Convective Paracellular Solute Flux

A Source of Ion-Ion Interaction in the Epithelial Transport Equations

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ABSTRACT   An electrolyte model of an epithelium (a cell and a tight junction in parallel, both in series with a lateral interspace basement membrane) is analyzed using the formalism of nonequilibrium thermodynamics. It is shown that if the parallel structures are heteroporous (i.e., reflection coefficients for two ion species differ between the components), then a cross-term will appear in the overall transport equations of the epithelium. Formally, this cross-term represents an ion-ion interaction. With respect to the rat proximal tubule, data indicating epithelial ionic reflection coefficients less than unity, together with the assumption of no transcellular solvent drag, imply the presence of convective paracellular solute flux. This means that a model applicable to a heteroporous structure must be used to represent the tubule, and, in particular, the cross-terms for ion-ion interaction must also be evaluated in permeability determinations. A series of calculations is presented that permits the estimation of the Na-Cl interaction for rat proximal tubule from available experimental data. One consequence of tubule heteroporosity is that an electrical potential may be substantially less effective than an equivalent concentration gradient in driving reabsorptive ion fluxes.

INTRODUCTION

Water flow, with convective solute flux, across epithelial tight junctions was first postulated to explain observations of anomalous solvent drag in amphibian skin preparations (Ussing, 1969; Ussing and Johansen, 1969). In these experiments, an outer bath made hypertonic with urea produced an increase in the sucrose flux from the outer to the inner bathing solution, as well as a substantial fall in the epithelial electrical resistance. It was proposed that although the bulk of water flow was transcellular and directed toward the outer bath, a component of water flow through opened tight junctions, toward the inner bath, might carry the sucrose convectively. Shortly after this proposal, a mathematical model of water and nonelectrolyte fluxes was offered that supported the feasibility of this

scheme (Patlak and Rapoport, 1971). The crucial feature of the model was the existence of two parallel pathways with different reflection coefficients for the important solutes, i.e., a "heteroporous" structure. Subsequent work has identified the impact of epithelial heteroporosity on unidirectional tracer fluxes and the relation between tracer and bulk solute permeability coefficients (Li and Essig, 1976; Caplan and Essig, 1983). Convective paracellular solute flux has also been suspected in certain experiments with leaky epithelia (Berry and Boulpaep, 1975; Munck and Rasmussen, 1977; Bomsztyk and Wright, 1986). Nevertheless, the existence of significant volume flow across the tight junction in vivo remains controversial (Berry, 1983; Preisig and Berry, 1985).

In the present work, the impact of convective paracellular solute flux on electrolyte transport is analyzed. The first objective is the description of the model of Spring (1973), which consists of cellular and tight-junctional pathways in parallel, both in series with an interspace basement membrane, using the membrane transport equations of Kedem and Leaf (1966). What is found is that when the paracellular pathway permits convective ion flux, the composite epithelial equations must contain a term that formally represents ion-ion interaction. The second objective is a limited attack on the inverse problem: resolving a set of aggregate epithelial parameters into the three component membranes' contributions. As a particular example, the micropuncture data of Fromter et al. (1973) for rat proximal tubule are reanalyzed and the magnitude of the Na-Cl interaction is estimated.

GLOSSARY

Constants

$RT = 1.93 \times 10^4$ (mmHg·cm^3/mmol)	gas constant·temperature
$RT = 2.57$ (J/mmol)	gas constant·temperature
$F = 96.5$ (C/meq)	Faraday
z_i	ion valence

Fluxes

J_v (ml/s·cm^2)	volume flux
J_i (mmol/s·cm^2)	solute flux
J_v^a (ml/s·cm^2)	metabolically driven volume flux
J_i^a (mmol/s·cm^2)	metabolically driven solute flux
I (A/cm^2)	current

Intensive Variables

C_s (mmol/cm^3)	salt concentration
ψ (V)	electrical potential difference
E (V)	Cl electromotive force
μ_i (J/mmol)	chemical potential difference
$\bar{\mu}_i$ (J/mmol)	electrochemical potential difference
P (mmHg)	hydrostatic pressure difference

Permeabilities

$\hat{L}_p$ (cm/s·osmol)   open-circuit water permeability
L_p (cm/s·osmol)   short-circuit water permeability
σ_i reflection coefficient
β (C/meq)   electro-osmotic coefficient
$RT\omega$ (cm/s)   salt permeability
τ Na transference number
κ [$(\Omega \cdot cm^2)^{-1}$]   electrical conductance
L_{ij} ($mmol^2$/J·s·cm^2)   Onsager coefficient
L_i^* ($mmol^2$/J·s·cm^2)   tracer permeability
$q_{ij} = L_{ij}/\sqrt{L_{ii}L_{jj}}$ coupling coefficient

MEMBRANE TRANSPORT EQUATIONS

Several systems of equations are available for representing epithelial volume and solute transport. For a binary solute system, the Kedem-Katchalsky (1963a) equations have been particularly useful:

$$J_v = \hat{L}_p \frac{P}{RT} - \hat{L}_p \sigma C_s \frac{\mu_s}{RT} + \beta I;$$

$$J_s = C_s(1 - \sigma)J_v + \omega C_s \mu_s + \tau \frac{I}{F}; \tag{1}$$

$$E = -RT \frac{\beta}{\hat{L}_p} J_v - \frac{\tau}{F} \mu_s + \frac{I}{\kappa}.$$

