Isosmotic Volume Reabsorption in Rat Proximal Tubule

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ABSTRACT A theoretical model incorporating both active and passive forces has been developed for fluid reabsorption from split oil droplets in rat intermediate and late proximal tubule. Of necessity, simplifying assumptions have been introduced; we have assumed that the epithelium can be treated as a single membrane and that the membrane “effective” HCO₃⁻ permeability is near zero. Based on this model with its underlying assumptions, the following conclusions are drawn. Regardless of the presence or absence of active NaCl transport, fluid reabsorption from the split oil droplet is isosmotic. The reabsorbate osmolarity can be affected by changes in tubular permeability parameters and applied forces but is not readily altered from an osmolarity essentially equal to that of plasma. In a split droplet, isosmotic flow need not be a special consequence of active Na transport, is not the result of a particular set of permeability properties, and is not merely a trivial consequence of a very high hydraulic conductivity; isosmotic flow can be obtained with hydraulic conductivity nearly an order of magnitude lower than that previously measured in the rat proximal convoluted tubule. Isosmotic reabsorption is, in part, the result of the interdependence of salt and water flows, their changing in parallel, and thus their ratio, the reabsorbate concentration being relatively invariant. Active NaCl transport can cause osmotic water flow by reducing the luminal fluid osmolarity. In the presence of passive forces the luminal fluid can be hypertonic to plasma, and active NaCl transport can still exert its osmotic effect on volume flow. There are two passive forces for volume flow: the Cl⁻ gradient and the difference in effective osmotic pressure; they have an approximately equivalent effect on volume flow. Experimentally, we have measured volume changes in a droplet made hyperosmotic by the addition of 50 mM NaCl; the experimental results are predicted reasonably well by our theoretical model.

INTRODUCTION

Isosmotic fluid reabsorption has been explained by osmotic coupling to active Na transport (13, 16, 56). We have previously shown that active salt transport makes only a small contribution (~10% or less) to volume flow in the rat intermediate and late proximal convoluted tubule (71, 72, 74, and footnote...
These observations suggest that an alternative mechanism is responsible for isosmotic flow in this tissue.

This paper presents a theoretical model and experimental investigation of fluid reabsorption from split oil droplets (22) in rat intermediate and late proximal convoluted tubule. The model incorporates neutral and electrogenic active transport, passive reabsorptive forces due to the asymmetrical salt solutions known to exist on the two sides of this epithelium (70), and oncotic and hydrostatic pressures. The model incorporates a number of simplifying assumptions, among them: the epithelium can be represented as a single membrane, and the "effective" HCO₃⁻ permeability of this membrane is near zero. This theoretical model has two advantages. The solution of the differential equations is in a mathematically closed form, which, compared with other theoretical analyses (16, 35, 56, 58, 59), provides a more direct assessment of the relative importance of the parameters affecting fluid reabsorption. The model also directly predicts the steady-state concentration of luminal and reabsorbate fluids. Using this model, we have evaluated previous proposals for the existence of ideal luminal hypotonicity in the presence of active transport (1, 2, 62); the model suggests that luminal hypotonicity is not required for volume flow in the presence of a difference in effective osmotic pressure across the epithelium. From previous demonstrations that volume flow in rabbit proximal convoluted and straight tubules can be accounted for by a high measured hydraulic conductivity and small osmolar differences between the luminal and peritubular solutions (1, 2, 60), one might conclude that isosmotic flow is simply the consequence of a very high hydraulic conductivity. Our model suggests that isosmotic reabsorption might still be obtained in tissues in which hydraulic conductivity is lower. The model suggests that isosmotic volume flow is not the result of a particular set of permeability properties and bathing solution composition (52) and need not be a special consequence of active Na transport (16, 35, 56); rather it suggests that isosmotic reabsorption is a simple consequence of the interdependent flow of salt and water, and that in the rat intermediate and late proximal tubule an isosmotic volume flow similar in magnitude to measured free-flow values can be obtained without incorporating active transport. We have investigated the implicit concept that isosmotic flow is characterized by a single reabsorbate concentration; the model suggests that, instead, a range of concentrations encompassing the plasma osmolarity may be obtained. We have evaluated the contribution of the difference in chloride and bicarbonate reflection coefficients to passive volume flow in the intermediate and late rat convoluted proximal tubule; the model suggests that reflection coefficient differences are not required to obtain a significant passive volume flow. The model suggests that small changes in some tubular permeability parameters can cause large changes in volume flow while maintaining the isotonic nature of the reabsorbate; modifications of these permeability parameters could, therefore, provide a sensitive control of proximal tubular reabsorption. The model

1 Warner, R. R., and C. Lechene. Analysis of standing droplets in rat proximal tubules. I. Na, Cl and raffinose limiting concentrations, solvent drag and active transport. Submitted for publication.
predicts our experimental measurements on volume changes in droplets made hypertonic by the addition of 50 mM NaCl. A preliminary report of some of these observations has been published (73).

**METHODS**

*Glossary of Symbols*

\( n_i \) moles of solute \( i \) in split droplets, where \( i = \) sodium ion (Na), chloride ion (Cl), or bicarbonate ion (HCO\(_3\))

\( n_{Cl,0} \) moles of chloride in droplet at \( t = 0 \)

\( V \) droplet volume

\( V_0 \) droplet volume at \( t = 0 \)

\( C_i \) concentration of solute \( i \) in luminal (droplet) fluid

\( C'_i \) concentration of solute \( i \) in peritubular fluid

\( C_{Cl} \) average concentration of chloride across tubular epithelium

\( C_{Cl}^{\infty} \) steady-state droplet (and reabsorbate) chloride concentration

\( P_{Cl} \) permeability of chloride

\( L_p \) hydraulic conductivity

\( \sigma_i \) reflection coefficient for solute \( i \)

\( \Delta P \) hydraulic pressure gradient across tubular epithelium

\( \Pi_{pr} \) oncotic pressure of peritubular protein

\( \Delta \psi \) electrical potential gradient across tubular epithelium

\( K_a \) rate of active (neutral) salt transport (dimensions same as permeability)

\( A \) area of droplet exposed to tubular epithelium (=\( 2V/r \))

\( r \) tubular radius

\( F \) Faraday constant

\( R \) gas constant

\( T \) absolute temperature, 310°K

\( m_1 \) rate constant for volume reabsorption (s\(^{-1}\))

\( m_2 \) rate constant for osmotic adjustment (s\(^{-1}\))

\( J_o \) \(-dV/dt\)

**Theoretical Model**

The theoretical model of solute and fluid reabsorption from split oil droplets exemplified by these used by Gertz (22) assumes for simplicity that the luminal fluid contains only Na and Cl, a reasonable approximation for intermediate and late proximal tubular fluid (40, 42, 43, and footnote 1) and an assumption frequently made in previous studies (16, 35, 52, 56, 74). The model assumes that the peritubular fluid contains only Na, Cl, HCO\(_3\) and protein, and that the permeability of protein is near zero.

Experimentally, the HCO\(_3\) concentration in the intermediate and late proximal tubular fluid is found to be low, only a few millimoles per liter (55), in spite of the presence of ~25 mM HCO\(_3\) in the peritubular fluid (55). As noted, our model assumes that the droplet HCO\(_3\) concentration can be neglected, a reasonable approximation considering the nearly 30-fold higher Cl concentration. For this model to be physiologically realistic, however, we must assume a mechanism for maintaining the droplet-plasma HCO\(_3\) gradient. It is likely that in vivo the HCO\(_3\) gradient is maintained by an active pump. However, not only is there insufficient experimental data to theoretically model such a pump, but its inclusion in our model would complicate a mathematical solution. We have instead assumed an effective HCO\(_3\) permeability that
is near zero. With this assumption we theoretically model the experimentally observed HCO₃⁻ gradient. It is unlikely that the active transport of a few millimoles per liter HCO₃⁻ is a process that per se is important for fluid reabsorption in the intermediate and late proximal tubule. If active pumps remove HCO₃⁻ in fixed proportion to the Cl⁻ concentration, this would constitute a negligible flux in relation to the NaCl flux and could reasonably be omitted from the equations of our model. Similarly, if active pumps remove additional HCO₃⁻ that has leaked into the droplet, this would not contribute to net HCO₃⁻ flux and would act as a futile cycle. We consequently feel it is reasonable to exclude HCO₃⁻ fluxes from our equations. We wish to emphasize, however, that the constant active reabsorption of the HCO₃⁻ leak is a likely mechanism necessary to maintain the passive forces of solute asymmetry.

