alterations have brought indirect evidence in favor of the intercellular spaces as the anatomical site of the middle compartment [8]. Direct demonstration of an hypertonic intercellular compartment has been given only for insect rectal pads [9].

With respect to the boundaries limiting the site of solute-solvent interaction Farqu-
har and Palade described the presence of specialized structures at the apical end of the lateral intercellular space [10]. Their work implied an area of fusion between cell membranes at the level of the zonula occludens. At the same time, Ussing and Windhager demonstrated that tight junctions exposed to hypertonic solutions become a shunt path for permeation of small solutes [11]. Electrophysiological studies from our laboratory have shown that the proximal tubule of the kidney in normal isosmotic conditions is characterized by two parallel pathways: one transcellular and one paracellular, consisting of the tight junction and the lateral interspace [12,13]. Of particular importance is the evidence that the paracellular path plays a dominant role in the transepithelial permeability for ions, small non-electrolytes and possibly water [13].

Direct evidence for a major leakage path to small ions shunting the cells comes from three types of studies. First, it was noted that electrically the cell membrane at the peritubular or serosal side of the cell did not behave independently of the cell barrier at the apical or luminal side. During ion substitutions at one face of the epithelium, the contralateral cell membrane was found to follow the primary membrane potential change elicited at the exposed cell membrane [12]. Second, electrical coupling between the peritubular and luminal cell membranes was detected directly in experiments where current was passed across one cell membrane only, resulting in nearly identical voltage deflections across both cell barriers [12,14]. The presence of a low resistance shunt bypassing the cells and allowing current flow across the contralateral cell membrane was the obvious explanation for these phenomena [12]. Thirdly, direct measurement of the cell membrane resistance in Necturus proximal tubule yielded values of 7,900 ohm cm² for the two cell membranes in series [15], whereas overall transepithelial resistance of the same epithelium was found to be two orders of magnitude smaller [16].

The paracellular pathway in proximal tubule was further localized morphologically by means of extracellular markers such as Lanthanum which could cross the tight junctions [17,14].

In addition to providing a leakage path for ions that are actively transported by the epithelium, the tight junction of the proximal tubule is quite permeable to lipid-insoluble non-electrolytes large enough not to penetrate the cell such as sucrose or raffinose [18,16]. Transepithelial permeability coefficients for small electrolytes do not deviate strikingly from those of non-electrolytes of similar size. Moreover the overall permeability ratios for small non-electrolytes which do not enter the cell are quite close to their free solution mobility ratio suggesting that movement of these non-electrolytes occurs via a poorly selective watery channel which bypasses the cells [18,16]. A selectivity pattern of this kind is created by the properties of both the tight junction and the lateral intercellular space [18].

It has not yet been resolved whether water movement occurs via a transcellular route alone, a transcellular path followed by the lateral intercellular space, or directly through the tight junction and the lateral intercellular space. Overall hydraulic conductivities of Necturus proximal tubule ranging over two to three order of magnitude have been reported depending on the type or magnitude of driving force and the experimental conditions of measurement [19,20]. On the other hand, osmotic water permeability of single cell membranes is at least one order of magnitude below the minimum estimate of transepithelial water permeability [19,21]. Thus the experimental data available for the proximal tubule are compatible with the view that at least a fraction of the water movement proceeds directly from the lumen to the lateral intercellular space crossing the zonula occludens [22,14].

Although the pathways of solute and water movement across the epithelium are not
fully understood, the paracellular pathway and the compartment constituted by the lateral intercellular space is presently seen to play a key role in the control of net solute absorption rates and in solute-solvent coupling or osmotic equilibration of the absorbate.

If solute is deposited in the lateral intercellular compartment then barrier “a” of Fig. 1 is made up of the apical cell membrane, the cellular cytoplasm and the basolateral cell membrane in parallel with another barrier the tight junction. Clearly membrane “a” has two parallel components: a transcellular path relatively tight for the transported solute and providing the active transport mechanism, and a paracellular passive leakage path. Therefore, the extent of diffusive backleak of, e.g., sodium, through the tight junction will greatly influence the efficiency of the system.