In this system, the fluxes of volume (J_v), salt (J_s), and current (I) are given as functions of the driving forces of hydrostatic (and oncotic) pressure difference (P), chemical potential difference of salt ($\mu_s = \mu_{Na} + \mu_{Cl}$), and the electromotive force ($EF = -\bar{\mu}_{Cl} = \psi F - \mu_{Cl}$). The water permeability here, $\hat{L}_p$ (in centimeters per second times osmoles), is that determined under open-circuit conditions. Alternatively, one can express the fluxes in terms of the electrochemical potential difference of each ion species (Sauer, 1973; Fromter, 1974).

$$J_v = L_p \frac{P}{RT} - L_p \sigma_{Na} C_{Na} \frac{\bar{\mu}_{Na}}{RT} - L_p \sigma_{Cl} C_{Cl} \frac{\bar{\mu}_{Cl}}{RT};$$

$$J_{Na} = C_{Na}(1 - \sigma_{Na})J_v + L_{Na}\bar{\mu}_{Na} + L_{NaCl}\bar{\mu}_{Cl}; \tag{2}$$

$$J_{Cl} = C_{Cl}(1 - \sigma_{Cl})J_v + L_{NaCl}\bar{\mu}_{Na} + L_{Cl}\bar{\mu}_{Cl}.$$

In this system of equations, the water permeability is determined under short-circuit conditions. Each system of equations contains six parameters, and the interconversion of these parameters is indicated in Table I.

For the saline system considered here, $C_{Na} = C_{Cl}$, and the nonconvective contribution to the solute fluxes of Eq. 2 can be rewritten as

$$J_{Na} = (L_{Na} + L_{NaCl})\mu_{Na} + (L_{Na} - L_{NaCl})F\psi; \tag{3}$$

$$J_{Cl} = (L_{Cl} + L_{NaCl})\mu_{Cl} + (L_{NaCl} - L_{Cl})F\psi \qquad (J_v = 0),$$

where ψ is the transmembrane electrical potential difference. It is apparent that whenever the cross-term $L_{NaCl} \neq 0$, a chemical potential difference and an electrical potential difference will not be equivalent in terms of their effect on the fluxes:

$$\frac{\partial J_{Na}}{\partial \mu_{Na}} - \frac{\partial J_{Na}}{\partial F\psi} = \frac{\partial J_{Cl}}{\partial \mu_{Cl}} + \frac{\partial J_{Cl}}{\partial F\psi} = 2L_{NaCl}. \tag{4}$$

With reference to Table I:

$$L_{Na} = L_{NaCl} + \frac{\kappa\tau}{F^2};$$

$$L_{Cl} = L_{NaCl} + \frac{\kappa(1-\tau)}{F^2}, \tag{5}$$

so that L_{NaCl} is a measure of the extent to which the partial ionic conductances underestimate the ionic permeabilities. Substitution of Eq. 5 into 3 with the

TABLE I
Conversion of Onsager Coefficients and Practical Coefficients

L_p	$= \hat{L}_p/(1 - RT\beta^2\kappa/\hat{L}_p)$	$\hat{L}_p$	$= L_p\left[1 + \dfrac{L_pC_s^2(\sigma_{Na} - \sigma_{Cl})^2}{RT\kappa/F^2}\right]^{-1}$
σ_{Na}	$= \sigma - \dfrac{RT\tau\kappa\beta}{F\hat{L}_pC_s}$	σ	$= \dfrac{(L_{Cl} - L_{NaCl})\sigma_{Na} + (L_{Na} - L_{NaCl})\sigma_{Cl}}{\kappa/F^2}$
σ_{Cl}	$= \sigma + \dfrac{RT(1-\tau)\kappa\beta}{F\hat{L}_pC_s}$	β	$= -\dfrac{\hat{L}_pC_s(\sigma_{Na} - \sigma_{Cl})}{RT\kappa/F}$
L_{Na}	$= \omega C_s + \kappa\tau^2/F^2$	ωC_s	$= \dfrac{L_{Na}L_{Cl} - L_{NaCl}^2}{\kappa/F^2}$
L_{Cl}	$= \omega C_s + \kappa(1-\tau)^2/F^2$	τ	$= \dfrac{L_{Na} - L_{NaCl}}{\kappa/F^2}$
L_{NaCl}	$= \omega C_s - \kappa\tau(1-\tau)/F^2$	κ/F^2	$= L_{Na} + L_{Cl} - 2L_{NaCl}$

conditions $\mu_{Na} = \mu_{Cl} = \mu_s/2$ and $J_{Na} = J_{Cl}$ leads to an expression for the dilution potential:

$$-F\psi = (\tau - \tfrac{1}{2})\,\mu_s. \tag{6}$$

The dilution potential can be used to estimate the Na transference number, irrespective of the degree of ion-ion interaction.

Two Membranes in Parallel

Consider a composite of two membranes in parallel, where the component membrane fluxes and permeabilities are distinguished by a prime and two primes and each obeys the flux equations

$$J_v = L_p\frac{P}{RT} - L_p\sum_i \sigma_iC_i\frac{\bar{\mu}_i}{RT};$$

$$J_i = C_i(1 - \sigma_i)J_v + \sum_j L_{ij}\bar{\mu}_j + J_i^a. \tag{7}$$

In this formulation, multiple solutes are permitted and J_i^a refers to metabolically driven transport of species i. The Onsager matrix, $L = (L_{ij})$, is assumed to be symmetric. Following standard methods (Kedem and Katchalsky, 1963b), the total fluxes across both components,

$$J = J' + J'', \tag{8}$$

can also be described by equations in the form of Eq. 7. The composite coefficients are

$$L_p = L_p' + L_p'';$$

$$\sigma_i = \sigma_i' \frac{L_p'}{L_p} + \sigma_i'' \frac{L_p''}{L_p}; \tag{9}$$

$$RT\,L_{ij} = RT\,L_{ij}' + RT\,L_{ij}'' + \frac{L_p'L_p''}{L_p}(\sigma_i' - \sigma_i'')(\sigma_j' - \sigma_j'')C_iC_j.$$

Note that even when both of the component membranes are "simple," i.e., without ion-ion interaction ($L_{ij}' = L_{ij}'' = 0$ for $i \neq j$), the composite membrane may not be simple. That is, even though chemical and electrical forces may be equivalent across each of the components, they may not be equivalent across the parallel array if ionic reflection coefficients for the two component membranes differ—i.e., if there is heteroporosity. When one membrane (e.g., the cell membrane) is tight to all ions ($\sigma_i'' = 1$ for all i), the paracellular convective flux generates a positive solute interaction ($L_{ij} > 0$).