The equations describing Na⁺, Cl⁻, and fluid movement with time from a split droplet in the late and intermediate proximal tubule are as follows:

\[ \frac{dn_{Cl}}{dt} = -AP_{Cl}(C_{Cl} - C_{Cl}^\infty) - \frac{F}{RT} \bar{C}_{Cl} \Delta \psi - AK_{Cl} C_{Cl} + \frac{dV}{dt}(1 - \sigma_{Cl}) \bar{C}_{Cl}, \]  

\[ \frac{dn_{Na}}{dt} = \frac{dn_{Cl}}{dt}, \text{ and} \]

\[ \frac{dV}{dt} = -AL_p(\Delta P + \Pi_{pr} - RT(\sigma_{Na} + \sigma_{Cl})(C_{Cl} - C_{Cl}^\infty) - [\sigma_{HCO_3} + \sigma_{Na}]C_{HCO_3}). \]

These equations assume that the epithelium can be treated as a single membrane. Because permeabilities, hydraulic conductivity, and reflection coefficients have previously been measured using this same assumption, their use in our model provides a consistent approach that would compensate for deviations from a single homogeneous membrane. This single-membrane assumption is not unique to our work and has been used in a number of previous studies (1, 21, 50, 57–59, and footnote 1). The single-membrane assumption can also be justified in that its application has led to successful predictions of experimental data (57–59). Furthermore, the observation of symmetrical NaCl dilution potentials in the rabbit proximal tubule is consistent with the electrical behavior of a single membrane rather than with two barriers of differing permeability (37, 59). The single-barrier model should adequately represent the proximal tubular epithelium to the extent that fluid and solute movement occur primarily via extracellular routes and are driven by differences in composition between the external bathing solutions, as has recently been suggested and explored (1, 2, 58, 59, 72, and footnote 1). It should be recognized, however, that in our model the effect of active transport is solely to remove solute from the droplet or to change the electrical potential; to the extent that fluid and solute movement are influenced by active transport into an extracellular compartment having a composition significantly different from that of plasma (13), our model should not be expected to accurately represent the reabsorptive process.

Eqs. 1, 2 and 3 include the passive forces of solute asymmetry and hydrostatic pressure, as well as a neutral mechanism for active Cl⁻ transport (46) that can be envisioned as the Na-Cl carrier found in Necturus proximal tubule (64) and responsible for active Cl⁻ transport in other tissues (18, 19). It is assumed that this process is operating well below saturation. These equations also include the coupling of Cl⁻ to active electrogenic Na transport via \( \Delta \psi \). Assuming the droplet to be a right circular cylinder, a reasonable approximation at least for large droplets (49), Eqs. 1 and 3
have a solution in closed form given by

\[ n_{Cl} = B e^{-m_{l}} - (B - n_{Cl,0}) e^{-m_{l}} \quad \text{and} \]
\[ V = C B e^{-m_{l}} - D (B - n_{Cl,0}) e^{-m_{l}}, \quad \text{where} \]
\[ m_{l} = 2(m_{2} - m_{l}) L_{p} R T \left( X - \frac{1 - \sigma_{Cl} C_{Cl}}{1 - \sigma_{Cl} C_{Cl}} \right) W \]
\[ B = \frac{m_{l} m_{2} (X - L_{p} R T \left[ 1 - \sigma_{Cl} C_{Cl} \right]) V_{0} - 2 L_{p} R T m_{l} ((\sigma_{Na} + \sigma_{Cl}) X - \left[ P_{Cl} + K_{e} + m_{l}/2 \right] W) n_{Cl,0}}{2(m_{2} - m_{l}) L_{p} R T \left[ X - \frac{1 - \sigma_{Cl} C_{Cl}}{1 - \sigma_{Cl} C_{Cl}} \right] W}, \]
\[ C = \frac{2 L_{p} R T \left[ (\sigma_{Na} + \sigma_{Cl}) X - \left[ P_{Cl} + K_{e} + m_{l}/2 \right] W \right]}{m_{l} (X - L_{p} R T \left[ 1 - \sigma_{Cl} C_{Cl} \right])}, \]
\[ D = \frac{2 L_{p} R T \left[ (\sigma_{Na} + \sigma_{Cl}) X - \left[ P_{Cl} + K_{e} + m_{l}/2 \right] W \right]}{m_{l} (X - L_{p} R T \left[ 1 - \sigma_{Cl} C_{Cl} \right])}, \]
\[ m_{l} = \frac{Y + \left( Y^{2} + 4 L_{p} R T [X - \left( P_{Cl} + K_{e} \right) W]^{1/2} / \right)}{2}, \]
\[ m_{2} = \frac{Y - \left( Y^{2} + 4 L_{p} R T [X - \left( P_{Cl} + K_{e} \right) W]^{1/2} / \right)}{2}, \]
\[ Y = L_{p} R T \left[ (\sigma_{Na} + \sigma_{Cl}) (1 - \sigma_{Cl} C_{Cl} - W) - P_{Cl} - K_{e}, \right] \]
\[ X = P_{Cl} C_{Cl} + P_{Cl} P_{Cl} C_{Cl} \Delta \psi / R T, \quad \text{and} \]
\[ W = (\sigma_{Na} + \sigma_{HCO_{3}}) C_{HCO_{3}} + (\sigma_{Na} + \sigma_{Cl}) C_{Cl} + \Pi_{pr} / R T + \Delta P / R T. \]

From Eq. 5, volume flow \((J_{v})\) from the split oil droplet can be calculated as a function of time, and from Eqs. 4 and 5, reabsorbate concentrations and droplet concentrations can also be calculated with time.

**Parameters of the Model**

The developed theoretical equations involve a number of parameters for tubular permeability properties, plasma concentrations, and hydrostatic and electrical forces. Although many of these parameters will be individually varied over wide ranges, with all other parameters kept constant, baseline values for these parameters have been chosen from the literature (Table 1). The active transport of chloride \((K_{e})\) via a neutral Na-coupled pump \((46, 64)\) is assumed to be zero in order to simplify the interpretation of the results. The chloride permeability \((P_{Cl})\) is chosen to be \(14.1 \times 10^{-5} \text{ cm/s} \) \((21 \text{ and footnote } 1)\). Although this chloride permeability has not been corrected for the presence of an electrical potential difference, we are not aware of more accurate determinations. The tubular radius was measured as described below and a mean value of \(16.9 \mu m \) \((\pm 0.24 \mu m, \text{ SEM, } n = 36)\) was obtained. The “average” chloride concentration \((\bar{C}_{Cl})\) is nearly equal to the arithmetic mean concentration across the epithelium \((C_{Cl} + C_{Cl})/2\), which, as will be shown, remains relatively constant under most conditions; \(C_{Cl}\) cannot a priori be precisely calculated because \(C_{Cl}\) is one of the unknowns. Consequently, \(\bar{C}_{Cl}\) was estimated, \(C_{Cl}\) calculated, and the process iterated until values for \(C_{Cl}\) (and consequently \(\bar{C}_{Cl}\)) converged. In nearly every case only a single iteration was required for convergence. All calculations were performed on a Hewlett-Packard 97 programmable calculator (Hewlett-Packard Co., Palo Alto, Calif.).

**Experimental Protocol**

This theoretical model was tested by applying it to experimental observations on hypertonic split oil droplets in the in vivo rat intermediate and late proximal tubule.
Female Wistar rats (Charles River Breeding Laboratories, Inc., Wilmington, Mass.), 152–203 g, were starved overnight, anesthetized with Inactin (Henley and Co., New York.) (155 mg/kg body wt), and prepared for micropuncture as previously described (39 and footnote 1). Experiments were conducted with 10 rats. Isotonic saline was infused via the jugular vein at 0.92 ml/h. Glomerular filtration rate was measured using [14C]inulin. Urine was collected from a ureteral catheter, and plasma was collected and blood pressure monitored via the femoral artery. Micropipettes with tip diameters of 5–8 μm were siliconized (Surfasil, Pierce Chemical Co., Rockland, Ill.) and prefilled with a single 500-pl droplet of the test solution containing [3H]methoxyinulin. The droplets were isolated by paraffin oil within the micropipettes, and neither the oil nor the droplets were stained with dyes. The pipettes were stored in a water-saturated atmosphere until use that day. We have previously shown that droplets do not concentrate when stored in this manner (41 and footnote 1).

