

Electro-osmosis and the Reabsorption of Fluid in Renal Proximal Tubules

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ABSTRACT The lateral intercellular spaces (LIS) are believed to be the final common pathway for fluid reabsorption from the renal proximal tubule. We postulate that electrogenic sodium pumps in the lateral membranes produce an electrical potential within the LIS, that the lateral membranes bear a net negative charge, and that fluid moves parallel to these membranes because of Helmholtz-type electro-osmosis, the field-induced movement of fluid adjacent to a charged surface. Our theoretical analysis indicates that the sodium pumps produce a longitudinal electric field of the order of 1 V/cm in the LIS. Our experimental measurements demonstrate that the electrophoretic mobility of rat renal basolateral membrane vesicles is 1 $\mu\text{m/s}$ per V/cm, which is also the electro-osmotic fluid velocity in the LIS produced by a unit electric field. Thus, the fluid velocity in the LIS due to electro-osmosis should be of the order of 1 $\mu\text{m/s}$, which is sufficient to account for the observed reabsorption of fluid from renal proximal tubules. Several experimentally testable predictions emerge from our model. First, the pressure in the LIS need not increase when fluid is transported. Thus, the LIS of mammalian proximal tubules need not swell during fluid transport, a prediction consistent with the observations of Burg and Grantham (1971, *Membranes and Ion Transport*, pp. 49–77). Second, the reabsorption of fluid is predicted to cease when the lumen is clamped to a negative voltage. Our analysis predicts that a voltage of -15 mV will cause fluid to be secreted into the *Necturus* proximal tubule, a prediction consistent with the observations of Spring and Paganelli (1972, *J. Gen. Physiol.*, 60:181).

INTRODUCTION

Spring and Ericson (1982) and Spring (1983) have lucidly summarized a paradigm for isotonic fluid reabsorption in epithelia classified as “leaky” by Frömter and Diamond (1972). Their model has four salient features. (a) Na^+ enters an epithelial cell through the apical membrane, moving passively down its electrochemical gradient. The influx of Cl^- through the apical membrane is coupled, either directly or indirectly, to the passive entry of Na^+ . (b) The influx of NaCl increases the osmolarity of the cytoplasm and water enters the cell through the

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apical membrane, moving passively down its chemical gradient. (c) The sodium pumps (Na,K-ATPases), which are distributed uniformly along the basolateral membranes, actively transport sodium out of the cell. Chloride follows sodium into the lateral intercellular spaces (LIS), where the resulting increase in osmolarity and the high permeability of the plasma membrane to water allow the solvent to rapidly follow the solutes across the lateral membrane. (d) A small hydrostatic pressure develops in the LIS, driving the essentially isotonic fluid out of the LIS and across the submucosal connective tissue.

In this report, we suggest that a mechanism other than pressure is responsible for the movement of fluid through the LIS of renal proximal tubules. We calculate theoretically that the electrogenic sodium pumps in the basolateral membranes produce an electrical potential within the LIS, demonstrate experimentally that the lateral membranes bear a net negative charge, and postulate that fluid moves parallel to the membranes because of electro-osmosis, the field-induced movement of fluid adjacent to a charged surface.¹

Our idea is not new. In a letter to Emil Du Bois-Reymond dated Sept. 19, 1850, Carl Ludwig suggested that electro-osmosis, which was then termed electrical endosmosis, might play a role in the secretion of fluid from the salivary gland (Du Bois-Reymond and Ludwig, 1982). In his reply, Du Bois-Reymond cautioned Ludwig "that it will hardly be possible to build up a hypothesis with any certainty on 'electrical endosmosis' . . . before a physicist has deigned to conduct a thorough investigation on the physical transport phenomena of the galvanic current." Although the physiologist-physicist Helmholtz published a thorough investigation of electro-osmosis in 1879, no quantitative treatment of the role that Helmholtz-type electro-osmosis might play in epithelial fluid movement has appeared in the intervening century.² We present a quantitative version of Ludwig's suggestion.

The essential feature of our hypothesis is that fluid can be moved along the LIS of epithelia by a gradient of voltage as well as by a gradient of pressure. This can be illustrated very simply (Fig. 1) by considering the two different ways in which fluid can be moved through a rigid cylindrical pipe.

The fluid velocity produced by a gradient of pressure was described quantitatively by Poiseuille. The velocity profile can be obtained by assuming that the

¹ The reader who is interested in a detailed modern discussion of electro-osmosis is referred to Overbeek and Wiersema (1967), Dukhin and Derjaguin (1974), and O'Brien and White (1978). A brief derivation of the Helmholtz-Smoluchowski equation can be found in textbooks by Shaw (1970) and Aveyard and Haydon (1973) and in a monograph by Hunter (1981). The history of electro-osmosis is discussed by Dukhin (1974). The ability of the Gouy-Chapman, Nernst-Planck, and Navier-Stokes equations to describe electro-osmotic flow in small pores has been investigated theoretically by Morrison and Osterle (1965) and tested experimentally by Anderson and his co-workers (Koh and Anderson, 1975; Westermann-Clark and Anderson, 1983).

² Papers by Hill (1975) and by Kuppens and Thurm (1980) deal with "Schmid-type" electro-osmosis, not the classical type of electro-osmosis considered here. Dainty (1963) and Dainty et al. (1963) discuss clearly the difference between Helmholtz-Smoluchowski and Schmid-type electro-osmosis. Schmid-type electro-osmosis arises when an electric field is applied across a membrane and the membrane has channels that are narrower than the Debye length (Schmid, 1950; Schmid and Schwarz, 1952). In a later paper, Hill (1980) discusses why fluid movement in epithelia probably does not result from this type of electro-osmosis.

fluid is incompressible, the flow is laminar, and the velocity is zero at the surface of the pipe. The velocity attains its maximum value in the center of the pipe and is described by a parabola (Fig. 1A). The flow (volume/time) is obtained by integrating this velocity profile over the cross-sectional area of the pipe:

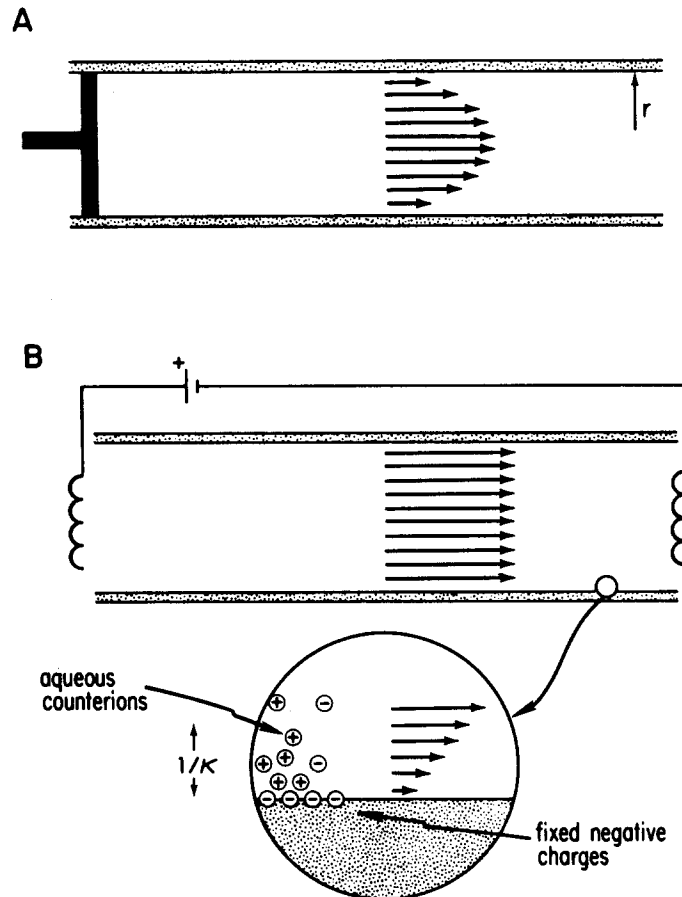


FIGURE 1. The velocity profiles that result when fluid is driven through a rigid cylindrical pipe of length l and radius r ($l \gg r$) by a gradient of either pressure or voltage. The steady state velocity of the fluid in the pipe is illustrated by the length of the arrows. (A) The pressure, p , is applied by a piston at one end of the pipe, $x = 0$. The other end of the pipe, located at $x = l$, is at atmospheric pressure, $p = 0$. (B) A voltage, ψ , is applied down the axis of the pipe by two nonpolarizable electrodes. The potential has a value of $+\psi$ at $x = 0$ and a value of 0 at $x = l$. The ends of the pipe at $x = 0, l$ are exposed to infinite reservoirs of fluid maintained at atmospheric pressure. The inset illustrates that the velocity is zero at the surface of the pipe, but increases to a maximum value within a few Debye lengths ($1/\kappa \approx 1$ nm for $[\text{NaCl}] = 0.1$ M). For a pipe with $r \gg 1/\kappa$, the velocity profile is constant throughout most of the pipe.

$$\text{flow} = -\frac{dp}{dx} \frac{\pi r^4}{8\eta}. \quad (1)$$

The flow is proportional to the pressure gradient, $-(dp/dx)$, inversely proportional to the viscosity, η , and proportional to the fourth power of the radius, r . If we divide the flow by the cross-sectional area, πr^2 , we can define an average velocity, \bar{u} , which depends on the square of the radius:

$$\bar{u} = -\frac{dp}{dx} \frac{r^2}{8\eta}. \quad (2)$$

There is another way to move fluid through a pipe. If the walls of the pipe bear a fixed negative charge, imposing an electric field parallel to the wall of the pipe causes the fluid to move as illustrated in Fig. 1B, a phenomenon termed electro-osmosis. Helmholtz's (1879) description of electro-osmosis is perhaps worth repeating. The phenomenon arises because the fixed charges on the surface "form an electric double layer along their boundary surface. . . . This layer has an extraordinarily small, but not disappearing, thickness. The side of this layer adjacent to the boundary surface clings immovably to the wall . . . a potential gradient directly propels the positively charged wall layer of the liquid. However, because of the internal friction of the liquid, the entire cross section of the tube assumes the same motion if no hydraulic back pressure results." Thus, the fluid velocity is constant throughout the pipe, except for an extremely thin region adjacent to the wall (see inset of Fig. 1B). The theoretical description of the electro-osmotic fluid velocity, u_{eo} , was given by Helmholtz (1879) and Smoluchowski (1921). The Helmholtz-Smoluchowski equation,¹

$$u_{eo} = -\frac{d\psi}{dx} \frac{\epsilon_r \epsilon_o \zeta}{\eta}, \quad (3)$$

relates the fluid velocity to the electric field along the axis of the tube, $-d\psi/dx$, the viscosity, η , the dielectric constant, ϵ_r , the permittivity of free space, ϵ_o , and the zeta potential, ζ , the electrostatic potential at the hydrodynamic plane of shear.³ Note that when the fluid is driven by a voltage gradient, the velocity does not depend on the radius (Eq. 3). When the fluid is driven by a pressure gradient, the average velocity is proportional to the square of the radius (Eq. 2). Thus, for given values of $d\psi/dx$, dp/dx , and ζ , the voltage gradient will be more important than the pressure gradient if the pipe is small enough.