This parallels the analysis of Patlak and Rapoport (1971), who derived a term similar to that in Eq. 9 for anomalous solvent drag in a nonelectrolyte model. It also reproduces the analysis of Li and Essig (1976), who found that convective fluxes in a heteroporous membrane might introduce a discrepancy between the tracer permeability (L_i^*) and the true solute permeability ($L_i = L_{ii}$). For a simple membrane, there is identity between the two permeabilities: $L_i = L_i^*$. Further, the tracer permeability of the composite membrane satisfies

$$L_i^* = L_i^{*\prime} + L_i^{*\prime\prime}. \tag{10}$$

Thus, the straight terms of Eq. 9 can be rewritten as

$$RT\,L_i = RT\,L_i^* + \frac{L_p'L_p''}{L_p}(\sigma_i' - \sigma_i'')^2 C_i^2, \tag{11}$$

as demonstrated by Li and Essig.

Two Membranes in Series

The analysis of two membranes in series is more difficult than for the parallel system, and is facilitated by adopting a vector notation for the flux Eq. 7. Across each component membrane and for the composite system, the vector of solute fluxes, $J = (J_1, \ldots, J_n)$ is written in the form

$$J = J_v C(1 - \sigma) + L\bar{\mu} + J^a, \tag{12}$$

where $1 - \sigma$ is understood as the vector $(1 - \sigma_1, \ldots, 1 - \sigma_n)$ and C is a diagonal matrix whose entries are the appropriate mean solute concentrations.

$$C = \begin{bmatrix} C_1 & & 0 \\ & \ddots & \\ 0 & & C_n \end{bmatrix}$$

For the composite system, the electrochemical driving forces, $\bar{\mu}$, are the sum of forces across the two components:

$$\bar{\mu} = \bar{\mu}' + \bar{\mu}''. \tag{13}$$

Thus, recognizing that, in a series system, the mass fluxes across each component are equal, consideration of the case when $J_v = 0$ reveals

$$L = [(L')^{-1} + (L'')^{-1}]^{-1} \tag{14}$$

and

$$J^a = L(L')^{-1}J^{a'} + L(L'')^{-1}J^{a''}. \tag{15}$$

Eq. 14 indicates that when one of the component membranes is simple (L'' is diagonal), the composite matrix is diagonal if and only if the other component is also simple. In particular, if L'' is thought to represent lumped interspace and basement membrane permeabilities, the presence of epithelial ion-ion interaction will be determined by the properties of the tight junction and cell in parallel.

Because of flow dependence of the mean membrane concentration, explicit expressions for the reflection coefficients and the water permeability are, in general, not feasible for the series system (Kedem and Katchalsky, 1963c; Patlak et al., 1963). For applications to leaky epithelia, however, where concentration differences are small, an isotonic convection approximation can be used to linearize the model equations, with little loss of accuracy (Weinstein and Stephenson, 1981; Weinstein, 1984). In this approximation, the mean membrane concentrations for each component, C' and C'', in the convective term of Eq. 12 are set equal to a constant reference concentration, C (typically the serosal or peritubular concentration):

$$C' = C'' = C. \tag{16}$$

With this approximation, the reflection coefficients are

$$C\sigma = L[(L')^{-1}C\sigma' + (L'')^{-1}C\sigma'']. \tag{17}$$

When the second membrane in series represents an interspace basement membrane, $\sigma'' = 0$, one can simplify Eq. 17 to

$$C\sigma = L(L')^{-1}C\sigma' \qquad (\sigma'' = 0). \tag{18}$$

The volume flow across each of the component membranes is, as indicated in Eq. 7,

$$J_v = L_p \frac{P}{RT} - L_p \sum_i \sigma_i C_i \frac{\bar{\mu}_i}{RT} = L_p \frac{P}{RT} - L_p \left\langle \sigma, C \frac{\bar{\mu}}{RT} \right\rangle,$$

where the summation over the solutes has been rewritten as a vector inner product for ease of notation. For the series membrane system, however, solute-linked water transport may occur when the two membranes have different reflection coefficients, so, for the composite system,

$$J_v = L_p \frac{P}{RT} - L_p \left\langle \sigma, C\frac{\bar{\mu}}{RT} \right\rangle, J_v^a. \tag{19}$$

Equating volume flows across each membrane, one obtains

$$L_p = \frac{L_p'''}{1 + L_p''' \langle C\Delta\sigma, (RTL' + RTL'')^{-1} C\Delta\sigma \rangle} \tag{20}$$

and

$$J_v^a = L_p \langle (RTL' + RTL'')^{-1} C\Delta\sigma, J^{a'} - J^{a''} \rangle, \tag{21}$$

where

$$L_p''' = \frac{L_p' L_p''}{L_p' + L_p''}$$

and

$$\Delta\sigma = \sigma' - \sigma''.$$

Eqs. 20 and 21 generalize those reported previously for a single nonelectrolyte (Weinstein and Stephenson, 1981). In particular, for a sufficiently large cell membrane water permeability (large L_p'), the composite epithelial water permeability of Eq. 20 is determined largely by the basement membrane solute permeabilities, L'' (solute polarization effect).