**TABLE I**

| CONSTANTS FOR EQUATIONS |  |
|--------------------------|---|
| \( C'c \) | 119 mmol/l (3, 77) |
| \( C'_{\text{CO}_2} \) | 25 mmol/l (55) |
| \( L_p \) | \( 18 \times 10^{-6} \text{ cm}^3/\text{cm}^2\cdot\text{s} \cdot\text{atm} \) (68) |
| \( \Delta p \) | 30 mm Hg (in split droplets) (25) |
| \( \Pi_p \) | 30 mm Hg (6) |
| \( \Delta \psi \) | 4-2 mV (intermediate and late proximal tubule) (20, 63) |
| \( r \) | 16.9 μm (measured) |
| \( K_a \) | 0 cm/s (assumed) |
| \( F_{\text{CO}_2} \) | \( 14.1 \times 10^{-4} \text{ cm}^3/\text{s} \) (21 and footnote 1) |
| \( \sigma_0 \) | 0.5 (21) |
| \( \sigma_{\text{CO}_2} \) | 0.96 (21) |
| \( \sigma_{
olongth} \) | 0.7 (21) |

With the kidney capsule intact, proximal tubules were punctured, a large oil block was injected, and the droplet was injected and isolated from the micropuncture site by further oil injection. The droplet was reaspirated into the same pipette after a predetermined length of time and isolated with oil from the tubular lumen. The oil-filled tubule was photographed for radius measurements. Collected droplets were immediately transferred to an oil-covered, siliconized concavity slide, replicate 49.1-pl aliquots were collected into a volumetric pipette (38), and the collected aliquots were injected into a vial of scintillation fluid for liquid scintillation counting. Immediately before and immediately after an experiment, a micropipette containing an uninjected droplet was randomly selected and replicate 49.1-pl aliquots of these droplets were likewise injected into a vial for liquid scintillation counting; results from the two determinations were averaged. By comparing the \(^3\text{H} \) counts per minute per aliquot of the injected droplet \( (A_i) \) with the \(^3\text{H} \) counts per minute per aliquot of the uninjected control \( (A_c) \), the fraction of the droplet remaining \( (V/V_0) \) can be calculated from the equation \( V/V_0 = A_i/A_c \). Plotting the natural logarithm of this fraction as a function of time, the resulting slope is the volume reabsorptive rate \( m_1(\text{s}^{-1}) \) and is obtained by
linear least squares fit. Volume flow (nanoliters per millimeter-minute) is then given by 0.6 πr²ml.

**Droplet Composition**

A solution was prepared to resemble late proximal tubular fluid (40, 42, 43, and footnote 1), to which NaCl was added to produce a solution of high osmolarity. [³H]methoxyinulin (New England Nuclear, Boston, Mass.) was added. The solution composition was measured using electron probe microanalysis and the liquid droplet technique (38). Measured composition (mM) is: Na, 202; Cl, 204; K, 3.2; PO₄, 1.5; Ca, 1.3; Mg, 1.0; and SO₄, 1.2. The osmolarity of the solution was 375 mosM/kg as measured by the procedure of Ramsay and Brown (54) on 49.1-μl aliquots of uninjected control droplets. The solution was stored frozen at ~80°C and used for all experiments.

**RESULTS**

**Volume Flow**

With chosen literature values for the parameters of the model (Table I), a steady-state volume flow (Jₑ) of 2.25 nl/mm-min is obtained. This value is within the range of reported experimental values for the rat intermediate and late proximal tubule (25-27, 47, 50, 76) and is obtained without incorporating active forces.

**Reabsorbate Concentration**

In the steady state, droplet concentration and reabsorbate concentration must be identical, and because mₑ (Eq. 8) will always be more negative than m₁ (Eq. 7), the steady-state reabsorbate (or droplet) chloride concentration will be given by 1/C (Eqs. 4, 5, and 6) or from equations 6, 7, 9-11,

\[
\frac{m₁\left(P_Cl(C_{Cl} + FC_{Cl}Δψ/RT) - Lₚ(ΔP + Π_{pr} + RT\left(σ_{Na} + σ_{Cl}\right)C_{Cl} + \left(σ_{Na} + σ_{HCO₃}\right)C_{HCO₃}\right)(1 - σ_{Cl})C_{Cl}\right)}{Lₚ\left(2P_Cl(σ_{Na} + σ_{Cl})(RTC_{Cl} + FC_{Cl}Δψ) - (2P_Cl + 2K_s + m₁)\right) - ΔP + Π_{pr} + RT\left(σ_{Na} + σ_{Cl}\right)C_{Cl} + \left(σ_{Na} + σ_{HCO₃}\right)C_{HCO₃}\right)}
\]

This rather lengthy equation states that “isosmotic flow” is not characterized by a single value for transported fluid osmolarity. Reabsorbate concentrations depend on a number of parameters, including hydrostatic pressure.

² Without convection coupling the equation is much simpler,

\[
\frac{C_{Cl}^{no\ convection}}{C_{Cl}^{no\ convection}} = \frac{2P_Cl(C_{Cl} + FC_{Cl}Δψ/RT)}{m₁ + 2P_Cl + 2K_s}
\]

where m₁ is now defined as \(-\left(P_Cl + K_s + LₚRTW\right)/r - \left(P_Cl + K_s + LₚRTW\right)^{1/2} + 4LₚRT\left(σ_{Na} + σ_{Cl}\right)X - \left(P_Cl + K_s\right)^{1/2}r\). Compared with Eq. 12, Eq. 13 permits an easier assessment of how changes in parameters will affect steady-state droplet concentrations.
peritubular bicarbonate and protein concentrations, and tubular permeabilities, all of which could vary with different physiological conditions. With the chosen parameter values, $C_{\text{PM}}^*$ (no active forces) is 150 mM.

Variation of Parameter Values (with Solute Asymmetry)

Taking literature values for the equation parameters, we obtain a steady-state droplet (and reabsorbate) osmolar concentration of 0.299, very close to the assumed plasma value of 0.290 (Table I), although slightly hypertonic. To investigate the effect of changes in the equation parameters on steady-state volume flow and droplet (and reabsorbate) concentrations in the late and intermediate proximal tubule, we have varied individual parameters of the theoretical model over a wide range, holding the remaining parameters constant. Although physiologically it may not be reasonable to vary a single parameter, holding all others constant, we choose this protocol to better dissect the dependence of the theoretical model predictions on parameter values. We will define droplet concentration to be "essentially isotonic" if it deviates by <10 mM from the isotonic concentration. With an assumed plasma osmolarity of 290 mosM (from Table I) and equal Na and Cl droplet concentrations, the isotonic concentration corresponds to a 145 mM droplet chloride concentration.

In Fig. 1, $L_p$ and $P_{\text{Cl}}$ have been varied over a 100-fold range about their base-line values. It might have been expected that increasing $L_p$ would directly and linearly increase $J_o$. However, as shown in Fig. 1 a, increasing $L_p$ above literature values causes only a small change in $J_o$. Large decreases in $L_p$ cause large decreases in the volume flow, but small decreases in $L_p$ from literature values result in small changes in $J_o$, illustrating that $J_o$ is relatively insensitive to $L_p$ over a considerable range of values. This is due to the interdependence of salt and water flows. An increase in $L_p$ increases water flow relative to that of salt; with more water leaving, droplet concentration increases, and the increasing osmolarity opposes and minimizes an increase in $J_o$. As a result, in spite of varying $L_p$ over two orders of magnitude, including a 10-fold reduction in its literature value, the droplet steady-state composition remains essentially isotonic, varying only from 135 to 155 mM (Fig. 1 b). Varying $P_{\text{Cl}}$ over two orders of magnitude likewise has only a small effect on droplet concentration, which varies over a range similar to that of Fig. 1 b, from 133 to 155 mM (Fig. 1 d). Changes in $P_{\text{Cl}}$ result in major changes in volume reabsorption (Fig. 1 c), indicating that $J_o$ is very sensitive to $P_{\text{Cl}}$ and that large changes in $J_o$ can occur without large changes in the reabsorbate concentration. An increase in $P_{\text{Cl}}$ increases salt flow relative to fluid movement, causing a decrease in droplet concentration; the decrease in droplet concentration becomes an additional force for volume flow that minimizes the change in droplet concentration.

Fig. 2 shows the changes in split oil droplet volume flow and concentration due to variations in the passive reabsorptive forces. In Fig. 2 a and b, the plasma bicarbonate concentration has been varied at the expense of the chloride concentration. With increasing peritubular bicarbonate concentration, and thus an increasing solute asymmetry, volume flow exhibits a marked increase (Fig. 2 a), but droplet concentration increases by only a few millimoles
per liter, remaining close to the isotonic concentration (Fig. 2 b). Increasing peritubular bicarbonate (decreasing peritubular chloride) results in an increased gradient for chloride efflux as well as an increase in the effective osmotic pressure$^3$ for volume flow (Eq. 3); with both salt and water flux changing in the same direction, droplet concentration tends to remain constant. Changing either the peritubular protein concentration or the hydrostatic pressure has only a minor effect on volume flow and, consequently, on droplet concentration (Fig. 2 c and d).