How small? In a pipe 50 nm in diameter, a voltage gradient of 1 V/cm will cause more flow than a pressure gradient of 100 mmHg/cm if the zeta potential is -10 mV. This simple calculation illustrates that electro-osmosis could be the dominant factor in the movement of fluid through many epithelia. However, this simple calculation cannot be applied directly to epithelia because the lateral membranes in a typical epithelium more closely resemble a pair of convoluted

³ The hydrodynamic plane of shear or slip plane is located 0.2 nm from the surface of a phospholipid bilayer exposed to a decimolar monovalent salt solution (Eisenberg et al., 1979; Alvarez et al., 1983).

parallel sheets than a cylindrical tube, and ionic current, solutes, and fluid all flow into the LIS perpendicular to the lateral membranes. The detailed analysis we present below indicates that the electric field in the LIS of a mammalian proximal tubule should be of the order of 1 V/cm. Our experimental measurements demonstrate that $\zeta = -10$ mV (electrophoretic mobility = 1 $\mu\text{m/s}$ per V/cm) for rat renal basolateral membranes. Thus, we hypothesize that electro-osmosis can produce a fluid velocity of ~ 1 $\mu\text{m/s}$ in the LIS, a value of the correct order to account for fluid reabsorption in mammalian renal proximal tubules.

The hypothesis should be easy to falsify. For example, if the electrodes in the pipe illustrated in Fig. 1B are reversed, the electro-osmotic flow will reverse direction. If electro-osmosis affects fluid flow in the LIS of renal proximal tubules, clamping the lumen to a negative potential should reverse the reabsorption of fluid. Furthermore, reversal of fluid flow should be instantaneous (< 1 s) on the time scale in which these measurements can presently be made (Donath et al., 1978). These predictions agree with the results obtained from *Necturus* proximal tubules. The fluid flow through the epithelium was reversed by clamping the lumen to a potential of -15 mV, a potential a few millivolts more negative than the spontaneous potential, and the new flow "usually became constant in the first few seconds and continued unchanged until the command was altered" (Spring and Paganelli, 1972; Spring, 1973a). Fluid flow can also be reversed in the rabbit gallbladder (Wedner and Diamond, 1969; van Os et al., 1976). When either the gallbladder or the *Necturus* proximal tubule is current-clamped, the fluid flow reaches a new steady state level only after several minutes have elapsed. This observation has been used repeatedly as an argument against electro-osmosis: "if electro-osmosis were the cause of water flow one would expect an instantaneous flow without transients" (van Os et al., 1976). The conclusion does not follow from the observation: it is based on the misconception that electro-osmotic flow is proportional to current. Eq. 3 illustrates that it is voltage rather than current that is responsible for the type of flow considered by Helmholtz.⁴

Diamond (1964), Wedner and Diamond (1969), Spring (1973a), van Os et al. (1976), Hill (1980), and Spring and Ericson (1982) have concluded that "Schmid-type" electro-osmosis² does not play a significant role in the transport of fluid through epithelia. We agree with their conclusion. However, the available experimental data do not rule out Helmholtz-type electro-osmosis.

GLOSSARY

c_o	solute concentration or osmolarity in lumen and interstitial fluid
$c_e(x)$	solute concentration in LIS
D_e	effective diffusion coefficient of solute in LIS (see Eq. 13)

⁴ For example, if the voltage gradient is constant and the current is reduced by decreasing the salt concentration in the pipe illustrated in Fig. 1B, the electro-osmotic fluid flow actually increases. This increase in flow occurs because the magnitude of the zeta potential increases when the salt concentration decreases (see Eq. 3). The increase in zeta potential can be accounted for by the Gouy-Chapman theory of the diffuse double layer (e.g., McLaughlin, 1977; Eisenberg et al., 1979).

D_s	diffusion coefficient of solute
F	Faraday constant
g_m	conductance per area of lateral membranes
g_t	conductance per area of tight junction
$\bar{i}_e(z)$	average current per area within LIS
$i_e(x)$	average current within LIS per area epithelium
i_m	current flux through lateral membranes
$i_z(y, z)$	z component of current per area within LIS
i_p	pump current per area through lateral membrane
I	see Eq. 35
$\bar{j}_e(z)$	average solute flow within LIS per area of LIS
$j_e(x)$	average solute flow within LIS per area of epithelium
j_m	solute flux through lateral membranes
$j_z(y, z)$	z component of flux of solute in LIS
J	see Eq. 36
h_e	average transepithelial electro-osmotic velocity per electric field (see Eq. 11)
K_e	average electro-osmotic velocity in LIS per electric field (see Eq. A17)
K_{sp}	specific conductance of solution
l	length of cells
L_m	hydraulic conductivity of lateral membranes
L_t	hydraulic conductivity of tight junction for a unit area of epithelium
$p_e(x)$	hydrostatic pressure in LIS
q	see Eq. 37
R_e	effective specific resistance of LIS (see Eq. A16)
R	gas constant
S_m/V_T	area of lateral membrane per volume of epithelium
T	absolute temperature
$\bar{u}_e(z)$	average fluid velocity within LIS
$u_e(x)$	average fluid flow per area of epithelium
$u_z(y, z)$	z component of fluid velocity in LIS
V_e/V_T	volume of LIS per volume of epithelium
w	width of LIS
ϵ	width of LIS per length of LIS
ϵ_o	permittivity of free space
ϵ_r	dielectric constant of fluid in LIS
ζ	zeta potential of lateral membranes
η	viscosity of fluid in LIS
η_e	see Eq. 10
κ	reciprocal of Debye length
λ	space constant for potential in LIS (see footnote 8)
ξ	length of LIS per length of cell
$\rho(y)$	space charge density in LIS
ρ_e	effective space charge density in LIS (see Eq. 14)
σ	surface charge density of lateral membranes
σ_t	reflection coefficient of the tight junction
τ	tortuosity factor equal to $1/\xi^2$ for isotropic, unbranched LIS

$\bar{\psi}(y)$	equilibrium electrostatic potential within LIS produced by fixed negative charges
ψ_a	electrical potential in lumen
$\psi_e(x)$	electrical potential within LIS
ω_t	permeability of the tight junctions in a unit area of epithelium

THEORY

Mathias (1985) has presented a detailed theoretical analysis of fluid flow in leaky epithelia; his model is similar to the one described qualitatively by Spring (1983). Here we extend Mathias' analysis to include the role of voltage and electro-osmosis in the movement of fluid through the LIS of epithelia. As illustrated in Fig. 2, the LIS is represented by two parallel membranes separated by a distance w . The membranes are convoluted,⁵ which increases the length of the LIS by a factor ξ . A local y - z coordinate system is constructed with the z axis following the axis of the LIS and the y axis normal to the lateral membranes, which are located at $y = \pm w/2$ (see inset of Fig. 2).

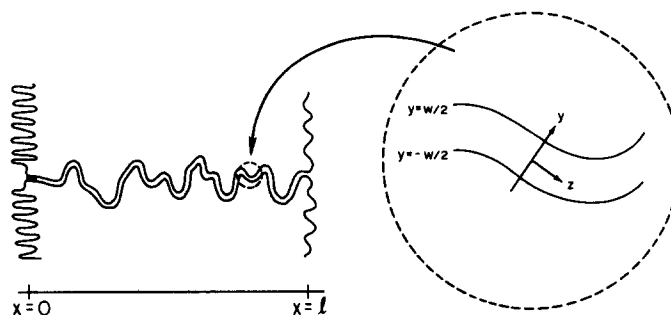


FIGURE 2. Sketch of an epithelium. The apical surface is located at $x = 0$, the basal surface at $x = l$. The insert illustrates the local y - z coordinate system constructed within the lateral intercellular space (LIS), which has a width w and a length ξl .

The lateral membranes lining the LIS bear a net negative charge that we assume is smeared uniformly over the surfaces located at $y = \pm w/2$. At equilibrium, these fixed negative charges produce an electrostatic potential, $\bar{\psi}(y)$, in the aqueous phase adjacent to the surface. We describe this potential using the Gouy-Chapman theory of the aqueous diffuse double layer (e.g., McLaughlin, 1977). The potential at the slip planes,³ which we assume are also located at $y = \pm w/2$, is by definition the zeta potential, ζ . For a single surface, the potential falls exponentially with distance from the surface when the zeta potential is small

⁵ We assume that the radius of curvature of the convolutions is smaller than the spatial changes in the flux of solutes and solvent, which implies that the tortuosity merely increases the membrane area and length of the LIS. We also assume that the radius of curvature is large compared with the Debye length in a physiological saline solution, $1/\kappa = 1$ nm, to derive the Helmholtz-Smoluchowski equation for electro-osmotic flow.