Application to a Proximal Tubule Experiment

Fromter et al. (1973) performed a series of experiments on rat proximal tubules to determine ionic reflection coefficients for this epithelium. In their preparation, the composition of the peritubular solution was controlled by capillary perfusion, and the rate of reabsorption from a luminal droplet, placed between oil blocks, was observed. Most importantly, they verified that with their choice of luminal solutions, there was little change in the luminal ion concentrations as reabsorption proceeded. The appropriate flux equations for this stationary state are

$$J_v C = J_v C(1 - \sigma) + L\bar{\mu} + J^a \tag{22}$$

or

$$0 = -J_v C\sigma + L\bar{\mu} + J^a.$$

By varying J_v (using raffinose), measuring $\bar{\mu}$, and assuming that J^a is constant with different solutions, the authors sought to evaluate σ. Eq. 22 was rewritten as

$$C\frac{\bar{\mu}}{RT} = J_v C \frac{L^{-1}}{RT} C\sigma - C\frac{L^{-1}}{RT} J^a, \tag{23}$$

and the data were plotted with $(C\bar{\mu})_i$ as a function of $J_v C_i$, for $i = $ Na, Cl, and HCO_3. The slopes of these curves, $m = (m_1, m_2, m_3)$, were related to the reflection coefficients by:

$$m = \frac{L^{-1}}{RT} C\sigma \tag{24}$$

or

$$C\sigma = RTLm.$$

In their analysis, the matrix L was assumed to be diagonal (no ion-ion interaction) and the permeability coefficients were identified with tracer permeabilities (which, themselves, agreed with the partial ionic conductances determined in a previous study).

It is possible, however, to evaluate the data for Na^+ and Cl^- transport from this experiment without the a priori assumption of a diagonal matrix. The analysis begins with the application of Eq. 24 to a family of permeability matrices, where the magnitude of ion-ion interaction is varied. One then tries to discern sets of coefficients, which can be derived from a composite membrane consisting of a tight junction and cell in parallel, both in series with an interspace basement membrane. The program of calculations consists of three steps.

(a) For the epithelial matrix, the magnitude of the Na-Cl interaction is identified by the degree of coupling, q (Essig and Caplan, 1968):

$$q = \frac{L_{NaCl}}{\sqrt{L_{Na}L_{Cl}}}. \tag{25}$$

Then, given the electrical conductance, κ, and the Na transference number, τ, the relations of Table I yield an equation for L_{Na}:

$$L_{Na}^2(1 - q^2) - L_{Na} \frac{\kappa}{F^2} [1 - (1 - q^2)(1 - 2\tau)] + \left(\frac{\tau\kappa}{F^2}\right)^2 = 0. \tag{26}$$

The two roots of Eq. 26 for $L_{Na} \gtrless \tau\kappa/F^2$ correspond to $q \gtrless 0$. In general, $-1 < q < 1$, and a suitable range within this interval is chosen for exploration. For each value of q, an epithelial matrix, L, is computed. Given the experimentally determined values of m, Eq. 24 permits the calculation of the reflection coefficients, σ.

(b) With an estimate of the basement membrane matrix, L^B, the luminal matrix, L^M, can be obtained from Eq. 14:

$$L^M = [L^{-1} - (L^B)^{-1}]^{-1}. \tag{27}$$

Here, L^M represents the properties of the cell and tight junction combined in parallel. Since $\sigma^B = 0$, the combination of Eqs. 18 and 24 yields

$$C\sigma^M = RTL^M m, \tag{28}$$

an evaluation of the luminal reflection coefficients, σ^M.

(c) Finally, the luminal properties are dissected into their cellular (C) and tight-junctional (J) components using Eq. 9. In particular, when both cell and junction are assumed to be simple ($L^C_{NaCl} = L^J_{NaCl} = 0$), one can write

$$RTL^M_{NaCl} = L^M_p \frac{L^C_p}{L^J_p} (\sigma^C_{Na} - \sigma^M_{Na})(\sigma^C_{Cl} - \sigma^M_{Cl})C_{Na}C_{Cl}. \tag{29}$$

(Although it is unlikely that $L^C_{NaCl} = 0$, it will certainly be relatively small when L^M_{NaCl} is comparable to the junctional permeabilities.) Thus, given estimates of cellular osmotic properties (L^C_p and, typically, $\sigma^C = 1.0$), Eq. 29 is solved for the fractional tight-junctional water permeability, L^J_p/L^M_p. The junctional reflection coefficients are obtained as

$$\sigma^C - \sigma^J = \frac{L^M_p}{L^J_p} (\sigma^C - \sigma^M). \tag{30}$$

The straight coefficients L^J_{Na} and L^J_{Cl} are estimated as

$$RTL^J_i = RTL^M_i - \frac{L^J_p L^C_p}{L^M_p} (\sigma^C_i - \sigma^J_i)^2 C^2_i, \tag{31}$$

again assuming that L^C_{Na} and L^C_{Cl} are both small relative to the junctional permeabilities.