Changes in split oil droplet volume flow and concentration resulting from variations in the active reabsorptive forces are shown in Fig. 3. Fig. 3 a and b

$^3$ In this paper, effective osmotic pressure is that pressure exerted across the membrane under consideration ($\Pi = oRTC$). Ideal osmotic pressure is that pressure exerted across an ideal semipermeable membrane ($\Pi = RTC$).
illustrates the effect of adding a coupled (electrically silent) Na-Cl active pump to the passive reabsorptive forces. With an increase in the active transport rate ($K_\alpha$), there is an augmented removal of salt from the droplet; droplet concentration falls and becomes hypotonic to the steady-state osmolality obtained in the presence of passive forces alone (Fig. 3 b). Effective hypotonicity causes volume flow to increase (Fig. 3 a), minimizing the decrease in droplet concentration. Effective hypotonicity exerts its effects even though the droplet is ideally hypertonic to plasma, as occurs for $K_\alpha < 1.8 \times 10^{-5}$ cm/s. Fig. 3 c and d illustrates the changes in $J_v$ and droplet concentration due to varying the electrical potential across the tissue. Because the plasma concentrations are kept constant and the droplet concentration does not change greatly, implying that diffusion potentials are constant, a change in $\Delta \psi$ can, thus, be seen primarily as a change in the contribution of an active electrogenic Na pump. Increasing the contribution of an electrogenic Na pump causes

![Graph](image-url)

**Figure 2.** Theoretical predictions for steady-state droplet volume flow and droplet chloride concentration with variation of passive forces. (a) Volume flow as a function of peritubular HCO$_3^-$, varied at the expense of peritubular Cl. (b) Droplet chloride concentration as a function of peritubular HCO$_3^-$, varied at the expense of peritubular Cl. (c) Volume flow as a function of the hydrostatic pressure gradient or oncotic pressure. (d) Droplet chloride concentration as a function of hydrostatic or oncotic pressure. The circles denote chosen literature values for peritubular HCO$_3^-$ (25 mM) and the hydrostatic pressure gradient (30 mm Hg) or oncotic pressure (30 mm Hg).
$\Delta \psi$ to become negative, enhancing the efflux of Cl and lowering droplet concentration. With an augmented removal of Na and Cl from the droplet, droplet concentration falls (Fig. 3 d), creating an additional force for volume movement (Fig. 3 c) and minimizing changes in the droplet concentration.

Fig. 4 illustrates the changes in split oil droplet volume flow and concentration resulting from variations in the chloride and bicarbonate reflection coefficients. Decreasing $o_{Cl}$ decreases the effective osmotic pressure of the chloride gradient opposing osmotic volume reabsorption, which results in relatively large increases in $J_v$ (Fig. 4 a). Decreasing $o_{Cl}$ also increases the contribution of solvent drag to chloride movement (Eq. 1), and the increase in $J_v$ occurs with little change in the droplet concentration (Fig. 4 b). Decreasing $o_{HCO_3}$ results in effects on $J_v$ (Fig. 4 c) opposite to those of Fig. 4 a; droplet concentrations do not deviate from essentially isotonic values (Fig. 4 d).
As shown in Fig. 5a, $J_v$ expressed per unit length varies directly with tubular radius; expressed per unit area, $J_v$ would have been invariant with $r$. Droplet concentration is not a function of tubular radius (Fig. 5b), and, as shown in Fig. 5c and d, neither $J_v$ nor droplet concentration is a function of the initial droplet volume (at constant $r$, initial droplet length).

![Graph](image)

**Figure 4.** Theoretical predictions for steady-state droplet volume flow and droplet chloride concentration with variation of chloride and bicarbonate reflection coefficients. (a) Volume flow as a function of the chloride reflection coefficient ($\sigma_{Cl}$). (b) Droplet chloride concentration as a function of $\sigma_{Cl}$. (c) Volume flow as a function of the bicarbonate reflection coefficient ($\sigma_{HCO_3}$. (d) Droplet chloride concentration as a function of $\sigma_{HCO_3}$. The circles denote chosen literature values for $\sigma_{Cl} (0.50)$ and $\sigma_{HCO_3} (0.96)$.

**Variation of Parameter Values (without Solute Asymmetry)**

To investigate isosmotic flow due solely to the presence of active forces, split oil droplet behavior in the late and intermediate proximal tubule has been modeled in the absence of solute asymmetry and hydrostatic and oncotic forces ($\Delta P = 0$, $\Pi_{pr} = 0$, $C_{HCO_3} = 0$, and $C_{Cl} = 145$ mM). These circumstances could occur with in vitro or in vivo renal perfusion procedures. These circumstances could also occur with conventional in vitro procedures for studying other epithelia, to which the concepts of this analysis might also apply.

The effect of active forces alone on $J_v$ and droplet concentration are
illustrated in Fig. 6, in which $K_s$ is varied with $\Delta \psi = 0$. As shown in Fig. 6 b, increasing $K_s$ results in a linear decrease in droplet osmolarity below that of plasma. The resulting ideal luminal hypotonicity becomes a force for volume flow, and $J_v$ varies directly with $K_s$ (Fig. 6 a). The results shown in Fig. 6 a and b are identical to the results of Fig. 3 a and b when the figures are transposed to the same intercept, illustrating that the effects of active and passive forces are additive and not synergistic. The effects of increasing $\Delta \psi$ in the absence of initial passive forces and with $K_s = 0$ can be inferred from Fig. 3 c and d and are similar to those for $K_s$ in Fig. 6 a and b. Because similar effects are obtained with $K_s$ or $\Delta \psi$, we will arbitrarily set $\Delta \psi = 0$ and $K_s = 2.4 \times 10^{-5}$ cm/s as base-line values for isosmotic flow resulting solely from the presence of active forces. These parameter values give a reabsorptive rate comparable to the base-line rate obtained with only passive forces.

Fig. 6 c and d illustrates the effects of varying $\sigma_{Cl}$ in the absence of initial passive forces. Decreasing $\sigma_{Cl}$ increases the salt removed from the droplet by
solvent drag (Eq. 1 and Fig. 6d), resulting in an increasing droplet hypotonicity and increasing force for osmotic volume flow (Fig. 6c).

Fig. 7 illustrates the effects of varying $L_p$ and $P_{Cl}$ in the absence of initial passive forces. As shown in Fig. 7a and b, varying $L_p$ results in effects similar to those observed with passive forces (Fig. 1a and b), with the exception that droplet concentrations are always ideally hypotonic. Increasing $L_p$ with constant active reabsorption increases water movement with respect to salt movement. The droplet concentration and $J_v$ both increase, but this increase is minimized by the decrease in force for water removal due to a smaller hypotonic gradient. Varying $P_{Cl}$ in the presence of active forces has effects opposite those observed in the absence of active forces (Fig. 1c and d). As shown in Fig. 7c and d, increasing $P_{Cl}$ results in a decrease in $J_v$ and an increase in $C_{Cl}$. With increasing $P_{Cl}$ there is an increase in chloride back-diffusion into the lumen; the luminal osmolarity rises, the force for fluid movement decreases, and $J_v$ decreases.

**Figure 6.** Theoretical predictions for steady-state droplet volume flow and droplet chloride concentration as a function of the active transport rate ($K_a$) and chloride reflection coefficient ($\sigma_{Cl}$) in the absence of peritubular HCO$_3$ and protein, a hydrostatic pressure gradient, and an electrical potential gradient. (a) Volume flow as a function of $K_a$. (b) Droplet chloride concentration as a function of $K_a$. (c) Volume flow as a function of $\sigma_{Cl}$. (d) Droplet chloride concentration as a function of $\sigma_{Cl}$. The circles denote the chosen value for $\sigma_{Cl}$ (0.50).
Volume Flow with Hypertonic Droplet

We have experimentally measured the change in droplet volume after injection of a droplet made hypertonic by 50 mM NaCl into intermediate and late proximal tubules. In these experiments, the rat mean blood pressure was 97.4 mm Hg; hematocrit was 0.45; and glomerular filtration rate was 1.17 ml/min. Fig. 8 a shows the experimental data (dots) for the change in droplet volume (plotted as the logarithmic ratio of droplet volume to initial volume \([\ln V/V_0]\)) with tubular contact time. Volume efflux does not initially occur from the hypertonic droplet; instead, there is a considerable influx of fluid into the droplet, and droplet volume increases by ~30% before net fluid efflux occurs. This volume influx is approximately that required to achieve isotonicity. By linear regression analysis, a straight line (not shown) can be fitted to the decreasing portion of the experimental data giving a slope \((m_1)\) of \(-0.032\) s\(^{-1}\) and a \(J_V\) of 1.70 nl/mm\(
\cdot\)min. The solid lines of Fig. 8 are the theoretical curves for the droplet volume ratio and droplet concentration obtained from

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**Figure 7.** Theoretical predictions for steady-state droplet volume flow and droplet chloride concentration as a function of hydraulic conductivity and chloride permeability in the presence of a neutral active salt pump (and absence of peritubular \(\text{HCO}_3\), protein, hydrostatic pressure gradient, and electrical potential gradient). (a) Volume flow as a function of hydraulic conductivity (\(L_p\)). (b) Droplet chloride concentration as a function of \(L_p\). (c) Volume flow as a function of the chloride permeability (\(P_{Cl}\)). (d) Droplet chloride concentration as a function of \(P_{Cl}\). The circles denote the chosen literature values for \(L_p\) and \(P_{Cl}\).
Eqs. 4 and 5 using the parameter values and initial droplet Cl concentration given in Methods (no active forces present). The parameter values were chosen independently of the Fig. 8 a data, and no fitting has been performed.

