Pressure Control of Sodium Reabsorption and Intercellular Backflux across Proximal Kidney Tubule

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ABSTRACT The magnitude of changes in luminal hydrostatic pressure (ΔPL), peritubular capillary hydrostatic pressure (ΔPPT), and peritubular capillary colloid osmotic pressure (Δσ) was determined in the Necturus kidney during volume expansion (VE). The specific effects of separate changes of each pressure parameter on proximal net sodium transport (JNS) were studied in isolated perfused kidneys. The combined effect of ΔPL, ΔPPT, and Δσ, of a magnitude similar to that induced by volume expansion, decreases JNS by 26% in the perfused kidney. A major portion of the natriuresis in VE is due to changes in intrarenal pressures. The effect of Δσ on the permeability characteristics of Necturus proximal tubule was studied. With increasing Δσ, the ionic conductance of the paracellular shunt pathway decreased, since transepithelial input and specific resistance rose significantly, whereas cellular membrane resistance remained unchanged. Transepithelial permeability coefficients for sodium chloride and raffinose changed inversely proportional to transepithelial resistance, indicating an alteration of a paracellular permeation route. Net passive sodium backflux and active transport flux components were calculated. Increased net sodium transport with rising Δσ is accompanied by a significant drop in passive back diffusion, without an increment in the active flux component. Change in passive sodium ion back diffusion thus appears to be a key physiological factor in the control of transepithelial sodium transport.

INTRODUCTION

Since the work of de Wardener, Mills, Clapham, and Hayter (1), it has become increasingly apparent that sodium excretion by the kidney can be regulated by factors other than changes in glomerular filtration and mineralocorticoid activity. A variety of additional mechanisms have been proposed. Recently, peritubular physical forces have been shown to be involved in the control of proximal sodium reabsorption (2). According to the sodium backflux hypothesis of Lewy and Windhager (3), changes in net sodium reabsorption can be caused by the extent to which ions, pumped actively into the intercellular spaces, leak back passively towards the lumen. In particular, it was proposed that peritubular colloid osmotic pressure changes affect the rate of removal of the reabsorbate from the basal side of the epithelium into peritubular capillaries. As a consequence, the volume of the intercellular spaces determines the extent of backflow of either bulk fluid or sodium ions.

In a study on the effects of volume expansion in Necturus kidney (4), electrophysiological evidence suggested that changes in paracellular pathway do indeed occur with changes in net solute and fluid reabsorption. Moreover, a distinction has been made between the several mechanisms by which changes in volume or pressure within the basolateral interspaces could be translated into the observed reduction in sodium reabsorption. The natriuretic effect of saline loading could be adequately accounted for by an increase in sodium ion backflux into the tubular lumen through the tight junction down its electrochemical gradient.

In the present study on Necturus proximal tubule, the contribution of separate changes in luminal hydrostatic pressure (P L), in peritubular hydrostatic pressure (PPT), and in capillary colloid osmotic pressure (σ) to sodium reabsorption were studied. This permitted an analysis of their relative importance during the reduction of net sodium transport during extracellular volume expansion. Since colloid osmotic pressure was found to be an im-

1 Abbreviations used in this paper: JNS, sodium reabsorption; Lapp, apparent hydraulic permeability; σ, capillary colloid osmotic pressure; PL, luminal hydrostatic pressure; PPT, peritubular hydrostatic pressure; PVP, polyvinylpyrroldone; Rm, specific membrane resistance.

portant determinant of sodium reabsorption, its mechanism of action on the regulation of sodium excretion was analyzed more extensively. It was found that changes in colloid osmotic pressure control the extent of sodium back diffusion. This backflux takes place through extracellular ion permeation paths.

**METHODS**

*Necturus* kidneys were used in vivo for the assessment of the physical factors altered during volume expansion. Doubly perfused isolated kidneys of *Necturus* offered an ideal model for the controlled induction of hydrostatic or colloid osmotic pressure changes.

**Preparation of the kidney.** Anesthesia of the animal and the preparation of the *Necturus* kidney in vivo was carried out as described earlier (4). Doubly perfused isolated kidneys were prepared by means of catherization of the upper aorta and caudal vein, effecting separate perfusion of glomeruli and renal portal system. Both circulations were drained through a cather placed in the postcaval vein, with an outflow opening level with the kidney surface. Lateral anastomoses between the portal renal veins and the femoral or ventral abdominal veins were interrupted by ligation of the pelvic arteries and veins. The lower segments of the intestine were removed with careful ligation of the mesenteric vasculature. The spinal chord was destroyed.