MODEL CALCULATIONS

Table II displays the standard set of model parameters used in the calculations that follow. The concentrations of Na^+ and Cl^- are those used by Fromter et al. (1973). The conductance, κ, and the transference number, τ, were chosen such that when ion-ion coupling is absent, the permeability coefficients correspond to the tracer permeabilities found by these workers ($P^*_{Na} = 15.5 \times 10^{-7}$ cm^2/s = 1.97×10^{-4} cm/s, and $P^*_{Cl} = 13.3 \times 10^{-7}$ cm^2/s = 1.69×10^{-4} cm/s). Thus, when $q = 0$, the reflection coefficients determined here will agree with those of Fromter et al. These electrical parameters differ only slightly from those reported previously [$\kappa = 0.2$ ($\Omega \cdot$cm^2)$^{-1}$, $\tau = 0.55$] by Fromter et al. (1971). The slopes of electrochemical driving force with respect to convective flux ($m_{Na} = 4.51 \times 10^5$ s/cm^2 and $m_{Cl} = 3.80 \times 10^5$ s/cm^2) were scaled to a 25-μm tubule diameter. The permeabilities assigned to the basement membrane correspond to the relative ionic mobilities in solution and a total electrical resistance of 0.8 $\Omega \cdot$cm^2 for the structure. When Eq. 20 is evaluated using these parameters, the solute polarization effect is substantial, and the water permeability of the model epithelium is well below that of the cell membranes (and approximately the measured epithelial L_p). The water permeability of the cell membranes, L^C_p, was obtained by multiplying the unit permeability, 5.4×10^{-4} cm/s$\cdot$osmol (Welling et al., 1983a), by an area amplification factor of 36 cm^2/cm^2 epithelium (Welling and Welling, 1975) and then dividing by 2 to adjust for the apical and lateral membranes in

series. The ionic reflection coefficients of unity for the cell membranes are suggested by the data of Gonzalez et al. (1982).[1]

Figs. 1 and 2 illustrate the program of calculations outlined above, for the standard model parameters and for several additional values of the epithelial conductance spanning the range reported by Seely (1973) for the rat proximal tubule [κ = 0.18, 0.16, 0.14, 0.12, and 0.10 ($\Omega \cdot cm^2)^{-1}$]. As the coupling coefficient is varied between 0.0 and 0.5, the associated permeability matrix is calculated using Eq. 26; the epithelial reflection coefficients (determined from Eq. 24) are plotted in the upper two panels of Fig. 1. In the curves corresponding to κ = 0.18, it can be seen that the points above q = 0 (σ_{Na} = 0.7 and σ_{Cl} = 0.5) reproduce the data analysis of Fromter et al. (1973). It should also be noted that, with this conductance, not all values of q yield reflection coefficients within the range 0 ≤ σ_i ≤ 1.0. In fact, as the calculation proceeds, progressively sharper limits on acceptable values for q will be obtained. Eq. 27 now permits the

TABLE II
Model Parameters

C_{Na}	0.146 meq/cm^3
C_{Cl}	0.125 meq/cm^3
κ	0.18 ($\Omega \cdot cm^2)^{-1}$
τ	0.58
m_{Na}	3,500 s/cm
m_{Cl}	3,000 s/cm
L^B_{Na}	6.75 × 10^{-5} mmol^2/J·s·cm
L^B_{Cl}	9.64 × 10^{-5} mmol^2/J·s·cm
L^C_p	0.01 cm/s·osmol
σ^C_{Na}	1.0
σ^C_{Cl}	1.0
RT	2.57 J/mmol
F	96.5 C/meq

subtraction of the basement membrane parameters from the epithelial matrix and the calculation (Eq. 28) of the composite luminal reflection coefficients, indicated in the middle panels of Fig. 1.

With the choice of cellular osmotic properties (L^C_p and σ^C) shown in Table II, Eq. 29 yields the fractional tight-junction water permeability [$L^J_p/(L^J_p + L^C_p)$]. This appears in the bottom panel of Fig. 1, and two important features can readily be seen. The first is that for each conductance, the permissible range for the coupling coefficient is quite restricted. [For example, when κ = 0.18 ($\Omega \cdot cm^2)^{-1}$, 0.075 ≤ q ≤ 0.10.] This means that the reflection coefficients can be fairly well determined. The second, and complementary, observation is that the value of

[1] It is acknowledged that Welling et al. (1983b) have measured a reflection coefficient of the basolateral membrane of rabbit proximal tubule for NaCl to be 0.5. Nevertheless, the whole epithelial reflection coefficient for this tubule is reported to be close to 1.0 (Jacobson et al., 1982). While it is possible to rationalize these two observations by supposing a relatively tight luminal cell membrane, the implications for the present considerations of the proximal tubule of the rat are uncertain.

the fractional tight-junction water permeability cannot be determined with any degree of certainty. This implies that the estimates of junctional properties that follow from this water permeability are quite crude.

As indicated above, there are narrow limits to the assignment of epithelial reflection coefficients and Onsager coefficients. The parameters in Table III were selected by taking the midpoint of each of the five curves in the bottom panel of Fig. 1 and reading the coupling coefficient from the abscissa. This
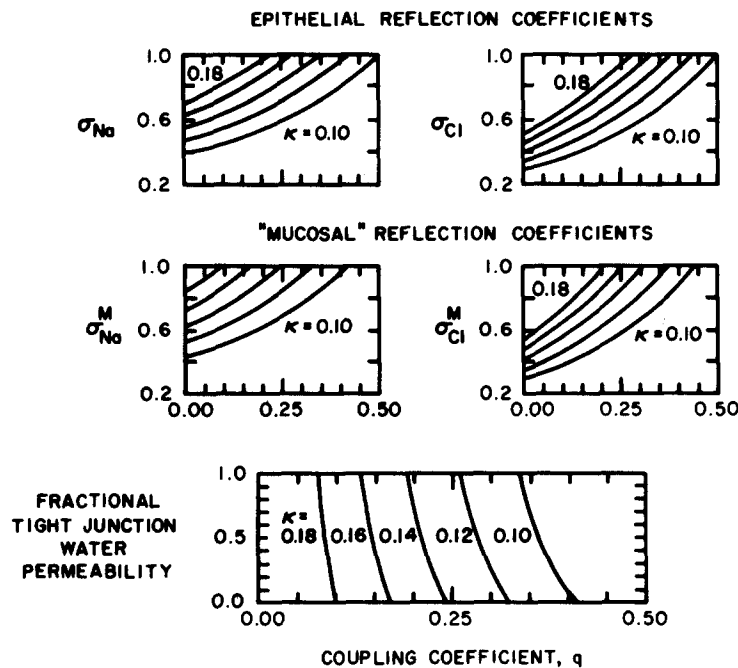