**Exponential Reabsorption**

Split oil droplet volume is presumed to decrease with time as a single exponential function (22, 30, 49). From Eq. 5, however, droplet volume in the

![Image of Figure 8](image_url)

**Figure 8.** (a) The ratio of droplet volume \(V\) to the initial droplet volume \(V_0\) is plotted vs. tubular contact time in seconds; the dots are experimental \(V/V_0\) ratios obtained from droplet \([\text{H}]\)inulin determinations; the solid line is a theoretical prediction based on our model evaluated without active forces. (b) Droplet chloride concentration vs. tubular contact time predicted by our theoretical model.

intermediate and late proximal tubule is given not by a single exponential term but by the sum of two exponential terms. It can be shown that with an initial droplet chloride concentration equal to the steady-state value, the term \(B - n_{c0}\) (Eq. 5) is zero and volume reabsorption proceeds as a single exponential. With any deviation of the initial droplet concentration from the steady-state concentration, there will be changes in droplet volume such that
it does not follow a single exponential until the steady-state concentration is reached. Furthermore, since \( m_2 \) of Eq. 5 will always be more negative than \( m_1 \) (the droplet reabsorptive rate), these alterations in droplet volume will proceed faster than the droplet reabsorption. The effect of the second exponential is, thus, to rapidly force the droplet to steady-state values, at which time reabsorption proceeds via a single exponential decrease. The effect of the second exponential term of Eq. 5 is clearly illustrated by comparing Fig. 8 a and Fig. 8 b; it is seen that single exponential absorption begins with the establishment of a steady-state concentration.

**Extracellular Osmolarity Dependence**

We have modeled the effect of increasing plasma osmolarity on volume flow and reabsorbate osmolarity from intermediate and late proximal tubular split oil droplets in the presence of either passive or active forces. As shown in Fig. 9 b, in the presence of passive forces the steady-state reabsorbate osmolarity varies directly with the plasma osmolarity (varied by increasing the NaCl content), and essentially isotonic transport is obtained at any plasma osmolarity. As shown in Fig. 9 a, steady-state volume flow decreases with increasing plasma osmolarity. As shown in Fig. 9 d, with an active transport system an essentially isotonic reabsorbate is also obtained by increasing the plasma osmolarity, with the predicted droplet concentration now falling slightly below the identity line. The effect of increasing plasma osmolarity has the opposite effect on volume flow (Fig. 9 c), and with increasing plasma osmolarity \( J_v \) increases rather than decreases.

**DISCUSSION**

**Assumptions of the Theoretical Model**

We wish to stress the assumptions on which our theoretical model is based. We have assumed that intermediate and late proximal tubular fluid contains only Na and Cl, that peritubular fluid contains only Na, Cl, HCO\(_3\) and protein, that the permeability of protein and effective permeability of HCO\(_3\) is near zero, that the proximal tubular epithelium can be treated as a single membrane, that the neutral pump for active Cl transport operates well below saturation, and that the split oil droplet can be represented as a right circular cylinder. These assumptions have been discussed under Methods. Our conclusions are valid only to the extent that these assumptions are not in error in a way important to fluid reabsorption.

**Parameters of the Model**

The theoretical model contains the major active, diffusional, osmotic, hydrostatic, and oncotic forces presumed to affect split droplet reabsorption in the late and intermediate proximal tubule. The chosen base-line values for the model parameters are reported values. \( P_{cl} \) has previously been measured under zero-volume flow conditions but without considering the existing electrical potential (21, 53, 69). As such, the permeability measurement might
include the effect of the electrical potential, and the use of this value in Eq. 1 would be incorrect. Attempts to recalculate $P_{\text{Cl}}$, $\sigma_{\text{Cl}}$, and $\sigma_{\text{Na}}$ from the data of Frömter et al. (21), accounting for an electrical potential of +1.9 mV (21, 63), led to $\sigma_{\text{Cl}} > \sigma_{\text{Na}}$, a situation not generally found in the kidney (21, 59). We have instead retained the reported values of Frömter et al. (21). The $P_{\text{Na}}/P_{\text{Cl}}$ ratio in the in vivo rat proximal tubule as evaluated by Frömter et al. (21) is 1.2, whereas in the in vitro intermediate superficial proximal tubule of the rabbit the $P_{\text{Na}}/P_{\text{Cl}}$ ratio is 0.56 (37). We are not aware of other determinations for $P_{\text{Cl}}$ in the rat proximal tubule.

Figure 9. Theoretical prediction of the effect of varying peritubular Cl ($C_{\text{Cl}}$) on steady-state volume flow and droplet concentration for an entirely passive and an entirely active system. For a and b, $C_{\text{HCO}_3}$ was kept constant at 25 mM. There was no HCO$_3$ in the peritubular fluid for c and d. (a) Volume flow as a function of $C_{\text{Cl}}$ for the passive system. (b) The solid line is a theoretical prediction for steady-state droplet chloride concentration as a function of $C_{\text{Cl}}$ for the passive system. The dashed line is the line of identity. (c) Volume flow as a function of $C_{\text{Cl}}$ for the active system. Note that the droplet chloride concentration is directly related to $C_{\text{Cl}}$ (d), and, although the active pump does not saturate with increasing $C_{\text{Cl}}$ (Eq. 1), volume flow does saturate. (d) Predicted steady-state droplet chloride concentration as a function of $C_{\text{Cl}}$; the dashed line is the line of identity.
Variation of Parameters

We have allowed only a single parameter of the theoretical model to vary at a time. This may be physiologically unrealistic, e.g., a change in chloride permeability might be expected to result in a change in the chloride reflection coefficient. The use of this protocol, however, is necessary to evaluate the role of individual parameters in our model.

Comparison with experimental data

The theoretical value for droplet volume flow in the late and intermediate proximal tubule obtained without invoking active forces is 2.25 nl/mm·min. This value is within the range of reported experimental values for volume flow in the rat proximal tubule (25–27, 47, 50, 76) and supports our previous conclusions that active salt transport directly accounts for, at most, only a small fraction of volume reabsorption in the intermediate and late proximal tubule.

The theoretical model predicts reasonably well our experimental data on volume transients in split oil droplets, as shown in Fig. 8, which illustrates the ability of this model to predict more than steady-state behavior. Initial volume flow from a significantly hypertonic solution (Fig. 8 a) was not observed, a result that was predicted by our theoretical model. Although the model overestimates the subsequent reabsorptive rate (Fig. 8), it may be that, instead, the split oil droplet experiments have underestimated the reabsorptive rate; a tendency for split oil droplet experiments to underestimate reabsorption is well recognized, although not understood (23, 24, 30, 49).

The theoretical model predicts available experimental split oil droplet data. The model predicts that reabsorption will be independent of initial volume or droplet length (Fig. 5 c), as has been experimentally observed (75). It predicts that reabsorption will vary directly with tubular radius (Fig. 5 a), as has been experimentally observed (31). And it predicts that reabsorption will vary directly with peritubular bicarbonate concentration (Fig. 2 a), as has been experimentally observed (67). The ability of the theoretical model to predict available split droplet data supports the validity of this model in describing the reabsorptive process.

Effect of Protein and Hydrostatic Pressure

The theoretical model predicts that protein and hydrostatic pressure will have a small effect on intermediate and late proximal tubular reabsorption (Fig. 2 c), as has been experimentally observed with split droplets (4, 25, 45) and with free-flow conditions (6, 11, 33, 34, 66). Nevertheless, others find that they have a large effect (8, 9, 27, 36, 44). If hydrostatic and oncotic forces are ultimately shown to have a large effect on tubular reabsorption, Fig. 2 c indicates that this cannot be explained by a direct osmotic effect of protein.

4 We cannot exclude the possibility that the observed initial volume increase is the result of a cellular osmotic response rather than a transepithelial transport response as assumed, although during the experiments cell shrinkage was not visually observed.
across a single barrier but instead a more complex epithelial model involving internal tissue compartments (27, 44, 65, 78) is required.

**Isosmotic Volume Flow**

As shown in Figs. 1-7, large variations in the model parameters generally result in large variations in volume flow but in only small changes in droplet concentration, the droplet osmolality seldom varying by more than 10 mM from plasma values. The essentially isotonic nature of this volume flow is observed with the use of only passive forces (Figs. 1, 2, 4, and 5), with the use of only active forces (Figs. 6 and 7), and with their combination (Fig. 3). Our theoretical model agrees with conclusions based on experiments with the rabbit gallbladder (14) and does not support a previous suggestion that isosmotic flow is the result of a particular set of membrane parameters and bathing solution osmolarities (52). The model predicts that isosmotic flow can be obtained in the absence of active reabsorption and, therefore, does not support the concept that isosmotic flow must be a special property of active Na reabsorption (16, 56).