This hypothesis was tested in conditions known to affect net transport of salt and water in the proximal tubule such as extracellular volume expansion in vivo [16] and modifications of colloid osmotic pressure in the peritubular capillaries [19]. Important physiological control on net reabsorption of solute and solvent was found to result from changes in solute permeability of the tight junction as measured from electrical conductance measurements and determinations of net solute fluxes [16,19]. Fig. 2 illustrates the effects of alterations in colloid osmotic pressure on net and unidirectional sodium flux components. Net fluxes were determined experimentally. Unidirectional diffusive Na fluxes across the tight junction were estimated in each condition from experimentally known electrochemical driving forces and ionic permeability coefficients. A progressive increase in peritubular colloid osmotic pressure from left to right in Fig. 2 enhances net transport. This is associated with a decrease from left to right of the two unidirectional fluxes illustrated by the dark arrows, and of the net passive ion backflow from 139 to 48 pEq cm⁻² sec⁻¹. On the other hand, the active flux component was not found to rise with increasing colloid osmotic pressure. On the basis of these [19] and similar findings [16] we have proposed a crucial regulatory role for the paracellular path in the control of net salt and water movement thus emphasizing the importance of the interspaces as the most plausible site for interaction between solute and solvent flows.

THE PROBLEM OF AN ISOSMOTIC SOLUTE TO SOLVENT FLUX RATIO

In addition to controlling net solute absorption rates, the paracellular pathway appears to play a key role in solvent to solute coupling. In the kidney proximal tubule, small intestine and gallbladder, solute-solvent interaction produces a net flux which is approximately isosmotic. Although the three compartment Curran model proposes a mechanism for the dependence of net water flux on active solute transport, it does not explain why solute and solvent should cross the epithelium in an isosmotic ratio [23].

Let Fig. 1 represent the proximal tubule epithelium of the kidney where C₁, C₃ and C₄ refer to luminal, interspace and peritubular space concentrations respectively. Since the basal end of the interspace probably offers very little resistance to Na and Cl ions, the salt reflection coefficient at this site, σ₀, must be close to zero. In the three compartment model the solute flux Jₛ is given by:

\[ J_s^b = J_s^c = \frac{C_o^b}{b} (1 - \sigma_b) J_s^b + 2 \omega_b \cdot RT(C_3 - C_4) \]  

where \( C_o^b \) is the average concentration of the solute in barrier \( b \), \( \omega_b \) solute diffusion coefficient across barrier \( b \), \( R \) the gas constant and \( T \) absolute temperature. The volume flux \( J_v \) is,

\[ J_v^c = J_v^c \]
EFFECT OF COLLOID OSMOTIC PRESSURE CHANGE ON SODIUM FLUXES

![Diagram showing sodium fluxes with different osmotic pressures](image)

FIG. 2. Estimates of sodium flux components in the proximal tubule and influence of alteration in paracellular permeability induced by changes of peritubular capillary colloid osmotic pressure. The length of the arrows together with the figures illustrate the magnitude of each component. Open arrows: active flux component. Dark arrows: unidirectional diffusive solute fluxes. Lower thin arrow: net passive solute flux. Upper thin arrow: net absorptive flux. Increase in net sodium flux from left to right is due to decrease of passive backflux from left to right (taken from reference 19).

Hence, the flux ratio is:

$$\frac{J_s}{J_v} = \frac{1}{2} (C_3 + C_4) (1 - \sigma_b) + \frac{2\omega_b \cdot RT(C_3 - C_4)}{J_v}$$

(3)

For $\sigma_b = 0$, $J_s/J_v > C_4 = C_1$ because $C_1 > C_4$.

Consequently, the model predicts that net transport cannot be isosmotic. Patlak, Goldstein and Hoffman also derived an expression for the ratio of net solute to solvent flux in a series membrane system [24]. Their model as well predicted a net hypertonic flux for $\sigma_b = 0$.