FIGURE 1.   Model parameters as a function of the epithelial coupling coefficient, q. For $\kappa = 0.10$, 0.12, 0.14, 0.16, and 0.18, and the remaining parameters as in Table II, the Onsager permeability matrix is calculated over a range of values of q. The epithelial reflection coefficients (from Eq. 24) appear in the upper two panels. Reflection coefficients defined for the cell and tight junction in parallel (Eq. 28) are plotted in the middle panels. $[L_p^J/(L_p^J + L_p^C)]$ is calculated from Eq. 29 and graphed at bottom.

corresponds to a fractional tight-junction water permeability of 0.5, or equal transcellular and transjunctional L_p values. As the assumed epithelial resistance is raised from 5.6 to 10 $\Omega \cdot cm^2$, the coupling coefficient increases fourfold, from 0.09 to 0.36. Nevertheless, there is little impact on the reflection coefficients computed for Na (0.76) or for Cl (0.65). The second panel in this table is derived from the three Onsager coefficients and estimates the measured ionic permeability when the driving force is either a concentration gradient or the electrical potential difference. Although there is little change in the concentration-associated permeabilities for Na and Cl, the effect of voltage falls to about half that of

an equivalent chemical potential difference when $q = 0.36$. Finally, Table III contains estimates for the passive fluxes that might be anticipated in a region of tubule with well-developed Cl and bicarbonate gradients. Given a net volume reabsorption of 42.4 nl/s·cm^2 (2.0 nl/min·mm), the total Na and Cl fluxes are 6.4 and 5.9 neq/s·cm^2. When the Na gradient is negligible, the Cl concentration difference is 20 meq/liter, and the electrical potential difference is 1.0 mV, one

TABLE III

Derived coefficients*

κ	σ_{Na}	σ_{Cl}	$L_{Na} \times 10^5$	$L_{Cl} \times 10^5$	$L_{NaCl} \times 10^5$	q
$(\Omega \cdot cm^2)^{-1}$			$mmol^2/J \cdot s \cdot cm^2$			
0.18	0.79	0.62	1.21	0.90	0.09	0.09
0.16	0.78	0.64	1.14	0.87	0.15	0.15
0.14	0.77	0.66	1.07	0.83	0.20	0.21
0.12	0.75	0.67	1.00	0.79	0.25	0.28
0.10	0.73	0.68	0.93	0.75	0.30	0.36

Associated permeabilities‡ (cm/s × 10^{-5})

$\dfrac{RT}{C_{Na}}(L_{Na} + L_{NaCl})$	$\dfrac{RT}{C_{Na}}(L_{Na} - L_{NaCl})$	$\dfrac{RT}{C_{Cl}}(L_{Cl} + L_{NaCl})$	$\dfrac{RT}{C_{Cl}}(L_{Cl} - L_{NaCl})$
22.3	19.2	19.6	16.0
22.1	17.0	20.2	14.2
21.8	14.9	20.4	12.5
21.4	12.8	20.6	10.7
21.1	10.8	20.8	8.9

Solution composition and net fluxes

J_v	C_{Na}^{LUM}	C_{Cl}^{LUM}	C_{Na}^{BLD}	C_{Cl}^{BLD}	ψ	J_{Na}^{TOT}	J_{Cl}^{TOT}
$nl/s \cdot cm^2$		$meq/liter$			mV	$neq/s \cdot cm^2$	
42.4	150	140	150	120	1.0	6.36	5.94

Passive fluxes§ (neq/s·cm^2)

J_{Na}^{CON}	$L_{Na}\bar{\mu}_{Na}$	$L_{NaCl}\bar{\mu}_{Cl}$	$J_{Na}^{PAS}/J_{Na}^{TOT}$	J_{Cl}^{CON}	$L_{Cl}\bar{\mu}_{Cl}$	$L_{NaCl}\bar{\mu}_{Na}$	$J_{Cl}^{PAS}/J_{Cl}^{TOT}$
1.32	1.17	0.27	0.43	2.10	2.70	0.09	0.82
1.40	1.10	0.43	0.46	1.99	2.60	0.14	0.80
1.49	1.03	0.60	0.49	1.90	2.49	0.19	0.77
1.60	0.96	0.76	0.52	1.81	2.38	0.24	0.75
1.71	0.89	0.91	0.55	1.74	2.26	0.29	0.72

* Assumes $\tau = 0.58$ and $L_p^J = L_p^C$.

‡ Assumes $C_{Na} = 150$ meq/liter and $C_{Cl} = 130$ meq/liter.

§ J_i^{CON}, J_i^{PAS}, and J_i^{TOT} denote convective, passive, and total flux of species i, respectively.

finds that about one-half the Na and three-fourths of the Cl are reabsorbed passively. The aggregate passive component of flux appears to be little influenced by the variation in the coupling coefficient.

Fig. 2 completes the program of calculations with the estimation of the tight-junctional parameters. These have been computed over the full range of fractional tight-junction water permeabilities. For each value of the junctional water

permeability, the bottom panel of Fig. 1 yields the value of the coupling coefficient q, and thus a set of epithelial and luminal parameters. Eqs. 30 and 31 then furnish the junctional coefficients. Although one cannot determine the precise value of these parameters, in some cases, the constraint that the thermodynamic coefficients be positive may have important consequences. In particular, when the epithelial resistance is <7 $\Omega \cdot cm^2$, the panel for L^J_{Cl} shows that the junctional water permeability must be at least 40% of the total. Further, since the junctional reflection coefficient is always <0.7, one is forced to the conclusion of significant paracellular convective Cl flux.