Our chosen literature value for \( L_p \) in the late and intermediate proximal tubule, \( 18 \times 10^{-5} \text{ cm}^2/\text{cm}^2/\text{s} \cdot \text{atm} \), is nearly half of that recently estimated (2), illustrating that an extremely high value for \( L_p \) is not required to achieve isosmotic flow. Furthermore, even lowering \( L_p \) by an order of magnitude to \( 2 \times 10^{-6} \text{ cm}^2/\text{cm}^2/\text{s} \cdot \text{atm} \) does not cause the droplet to deviate appreciably from isotonic concentrations in the presence of passive forces, as shown in Fig. 1 \( b \), in which the droplet concentration is seen to be lowered to only 135 mM. Lowering \( L_p \) has more drastic effects on droplet concentrations when only active forces are involved, as shown in Fig. 7 \( b \), in which it can be seen that lowering \( L_p \) to \( 2 \times 10^{-5} \text{ cm}^2/\text{cm}^2/\text{s} \cdot \text{atm} \) lowers the droplet concentration to 128 mM. We have also investigated the effect of varying the salt permeability with a lowered hydraulic conductivity (\( L_p = 2 \times 10^{-5} \text{ cm}^2/\text{cm}^2/\text{s} \cdot \text{atm} \)). As shown in Fig. 10, the resulting curves are qualitatively similar to those obtained with \( L_p = 18 \times 10^{-5} \text{ cm}^2/\text{cm}^2/\text{s} \cdot \text{atm} \) (Fig. 1 \( c \) and \( d \) with passive forces and Fig. 7 \( c \) and \( d \) with active forces). As shown in Fig. 10 \( a \) and \( b \), in the presence of only passive forces the droplet concentration remains reasonably close to isotonic values over the given range for \( P_Cl \). Although this low value for \( L_p \) restricts the magnitude of \( J_v \) to values less than approximately 1.0 nl/mm/min, Fig. 10 \( a \) and \( b \) shows that with \( P_Cl = 14.1 \times 10^{-5} \text{ cm/s} \), near maximum values are obtained for \( J_v \) with essentially isotonic droplet concentrations. This is not observed in the presence of only active forces, as shown in Fig. 10 \( c \) and \( d \); although essentially isotonic concentrations can be obtained with very high values for \( P_Cl \) (\( P_Cl > 30 \times 10^{-5} \text{ cm/s} \)), this results in a considerable decrease in volume flow (\( J_v < 0.4 \text{ nl/mm/min} \)). For reabsorption involving only active transport, using measured values for \( P_Cl \), values for \( L_p \) in the vicinity of \( 10 \times 10^{-5} \text{ cm}^2/\text{cm}^2/\text{s} \cdot \text{atm} \) are required to obtain a volume flow with essentially isotonic concentrations (Fig. 7 \( b \)). Nevertheless, even with active transport, an \( L_p \) approximately half of that previously measured for the proximal tubule (2, 68) will produce isosmotic flow.

There are four basic factors responsible for the isotonic nature of volume
flow. (a) $L_p$ is reasonably high ($>2 - 10 \times 10^{-5}$ cm$^3$/cm$^2$.s.atm), with isotonic volume flow due to active forces requiring a higher $L_p$ than that due to passive forces. (b) The forces and flows for salt and water are interdependent. Any change in forces or membrane parameters that alter salt or water removal from the droplet will change the droplet salt concentration; the change in droplet concentration will create a force opposing and limiting its change, so that variations in droplet concentrations are minimized. This effect was described in Results with the presentation of Figs. 1–7. (c) Pertaining solely to reabsorption due to passive forces, the major passive force affecting droplet reabsorption is that of solute asymmetry (50, 74, and footnote 1), which is a force for salt movement as well as water movement. A change in this force will cause a parallel change in salt movement and water movement, which will act to minimize changes in droplet concentration. (d) The concentration of transported fluid is determined by the ratio of salt movement to water movement. The forces for salt and water movement change in parallel, and, consequently, this ratio is relatively insensitive to any change.
Droplet Reabsorption

The theoretical model provides a number of insights into the mechanism of volume reabsorption from split oil droplets. As shown in Fig. 3 b and d and Fig. 6 b, the ability of active NaCl transport to lower droplet concentration and provide an osmotic force for volume efflux, as previously proposed (1, 2, 62), is theoretically verified. The droplet concentration, however, need not be ideally hypotonic, as has been proposed (1, 2, 62). As illustrated in Fig. 3 b, with active as well as passive reabsorptive forces, droplet osmolarity could be ideally hypertonic, isotonic, or hypotonic to plasma osmolarity. The presence of both active and passive reabsorptive forces would minimize deviations from plasma osmolarity, since active forces produce a hypotonic droplet concentration, whereas passive forces tend to produce a hypertonic droplet concentration. Using only passive forces and literature constants, our theoretical model predicts that the droplet steady-state concentration will be ideally hypertonic to that of plasma (see, for example, Fig. 1 b). Recent measurements have found rat proximal tubular fluid to be hypertonic to the peritubular capillary plasma by 4 mosmol/kg H2O (3), which supports our previous observations that passive forces predominate in the intermediate and late proximal tubule.

Passive volume flow in the rat intermediate and late proximal tubule is the result of two forces: differences in ideal osmotic pressure due to salt leaving the droplet by diffusion down electrochemical gradients, tending to make the ideal osmotic pressure of the droplet slightly hypotonic to that of plasma, and differences in effective osmotic pressure due to different reflection coefficients for chloride and bicarbonate in the presence of solute asymmetry, making the effective osmotic pressure of the droplet lower than that of plasma. The latter force is independent of the movement of solute and tends to make the ideal osmotic pressure of the droplet hypertonic to that of plasma. The relative importance of these two forces to passive volume flow has not previously been addressed. As shown in Fig. 4 a and c, a difference between bicarbonate and chloride reflection coefficients is not essential for passive volume flow. As shown in Fig. 4 a, increasing the value of $\sigma_{Cl}$ to that of $\sigma_{HCO_3}$, 0.96, halves the volume flow; conversely, decreasing the value of $\sigma_{HCO_3}$ to that of $\sigma_{Cl}$, 0.5, results in a 30% reduction in volume flow. Although passive volume reabsorption is more efficient with a difference in reflection coefficients, the chloride gradient alone is a major passive force for volume flow. This result should be obtained as long as the chloride permeability exceeds the bicarbonate permeability.

Control of Volume Flow

The theoretical model provides a number of insights into possible mechanisms for effecting a control of intermediate and late proximal tubular reabsorption. It is seen from Fig. 1 c and d and Fig. 4 that small changes in the chloride permeability or reflection coefficient result in large changes in volume flow, with only minor changes in reabsorbate concentration. Modifiers of these membrane parameters could provide a sensitive means for controlling proxi-
mal tubular reabsorption. There is experimental support for this possibility.
Saline expansion causes decreased proximal tubular reabsorption (12, 48),
and, in Necturus proximal tubule, it has been suggested that this decrease is the
result of an increase in salt permeability, leading to an increased salt backflux
into the lumen, which diminishes reabsorption (7). It should be noted that
our theoretical model supports this hypothesis for reabsorption involving
primarily active forces (Fig. 7 c), but, for reabsorption involving primarily
passive forces, the opposite effect would occur, and a permeability increase
would augment rather than diminish net reabsorption (Fig. 1 c). Furthermore,
saline expansion has been reported to decrease the salt reflection coefficient in
Necturus and to result in an increase in osmotic water flow (5), as is predicted
by our model (Figs. 4 a and 6 c). As shown in Fig. 6 c and d and Fig. 7 c and
d, for reabsorption involving active forces, an increase in permeability and a
decrease in the reflection coefficient would have opposing effects on volume
flow and droplet concentrations, and the net response would depend on the
magnitude of the parameter changes. The theoretical model thus illustrates
the possibility that changes in a tubular parameter can effect large changes in
volume flow with only small changes in droplet concentration, and, further-
more, that changes in several tubular parameters could separately modify $J_v$
and the reabsorbate osmolarity.

Application to Other Tissues
With higher values for $L_p$ being reported in a variety of tissues (60, 79) and
projected for most tissues (15), it is becoming easy to obtain isosmotic
transport. Extracellular regions that are ideally hypotonic and intercellular
regions that are ideally or effectively hypertonic will all suffice to produce
essentially isotonic coupling of solute and water fluxes (1, 10, 13, 16, 35, 56,
58). We have obtained, with a simple single membrane model, essentially
isosmotic flow with many values for membrane parameters that currently
would be applicable to a variety of leaky epithelia. It should be noted,
however, that our model would not predict earlier observations in other
epithelia of volume flow from solutions made significantly hypertonic by NaCl
addition (28, 29, 32, 51).

Our previous observation that passive forces are predominantly responsible
for volume flow in the intermediate and late proximal tubule, combined with
our present conclusions that isosmotic flow can be obtained solely by passive
forces, strengthens the possibility that, in other tissues, passive forces may
participate in the movement of salt and water. With high values for $L_p$ it
becomes difficult to discriminate between the contributions of passive and
active forces to volume flow based on concentration or osmolarity measure-
ments. Our theoretical analysis suggests that discrimination might be obtained
from volume flow measurements. Our model predicts opposite effects of active
and passive forces on volume flow due to changes in salt permeability (Figs.
1 c and 7 c) and due to changes in bathing solution (or plasma) osmolarity
(Fig. 9 a and c). For example, the biphasic response of volume flow to changes
in the external solution osmolarity in the gallbladder are compatible with the
participation of both active and passive forces in promoting volume flow (14).
Conclusions

A theoretical model for fluid reabsorption from split droplets in the rat intermediate and late proximal tubule has been developed. The applicability of this model depends on the validity of its simplifying assumptions. The major assumptions we have made are that the epithelium can be treated as a single membrane and that the effective permeability of HCO₃⁻ is near zero.