Perfusion rates were controlled by means of rotameters and maintained at a steady level of 1.5 and 1.0 ml/min through the aorta and caudal vein, respectively. Homogenous distribution of the perfusate to the kidney was checked at the start of the experiment by single injections of small amounts of buffered lissamine green solution. The composition of the perfusion fluid was similar to the Ringer solution used before (4) with the addition of 0.4 g/liter glucose, 20 g/liter polyvinylpyrrolidone (PVP) of an average molecular weight of 40,000 (Plasdone C, GAF Corp., Dye-stuff & Chemical Div., New York), 66 mg/liter tricaine methanesulfonate (Ayerst Laboratories, New York) and 2000 U USP of heparin. Ringer solution (4) was also dripped on the kidney surface. All solutions were equilibrated with a gas mixture of 97.5% O2 and 2.5% CO2 and had a pH of 7.4.

**Intravenous saline loading** was performed as described previously (4). Control infusion rates were 0.1 μl min⁻¹ kg⁻¹ body wt and during saline loading 1.0 μl min⁻¹ kg⁻¹ body wt. Intrarenal pressures were measured after 1 h of control infusion or after 2 h of saline loading.

**Induced hydrostatic pressure changes.** Preferential increments in hydrostatic pressure in either luminal or peritubular capillary pressure were induced in three different groups of experiments.

In group A (data for these groups are in Table II), Pbeat was altered in vivo as described previously (5). Essentially, different levels of intraluminal pressure were obtained in a split-drop and monitored by means of a servocontrol nulling technique. Hydrostatic pressure was altered by means of a controlled injection of Ringer's into the tubule up-stream of the proximal oil block confining the droplet. This intraluminal pressure-clamp technique has been shown earlier to allow the maintenance of steady high intraluminal pressure levels (5).

In group B, the Pbeat in the perfused kidney was selectively altered by an elevation of the outflow opening of the postcaval vein to 6 cm above the kidney surface. Perfusion flow rates in both circulations were kept constant with respect to the control value.

In group C, an attempt was made to reverse the normal transepithelial pressure gradient in one perfused kidney. This was accomplished by perfusing the right renal portal vein at an increased rate of 3-6 ml min⁻¹. In addition, the postcaval vein was connected to a high pressure reservoir so that some retrograde flow occurred from the postcaval vein to the peritubular capillaries of the renal portal system. The outflow resistance of the renal peritubular circulation was thus drastically increased, and perfusion fluids could only leave the circulations by either the anastomoses between portal and arterial circulation, or the connection between renal portal and postcardinal vein. No infusion was made through the aortic cather, which was left open for the perfusion fluids to escape. Again, perfusion rates from both renal portal system and postcaval vein were adjusted to maintain an adequate superficial capillary perfusion in the kidney similar to control conditions. This was checked by determining the lissamine green transit time from the lateral border to the medial border of the kidney. Due to retrograde glomerular capillary perfusion from postcaval vein to aorta, glomerular filtration was not abolished in kidneys undergoing the treatment of group C and Pbeat rose also. Therefore, the changes in Pbeat elicited in this method are preferential changes in Pbeat as compared to only small modifications of Pbeat. Intraluminal hydrostatic pressure was further decreased in this group of experiments by an incision in the Bowman capsule of the nephrons under study.

*Induced colloid osmotic pressure changes in peritubular capillaries* were obtained by changing the PVP content of the perfusate from 20 g/liter to 0 and 40 g/liter. The colloid osmotic pressure was estimated in vitro with a membrane osmometer (Dialytone 100, Amicon Corp., Lexington, Mass). Aqueous solutions of 0, 20, and 40 g/liter PVP gave colloid osmotic pressures of 0, 120, and 272 mm H2O.