($\zeta < RT/F = 25$ mV). By following Overbeek (1952), it is easy to show that the potential between two parallel surfaces, $\bar{\psi}(y)$, is:⁶

$$\bar{\psi}(y) = \frac{\zeta \cosh(\kappa y)}{\sinh(\kappa w/2)}, \quad (4)$$

when the zeta potential is small ($\zeta < 25$ mV). $1/\kappa$ is the Debye length. Fig. 3 shows a graph of Eq. 4 when $w = 20$ nm; this value of w is characteristic of the narrowest LIS, such as those found in mammalian renal proximal tubules (e.g.,

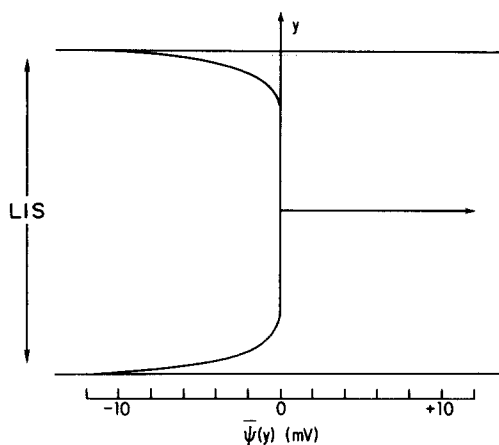


FIGURE 3. The equilibrium electrostatic potential, $\bar{\psi}(y)$, produced within the LIS by fixed charges located on the interfaces ($y = \pm w/2$). The potential at $y = \pm w/2$ is -11 mV, the experimentally determined value for the zeta potential of basolateral membranes from rat renal proximal tubule cells. The distribution of potential within the LIS is given by the Gouy-Chapman theory of the diffuse double layer, Eq. 4. The width of the LIS is assumed to be 20 nm and the aqueous phases are assumed to contain 0.15 M NaCl ($1/\kappa = 0.78$ nm). Note that the potential falls in an approximately exponential manner with distance from the surfaces and that the potential is essentially zero over most of the LIS.

Burg and Grantham, 1971; Berridge and Oschman, 1972; Tisher and Kokko, 1974). Note that even in the narrowest LIS there is essentially no overlap of the two diffuse double layers. The concentrations of Na^+ and Cl^- in the diffuse double layers can be calculated from the potential illustrated in Fig. 3 using the Boltzmann relation.

In our model, the zeta potential is proportional to the surface charge density, σ :

$$\zeta = \sigma / \kappa \epsilon_r \epsilon_0, \quad (5)$$

⁶ Eq. 4 may also be obtained from the more general Eqs. 13–15 of Parsegian and Gingell (1972) by assuming that the charge densities on the two surfaces are small and equal. Ninham and Parsegian (1971) have considered the case in which the surfaces contain ionizable groups that can be titrated by ions in the aqueous phase.

where ϵ_r is the dielectric constant and ϵ_0 is the permittivity of free space.⁷ The electro-osmotic flow in the LIS depends on both the zeta potential, which we determine experimentally from electrokinetic mobility measurements on vesicles formed from basolateral membranes, and the electric field parallel to the membrane, which we estimate from theoretical considerations.

In the Appendix, we show that both the potential in the LIS due to the electrogenic sodium pumps, $\psi_e(y, z)$, and the pressure in the LIS, $p_e(y, z)$, are primarily functions of z rather than y because the aspect ratio, w/l , is small. We demonstrate that the fluid velocity down the axis of the LIS, $u_z(y, z)$, is

$$u_z(y, z) = \frac{1}{\eta} \left(\frac{y^2}{2} - \frac{w^2}{8} \right) \frac{\partial p_e(z)}{\partial z} - \frac{\epsilon_0 \epsilon_r \zeta}{\eta} \left(\frac{\cosh \kappa y - \cosh \kappa w/2}{\sinh \kappa w/2} \right) \frac{\partial \psi_e(z)}{\partial z}. \quad (6)$$

When either the zeta potential or the longitudinal component of the electric field in the LIS is zero, the fluid velocity is described by the first term in Eq. 6, which is the Poiseuille equation for laminar fluid flow between two parallel plates. The parabolic dependence of the fluid velocity on the y coordinate is illustrated in Fig. 4A. When the pressure gradient, dp_e/dz , is zero, the fluid velocity is described by the second term in Eq. 6. This electro-osmotic fluid flow is graphed in Fig. 4B. Note that the fluid velocity is constant over most of the width of the interspace and that this velocity is described by the Helmholtz-Smoluchowski equation (compare the second term in Eq. 6 with Eq. 3). In general, both pressure and voltage drive the fluid longitudinally in the LIS and both terms in Eq. 6 are important.

We also derive equations in the Appendix for the flux of current and solute in the LIS. We integrate Eq. 6 and these equations over the width of the LIS and obtain expressions for the average fluid velocity, average current flux, and average solute flux in the LIS. We then make a geometrical transformation (Mathias, 1983) and obtain the average fluid velocity, $u_e(x)$, the average current flux, $i_e(x)$, and average solute flux, $j_e(x)$, per unit cross-sectional area of tissue:

$$u_e(x) = \frac{1}{\eta_e} \frac{dp_e}{dx} - k_e \frac{d\psi_e}{dx}; \quad (7)$$

$$i_e(x) = -k_e \frac{dp_e}{dx} - \frac{1}{r_e} \frac{d\psi_e}{dx}; \quad (8)$$

$$j_e(x) = c_o u_e(x) - D_e \left(\frac{dc_e}{dx} + \frac{\rho_e}{RT} \frac{d\psi_e}{dx} \right); \quad (9)$$

⁷ We note that the potential profile between two biological membranes is not accurately described by Eq. 4 and that the relationship between the zeta potential and charge density is more complex than expressed by Eq. 5 (Donath and Pastushenko, 1979; Wunderlich, 1982; Levine et al., 1983; McDaniel et al., 1984). However, the simplifications inherent in Eqs. 4 and 5 do not introduce serious errors into our model. We show (see Eq. 11) that the exact shape of the potential profile adjacent to the surface has little effect on the analysis. Furthermore, we do not calculate the zeta potential from Eq. 5; we determine it experimentally.

$$\frac{1}{\eta_e} = \frac{w^2}{12\eta} \left[\frac{\tau V_e}{V_T} \right]; \quad (10)$$

$$k_e = \frac{-\epsilon_r \epsilon_0 \zeta}{\eta} (\coth \kappa w / 2 - 2 / \kappa w) [\tau V_e / V_T]; \quad (11)$$

$$\frac{1}{r_e} = \frac{F^2 c_0 D_s}{RT} \left\{ 1 + \frac{F^2 c_0 \zeta^2}{RT w \kappa^3 D_s \eta} \left[\frac{\sinh(\kappa w / 2) \cosh(\kappa w / 2) - \kappa w / 2}{\sinh^2(\kappa w / 2)} \right] \right\} (\tau V_e / V_T); \quad (12)$$

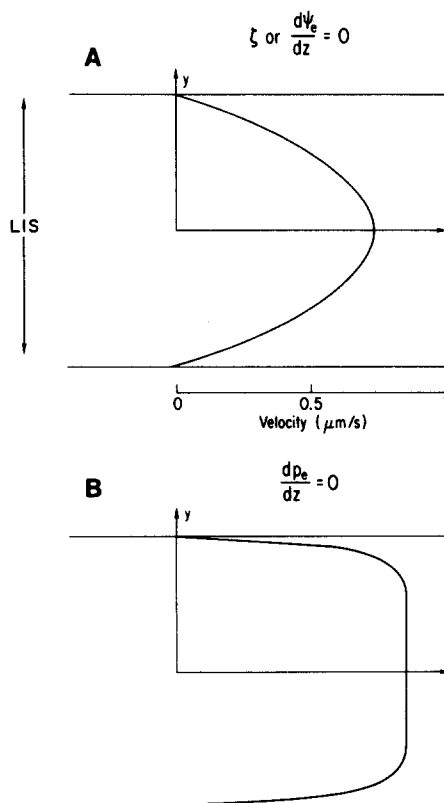


FIGURE 4. The fluid velocity in the LIS when either the voltage gradient is zero (A) or the pressure gradient is zero (B). The fluid velocity is plotted on the abscissa. (A) The velocity, $u_x(y, z)$, predicted by Eq. 6 when $d\psi_e/dz = 0$. The width of the LIS is assumed to be 20 nm and the pressure gradient is assumed to be -0.1 mmHg/ μm . (B) The velocity predicted by Eq. 6 when $dp_e/dz = 0$. The zeta potential is assumed to be -11 mV, the width of the LIS is assumed to be 20 nm, and the aqueous solution is assumed to contain 0.15 M NaCl ($1/\kappa = 0.78$ nm), as in Fig. 3. The longitudinal voltage gradient, $d\psi_e/dz$, is assumed to be -1 V/cm = -0.1 mV/ μm .

$$D_e = D_s(\tau V_e/V_T); \quad (13)$$

$$\rho_e = -2\sigma/w. \quad (14)$$

c_o is the total solute concentration at equilibrium. The terms defined in Eqs. 10–14 are all positive when the zeta potential is negative.

Conservation of mass requires that the divergence of fluid velocity be zero in the steady state. In other words, the longitudinal flow of fluid in the LIS must be coupled to the flow of fluid across the lateral membranes. The divergence of the current and solute flow are also zero in the steady state.

$$\frac{du_e(x)}{dx} = \frac{S_m}{V_T} L_m [p_i - p_e + RT(c_e - c_i)]; \quad (15)$$

$$\frac{di_e(x)}{dx} = \frac{S_m}{V_T} i_m; \quad (16)$$

$$\frac{dj_e(x)}{dx} = \frac{S_m}{V_T} j_m, \quad (17)$$

where S_m/V_T is the area of lateral membrane per volume of tissue and L_m is the hydraulic conductivity of the lateral membranes. The pressure and total concentration of solutes inside the cell, p_i and c_i , should be approximately constant because the intracellular dimensions are relatively large and the resistances to flows are relatively small (Mathias, 1985). i_m is the current flux and j_m is the solute flux through the lateral membranes.