In 1967, Diamond and Bossert developed a model for salt and water coupling [25] which treated the interspace as an unstirred continuum rather than a single homogeneous compartment as in Curran's model. Active transport of solute across the lateral cell membranes makes the interspace hypertonic enough for a net transport of water to occur between solutions of equal pressures and osmolarities. By exploiting the inhomogeneity of the interspace, the Diamond and Bossert model can produce a flux ratio which is nearly isotonic, i.e., $J_s/J_v \approx C_1 = C_4$. However, in order to do this all the solute pumps have to be confined to a small region at the apical end of the interspace. In this manner, fluid which is strongly hypertonic at the apical end becomes diluted by cellular water influx along the length of the interspace. However, except for certain degenerate cases, the Diamond and Bossert model always predicts a reabsorbate which is slightly hypertonic [22].

Diamond and Bossert's model involves a number of important assumptions, two of which have not stood up to experimental verification in recent years: first, that the intercellular spaces are closed at the luminal end, and second, that active transport is confined to a small region at the apical end of the interspace. As pointed out above, tight junctions are permeable to ions and small non-electrolytes and most likely water. As for the second assumption, there is no substantial evidence to indicate a localization...
of active pump sites to a region near the apical end of the interspace. In electron micrographs, the lateral cell membranes appear structurally uniform. Furthermore there is direct evidence on the distribution of ATPase sites. First, Stirling found a uniform distribution of labelled ouabain in autoradiographs of rabbit intestine [26]. Second, Farquhar and Palade also found uniform staining in frog skin membranes using lead sulfate precipitation to mark ATPase sites [10].

A uniform distribution of solute pumps has two important implications for Diamond and Bossert's theory. First, it requires Diamond and Bossert's model to always predict both a hypertonic emergent concentration and a significant violation of mass balance [22]. The second implication is that even if different boundary conditions are used in Diamond and Bossert's theory, it is impossible to achieve exact isotonic reabsorption with this class of models.

In view of these implications, two associated models of salt and water coupling were developed. In addition, an unstirred layer in the peritubular compartment beyond the lateral interspace was incorporated into the analysis to avoid a violation of mass balance. Since isosmotic reabsorption was not an a priori assumption, the models were used to investigate the dependence of the net solute to solvent flux ratio ($J_s/J_v$) on the transport parameters of the epithelium. In this manner we could ascertain whether the degree of predicted hypertonicity is small enough to be experimentally indistinguishable from isotonic transport. Although both models deal specifically with the Necturus proximal tubule, they may also be applicable to other leaky epithelia such as the gallbladder and small intestine.

THE CONTINUOUS MODEL

In the first model or "continuous version" we modified Diamond and Bossert's theory to include a permeable tight junction and a uniform distribution of active pumps. We also moved the solute boundary condition from the mouth of the interspace to the capillary lumen so that $C$ (at the capillary) = $C_o$, the luminal and plasma salt concentration. Solute concentration profiles can be generated by numerically integrating the differential equations between the tubular lumen and the peritubular capillaries.

As with the Diamond and Bossert model, the basic equations are one-dimensional. Although this is appropriate for a long narrow channel like the interspace, it cannot characterize the precise solute-solvent mixing which occurs when fluid from a narrow channel like the interspace enters a large peritubular region. By analogy with heat conduction in a stream of fluid we have determined the general two-dimensional spread of the peritubular space concentration profiles. This is shown in Fig. 3. where solute concentration is plotted as a function of position in the $x$--$y$ plane. However, the fundamental problem with any two-dimensional analysis is the necessity for information about differences in solute concentration between two regions of fluid that are less than a micron apart. In this realm one is confronted with the problem that the process of measurement necessarily perturbs the system. Although Fig. 3 gives a general idea of the real concentration profile, it is clear that numerical values will have to come from a one-dimensional analysis.