The model predicts available split oil droplet experimental data. Based on this model, isosmotic flow is not characterized by a single reabsorbate concentration but by a range of concentrations that can be affected by changes in tubular permeability parameters and applied forces. It is nevertheless difficult to obtain a reabsorbate osmolality that differs markedly from plasma. Isosmotic flow is not the result of a particular set of permeability properties and bathing solution composition and need not be a special consequence of active Na transport. Isosmotic flow requires a high but not exceptionally high $L_p$.

Isosmotic flow is a simple consequence of the interdependence of forces and flows for salt and water, and of salt and water movement always changing in parallel; the ratio of salt flow to water flow, or reabsorbate concentration, is thus a slowly varying function. Active NaCl transport can lower the tubular fluid concentration, but the ideal osmolarity of the tubular fluid need not be hypotonic to that of plasma. The ability to obtain, without incorporating active NaCl transport, an isosmotic volume flow similar in magnitude to free-flow values supports our previous observations that active NaCl transport may play a minor role in the intermediate and late proximal tubule. The chloride gradient and the difference in effective osmotic pressure have an approximately equal effect on volume flow. Passive volume flow does not require a difference in chloride and bicarbonate reflection coefficients. Modifications in the chloride permeability or reflection coefficient result in large changes in volume flow, with only minor changes in droplet concentration, and could provide a sensitive control of proximal tubular reabsorption.

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REFERENCES

1. Andreoli, T. E., and J. A. Schafer. 1978. Volume absorption in the pars recta. III. Luminal hypotonicity as a driving force for isotonic volume absorption. Am. J. Physiol. 234:F349-F355.

2. Andreoli, T. E., J. A. Schafer, and S. L. Troutman. 1978. Perfusion rate-dependence of transepithelial osmosis in isolated proximal convoluted tubules: estimation of the hydraulic conductance. Kidney Int. 14:263–269.

3. Atherton, J. C. 1977. Comparison of chloride concentration and osmolality in proximal tubular fluid, peritubular capillary plasma and systemic plasma in the rat. J. Physiol. (Lond.). 273:765–773.
4. BALDAMUS, C. A., K. HIERHOLZER, G. RUMRICH, H. STOLTE, E. UHLICH, K. J. ULLRICH, and M. WIEDERHOLT. 1969. Natriumtransport in den proximalen Tubuli und den Sammelröhren bei Variation der Natriumkonzentration im umgebenden Interstitium. Pfluegers Arch. Eur. J. Physiol. 310:354–368.

5. BENTZEL, C. J., and P. R. RECZEK. 1978. Permeability changes in Necturus proximal tubule during volume expansion. Am. J. Physiol. 234:F225–F234.

6. BLANTZ, R. C., and B. J. TUCKER. 1975. Determinants of peritubular capillary fluid uptake in hydropenia and saline and plasma expansion. Am. J. Physiol. 228:1927–1935.

7. BOULSAEP, E. L. 1972. Permeability changes of the proximal tubule of Necturus during saline loading. Am. J. Physiol. 222:517–531.

8. BRNNER, B. M., and J. L. TROY. 1971. Postglomerular vascular protein concentration: evidence for a causal role in governing fluid reabsorption and glomerulotubular balance by the renal proximal tubule. J. Clin. Invest. 50:336–349.

9. BRNNER, B. M., J. L. TROY, and T. M. DAUGHARTY. 1971. On the mechanism of inhibition in fluid reabsorption by the renal proximal tubule of the volume-expanded rat. J. Clin. Invest. 50:1596–1602.

10. BRESLER, E. H. 1978. A model for transepithelial fluid transport. Am. J. Physiol. 235:F626–F637.

11. CONGER, J. D., E. BARTOLI, and L. E. EARLEY. 1976. A study ‘in vivo’ of peritubular oncotic pressure and proximal tubular reabsorption in the rat. Clin. Sci. Mol. Med. 51:379–392.

12. CORTNEY, M. A., M. MYLLLE, W. E. LASSITER, and C. W. GOTTSCALK. 1965. Renal tubular transport of water, solute and PAH in rats loaded with isotonic saline. Am. J. Physiol. 222:1199–1205.

13. CURRAN, P. F., and J. R. McINTOSH. 1962. A model system for biological water transport. Nature (Lond.). 193:347–348.

14. DIAMOND, J. M. 1964. The mechanism of isotonic water transport. J. Gen. Physiol. 48:15–42.

15. DIAMOND, J. M. 1978. Solute-linked water transport in epithelia. In Membrane Transport Processes I J. F. Hoffman, editor, Raven Press, New York. 257–276.

16. DIAMOND, J. M., and W. H. BOSSERT. 1967. Standing-gradient osmotic flow. J. Gen. Physiol. 50:2061–2083.

17. DuBose, T. D. 1977. Determination of in situ pCO₂ in the rat nephron. Kidney Int. 12:555. (Abstr.).

18. FRIZZELL, R. A., M. DUGAS, and S. G. SCHULTZ. 1975. Sodium chloride transport by rabbit gall bladder: direct evidence for a coupled NaCl influx process. J. Gen. Physiol. 65:769–795.

19. FRIZZELL, R. A., M. FIELD, and S. G. SCHULTZ. 1979. Sodium-coupled chloride transport by epithelial tissues. Am. J. Physiol. 236:F1–F8.

20. FRÖMTER, E., and K. GESSNER. 1974. Free-flow potential profile along rat kidney proximal tubule. Pfluegers Arch. Eur. J. Physiol. 351:69–83.

21. FRÖMTER, E., G. RUMRICH, and K. J. ULLRICH. 1973. Phenomenologic description of Na⁺, Cl⁻ and HCO₃⁻ absorption from proximal tubules of the rat kidney. Pfluegers Arch. Eur. J. Physiol. 343:189–220.

22. GERTZ, K. H. 1963. Transtubulare Natriumchloridflüsse und Permeabilität für Nichtelektrolyte im proximalen und distalen Konvolut der Rattenneire. Pfluegers Arch. Eur. J. Physiol. 276:336–356.

23. GERTZ, K. H. 1972. Stationary perfusion methods. Yale J. Biol. Med. 45:265–268.

24. GOTTSCALK, C. W., and W. E. LASSITER. 1973. Micropuncture methodology. In Handbook
25. Grandchamp, A., J. R. Scherrer, D. Scholer, and J. Bornand. 1975. Role of luminal hydrostatic pressure in proximal tubular fluid reabsorption in the rat. Am. J. Physiol. 229:813–819.

26. Green, R., and G. Giebisch. 1975. Ionic requirements of proximal tubular sodium transport. I. Bicarbonate and chloride. Am. J. Physiol. 229:1205–1215.

27. Green, R., E. E. Windhager, and G. Giebisch. 1974. Protein oncotic pressure effects on proximal tubular fluid movement in the rat. Am. J. Physiol. 226:265–276.

28. Grim, E. 1962. Water and electrolyte flux rates in the duodenum, jejunum, ileum and colon, and effects of osmolarity. Am. J. Dig. Dis. 7:17–27.

29. Grim, E., and G. A. Smith. 1957. Water flux rates across dog gallbladder wall. Am. J. Physiol. 191:555–560.

30. Gyory, A. Z. 1971. Reexamination of the split oil droplet method as applied to kidney tubules. Pfluegers Arch. Eur. J. Physiol. 324:328–343.

31. Gyory, A. Z. 1972. Sources of error in and limitations in the use of t1/2 as a measure of tubular reabsorptive capacity. Yale J. Biol. Med. 45:269–274.

32. Hakim, A., R. G. Lester, and N. Lifson. 1963. Absorption by an in vitro preparation of dog intestinal mucosa. J. Appl. Physiol. 18:409–413.

33. Holzgreve, H., and R. W. Schrier. 1975. Variation of proximal tubular reabsorptive capacity by volume expansion and aortic constriction during constancy of peritubular capillary protein concentration in rat kidney. Pfluegers Arch. Eur. J. Physiol. 356:73–86.

34. Horster, M., M. Burg, D. Potts, and J. Orloff. 1973. Fluid absorption by proximal tubules in the absence of a colloid osmotic gradient. Kidney Int. 4:6–11.

35. Huss, R. E., and D. J. Marsh. 1975. A model of NaCl and water flow through paracellular pathways of renal proximal tubules. J. Membr. Biol. 23:305–347.

36. Imai, M., and J. P. Kokko. 1974. Transtubular oncotic pressure gradients and net fluid transport in isolated proximal tubules. Kidney Int. 6:138–145.

37. Jacobson, H. R., and J. P. Kokko. 1976. Intrinsic differences in various segments of the proximal convoluted tubule. J. Clin. Invest. 57:818–825.