The uncertainty in the assignment of the transcellular water permeability is considered in Fig. 3, where the calculations were repeated for $L^C_P = 0.01$, 0.005,

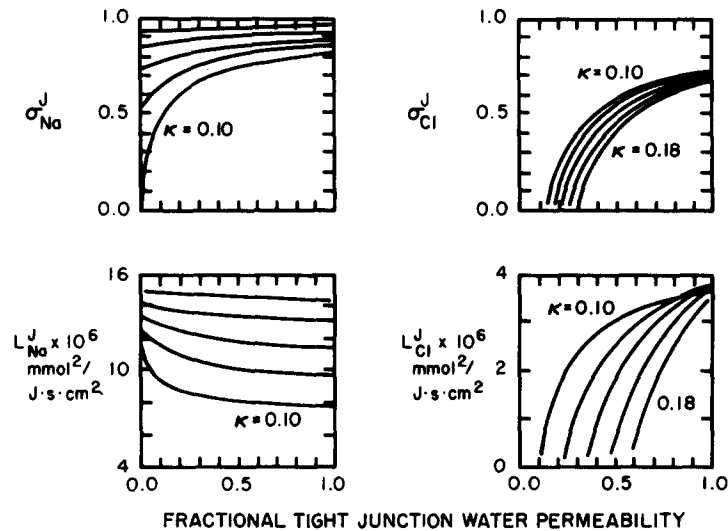FRACTIONAL TIGHT JUNCTION WATER PERMEABILITY

FIGURE 2.   Tight-junctional parameters as a function of fractional tight-junction water permeability. For the calculations of Fig. 1, the choice of a fractional tight-junction water permeability prescribes a unique coupling coefficient and permeability matrices. Eq. 30 is used to calculate the tight-junctional reflection coefficients (upper panels) and Eq. 31 yields the Onsager coefficients (lower panels).

0.002, and 0.001 cm/s·osmol. Since L^C_P does not appear in the computation of the epithelial or luminal parameters, these parameters are identical to those in Fig. 1, so that only the junctional parameters need be shown. From this figure, it is clear that the condition that the Cl reflection coefficient be positive requires a junctional water permeability $>30\%$ of the total. Again, since $\sigma^J_{Cl} < 0.7$, paracellular convective Cl flux is implied.

This somewhat convoluted series of calculations can be summarized in more intuitive terms. The epithelial conductance and the transference number are taken as firm experimental data. It has been indicated (Eq. 6) that regardless of the degree of ion-ion interaction, it is still valid to use the dilution potential to estimate the transference number. In a simple membrane, where ion-ion interaction is absent, these two parameters determine the Na and Cl permeabilities.

However, when the magnitude of the interaction is unknown, the ionic permeabilities cannot be computed, and one must obtain a third piece of experimental data to completely describe the system. Ideally, this would be the difference between an electrical and chemical potential in driving ionic fluxes (Eq. 4). Since this additional datum is not available, one can only calculate the epithelial permeabilities as a function of the assumed Na-Cl coupling coefficient. Furthermore, since these permeabilities are required to analyze the experimental data of Fromter et al. (1973), the uncertainty of the ion-ion interaction extends to the epithelial reflection coefficients. What has been done, however, in the calculations of Fig. 1 is that the epithelial parameters have been computed for

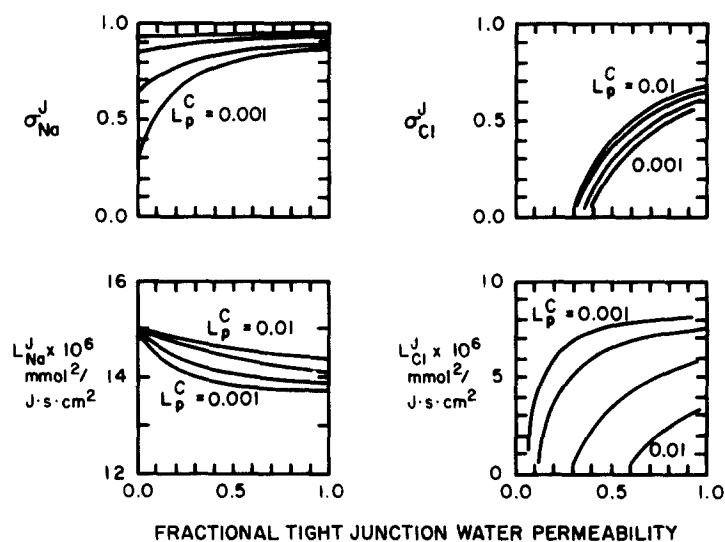FRACTIONAL TIGHT JUNCTION WATER PERMEABILITY

FIGURE 3.   Tight-junctional parameters as a function of fractional tight-junction water permeability. The calculations of Figs. 1 and 2 are repeated for cell membrane water permeability, $L_p^C = 0.01$, 0.005, 0.002, and 0.001 cm/s·osmol, and remaining parameters as in Table II.

each degree of coupling. One then asks (after subtracting off the interspace basement membrane) whether this yields a realistic description for the cell and the tight junction in parallel (Fig. 2). It appears that this additional constraint may suffice to put narrow bounds on the overall epithelial parameters.