The transition between the narrow interspace and the large peritubular region could be represented simply by a discontinuity in cross sectional area at the end of the interspace ($x = L$). Specifically, one can assume that $A(L') \gg A(L)$ where $A$ is the cross sectional area $L'$, and $L'$ are the right- and left-sided limits about the point $L$ respectively. In a one-dimensional analysis this assumption requires the fluid just outside the
peritubular membrane to have the same concentration as fluid just outside the basal end of the interspace. This is clearly unreasonable since the general profiles in Fig. 3 indicate that unstirred layers probably extend in two dimensions from the mouth of the interspace.

In order to retain a one-dimensional form of analysis we chose an approximation where the inhomogeneous peritubular space was represented as two homogeneous but non-interacting regions (Fig. 4B). The solute concentration in the shaded region of peritubular space is independent of $y$. Its $x$ dependence is calculated by extending the one-dimensional convection-diffusion analysis used to calculate the interspace profiles. The rest of the peritubular space was represented as a surrounding region of solute concentration, $C_0$, equal to capillary concentration. It now becomes relatively straightforward to generate concentration profiles along the entire region between the apical end of the interspace and capillary lumen.

Fig. 4A illustrates 4 different profiles which depend on the choice of transport parameters. The main features of the profiles are:

1. a remarkably flat shape compared to those of Diamond and Bossert.
2. a slight dip at the apical end of the interspace arising from a permeable tight junction.
3. little or no drop in the peritubular space concentration.
4. a steep drop in concentration across the capillary wall.

There are two reasons why the concentration profiles of Fig. 4A do not decrease in the peritubular space. First, no solute resistance was assumed at the basement membrane. Second, Fig. 4A represents only the results of a one-dimensional analysis where the peritubular space is treated as two non-interacting regions as shown in Fig. 4B. If these two regions were allowed to interact, they would produce a two-dimensional profile similar to Fig. 3.

The reason for the abrupt concentration drop at the capillary wall follows directly from mass balance. Conservation of volume and continuity of concentration require the convective solute transport to be continuous at the outside capillary wall, i.e., $C(M)v(M^+)A(M^)-C(M^)v(M^-)A(M^-)$ where $v$ is linear velocity of the solvent at any point $x$, $M^+$ and $M^-$ are the right- and left-sided limits about the point $M$. Since the
convective solute flux and the sum of both convective and diffusive flux, i.e., the total solute flux, are continuous at the outside capillary wall ($x = M$), it follows that the diffusive flux, $-A \cdot D \cdot (dC/dx)$ must also be continuous. If the capillary endothelium contains pores over 0.1% of its histological area, an abrupt decrease in available area “$A$” occurs as fluid enters the capillary wall at $x = M$ which requires a corresponding increased negativity in the slope $dC/dx$ at that point.

**THE COMPARTMENT MODEL**

The flat shape of the interspace concentration profiles suggests that a well stirred model of the interspace may not be a bad approximation. Hence, we developed the series-parallel compartment system shown in Fig. 5. It consists of separate homogeneous compartments for the lumen, cell, interspace, peritubular space and capillary, and includes the basic driving forces of the continuous model with the following additions:

(a) inclusion of electrical driving forces
(b) consideration of individual ionic fluxes rather than neutral salt fluxes
(c) inclusion of interactions between cell and interspace where the flows and forces across both the luminal and lateral cell membrane are considered.

The conceptual simplification of a compartmental model is appropriate from an experimental standpoint. Even the electron probe or the most refined sampling techniques may only be able to determine average interspace concentrations. It is unlikely that gradients of concentration could ever be measured in a region as small as the cellular interspace. As it turns out, the two most verifiable predictions, the average interspace salt concentration and the reabsorbate osmolarity do not differ appreciably for compartmental and continuous models.

In Fig. 5 the direction of the arrows defines positive flows although negative flows may also occur. The transcellular fluxes not entering the interspace were neglected because the two-dimensional profile of Fig. 2 indicates very little concentration difference between fluid adjacent to the basal cell membrane and fluid within the cell. This would lead to very little osmotic driving force for water across the basal side of the cell. A direct path for salt and water flow which doesn't cross the interspace can be included but it does not appreciably enhance the predictive power of the model since it introduces a number of unknown quantities.