38. Lechene, C. 1974. Electron probe microanalysis of picoliter liquid samples. In Microprobe Analysis as Applied to Cells and Tissues. T. Hall, P. Echlin, and R. Kaufman, editors. Academic Press, Inc., New York. 351–367.

39. Lechene, C., F. Morel, M. Guinnebault, and C. De Rouffignac. 1969. Étude par microponction de l'élaboration de l'urine. Nephron. 6:457–477.

40. Lechene, C., E. Smith, and K. Blough. 1974. Site of sulfate reabsorption along the rat nephron. Kidney Int. 6:65A. (Abstr.)

41. Lechene, C., and R. Warner. 1979. Electron probe analysis of liquid droplets. In Microbeam Analysis in Biology. C. Lechene and R. Warner, editors. Academic Press, Inc., New York. 279–298.

42. Le Grimmellec, C. 1975. Micropuncture study along the proximal convoluted tubule. Pfluegers Arch. Eur. J. Physiol. 354:133–150.

43. Le Grimmellec, C., N. Roine, and F. Morel. 1973. Simultaneous Mg, Ca, P, K, Na and Cl analysis in rat tubular fluid. I. During perfusion of either inulin or ferrocyanide. Pfluegers Arch. Eur. J. Physiol. 340:181–196.

44. Lewy, J. E., and E. E. Windhager. 1968. Peritubular control of proximal tubular fluid reabsorption in the rat kidney. Am. J. Physiol. 214:943–954.

45. Lowitz, H.-D., K. O. Stumpe, and B. Ochwaldt. 1969. Micropuncture study of the action
of angiotensin II on tubular sodium and water reabsorption in the rat. *Nephron.* 6:173-187.

46. MAUDE, D. L. 1970. Mechanism of salt transport and some permeability properties of rat proximal tubule. *Am. J. Physiol.* 218:1590-1595.

47. MOREL, F., and Y. MURAYAMA. 1970. Simultaneous measurement of unidirectional and net sodium fluxes in microperfused rat proximal tubules. *Pfluegers Arch. Eur. J. Physiol.* 320:1-23.

48. MORGAN, T., and R. W. BERLINER. 1969. In vivo perfusion of proximal tubules of the rat: glomerulotubular balance. *Am. J. Physiol.* 217:992-997.

49. NAKAJIMA, K., J. R. CLAPP, and R. R. ROBINSON. 1970. Limitations of the shrinking-drop micropuncture technique. *Am. J. Physiol.* 219:345-357.

50. NEUMANN, K. H., and F. C. RECTOR, Jr. 1976. Mechanism of NaCl and water reabsorption in the proximal convoluted tubule of rat kidney. *J. Clin. Invest.* 58:1110-1118.

51. PARSONS, D. S., and D. L. WINGATE. 1961. The effect of osmotic gradients on fluid transfer across rat intestine in vitro. *Biochim. Biophys. Acta.* 46:170-183.

52. PATLAK, C. S., D. A. GOLDSTEIN, and J. F. HOFFMAN. 1963. The flow of solute and solvent across a two-membrane system. *J. Theor. Biol.* 5:426-442.

53. RADTKE, H. W., G. RUMRICH, S. KLOSS, and K. J. ULLRICH. 1971. Influence of luminal diameter and flow velocity on the isotonic fluid absorption and $^{36}$Cl permeability of proximal convolution of the rat kidney. *Pfluegers Arch. Eur. J. Physiol.* 324:288-296.

54. RAMSAY, J. A., and R. H. J. BROWN. 1955. Simplified apparatus and procedures for freeze-point determinations upon small volumes of fluid. *J. Sci. Instrum.* 32:372-375.

55. RECTOR, F. C., Jr., H. A. BLOOMER, and D. W. SELDIN. 1964. Effect of potassium deficiency on the reabsorption of bicarbonate in the proximal tubule of the rat kidney. *J. Clin. Invest.* 43:1976-1982.

56. SACKIN, H., and E. L. BOULPAEP. 1975. Models for coupling of salt and water transport. *J. Gen. Physiol.* 66:671-733.

57. SCHAFFER, J. A., and T. E. ANDREOLI. 1976. Anion transport processes in the mammalian superficial proximal straight tubule. *J. Clin. Invest.* 58:500-513.

58. SCHAFFER, J. A., C. S. PATLAK, and T. E. ANDREOLI. 1975. A component of fluid absorption linked to passive ion flows in the superficial pars recta. *J. Gen. Physiol.* 66:445-471.

59. SCHAFFER, J. A., C. S. PATLAK, and T. E. ANDREOLI. 1977. Fluid absorption and active and passive ion flows in the rabbit superficial pars recta. *Am. J. Physiol.* 233:F154-F167.

60. SCHAFFER, J. A., C. S. PATLAK, S. L. TROUTMAN, and T. E. ANDREOLI. 1978. Volume absorption in the pars recta. II. Hydraulic conductivity coefficient. *Am. J. Physiol.* 234:F340-348.

61. SCHAFFER, J. A., S. L. TROUTMAN, and T. E. ANDREOLI. 1974. Volume reabsorption, transepithelial potential differences, and ionic permeability properties in mammalian superficial proximal straight tubules. *J. Gen. Physiol.* 64:582-607.

62. SCHAFFER, J. A., S. L. TROUTMAN, M. L. WATKINS, and T. E. ANDREOLI. 1978. Volume absorption in the pars recta. I. "Simple" active Na$^+$ transport. *Am. J. Physiol.* 234:F332-F339.

63. SEELY, J. F., and E. CHIRITO. 1975. Studies of electrical potential difference in rat proximal tubule. *Am. J. Physiol.* 229:72-80.

64. SPRING, K. A., and G. KIMURA. 1978. Chloride reabsorption by renal proximal tubules of *Necturus. J. Membr. Biol.* 38:233-254.

65. TISHER, C. C., and J. P. KOKKO. 1974. Relationship between peritubular oncotic pressure gradients and morphology in isolated proximal tubules. *Kidney Int.* 6:146-156.
66. Tucker, B. J., and R. C. Blantz. 1978. Determinants of proximal tubular reabsorption as mechanisms of glomerulotubular balance. *Am. J. Physiol.* 235:F142–F150.

67. Ullrich, K. J., H. W. Radtke, and G. Rumrich. 1971. The role of bicarbonate and other buffers on isotonic fluid absorption in the proximal convolution of the rat kidney. *Pfluegers Arch. Eur. J. Physiol.* 330:149–161.

68. Ullrich, K. J., G. Rumrich, and G. Fuchs. 1964. Wasserpermeabilität und transtubularer Wasserfluss cortikaler Nephronabschnitte bei verschiedenen Diuresezuständen. *Pfluegers Arch. Eur. J. Physiol.* 280:99–119.

69. von Baumann, K., H. Holtzgreve, F. Kolb, R. Peters, G. Rumrich, and K. J. Ullrich. 1966. Unidirektionale Flüsse für 24Na, 42K, 36Cl, 86Br, und 123I im proximalen Konvolut der Rattenniere. *Pfluegers Arch. Eur. J. Physiol.* 289:R77–R78.

70. Walker, A. M., P. A. Bottr, J. Oliver, and M. C. MacDowell. 1941. The collection and analysis of fluid from single nephrons of the mammalian kidney. *Am. J. Physiol.* 134:580–595.

71. Warner, R., and C. Lechene. 1977. Analysis of standing droplets. *Kidney Int.* 12:576. (Abstr.)

72. Warner, R., and C. Lechene. 1978. Analysis of proximal tubule standing droplets. *Fed. Proc.* 37:728. (Abstr.)

73. Warner, R. R., and C. P. Lechene. 1978. Isosmotic volume flow. *Kidney Int.* 14:784. (Abstr.)

74. Warner, R. R., T. Strunk, and C. Lechene. 1979. Analysis of proximal tubule salt and water transport in standing droplets. *J. Theor. Biol.* 77:453–471.

75. Weinman, E. J., R. J. Hardy, M. Kashgarian, and J. P. Hayslett. 1972. Examination of the Gertz technique as applied to the proximal tubule of the rat kidney. *Yale J. Biol. Med.* 45:289–298.

76. Weinman, E. J., W. N. Suki, and G. Eknoyan. 1976. d-Glucose enhancement of water reabsorption in proximal tubule of the rat kidney. *Am. J. Physiol.* 231:777–780.

77. Weinstein, S. W., and J. Szyjewicz. 1976. Early postglomerular plasma concentrations of chloride, sodium and inulin in the rat kidney. *Am. J. Physiol.* 231:822–831.

78. Welling, D. J., L. W. Welling, and J. J. Hill. 1978. Phenomenological model relating cell shape to water reabsorption in proximal nephron. *Am. J. Physiol.* 234:F308–F317.

79. Wright, E. M., A. P. Smulders, and J. M. Tormey. 1972. The role of the lateral intracellular spaces and solute polarization effects in the passive flow of water across the rabbit gall bladder. *J. Membr. Biol.* 7:198–219.