Eqs. 7–9 and 15–17 constitute a set of six ordinary differential equations with constant coefficients. Six boundary conditions are required to solve the equations. We ignore the resistance of the basement membrane to fluid flow (Welling and Grantham, 1972) and current flow and assume that both the pressure and electrical potential at $x = l$ are equal to zero:

$$p_e(l) = 0; \quad (18)$$

$$\psi_e(l) = 0, \quad (19)$$

and that the solute concentration in the LIS is equal to the concentration in the lumen:

$$c_e(l) = c_o. \quad (20)$$

For a tight junction that is nonselective with respect to ions, the general boundary conditions at $x = 0$ within the LIS are

$$u_e(0) = -L_t \{p_e(0) - \sigma_t RT[c_e(0) - c_o]\}; \quad (21)$$

$$i_e(0) = -g_t[\psi_e(0) - \psi_a]; \quad (22)$$

$$j_e(0) = -\omega_t[c_e(0) - c_o] + (1 - \sigma_t)c_o u_e(0), \quad (23)$$

where L_t is the hydraulic conductivity of the tight junction, σ_t is the reflection coefficient of the tight junction, g_t is the conductance per unit area of the tight junctions, and ω_t is the permeability of the tight junctions. ψ_a is the electrical potential on the mucosal side of the epithelium, measured with respect to the potential on the serosal side, which is defined to be zero (Eq. 19). The conductance and the permeability are most simply related by (Hodgkin and Katz, 1949):

$$g_t/c_o = F^2\omega_t/RT. \quad (24)$$

Although a general solution to the six differential equations (Eqs. 7–9, 15–17) with the associated boundary conditions (Eqs. 18–23) exists, we prefer to simplify the equations before solving them. Our approximations are based on experimental rather than rigorous mathematical arguments.

The first term in Eq. 7 describes the average fluid velocity when the fluid is driven only by a gradient of pressure; the coefficient $1/\eta_e$ is defined in Eq. 10. The bracketed term in Eq. 10, $\tau V_e/V_T$, where $\tau = 1/\xi^2$ (Fig. 2) and V_e/V_T is the volume of the LIS per unit volume of tissue, is a scaling factor that Mathias (1983) discussed in more detail. If we ignore this scaling factor, it is apparent from Eqs. 7 and 10 that the average velocity of pressure-driven fluid in an LIS of width w differs by only a factor of 2/3 from the analogous expression for Poiseuille flow in a cylindrical pipe of radius w (Eq. 2). The second term in Eq. 7 describes the average velocity due to electro-osmotic flow; the coefficient k_e is defined in Eq. 11. If we ignore the scaling factor in brackets, it is easy to demonstrate that the Helmholtz-Smoluchowski equation (Eq. 3), which describes the electro-osmotic fluid velocity several Debye lengths from a surface of arbitrary geometry, closely approximates the second term in Eq. 7. For example, if the width of the LIS is >20 nm and the aqueous solution contains 0.15 M NaCl ($1/\kappa = 0.78$ nm), an error of $<10\%$ is introduced by equating the term in parentheses in Eq. 11 to unity, a procedure we adopt in the analysis that follows.

We now consider Eq. 8, which describes the average current per unit cross-sectional area of tissue. The coefficient k_e in Eq. 8 is identical to the coefficient k_e in Eq. 7 because the equations have been written so that the sum of the products of the generalized flows and forces equals the entropy production; in this case, Onsager's reciprocal relationship is valid (e.g., Katchalsky and Curran, 1965, pp. 85–97). Eq. 8 can also be derived without invoking Onsager's relationship (Appendix). The first term of Eq. 8 is only $\sim 1\%$ of the second term if $\zeta = -10$ mV, $c_o = 0.3$ M, $dp_e/dx = 0.1$ mmHg/ μm , and $d\psi_e/dx = 0.1$ mV/ μm . In other words, the excess charge in the double layer moved by convection does not contribute significantly to the current flow. Thus, we ignore this term in the analysis that follows. The second term in Eq. 8 describes the current that results directly from the voltage gradient in the LIS, averaged over a unit cross-sectional area of epithelia. The term in front of the braces in Eq. 12, $F^2c_oD_s/RT = K_{sp}$, is simply the specific conductance (conductivity) of an electrolyte solution encased by walls bearing no net charge. (For a decimolar salt solution, $1/K_{sp} \approx 10^2 \Omega \cdot \text{cm}$.) The term inside the braces in Eq. 12 is related to the surface conductance (Duhkin and Derjaguin, 1974); if $\zeta = -10$ mV, $w > 20$ nm, and $1/\kappa = 0.8$ nm, this term differs from unity by $<1\%$, and will be assumed to be unity in the

analysis that follows. The term in the last set of parentheses in Eq. 12 scales the current flux in the LIS to a unit cross-sectional area of tissue.

Eq. 9 describes the average longitudinal flux of solute (Na + K + Cl) down the LIS, scaled to a unit cross-sectional area of tissue. The first term represents the convection of solute, the second term the diffusion of solute down a concentration gradient, and the third term the conduction of solute by the longitudinal electric field. The third term is proportional to the average space charge density in the LIS, ρ_e , which is equal to twice the surface charge density divided by the width of the LIS, $-2\sigma/w$. This conduction term is most important in epithelia with narrow LIS (small w) and high surface charge densities (large σ). For a typical epithelium, the convection term dominates both the diffusion and the conduction terms in Eq. 9. Specifically, the diffusion and conduction terms are <2% and <10% of the convection term if we assume that $\Delta p_e = 5$ mmHg, $\Delta\psi_e = 10$ mV across the epithelium, $w > 20$ nm, and $\zeta = -10$ mV. We ignore the diffusion and conduction terms in Eq. 9 in the analysis that follows.

Thus, Eqs. 7-9 reduce to the approximate Eqs. 25-27:

$$u_e(x) = \frac{-1}{\eta_e} \frac{dp_e}{dx} - k_e \frac{d\psi_e}{dx}; \quad (25)$$

$$i_e(x) \approx \frac{-1}{r_e} \frac{d\psi_e}{dx}; \quad (26)$$

$$j_e(x) \approx c_o u_e(x), \quad (27)$$

where

$$k_e \approx \frac{-\epsilon_r \epsilon_o \zeta}{\eta} \left(\frac{\tau V_e}{V_T} \right); \quad (28)$$

$$\frac{1}{r_e} \approx \frac{F^2 c_o D_s}{RT} \left(\frac{\tau V_e}{V_T} \right) = K_{sp} \left(\frac{\tau V_e}{V_T} \right). \quad (29)$$

Eqs. 7 and 9 are linearly independent; Eqs. 25 and 27 are not. We have reduced the number of generalized forces from three to two by assuming that diffusion can be neglected. This assumption reduces the number of independent equations from six to four (e.g., Eqs. 15, 16, 25, and 26). We must also reduce the number of boundary conditions (Eqs. 18-23) from six to four to determine the pressure and velocity profiles. However, we can determine the profile of the electrical potential in the LIS without additional consideration of the boundary conditions.

To simplify the discussion that follows, we assume that the conductance of the lateral membranes is low and the current through the lateral membranes is dominated by the pump current.⁸ In other words, we assume that i_m in Eq. 16

⁸ Specifically, we assume that $l/\lambda \ll 1$, where $1/\lambda^2 = r_e g_m S_m / V_T$ and g_m is the conductance of a unit area of lateral membrane. Eq. 32 follows when $\psi_e(0) = 0$. Conversely, when the conductance of the lateral membranes is large, $l/\lambda \gg 1$, $\psi_e(x)$ can be shown (R. T. Mathias, unpublished data) to be proportional to $1 - \exp[-(l-x)/\lambda] - \exp(-x/\lambda)$ when $\psi_e(0) = 0$.

can be equated to the pump current, i_p , which we assume is constant because the sodium pumps are distributed uniformly along the basolateral membranes (Kyte, 1976; DiBona and Mills, 1979). We combine Eqs. 16 and 26 and integrate the resulting equation. The solution that satisfies the boundary conditions (Eqs. 19 and 22) is

$$\psi_e(x) = \frac{l^2}{2} r_e \frac{S_m}{V_T} i_p \left[1 - \frac{x^2}{l^2} - \frac{r_e g_i l}{1 + r_e g_i l} (1 - x/l) \right] + \frac{r_e g_i l}{1 + r_e g_i l} (1 - x/l) \psi_a. \quad (30)$$

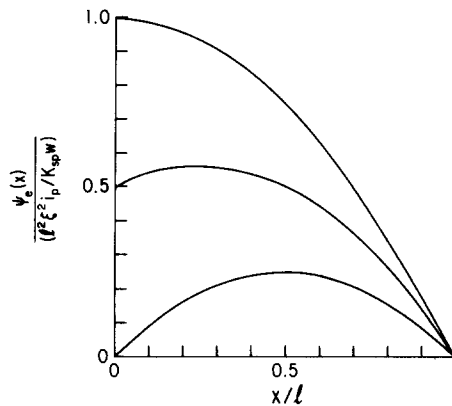


FIGURE 5. Profiles of the electrical potential in the LIS. The abscissa is the distance through the epithelium, x (see Fig. 2), divided by the length of the cells, l . The ordinate is the potential, $\psi_e(x)$, normalized by the factor $(l^2/2)r_e i_p (S_m/V_T) = l^2 \xi^2 i_p / (K_{ap} w)$. This normalization factor equals 2 mV if $w = 20$ nm, $i_p = 10 \mu\text{A}/\text{cm}^2$, $1/K_{ap} = 100 \Omega \cdot \text{cm}$, $l = 10 \mu\text{m}$, and $\xi = 2$. The lower curve illustrates the potential profile when the conductance of the tight junction is high. The upper curve illustrates the potential when the conductance of the tight junction is zero. The middle curve illustrates the potential profile when the conductance of the tight junction is equal to the conductance of the remainder of the LIS, $g_t = 1/(r_e l)$.