Since the compartment model is a one-dimensional form of analysis, it cannot precisely describe the two-dimensional solute profile in the peritubular space. Consequently, an approximation was developed similar to that used in the continuous model where the peritubular space was represented as two discrete homogeneous regions:

(a) a narrow band of fluid originating from the interspace and having a constant width \( W_i \).

(b) a surrounding bulk of fluid taken as isosmotic to capillary plasma (Fig. 4B).

There is assumed to be no interaction or flow between these two regions of fluid. The average interspace concentration \( C_i \) predicted by the compartment model is in the same range as the profiles shown in Fig. 4A. The specific values under different conditions are given in reference [22].
DEPENDENCE OF SOLUTE TO SOLVENT FLUX RATIO
ON THE TRANSPORT PARAMETERS

The input parameters to both the continuous model and the compartment model were calculated from data on the Necturus proximal tubule. Quantities which were not known precisely were varied over the range of experimentally observed values.

In the physiological range, the transport parameters which had the largest effect on the flux ratio were the hydraulic conductivities of the tight junction and lateral cell membrane, the interspace length, and the reflection coefficient of the tubular basement membrane. In both models, the tight junction $L_p$'s were estimated by assuming two parallel pathways for water movement across the epithelium. From values of the overall $L_p$ and the cellular $L_p$ it is possible to estimate the hydraulic conductivity of the tight junction, $L_p^0$.

Experiments by Whittembury indicate an $L_p^0$ of $6.1 \times 10^{-6}$ cm sec$^{-1}$ (cm H$_2$O)$^{-1}$ whereas experiments from our laboratory indicate a much higher value of $2.6 \times 10^{-4}$ cm sec$^{-1}$ (cm H$_2$O)$^{-1}$ (see footnote 2). Hence, there is a sizable spread in possible values. Fig. 6 shows the dependence of the flux ratio on the $L_p^0$ of the tight junction. Curve 1 (closed circles) was generated by the continuous model whereas curve 1a was predicted by the compartment model. Since the two models involve different assumptions, they should not be expected to produce identical results. For a low tight junction $L_p$ (left side of graph) the flux ratio is between 16 and 18% hypertonic to both luminal and capillary fluid. At large tight junction $L_p$'s (right side of graph) the same net fluid reabsorption occurs at a much lower hypertonicity.

The effect of variations in the lateral membrane $L_p$ are shown in Fig. 7. Curves 1 and la illustrate the predicted flux ratios for the two models under the assumption of a low hydraulic conductivity at the tight junction. The dashed curves, 2 and 2a, illustrate the predictions when a high $L_p$ is assumed for the tight junction.

Based on data from Necturus kidney slices, Whittembury [20,21] has calculated an effective cell membrane $L_p$ ($L_p^0$) of $2.8 \times 10^{-10}$ cm sec$^{-1}$ (cm H$_2$O)$^{-1}$. This corresponds to the leftmost point of each curve. In this range, the predicted flux ratio is quite sensitive to the estimate of tight junction conductivity which spans a wide range of values. As shown in Fig. 7, a cell membrane $L_p$ larger than that calculated by Whittembury leads to a dramatic reduction in the reabsorbate hypertonicity for the low $L_p^0$ case (curves 1 and 1a).

Fig. 8 illustrates the dependence of the predicted solute to solvent flux on the assumed interspace length. A standard interspace length of 25 microns was taken for the Necturus proximal tubule based on work by Bentzel [27] and Claude [28]. For a low tight junction $L_p$, both models predict a similar decline in the solute to solvent flux ratio with longer interspaces (curves 1 and 1a). It is interesting that for a low $L_p^0$, even extremely long interspaces would not produce an isosmotic reabsorbate provided the solute pumps are distributed uniformly along the lateral cell membranes. For the high $L_p^0$ condition (curves 2 and 2a) both models predict low flux ratios which are practically independent of assumed interspace length.