**Electrical measurements.** Fig. 1 illustrates the techniques used for measurements of potential differences and resistance. The techniques for the measurements of peritubular membrane potential and cellular input resistance (technique A, Fig. 1), and for transepithelial potential and input resistance (technique B, Fig. 1) were described earlier (4). The previous method (4) for cable analysis was also used (technique C, Fig. 1) and consists of the injection of current by one microelectrode at distance x = 0, while a potential pick-up electrode is impaled successively at different distances from the current injection site. Time-dependent changes of the apparent input impedance at the current injection site sometimes occurred during the time interval between successive impalements of the potential pick-up microelectrode assessing the voltage attenuation of the applied signal along the cable. Therefore, only those observations were included in the results in which the input impedance at the site of current injection remained unchanged. In addition, a second method of applying technique C was used. It consisted in the simultaneous impalement by two single-barreled microelectrodes placed at two different distances from the current injection for the recording of the electrotonic potential. The latter method offers the advantage of excluding any time-dependent changes of the current signal, which may occur in the first method. Since the results of the two methods were not statistically different, pooled data will be given. Spe-
specific membrane resistance (R_m) was calculated as described earlier, from length constant and tubular geometry (4).

Transepithelial potential and resistances were determined in stop flow proximal tubules, with a small oil block placed in the late segment of the proximal tubule and in the intermediate segment. This condition was shown to minimize changes in tubule diameter (4), and to mimic the pressure conditions prevailing during determinations of fluid reabsorption in split-drop experiments (5).

**Micro puncture experiments.** P_l and P_{PT} were determined with the servo control nulling technique of Wiederholm. Some measurements were also performed by the method of Landis. The two methods have been discussed earlier and no significant differences were found with either method (5). Accordingly, pooled results of both methods are presented here. Protein concentrations in peritubular capillaries and aorta were determined using a micro adaptation of the method of Lowry (6). Repeated measurements testing the reproducibility of the method gave a SEM of ±1.45% (n = 16). r was calculated according to the formula of Landis and Pappenheimer (7). No correction for room temperature was made.

Split-drop experiments with Ringers or isotonic raffinose solutions were performed in stop-flow conditions as defined by technique A of reference 5. Net sodium reabsorptive rates were calculated as described earlier (4, 5). Permeability coefficients for sodium chloride and raffinose were calculated on the basis of the parameters obtained with the isotonic raffinose split-drop as reported previously (4).

In all experiments (except those of Table II, group C), experimental manipulations of intrarenal pressure were preceded or followed by a control period, during which identical parameters were measured.

\[ R_m = \frac{(2 \pi \lambda^2)}{a} \]

where \( R_m \) = specific transverse resistance in \( \Omega \text{ cm}^2 \), \( \pi \) = volume resistivity of the luminal fluid in \( \Omega \text{ cm} \), \( \lambda = \) length constant in \( \text{cm} \), and \( a = \) tubular radius of the lumen in \( \text{cm} \).

All values are expressed as means ±SEM, followed by the number of observations (n).

**RESULTS**

**Effect of intravenous saline loading on intrarenal pressures.** The results of hydrostatic pressure measurements in *Necturus* kidneys undergoing saline loading in vivo are shown in Table I. An increase of hydrostatic pressure was observed in the aorta, measured by an implantation with a large micropipette. Pressure also rose in glomerular capillaries, in the peritubular capillaries (average diameter between 30 and 100 \( \mu \text{m} \), halfway between the medial and lateral borders of the kidney). Luminal free-flow and stop-flow pressures were also elevated during saline infusions. With the exception of hydrostatic pressures measured during stop-flow condi-

<table>
<thead>
<tr>
<th>TABLE I</th>
<th>Effect of Intravenous Saline Loading on Intrarenal Pressures</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Pressure ( \text{mm H}_2\text{O} )</td>
<td></td>
</tr>
<tr>
<td>Aortic</td>
<td>102.3±7.2 (11)</td>
</tr>
<tr>
<td>P_l</td>
<td>74.4±3.3 (10)</td>
</tr>
<tr>
<td>Stop-flow P_l</td>
<td>57.1±2.1 (48)</td>
</tr>
<tr>
<td>Free-flow P_l</td>
<td>24.9±1.2 (60)</td>
</tr>
<tr>
<td>P_{PT}</td>
<td>22.3±0.9 (87)</td>
</tr>
<tr>
<td>r</td>
<td>92.9±4.6 (26)</td>
</tr>
</tbody>
</table>

P₀, P_l, P_{PT}, and r are glomerular capillary hydrostatic, proximal luminal hydrostatic, peritubular capillary hydrostatic, and peritubular capillary colloid oncotic pressures, respectively.

Values are means ±SEM, and (n).

NS, difference not significant at the level of \( P = 0.05 \).
tions, the pressure increments were all statistically significant.