DISCUSSION

This investigation was prompted by a series of calculations (unpublished) using an electrolyte model of the rat proximal tubule epithelium (Weinstein, 1985) in which a Cl concentration gradient was found to be more effective at driving Cl flux than an equivalent electrical potential difference. With reference to linear nonequilibrium thermodynamic theory (Kedem and Leaf, 1966), this occurs when there is a positive interaction between the fluxes of Na^+ and Cl^-. The present work provides an interpretation for this interaction in the case of two simple membranes combined in parallel. When the composite membrane is

heteroporous (i.e., when the reflection coefficients for two ion species differ between the two component membranes), then cross-terms will appear in the overall transport equations of the composite membrane. The imposition of an additional simple membrane in series neither introduces nor abolishes such a cross-term. With respect to rat proximal tubule, the finding of ionic reflection coefficients less than unity (Fromter et al., 1973), together with the assumption of no solvent drag through cell membranes, requires paracellular convective solute flux. In particular, this implies that a term for ion-ion interaction must be included in the analysis of such data. The calculations presented suggest that, in some cases, the additional term still permits the assignment of the epithelial coefficients within a narrow range.

The intuitive description of solute fluxes across a heteroporous membrane has already been given in discussions of anomalous solvent drag (Ussing and Johansen, 1969; Patlak and Rapoport, 1971) and isotope interaction (Li and Essig, 1976). The present work provides an extension to electrolyte fluxes in the context of proximal tubule transport. When water flux is driven by oncotic or hydrostatic forces across the cell and tight junction in parallel, the flows partition according to the water permeabilities of the two component structures. Thus, the aggregate solvent drag is represented by an average of the two reflection coefficients, weighted according to the individual L_p's. However, when there is water flux driven by oppositely directed Cl and bicarbonate gradients (Schafer et al., 1975), the force for water flow is solely across the tight junction (assuming unity reflection coefficients for the transcellular pathway). Thus, the convective flux of Na is determined by the sieving properties of only the paracellular path, and should exceed that expected from a composite reflection coefficient. This appears in the transport equations as a Cl gradient driving net Na flux, i.e., a term for ion-ion interaction. It should be clear that the prerequisites for such an effect are different reflection coefficients for Na, as well as for Cl, across cellular and paracellular pathways. It is also important to note (Fig. 1) that the finding of aggregate reflection coefficients close to unity does not preclude the presence of significant ion-ion coupling. Thus, the lack of solvent drag of NaCl found across rabbit proximal tubule using raffinose (Jacobson et al., 1982) does not rule out important paracellular convection when Cl and bicarbonate gradients are present.

In the model calculations motivating this study, the water permeability of the junctional and cellular pathways had been set equal (Weinstein, 1985). It should be noted that all previous electrolyte models of the proximal tubule epithelium (Spring, 1973; Sackin and Boulpaep, 1975; Schafer et al., 1975) had required virtually all of the transepithelial water flow to be transjunctional. Thus, these earlier efforts had, in effect, fashioned a simple membrane for the cell and tight junction in parallel, and could not have yielded the type of convective interaction described here. It is especially intriguing that Schafer et al. commented that their model could not accommodate any transcellular water flux and still fit the observed data (reabsorptive solute fluxes, transepithelial gradients, and tracer permeabilities for Na^+ and Cl^-). Although it is not clear how to resolve this numerical difficulty, it is important to indicate that in a composite system of membranes in parallel, the Cl tracer permeability, the partial ionic conductance

$[(L_{Cl} - L_{NaCl})/F]$, and the ionic permeability $[RT(L_{Cl} + L_{NaCl})/C_{Cl}]$ are all distinct quantities. Indeed, Caplan and Essig (1983) have emphasized that the tracer permeability determined in an exchange experiment may also be quite different from that obtained when ionic gradients are present. From the present analysis, it can be seen that when $\sigma_{Na}^l = \sigma_{Cl}^l$, the tracer permeability and the partial ionic conductance may be in good agreement (as observed experimentally by Fromter et al., 1973, and by Schafer et al., 1975) but still be less than the ionic permeability.

Recently, Alpern et al. (1985) have re-examined the dependence of proximal tubule Cl fluxes on transepithelial gradients in a microperfusion study of the rat kidney. Luminal perfusates contained normal or low NaCl (with raffinose replacement) and standard or low bicarbonate, yielding four data points. From the perspective of the present work, the measured Cl fluxes would be represented as

$$J_{Cl} = J_v(1 - \sigma_{Cl})C_{Cl} + L_{Cl}\bar{\mu}_{Cl} + L_{NaCl}\bar{\mu}_{Na} + J_{Cl}^a.$$

The values of J_{Cl}, J_v, $\bar{\mu}_{Cl}$, and $\bar{\mu}_{Na}$ for the four perfusions are thus required to solve for the four unknown parameters: σ_{Cl}, L_{Cl}, L_{NaCl}, and J_{Cl}^a. In their analysis of their data, Alpern et al. assumed $\sigma_{Cl} = 1.0$ and $L_{NaCl} = 0.0$ to obtain a best fit for L_{Cl} and J_{Cl}^a. It is clear that the determination of Na fluxes during these same perfusions would provide an important test of the representation of the epithelium as a composite membrane and, in particular, of the validation of the symmetry of ion-ion interaction.

The documentation of ion-ion interaction in proximal tubule will, at best, be difficult. Perhaps a study of a flat epithelium, especially one in which tight junctions are thought to be opened (e.g., the preparation of Munck and Rasmussen, 1977), might be more rewarding. Here, the critical experiment will be the comparison of an electrical potential difference and an equivalent chemical potential difference in driving transepithelial Na flux. For renal tubules, notwithstanding the technical challenge, the establishment of the magnitude of ion-ion interactions is required for an accurate description of transport, especially if alterations in paracellular convective ion fluxes are implicated in the regulation of proximal Na reabsorption.

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