In all previous figures it was assumed that the basement membrane reflection coefficient was zero. Fig. 9 shows the effect of a non-zero reflection coefficient at the tubular basement membrane. The dependence of the flux ratio on reflection coefficient is essentially linear, and a reflection coefficient of only 0.19 can produce isosmotic

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1If the shunt pathway and the cell membranes constitute two parallel paths for water flow across the epithelium, the $L_p$ of the tight junction can be estimated by $L_p = (A_{cell}/A') (L_p^0 - L_p^{int})$ where $(A_{cell}/A')$ is the ratio of total luminal membrane area to total tight junction cross sectional area. $L_p^{int}$ was taken from Whittembury et al. [20,21]. Extreme values for the transepithelial filtration coefficient are $15 \times 10^{-10}$ cm sec$^{-1}$ (cm H$_2$O)$^{-1}$ [20] and $520 \times 10^{-10}$ cm sec$^{-1}$ (cm H$_2$O) [19].
FIG. 6. The effect of the tight junction filtration coefficient $L_p$ on the solute to solvent flux ratio (emergent concentration) for control Necturus proximal tubule. Curve 1 = continuous model, curve 1a = compartment model (taken from reference 22).

FIG. 7. The effect of the lateral cell membrane coefficient $L_p$ on the solute to solvent flux ratio (emergent concentration) for control Necturus proximal tubule. Curve 1 continuous model for low $L_p$ and curve 2 for high $L_p$. Curve 1a compartment model for low $L_p$ and curve 2a for high $L_p$ (taken from reference 22).

transport even though the interspace salt concentration is 118 mM. However, the finding that the basement membrane of isolated rabbit tubules has a significant permeability to albumin [29] suggests that the NaCl reflection coefficient is probably very close to zero.

Neither the continuous nor the compartment model consider transport across the
FIG. 8. The effect of interspace length $L$ on the solute to solvent flux ratio (emergent concentration) for control Necturus proximal tubule. Curves 1 and 2, continuous model for respectively low and high $L$; curves 1a and 2a, compartment model for respectively low and high $L$ (taken from reference 22).

FIG. 9. The effect of basement membrane reflection coefficient for sodium $\sigma_{Na}$ on the solute to solvent flux ratio (emergent concentration).

basal side of the cell. Consequently, it could be argued that ion and water fluxes across this barrier might affect the net solute to solvent flux ratio. If the basal labyrinth contained a hyperosmotic salt solution, it could draw water out of the cell. However, the tubule basement membrane reflection coefficient for NaCl is probably close to 0, and fluxes originating from a hyperosmotic labyrinth would always cross the basement membrane in a hypertonic ratio. Alternatively, peritubular colloid present in the basal
labyrinth could selectively draw water across the basal side of the cell since the cell membrane salt reflection coefficient is close to 1.0. However, the labyrinth salt concentration could still be no less than 1 mM hyperosmotic to the cell. Salt concentrations lower than this would offset the maximal colloid osmotic pressure and produce a reverse water flux from basal labyrinth to cell. Hence, the reabsorbate originating from the basal cell membrane would be no less than 1 mM hyposmotic since the basement membrane offers little restriction to salt. Since the basal and lateral cell membrane have similar effective surface areas, the combination of equal transcellular and interspace fluxes could at best only half the deviation from isotonicity. For example, in curve 1 of Fig. 4A the presence of protein in the basal labyrinth could lower the predicted flux ratio from 16% to 8% hypertonic.

CONSEQUENCES OF THE PROPOSED MODELS

The two models developed and applied to the Necturus proximal tubule, expand on the theories proposed by Curran, Diamond and Bossert and their colleagues [1,23,25,6,24]. The main original assumptions underlying both the continuous model and the compartment model are that, first, the tight junction is permeable to ions and water, and second, that solute pumps are distributed uniformly along the lateral cell membrane. Two general predictions follow from these models: (a) The salt concentration profiles are practically uniform, with no standing gradients as in Diamond and Bossert’s model [25]. This allows the lateral interspace to be approximated as a single compartment with a uniform salt concentration. (b) Osmotic equilibration is not achieved by the time fluid reaches the end of the interspace. Consequently, the reabsorbate is always hypertonic to some degree.