Blood samples were collected halfway between medial and lateral border of the kidney from surface capillaries in control and after 2 h of volume expansion for microdetermination of the protein concentration. At the same time, aortic protein levels were determined. Peritubular and aortic protein concentration were in control conditions 2.6±0.2 (n = 9) and 2.6±0.1 (n = 26) g/100 ml. These data are at variance with a report of about 5 g/100 ml (8), with an unexplained large difference between aortic and renal portal blood protein concentration. After volume expansion, peritubular and aortic protein concentration dropped to 1.2±0.2 (n = 8) and 1.1±0.1 g/100 ml, (n = 24) respectively. According to the formula of Landis and Pappenheimer (7), the observed changes in peritubular protein concentrations represent a drop of colloid osmotic pressure of 58.1 mm H2O. Among the intrarenal physical forces that might influence net reabsorption or passive permeability of the proximal tubule during volume expansion (4), three parameters, stop-flow Pp, Prt, and τ, are of importance. A comparison of the absolute change of each parameter shows that alterations in τ are by far the most significant.

Effect of hydrostatic pressure change on net sodium reabsorption. Stop-flow Pp and Prt, were varied experimentally and their effect on sodium reabsorption, Jw, is shown in Table II and Fig. 2. The mean values of the effect of selective changes in luminal pressure, are given in Table II, part A, and on the left of Fig. 2. A fourfold rise of Pp above the control stop-flow level

A. Increase in luminal hydrostatic pressure

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Experimental</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pressure, mm H2O</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stop-flow Pp</td>
<td>60.3±5.8 (16)</td>
<td>249.3±18.1 (14)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ringer split drop</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium flux, 10^-11 eq cm^-2 s^-1</td>
<td>62±7 (16)</td>
<td>620±22 (14)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Net flux, 10^-4 liter cm^-2 s^-1</td>
<td>0.62±0.07 (16)</td>
<td>0.60±0.022 (14)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Rate constant, 10^-3 min^-1</td>
<td>14.3±1.6 (16)</td>
<td>33.6±4.8 (14)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>t0, min</td>
<td>77.7±25.9 (16)</td>
<td>27.2±4.2 (14)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Tubular diameter, 10^-4 cm</td>
<td>105.5±24.6 (16)</td>
<td>115.6±3.9 (14)</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

B. Increase in peritubular capillary hydrostatic pressure (first series)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Experimental</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pressure, mm H2O</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pp</td>
<td>20.8±1.5 (75)</td>
<td>44.6±1.4 (53)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Stop-flow Pp</td>
<td>39.1±1.6 (46)</td>
<td>49.8±1.6 (53)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ringer split drop</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium flux, 10^-11 eq cm^-2 s^-1</td>
<td>63±7 (34)</td>
<td>51±4 (33)</td>
<td>NS</td>
</tr>
<tr>
<td>Net flux, 10^-4 liter cm^-2 s^-1</td>
<td>0.63±0.07 (34)</td>
<td>0.51±0.04 (33)</td>
<td>NS</td>
</tr>
<tr>
<td>Rate constant, 10^-1 min^-1</td>
<td>21.4±5.5 (34)</td>
<td>12.0±1.0 (33)</td>
<td>NS</td>
</tr>
<tr>
<td>t0, min</td>
<td>63.2±5.2 (34)</td>
<td>74.2±6.9 (33)</td>
<td>NS</td>
</tr>
<tr>
<td>Tubular diameter, 10^-4 cm</td>
<td>107.3±3.4 (34)</td>
<td>105.6±3.5 (33)</td>
<td>NS</td>
</tr>
</tbody>
</table>

C. Increase in peritubular capillary hydrostatic pressure (second series)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Experimental</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pressure, mm H2O</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pp</td>
<td>20.8±1.5 (75)</td>
<td>87.5±14.7 (10)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Stop-flow Pp</td>
<td>39.1±1.6 (46)</td>
<td>81.7±12.3 (10)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ringer split drop</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium flux, 10^-11 eq cm^-2 s^-1</td>
<td>63±7 (34)</td>
<td>(-)280±110 (10)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Net flux, 10^-4 liter cm^-2 s^-1</td>
<td>0.63±0.07 (34)</td>
<td>(-)2.8±1.1 (10)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Rate constant, 10^-3 min^-1</td>
<td>21.4±5.5 (34)</td>
<td>(-)62.2±25.0 (10)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Tubular diameter, 10^-4 cm</td>
<td>107.3±3.4 (34)</td>
<td>107.8±5.7 (10)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ±SEM, and (n). Abbreviations: see Table I.