The potential at $x = 0$ within the LIS is

$$\psi_e(0) = \frac{l^2}{2} r_e \frac{S_m}{V_T} i_p / (1 + r_e g_i l) + \frac{r_e g_i l}{1 + r_e g_i l} \psi_a. \quad (31)$$

To illustrate the dependence of $\psi_e(x)$ on x , we set $\psi_a = 0$. When the tight junction is electrically very leaky, $g_t \gg 1/r_e l$, $\psi_e(0)$ reduces to zero, and the potential profile in the LIS is symmetrical about $x = l/2$, as illustrated by the lower curve in Fig. 5 and Eq. 32:

$$\psi_e(x) = \frac{l^2}{2} r_e \frac{S_m}{V_T} i_p \left(\frac{x}{l} - \frac{x^2}{l^2} \right). \quad (32)$$

When the conductance of the tight junction is much lower than the conductance of the remainder of the LIS, $g_t \ll 1/r_e l$, the potential at $x = 0$ attains its maximum value, $\psi_e(0) = (l^2/2)r_e i_p (S_m/V_T)$, and $\psi_e(x)$ decreases with x along the LIS, as illustrated by the upper curve in Fig. 5. The middle curve in Fig. 5 illustrates the potential profile when the tight junction and LIS offer equal resistances to current flow, $g_t = 1/r_e l$.

If we note that $(l^2/2)r_e i_p (S_m/V_T) = l^2 \xi^2 i_p / (K_{sp} w)$, is it apparent from Eqs. 30 and 32 that the potential and the potential gradient will be largest for epithelia with long cells (large l), narrow tortuous lateral intercellular spaces (small w , large ξ), and a high density of sodium pumps (large i_p).

We now examine the magnitude of the potential and potential gradient in the LIS of mammalian renal proximal tubules. We assume that the length of the cells is $l = 10 \mu\text{m}$ and that the width of the LIS is $w = 20 \text{ nm}$ (Burg and Grantham, 1971; Tisher and Kokko, 1974).⁹ We also assume that the length of the LIS divided by the length of the cells is $\xi = 2$, that the current produced by the electrogenic sodium pumps is $i_p = 10 \mu\text{A}/\text{cm}^2$, and that the resistivity of the solutions is $1/K_{sp} = 100 \Omega \cdot \text{cm}$. The collection of terms in front of the parentheses in Eq. 32, which is also the normalization factor in Fig. 5, then has a value of 2 mV. For a leaky epithelium, the potential at $x = l/2$ is one-quarter this value (Eq. 32), or 0.5 mV. It is apparent from Fig. 5 that the electric field at $x = l$ is lowest when the tight junctions are electrically leaky. For the above choice of parameters, the electric field at $x = l$ within the LIS is $d\psi_e/dz = d\psi_e/d\xi x = 1 \text{ V/cm}$ (Eq. 32) when the junctions are electrically leaky. An electric field of 1 V/cm is capable of producing the electro-osmotic fluid flow illustrated in Fig. 4B, a flow that is of the correct magnitude to account for the reabsorption of fluid in renal proximal tubules. In the steady state, of course, the flow of fluid from the LIS is precisely coupled to the flow of fluid across the lateral membranes. If the field produced by the electrogenic sodium pumps is more than sufficient to drive the fluid from the LIS by means of electro-osmosis, a negative pressure will build up in the LIS to couple the efflux of solvent from the LIS to the influx across the lateral membranes. We illustrate this point by considering the simple case where the tight junctions offer resistance to neither current nor solute nor fluid flow.

The four boundary conditions we require are Eq. 18, Eq. 19, $p_e(0) = 0$, and $\psi_e(0) = \psi_a = 0$. We combine Eqs. 15 and 25 and obtain

$$\frac{-1}{\eta_e} \frac{d^2 p_e}{dx^2} - k_e \frac{d^2 \psi_e}{dx^2} = \frac{S_m}{V_T} L_m [p_i - p_e + RT(c_e - c_i)]. \quad (33)$$

We integrate Eq. 33 to obtain the pressure profile. The term in Eq. 33 that is proportional to the second derivative of the voltage is a constant because we assume that $i_m = i_p = \text{constant}$ (see Eqs. 16 and 26). From Eqs. 17 and 27, it is

⁹ Burg and Grantham (1971) state that the LIS are of the order of 20 nm wide, whereas Tisher and Kokko (1974) estimate w to be 33 nm. To calculate the electric field, we require the effective resistance of the LIS, r_e , which depends on the integral of the reciprocal of the width. Thus, any narrowing of the LIS will produce a large resistance: we take the effective width to be $w = 20 \text{ nm}$.

apparent that the right-hand side of Eq. 33 is equal to $(j_m/c_o)(S_m/V_T)$. If we assume that j_m is a constant, an assumption consistent with our postulate that i_m is a constant, we can integrate Eq. 33 directly.¹⁰ In this simple case, the pressure and potential profiles have the same mathematical form:

$$p_e(x) = \frac{l^2}{2} (J - qI) \left(\frac{x}{l} - \frac{x^2}{l^2} \right), \quad (34)$$

where

$$I = r_e i_p S_m / V_T; \quad (35)$$

$$J = (\eta_e j_m / c_o)(S_m / V_T); \quad (36)$$

$$q = \eta_e k_e. \quad (37)$$

We consider three specific cases. (a) Positive pressure in the LIS: $qI < J$, $p_e(x) > 0$. When either the zeta potential of the lateral membranes is zero ($q = 0$) or the current flux through the lateral membranes and the electric field in the LIS is zero ($I = 0$), there is no electro-osmosis. The pressure profile predicted by Eq. 34 when $qI = 0$ is illustrated by the upper curve in Fig. 6. Specifically, if $\zeta = 0$ and $i_p \neq 0$, there is no electro-osmosis but there is a potential in the LIS, illustrated by the lower curve in Fig. 5. Consider the following thought experiment: add fixed negative charges to the walls of the LIS and make the zeta potential negative. An examination of Eq. 34 indicates that the pressure in the LIS becomes less positive as qI increases. (b) Zero pressure in the LIS: $qI = J$, $p_e(x) = 0$. At a zeta potential for which $qI = J$, the pressure in the LIS reduces to zero and electro-osmosis alone drives from the LIS all the fluid that crosses the lateral membranes. The line in Fig. 6 illustrates the zero pressure that exists when the condition $qI = J$ is satisfied. (c) Negative pressure in the LIS: $qI > J$, $p_e(x) < 0$. If we add still more fixed negative charge to the lateral membranes, the zeta potential becomes sufficiently negative that $qI > J$, and electro-osmosis more than suffices to drive all the fluid crossing the lateral membranes from the LIS. In this case, a negative pressure will develop in the LIS to retard the electro-osmotic flow of fluid from the LIS. For example, if $qI = 2J$, the pressure profile will be given by the lower curve in Fig. 6.

The profile of the average velocity, $u_e(x)$, is independent of the degree to which electro-osmosis occurs; it is coupled solely to the solute flux, which we assume is constant along the LIS. In other words, fluid must be conserved in the steady state, and the divergence of flow must be zero whether the fluid is driven by a pressure gradient, a voltage gradient, or a combination of the two gradients.

¹⁰ The assumption that j_m is a constant implies that the fluid flow across the lateral membranes does not vary with distance along the LIS. The fluid follows the solute across the lateral membranes because we have assumed that the water permeability of these membranes is large (Spring, 1983; Carpi-Medina et al., 1983) and that the volume of the LIS is small (Welling and Welling, 1976). Stated mathematically, the assumption that j_m is a constant implies that $p_e(x) = RTc_e(x) + \text{constant}$ (Eqs. 15, 17, and 27). It follows from Eqs. 18 and 20 that $p_e(x) = RT[c_e(x) - c_o]$, which Mathias (1985) refers to as the "balanced gradient" condition.

The average velocity in the LIS is given by Eq. 25. If we substitute into Eq. 25 from Eqs. 32 and 34–37, we obtain

$$u_e(x) = \frac{-lJ}{2\eta_e} \left(1 - \frac{2x}{l} \right), \quad (38)$$

which is independent of the degree to which electro-osmosis occurs. Eq. 38 is graphed in Fig. 7; it is apparent that the average velocity is zero at $x = l/2$, and has maximal positive and negative values at $x = l$ and $x = 0$, respectively. Half the fluid that enters the LIS leaves through the apical end, because we assume

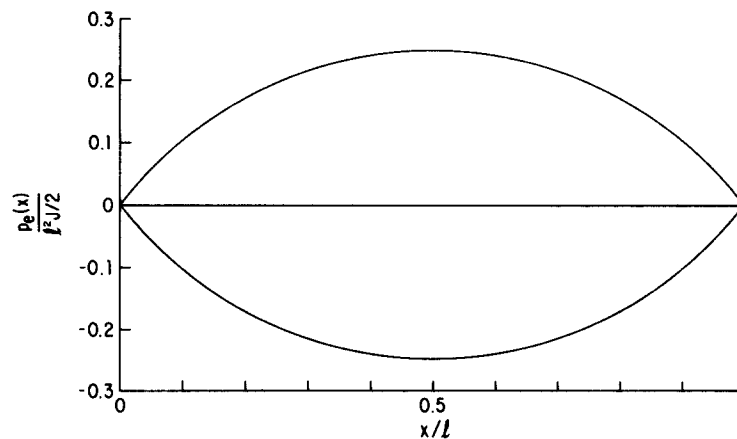


FIGURE 6. Profiles of the pressure in the LIS of an epithelium with a tight junction that is leaky to current, solutes, and water. The abscissa is the distance through the epithelium, x , normalized to the length of the cells, l . The ordinate is the pressure, $p_e(x)$, normalized by the factor $l^2 J / 2$ (see Eq. 34). The upper curve illustrates the pressure when there is no electro-osmosis, $qI = 0$. The line illustrates that when $J = qI$, the pressure in the LIS is zero: all the fluid that enters the LIS through the lateral membranes is driven from the LIS by means of electro-osmosis. The lower curve illustrates that when $qI > J$, a negative pressure builds up in the LIS to counter the electro-osmotic flow of fluid. The lower curve is drawn with $qI = 2J$.

the junctions are very leaky, and the other half leaves through the basal end; a net reabsorption of fluid occurs because fluid enters the cells primarily through the apical membranes.