In addition, both the continuous and compartment models are useful in predicting values of certain transport parameters. Finally, using the known alterations in paracellular pathway following changes in peritubular colloid osmotic pressure, the models suggest an intracellular mechanism for volume expansion.

With respect to the first prediction, a definitive verification of either our proposed models or the model of Diamond and Bossert [25] requires direct experimental information about the interspace concentration profiles. Micropuncture samples obtained from insect rectal pads [9] indicate an hyperosmotic compartment between the cells. However, “standing gradients” have never been reported in a cellular interspace. Final resolution of this issue must await the development of techniques to determine solute concentrations at two points within the interspace.

The second prediction of both the continuous and compartment models: that net reabsorption is not exactly isotonic seems more testable. Simultaneous measurements of solute flux $J_s$ and solvent flux $J_o$ have been usually performed over a wide range of solute concentrations in the tubular lumen. Plotting $J_s$ against $J_o$ led to the conclusion that the absorbate is always isotonic [30]. Since the hypertonicity could be very slight, a large number of measurements would have to be performed in order to detect a significant deviation from isotonicity in such plots. Supporting this point is a re-analysis by Whittenbury of some earlier data which suggests that fluid reabsorption in the proximal tubule may be slightly hyperosmotic [20]. Powell and Malawer [31] have also obtained evidence that net transport in the rat ileum can be hypertonic, and experiments by Wheeler [32] suggest a slightly hypertonic net transport in the rabbit gallbladder although Diamond did not find this [4].

An alternate means of testing the prediction of hypertonic reabsorption is possible in the proximal tubule since fluid transport unlike other epithelia occurs out of a region of
finite volume. Consequently, hypertonic transport along the length of the tubule should result in a hypotonic luminal fluid by the end of the proximal tubule. It is interesting to consider whether this drop in concentration could be detected with available methods.

End proximal \((TF/P)_{osm}\) during hypertonic reabsorption can be calculated from a simple mass balance analysis. The following assumptions are used:

(a) Volume reabsorption per cm\(^2\) tubular epithelium is assumed to be the same all along the tubule

(b) The ratio of salt to volume reabsorption at any point along the tubule is assumed to be hypertonic to luminal fluid, \(C\), by a factor \(\alpha\), \((\alpha > 1)\); i.e.,

\[
J_s(\gamma)/J_v = \alpha \cdot C(\gamma)
\]

where \(C(\gamma)\) is now the luminal salt concentration at position \(\gamma\) along the tubule (mosmoles ml\(^{-1}\)), \(J_s(\gamma)\) is the salt reabsorption at any point (mosmoles cm\(^{-2}\)sec\(^{-1}\)) and \(J_v\) is the volume reabsorption (ml cm\(^{-2}\)sec\(^{-1}\)).

(c) Salt diffusion along the tubule axis is neglected because it requires a knowledge of the salt concentration gradient, \(dC/d\gamma\), at the start of the proximal tubule. This derivative depends on the microscopic transition between filtering the reabsorbing surfaces at the start of the nephron. It cannot be taken a priori as zero.

Equations (4) and (5) are the simplified mass balance relations arising from these assumptions.

\[
\frac{dv}{d\gamma} = -\frac{2}{r} J_v
\]

Linear fluid velocity (cm sec\(^{-1}\)) is \(v\), distance along the tubule (cm) is \(\gamma\) and tubule radius (cm) is \(r\),

\[
v \frac{dC}{d\gamma} + C \frac{dv}{d\gamma} = -\frac{2}{r} J_s(\gamma)
\]

Since \(J_v\) is assumed independent of \(\gamma\), the integration of Eqs. (4) and (5) is straightforward and end proximal \(TF/P\) osmolarity is given by the simple expression:

\[
(TF/P)_{osm} = (TF/P)_{salt} = (1 - \lambda)^{\alpha-1}
\]

where \(\alpha\) is the hypertonicity factor, \(\lambda\) is the fractional reabsorption at the end of the proximal tubule \(= (2\pi r J_v \cdot L)/SNGFR\), \(L\) is the length of the proximal segment, and \(SNGFR\) is the glomerular filtration rate of a single nephron.