We now calculate the transepithelial potential, ψ_a , required to reverse the flow of fluid in the LIS and prevent the reabsorption of fluid by the proximal tubule. It is intuitively apparent (see Fig. 1) that if the lateral membranes bear a negative charge, the lumen must be clamped to a negative potential to induce a net flow of fluid toward the lumen. To simplify the calculation, we assume that the tight junctions are leaky to current, solutes, and fluid. The potential in the LIS, when $g_i \gg 1/r_e$, is (Eq. 30):

$$\psi_e(x) = \frac{l^2 I}{2} \left(\frac{x}{l} - \frac{x^2}{l^2} \right) + \psi_a(1 - x/l). \quad (39)$$

The first term in Eq. 39 is the parabolic potential profile produced in the LIS by the sodium pumps (see Eq. 32 and the lower curve in Fig. 5). The second term is the linear potential profile produced in the LIS by the voltage clamp. The potential produced by the voltage clamp and the resulting electro-osmotic flow do not affect the pressure profile, which is given by Eq. 34.

When the application of a negative voltage ψ_a induces an electro-osmotic flow of fluid toward the lumen that is just sufficient to prevent the normal reabsorption

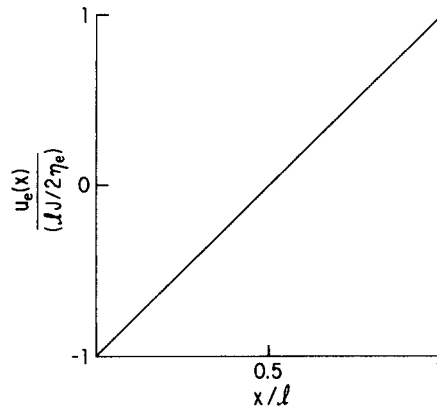


FIGURE 7. The velocity of fluid in the LIS of an epithelium with a tight junction that is leaky to current, solutes, and fluid. The abscissa is the distance through the epithelium, x , normalized to the length of the cells, l . The ordinate is the fluid velocity, normalized to the factor $lj/(2\eta_e) = (lj_m/2c_o)(S_m/V_T)$ = the volume of fluid that flows out of the epithelium per unit cross-sectional area per unit time. For *Necturus* renal proximal tubules (Spring, 1973*b*), the normalization factor is $\sim 7 \times 10^{-7}$ cm/s. If we take the cross-sectional area of the LIS per cross-sectional area of epithelium to be 0.016 and the factor ξ to be 2.3 (Spring, 1973*b*), then the average velocity of the fluid in the z direction within the LIS at $x = l$ is $1 \mu\text{m/s}$. The fluid velocity in the LIS of rabbit renal proximal tubules, as calculated from the measured rate of fluid reabsorption (Schafer et al., 1981) and the morphology of the tissue (Welling and Welling, 1975, 1976), is also of the order of $1 \mu\text{m/s}$. This corresponds approximately to the fluid velocities illustrated in Fig. 4.

of fluid, the fluid velocity in the LIS at $x = l$ is zero, $u_e(l) = 0$. Equivalently, the velocity at $x = 0$ is twice its normal value.

We substitute Eqs. 34 and 39 into Eq. 25, the general expression for the fluid velocity, and set $u_e(l) = 0$. This condition is satisfied when

$$\psi_a = -Jl^2/2q. \quad (40)$$

Using Eqs. 28 and 37, we can write Eq. 40 as

$$\left(\frac{\psi_a}{\xi l}\right)\left(\frac{\zeta \epsilon_r \epsilon_o}{\eta}\right) = \left(\frac{-j_m \xi l}{2c_o}\right)\left(\frac{S_m}{V_c}\right). \quad (41)$$

Eq. 41 has the following simple interpretation. The left-hand side is the fluid velocity in the LIS produced by the voltage clamp because $-\psi_a/\xi l$ is the electric

field in the LIS produced by the voltage clamp and $\zeta\epsilon_r\epsilon_0/\eta$ is the fluid velocity per unit field. The right-hand side of Eq. 41 is the fluid velocity at $x = l$ when $\psi_a = 0$ because $l\xi S_m/V_e$ is the area of lateral membrane per cross-sectional area of the LIS. We now insert values appropriate to the *Necturus* proximal tubule into Eq. 41. The right-hand side is obtained by dividing the fluid flow per unit cross-sectional area of epithelium measured under short-circuit conditions, 2×10^{-6} cm³/cm²·s (Spring and Paganelli, 1972), by the calculated cross-sectional area of the LIS, 1.6×10^{-2} cm²/cm² epithelium (Spring, 1973*b*), and multiplying by the factor $\xi = 2.3$ (Spring, 1973*b*). The fluid velocity at $x = l$ in the LIS is 2.8 $\mu\text{m/s}$. We found that $\zeta\epsilon_r\epsilon_0/\eta = 1 \mu\text{m/s}$ per V/cm for rat basolateral renal proximal tubule membranes (see Results); we assume a similar value for *Necturus* membranes. The length of the cells is $l = 25 \mu\text{m}$ (Spring, 1973*b*). Eq. 41 is satisfied when $\psi_a = -16$ mV. This theoretically predicted value of ψ_a agrees very well with the experimental observation. Spring and Paganelli (1972) observed that fluid reabsorption ceased when the potential in the lumen was clamped to a value of about -15 mV (see their Fig. 6); the quantitative agreement between theory and experiment is probably fortuitous. It is probably unrealistic to assume that the basement membranes and tight junctions exert no resistance to the flow of current, solutes, and fluid (e.g., Welling and Grantham, 1972; Maunsbach and Boulpaep, 1983), to assume that the conductance of the lateral membranes is negligible, and to ignore both the ionic selectivity of the tight junctions and transport number effects in our theoretical calculations. Nevertheless, this simple calculation demonstrates that electro-osmotic flow is of the correct order of magnitude to account for the fluid movement observed in leaky epithelia under current- and voltage-clamp conditions.

MATERIALS AND METHODS

The electrophoretic mobility was determined by observing a single vesicle with a microscope and measuring its velocity in a known electric field with a stop watch. Measurements were made in tubes ~ 15 cm in length and 1 mm in radius. The mobility was independent of the applied electric field, which was < 3 V/cm. Identical results were obtained with Rank Bros. Mark I and Mark II (ultramicroscope) microelectrophoresis machines (Bottis-ham, Cambridge, UK). Care was taken to focus on vesicles at the stationary layer (Henry, 1938) and to monitor the current to ensure that no significant electrode polarization occurred. The main source of error was the settling of vesicles at the bottom of the tube, which changes the position of the stationary layer. Details of the experimental techniques are discussed in standard texts (e.g., Hunter, 1981).

Measurements were made at 25°C in 0.15 M NaCl buffered to pH 7.4 with 0.003 M 3-(*N*-morpholino)-propanesulfonic acid (MOPS). The water was purified with a Super-Q system (Millipore Corp., Bedford, MA), and then double-distilled in a quartz still. Frozen rat renal basolateral membrane vesicles were supplied by R. Kinne (Albert Einstein College of Medicine, Bronx, NY). Fresh rat renal basolateral and brush border membranes were supplied by G. Kaloyanides (SUNY, Stony Brook, NY). The vesicles were isolated by differential centrifugation and either free-flow electrophoresis (Keljo et al., 1978) or Percoll gradients. We obtained identical results with the frozen and fresh basolateral membranes. There was no significant dependence of mobility on vesicle size. Although only $\sim 60\%$ of the basolateral membrane vesicles are oriented right side out (Kinne and Schwartz, 1978), we detected no bimodal distribution of mobilities in our measurements: the right-side-out and inside-out vesicles presumably have similar mobilities.

The rabbit small intestine basolateral membrane vesicles were a gift from E. Wright (University of California, Los Angeles, CA). The erythrocytes were obtained from a fresh drop of human blood. Egg phosphatidylcholine vesicles were prepared according to Bangham et al. (1974). Polylysine (4,000–15,000 mol wt) and protamine were obtained from Sigma Chemical Co. (St. Louis, MO).

RESULTS

The electrophoretic mobilities of fresh and frozen basolateral membrane vesicles from rat renal proximal tubules were -0.89 ± 0.12 (\pm SD, $n = 50$) and -0.87 ± 0.19 (\pm SD, $n = 39$) $\mu\text{m/s}$ per V/cm, respectively, in 0.15 M NaCl (pH 7.4). The zeta potential, calculated from Eq. 3, is -11.3 mV. Brush border (apical) membrane vesicles from rat renal proximal tubules have a similar mobility and zeta potential, -10.0 ± 1.0 (\pm SD, $n = 40$) mV. The zeta potential of the basolateral vesicles is also similar to the zeta potential of fresh human (S. McLaughlin) erythrocytes (-13.8 ± 0.7 [\pm SD, $n = 18$] mV) and basolateral membrane vesicles from rabbit small intestine (-14.3 ± 0.9 [\pm SD, $n = 20$] mV).