Table 1 illustrates some representative values for fractional reabsorptions of 25 and 50%. In the Necturus a 25% reabsorption by the end of the proximal tubule is quite reasonable [33]. In mammals, end proximal reabsorption would probably be closer to 50%-70%.

For a 10% hypertonic reabsorption in the Necturus \((\alpha = 1.10)\), the end proximal \(TF/P\) ratio would be just barely detectable with existing methods. Reabsorption which is hypertonic by 5% \((\alpha = 1.05)\) would produce luminal fluid that is only 1% hypotonic by the end of the proximal segment. At earlier sites along the tubule, fluid would be less than 1% hypotonic.

Mammalian tubules which reabsorb a larger fraction of proximal fluid would have a measurable value of end \((TF/P)_{osm}\) if reabsorption exceeded 2% hypertonic. However, the low electrical resistance of mammalian proximal tubules compared to Necturus suggests a significant tight junction water permeability which might allow reabsorption to occur at interspace hypertonicities in the range of 2% (see Fig. 5). The available
TABLE 1

| \( \alpha \)  | End proximal fraction reabsorption = 25\% (Necturus) | End proximal \((TF/P)_{\text{osm}}\) |
|------------|-------------------------------------------------|-------------------------------|
| 1.10      |                                                 | 0.972                         |
| 1.05      |                                                 | 0.986                         |
| 1.02      |                                                 | 0.994                         |

| \( \alpha \)  | End proximal fractional reabsorption = 50\% (Mammalian) | End proximal \((TF/P)_{\text{osm}}\) |
|------------|-------------------------------------------------|-------------------------------|
| 1.10      |                                                 | 0.933                         |
| 1.05      |                                                 | 0.966                         |
| 1.02      |                                                 | 0.986                         |

Techniques for the determination of free flow \((TF/P)_{\text{osm}}\) ratios have failed to indicate significant deviations from unity along the proximal tubule of mammalian and Necturus kidneys [34,35,36].

In summary, we have developed two associated models for salt and water transport across the proximal tubular epithelium of the Necturus which might also be applicable to other leaky epithelia such as the gallbladder and small intestine. Although the proposed models do not completely resolve the problem of salt and water coupling, they suggest some possible explanations. Both models predict a ratio of net solute to solvent flux which deviates from exact isotonicity, except when the basement membrane has an appreciable salt reflection coefficient. Analysis of the models indicates that the flux ratio depends on a few key transport parameters whose values remain controversial. For some of these values, the predicted degree of hypertonicity is small enough to be experimentally indistinguishable from isotonic transport.

**NOTE:**

Since the presentation of this symposium at Albany, New York, in August 1974, a number of models for epithelial salt and water transport have appeared in the literature: A.E. Hill [37,38], Schafer, Patlak and Andreoli [39], Huss and Marsh [40].

The paper by A.E. Hill [37] concludes that the published values of membrane hydraulic conductivity require a hyperosmotic reabsorbate. This is consistent with the predictions of our model. However, we do not embrace his belief that, for this reason, the intercellular spaces cannot be the site of solute-solvent coupling. The model proposed by Schafer, Patlak and Andreoli [39] applies to the mammalian pars recta. The authors contend that all fluid transport occurs across the tight junction with the osmolarity of the intercellular spaces equal to or less than that of lumen and bath. Our model requires only part of the water flux to cross the tight junction and always requires a small hypertonicity in the interspace. A direct comparison cannot be made between these two theories because different driving forces, compartments and barriers are involved. In our model of the Necturus convoluted proximal tubule, solutions of identical ionic composition are on both sides of the epithelium. This is not the case for mammalian straight proximal tubule. Huss and Marsh [40] propose a model which explicitly examines the modulating role of hydrostatic pressure differences between tubule lumen and interstitium. This differs from our analysis where, during normal reabsorption, we assign a negligible role to transmural hydrostatic pressure compared to osmotic driving forces.
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