We are interested in substances that change the electrophoretic mobility of the vesicles because our hypothesis predicts that these substances should change the pressure in the LIS. If a cation reverses the charge on the renal basolateral membrane vesicles, it should, in terms of our model, reverse the direction of electro-osmotic flow in the LIS of the renal proximal tubule, cause the pressure in the LIS to become positive, and induce the LIS to swell. We measured the effect of calcium and the polycationic antibiotic gentamicin on the zeta potential of these vesicles. Gentamicin, at a concentration of 3 mM, reduces the zeta potential from -11 to -8 mV. Calcium has a similar effect: a concentration of 20 mM reduces the zeta potential to -6 mV.

We also investigated the effect of uranyl nitrate on the zeta potential. The uranyl ion, UO_2^{2+} , binds strongly to the phosphate groups of phospholipids (Bangham et al., 1967; Sukharev et al., 1981) and, in unbuffered solutions (acid pH), uranyl nitrate reverses the charge of erythrocytes, lymphocytes, and platelets (Bangham et al., 1958). To our surprise, we found that uranyl nitrate caused the zeta potential of basolateral membranes to become more, not less, negative at physiological pH. The zeta potential of rat renal basolateral membranes in 0.15 M NaCl buffered to pH 7.4 with 0.003 M MOPS changed from -11.0 ± 1.3 mV ($n = 10$) to -13.5 ± 0.8 mV ($n = 10$) upon addition of 0.1 mM uranyl nitrate and to -16.8 ± 0.9 mV ($n = 10$) upon addition of 1 mM uranyl nitrate. We observed similar results with rabbit small intestine basolateral membranes and human erythrocytes in 0.15 M NaCl at pH 7.4. For example, 0.3 mM uranyl nitrate changed the zeta potential of the rabbit vesicles from -14.3 ± 0.9 to -18.4 ± 1.2 mV ($n = 10$). Experiments with artificial phospholipid vesicles suggest that these results are probably due to the adsorption of some negatively charged complex of uranyl, such as a hydroxide, to the membranes rather than to uranyl inducing a structural change in the membranes.¹¹

¹¹ Multilamellar vesicles formed from egg phosphatidylcholine, a zwitterionic phospholipid, have zero electrophoretic mobility in 0.15 M NaCl for $4 < \text{pH} < 12$. The vesicles become positive upon addition of uranyl nitrate when the pH is 6.0 or lower, but become negative upon addition of uranyl nitrate when the pH is 6.5 or greater: specifically, 0.1 mM uranyl

Thorium, at concentrations below 0.1 mM, reverses the mobility of erythrocytes in unbuffered (acid) solutions (Bangham et al., 1958). However, 0.1 mM thorium had no significant effect on the electrophoretic mobility of rabbit small intestine and rat renal basolateral membrane vesicles in our buffered (pH 7.4) 0.15 M NaCl solutions. Pentalysine (0.4 mM) also had no effect on the mobility of rat renal basolateral vesicles.

TABLE I
*Effect of Poly-L-Lysine Hydrobromide, Protamine Chloride, and Lanthanum Chloride on the Zeta Potentials of Rat Renal Basolateral Membrane Vesicles and Human Erythrocytes**

[Polylysine]	Zeta potential + SD	
	Basolateral vesicles	Erythrocytes
mg/ml	mV	mV
0.0	-11.5±1.7 (n = 20)	-14.1±1.8 (n = 22)
0.001	-10.9±1.3 (n = 20)	-3.1±1.3 (n = 19)
0.01	-7.5±0.5 (n = 20)	+9.9±0.8 (n = 20)
0.05		+15.7±0.6 (n = 20)
0.1	-3.9±0.7 (n = 20)	
0.3	+1.2±1.0 (n = 20)	
0.5		+21.6±1.1 (n = 20)
1.0	+7.8±0.6 (n = 20)	
[Protamine]		
mg/ml		
0.0	-11.5±1.7 (n = 20)	-13.9±1.0 (n = 20)
0.01		-7.7±0.8 (n = 20)
0.1	-5.7±0.5 (n = 20)	-3.9±0.4 (n = 20)
1.0	-2.5±0.8 (n = 20)	+0.4±0.6 (n = 20)
10.0	+3.5±0.8 (n = 20)	+4.6±1.2 (n = 20)
[Lanthanum]		
M		
0.0	-11.5±1.7 (n = 20)	-14.0±0.7 (n = 20)
0.001	-6.0±0.3 (n = 10)	-5.5±1.4 (n = 51)
0.01	+1.7±0.5 (n = 10)	+4.1±3.6 (n = 20)

* The aqueous solutions contained 0.15 M NaCl buffered to pH 7.4 at 25°C with 0.003 M MOPS. The figure in parentheses represents the number of measurements.

We found three cations that did reverse the electrophoretic mobility of rat renal basolateral membrane vesicles. The concentrations of polylysine (4,000–15,000 mol wt), protamine, and lanthanum required to produce charge reversal are ~0.3 mg/ml, 3 mg/ml, and 10 mM, respectively. The detailed results obtained with these three cations are listed in Table I. Polylysine is about two orders of magnitude more effective in reversing the charge of an erythrocyte than a basolateral membrane vesicle, whereas protamine (and lanthanum) bind equally well to the two membranes.

nitrate produces zeta potentials of -40 mV at pH 4 and 5, -20 mV at pH 6, and +20 mV at pH 6.5, 7.0, 7.5, and 8.0.

DISCUSSION

We have demonstrated experimentally that the electrophoretic mobility of membrane vesicles from rat renal proximal tubules is $\sim 1 \mu\text{m/s}$ per V/cm : this is also the electro-osmotic fluid velocity induced in the LIS by an electric field of 1 V/cm (e.g., Balasubramanian and McLaughlin, 1982). From the measured rate of fluid reabsorption in the rabbit renal proximal tubule ($0.2 < J_v < 0.6 \text{ nl min}^{-1} \text{ mm}^{-1}$; Schafer et al., 1981) and the known morphology of the tissue (Welling and Welling, 1975, 1976), the fluid velocity in the LIS can be calculated to be of the order of $1 \mu\text{m/s}$, assuming the lateral spaces constitute the final common pathway for fluid reabsorption. Thus, the final step in the reabsorption of fluid, the flow of fluid from the LIS, must be influenced by electro-osmosis if a field of the order of 1 V/cm exists in the LIS.

We calculated theoretically that the electric field produced in the LIS of mammalian proximal tubules by the electrogenic sodium pumps should be of the order of 1 V/cm . Of course, the model we presented above is highly oversimplified. For example, it ignores the conductance of the lateral membranes, the current loops that must exist in epithelia, the resistance to fluid flow exerted by the basement membrane, the ion selectivity of the tight junctions, and the different composition of the solutions on the apical and basal sides of the epithelia. The point we wish to raise in this paper is that the situation is too close to call: the possibility that electro-osmosis accounts for fluid flow in the LIS of renal proximal tubules can be neither ruled out nor proved on the basis of simple theoretical arguments.

In our opinion, a simple theoretical analysis of a complex physiological problem is useful only if it suggests new explanations for old observations or provides the impetus for new experiments. Our analysis does suggest a new explanation for the reversal of fluid reabsorption observed when the lumen of a renal proximal tubule is clamped to a negative voltage. The critical assumptions in our analysis were that the tight junctions of *Necturus* are leaky to ions, solutes, and fluid, that the conductance of the lateral membranes is negligible, and that the zeta potential of the lateral membranes is similar to the value we observed for rat renal proximal tubules. From the known morphological properties of the tissue and the rate of fluid reabsorption, we calculated that a voltage clamp of -15 mV (lumen negative) should reverse reabsorption of fluid in the *Necturus* proximal tubule by inducing an electro-osmotic flow of fluid in the LIS toward the lumen. This prediction agrees quantitatively with the experimental observations of Spring and Paganelli (1972), which were previously interpreted as accumulation/depletion (diffusion polarization) effects.

In the *Necturus* proximal tubule and other leaky epithelia, the current decreases with time under voltage-clamp conditions and the voltage increases with time (over several minutes) under current-clamp conditions, probably because of salt polarization (accumulation/depletion) effects (Diamond, 1979). If the fluid flow observed under these conditions is due to electro-osmosis, we would expect the flow to change essentially instantaneously under voltage-clamp conditions but to follow the same time course as the voltage under current-clamp conditions. This prediction is consistent with the experimental observations (Spring and Paganelli,

1972). It should be easy to falsify our model if it is incorrect. For example, if a leaky epithelium is exposed to a substance that reduces the zeta potential of the basolateral membranes to zero (e.g., polylysine, protamine, lanthanum), we predict that a voltage clamp should produce no instantaneous change in the flow of fluid across the epithelia.

Although our theoretical analysis provides a quantitative, and experimentally testable, explanation for the change in fluid flow observed across leaky epithelia under current- and voltage-clamp conditions, it will be much more difficult to determine directly whether the electric field produced within the LIS by the sodium pumps on the lateral membranes is sufficient to induce a significant electro-osmotic flow. However, our simple model predicts that if the sodium pumps produce a positive electric field $>1 \text{ V/cm} = 0.1 \text{ mV}/\mu\text{m}$ in the LIS of a renal proximal tubule, the pressure will be negative (subatmospheric) when fluid reabsorption occurs. The negative pressure impedes the movement of fluid from the LIS and allows the exit of fluid to be coupled to the entry of fluid across the lateral membranes. Thus, the LIS should not swell when fluid is transported across the proximal tubule. Burg and Grantham (1971) examined isolated rabbit renal proximal tubules and reported: "It is noteworthy that these intercellular spaces show no dilation or deformation attendant on the transcellular fluid transport." The lateral spaces in the rabbit proximal tubule dilate under other circumstances: the width of the LIS increases when fluid flow is augmented by increasing the protein concentration in the bath (Tisher and Kokko, 1974). Oschman and Berridge (1970) also reported that when 5-hydroxytryptamine induces a 60-fold increase in the rate of fluid secretion in insect salivary glands, there is no obvious distension of the secretory canaliculi or basal infolds. The observation that these narrow lateral spaces do not swell during fluid transport is consistent with our hypothesis, but could be due to a fixation artifact: results with modern, fast-freeze electron microscopy techniques would be valuable. We also note that the lateral spaces of gallbladders do swell during transport (Tormey and Diamond, 1967; Spring and Hope, 1979). Thus, it would appear that electro-osmosis does not play the major role in fluid reabsorption in this tissue. This result is not unexpected. Gallbladder lateral spaces are large, of the order of $1 \mu\text{m}$ in width, and the electric field that could be generated within the LIS by the sodium pumps on the lateral membranes is small.

Our hypothesis could be tested in several other ways. For example, if a substance (e.g., polylysine) reduces the zeta potential of the renal proximal tubule basolateral membranes to zero, it will prevent electro-osmosis. However, the volume of fluid that crosses the lateral membrane and enters the LIS per unit time should not be altered by polylysine, and the hydrostatic pressure must increase to drive from the LIS the fluid that was driven previously by electro-osmosis. Thus, if electro-osmosis normally assists fluid reabsorption to a significant degree, the LIS should swell in the presence of polylysine.

In conclusion, we note that electro-osmosis could be important in many tissues other than renal proximal tubules. All biological membranes that have been examined to date have fixed negative charges and negative zeta potentials. Many tight epithelia have intercellular spaces that are longer and narrower than those

in the renal proximal tubule and some syncytial tissues have long intercellular spaces that support large voltage gradients. For example, the intercellular spaces between fiber cells of the lens are ~20 nm wide and several millimeters long. Rae (1974) reported steady state voltage gradients along these clefts of the order of 0.3 V/cm; electro-osmosis may play a central role in the steady state circulation of fluid in this tissue (Rae and Mathias, 1985).

APPENDIX

In this appendix, the partial differential equations that describe voltage gradients, hydrostatic gradients, and diffusion along the intercellular spaces of a tissue will be presented and simplified. The simplifications are based on two assumptions: (a) the fluxes of ions and water along the lateral intercellular spaces of epithelia are small perturbations from the equilibrium state where there is no transport, and (b) the aspect ratio of LIS width to length, w/l , is small. The definitions of w , l , and other coordinates are illustrated in Fig. 2 of the text.

Equilibrium

We will define equilibrium as the state where there is no water flow and the net flux of all solutes is zero. The membranes lining the LIS are assumed to have a uniformly smeared charge density, σ (coulombs per square meter), which causes a small charge imbalance, $\rho(y)$ (coulombs per cubic meter), in the layer of fluid adjacent to the membrane. Hence, there will be an equilibrium voltage, $\bar{\psi}(y)$, in this layer. $\bar{\psi}(y)$ is described by Eqs. 4 and 5 of the text and the relevant definitions and assumptions are discussed nearby. The net charge density is determined from the Boltzman relationship such that

$$\rho(y) = -c_o F \sinh F\bar{\psi}/RT,$$

but in accordance with the discussion in the text, we assume that $\bar{\psi}$ is small in comparison with RT/F , so we adopt the approximation

$$\rho(y) \approx -c_o F^2 \bar{\psi}(y)/RT. \quad (\text{A1})$$

These equations describe the voltage and charge distribution at equilibrium. We next consider epithelial transport, but we assume that the various flows are sufficiently small that the equations can be linearized about the equilibrium solutions.

Linearization

The nonequilibrium perturbations to the voltage and hydrostatic pressure within the LIS are given by ψ_e and p_e . In the nonequilibrium state, the voltage within the LIS is given by $\bar{\psi} + \psi_e$. Since $\bar{\psi}$ satisfies Poisson's equation for the excess positive charge near the membrane, the perturbation ψ_e must satisfy Laplace's equation:

$$\nabla^2 \psi_e = 0.$$

The normalization

$$Z = z/l, \quad (\text{A2})$$

$$Y = y/w \quad (\text{A3})$$

yields

$$\epsilon^2 \frac{\partial^2 \psi_e}{\partial Z^2} + \frac{\partial^2 \psi_e}{\partial Y^2} = 0, \quad (\text{A4})$$

where

$$\epsilon = w/l. \quad (\text{A5})$$

Because ϵ is small, we can make the approximation

$$\frac{\partial^2 \psi_e}{\partial Y^2} = 0.$$

Given that ψ_e is a symmetrical function of Y , the above result implies that to a first approximation ψ_e is independent of Y and varies primarily in the axial direction.

We now turn to the description of fluid flow. Fluid movement must obey the laws of continuum mechanics (Landau and Lifshitz, 1959) and a version of the Navier-Stokes equation should describe the flow. The Navier-Stokes equation is a form of Newton's law: mass \times acceleration = sum of the forces. In general, the forces will be hydrostatic pressure and viscous drag, but because of the excess charge $\rho(y)$, if there is a voltage within the LIS there will be a force acting on each element of fluid equal to the electric field times the charge contained within the element.

By arguments similar to those for ψ_e , one finds that the pressure, p_e , also varies primarily in the axial direction and the y -directed component of water flow is negligible when compared with the z component (see Mathias, 1985). Moreover, for low-velocity, small Reynolds number flow, the nonlinear terms describing the acceleration of a fluid element can be neglected. The resulting version of the Navier-Stokes equation is:

$$0 = -\frac{1}{\eta} \frac{\partial p_e(z)}{\partial z} + \frac{\partial^2 u_z(y, z)}{\partial y^2} - \frac{\rho(y)}{\eta} \frac{\partial \psi_e(z)}{\partial z}, \quad (\text{A6})$$

where η is the viscosity of water. We assume that the boundary conditions at the membrane solution interface are

$$u_z(\pm w/2, z) = 0. \quad (\text{A7})$$

Integrating Eq. A6 twice over y and invoking boundary conditions (Eq. A7) yields

$$u_z(y, z) = \frac{1}{\eta} \left(\frac{y^2}{2} - \frac{w^2}{8} \right) \frac{\partial p_e(z)}{\partial z} - \frac{F^2 c_o \zeta}{RT \kappa^2 \eta} \left(\frac{\cosh \kappa y - \cosh \kappa w/2}{\sinh \kappa w/2} \right) \frac{\partial \psi_e(z)}{\partial z}, \quad (\text{A8})$$

where ζ is defined in Eq. 5 of the text and $1/\kappa$ is the Debye length.

Consider next the flux of a univalent cation S . The flux vector J_s (moles per square meter per second), describing the movement of S (moles per cubic meter), depends on convection, conduction, and diffusion, so

$$J_s = Su - D_s \left(\frac{F}{RT} S \nabla \psi_e + \nabla S \right), \quad (\text{A9})$$

where D_s (square meters per second) is the diffusion coefficient.

If the flux equations for anions and cations are added, one obtains an expression for total solute flow. Because of the excess charge in the LIS, macroscopic electroneutrality is violated and the solute flux depends on voltage. Several investigators (Huss and Marsh, 1975; Sackin and Boulpaep, 1975; Weinstein and Stephenson, 1979; Mathias, 1985) have calculated that standing osmotic gradients within the LIS will be quite small when compared with the total osmolarity, c_o . Hence, as in the perturbation analysis by Segel (1970) or Weinstein and Stephenson (1981), we make the linearized approximation that if $c_e(z)$ is the osmolarity of the LIS, then $c_e = c_o + \delta c$ and $c_e u \approx c_o u$. In other words, the amount of solute being convected is given approximately by the flow velocity times bulk osmolarity. The axial solute flux $j_z(y, z)$ is then

$$j_z(y, z) = c_o u_z(y, z) - D_s \left[\frac{\rho(y)}{RT} \frac{\partial \psi_c(z)}{\partial z} + \frac{\partial c_c(z)}{\partial z} \right]. \quad (\text{A10})$$

Once again, owing to the smallness of w/l , the variation in c_c is predominantly axial.

If Eq. A9 for anions is subtracted from Eq. A9 for cations, the result is the ionic current flux $i_z(y, z)$ along the LIS,

$$i_z(y, z) = \rho(y) u_z(y, z) - D_s \frac{F^2 c_o}{RT} \frac{\partial \psi_c(z)}{\partial z}. \quad (\text{A11})$$

The average of any flux $f = [u, j, i]$ is defined by

$$\bar{f}_c(z) = \frac{1}{w} \int_{-w/2}^{w/2} f_z(y, z) dy. \quad (\text{A12})$$

In order to compute the average flux of water, solute, and ionic current along our generic cleft, we substitute Eq. 4 of the text and Eq. A1 into Eq. A11, and then average Eqs. A8, A10, and A11 to yield

$$\bar{u}_c(z) = -\frac{w^2}{12\eta} \frac{dp_c}{dz} - K_c \frac{d\psi_c}{dz}; \quad (\text{A13})$$

$$\bar{i}_c(z) = -K_c \frac{dp_c}{dz} - \frac{1}{R_c} \frac{d\psi_c}{dz}; \quad (\text{A14})$$

$$\bar{j}_c(z) = c_o \bar{u}_c(z) + D_s \left[\frac{F^2 c_o \zeta}{R^2 T^2 \kappa w / 2} \frac{d\psi_c}{dz} - \frac{dc_c}{dz} \right], \quad (\text{A15})$$

where

$$\frac{1}{R_c} = \frac{F^2 c_o D_s}{RT} \left[1 - \frac{F^2 c_o \zeta^2}{RT w \kappa^3 D_s \eta} \left(\frac{\kappa w / 2 - \sinh \kappa w / 2 \cosh \kappa w / 2}{\sinh^2 \kappa w / 2} \right) \right]; \quad (\text{A16})$$

$$K_c = \frac{F^2 c_o \zeta}{RT \kappa^2 \eta} \left(\frac{1}{\kappa w / 2} - \coth \kappa w / 2 \right). \quad (\text{A17})$$

Notice that the coefficient for voltage in Eq. A13 is the same as the coefficient for pressure in Eq. A14. Hence, the Onsager reciprocal relationship is established.

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