* Negative signs indicate that the net flux was reversed as compared to control.

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significantly increases the net sodium and fluid reab-
sorption. This observation differs from recent findings of
Grantham, Qualizza, and Welling (9). Measuring over a
similar range of pressure values, these investigators
found no effect on fluid reabsorption of intraluminal
pressure changes in isolated rabbit proximal convoluted
tubules. However, in an earlier work the same authors
found significantly increased net water flow as luminal
hydrostatic pressure rose to four times the control
value (10).

Preferential changes in $P_{\text{tr}}$ and their effects on net
sodium and fluid reabsorption are reported in Table II,
groups B and C, and in the middle panel of Fig. 2. In
group B, only small peritubular pressure changes were
induced and the normally occurring transepithelial pres-
sure gradient directed from lumen to blood, although
reduced, was maintained (5). This results in a small
decrease of sodium reabsorption just below the significance
level. In an in vivo study on Necturus, reductions in
net reabsorption have been reported when changes in
hydrostatic pressure $P_{\text{tr}}$ of similar magnitude were per-
formed (8). As mentioned in the techniques, all the
present split-drop reabsorptive studies were performed
under stop-flow conditions, rather than an undefined
pressure ranging between free-flow and stop-flow pres-
sure used in most reported studies (5). In group C, a
large increase of $P_{\text{tr}}$ was obtained, resulting in an un-
usual, reversed transepithelial hydrostatic pressure gradi-
ent, the peritubular pressure exceeding the luminal one
by 6 mm H$_2$O. The proximal tubular walls did not col-
lapse under this reversed gradient and the tubular diam-
eters were not found significantly different from control
values. Under these conditions, reversed fluid movement
from capillary to lumen was observed. Similar decreases
in fluid reabsorption, but no reversal of flow, have been
found when renal perfusion of venous pressure is altered
(9, 11, 12), or after modifications in the capillary
perfusion rates (13).

*Effect of colloid osmotic pressure changes on net so-
dium reabsorption.* Relevant data are summarized in
Table III. Changes in the peritubular capillary colloid
osmotic pressure were induced by changes in the PVP
content of the perfusion fluid in the systemic and portal
circulation. In vitro estimates of the colloid osmotic
pressure of the perfusion fluids used are also given in
Table III. The actual colloid osmotic pressure difference
at the level of the epithelium might be lower than the
in vitro estimate if the reflection coefficient of the appro-
priate barrier for PVP in vivo is lower than that of the
Diaflo PM-10 membrane. The given values for $\pi$ of
Table III are thus indicative only of the maximum
changes in $\pi$ actually induced. Such approximation is
adequate in view of the lack of information about the
proper ultrastructural localization of the oncotic pressure
gradient in vivo.

Net sodium flux, as measured in split-drop experi-
ments, was found to be altered significantly when the
colloid osmotic pressure was varied from 0 to 120 mm
H$_2$O, and from 0 to 272 mm H$_2$O. Smaller but not sig-
nificant changes in sodium flux were observed with
pressure changes from 120 to 272 mm H$_2$O. Conse-
quently, as shown in Fig. 2, the sodium reabsorption is

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**Figure 2.** Effect of pressure change on net sodium reabsorption. Left panel, change in luminal
hydrostatic pressure; middle panel, change in peritubular hydrostatic pressure; right panel,
change in peritubular capillary colloid osmotic pressure. For comparison, the spontaneous
variations observed during volume expansion are indicated by the open arrows.
not a linear function of \( \pi \). Similar findings were observed in the isolated rabbit tubule (9, 14).

For comparison, the range and the direction of pressure changes during volume expansion have been illustrated by arrows in Fig. 2. From the specific effects of hydrostatic and colloid osmotic pressure shown in Fig. 2, it is feasible to calculate the flux variations predictable from the observed pressure alterations during volume expansion. As shown in Table I, in volume expansion a change of stop-flow \( P_L \), \( P_T \), and \( \pi \) occurs of \(+3.7, +11.4, \) and \(-58.1 \text{ mm H}_2\text{O} \), respectively. With the corresponding slopes of the plots of Fig. 2, changes in \( J_{Na} \) of \(+1.9, -5.8, \) and \(-12.1 \times 10^{-12} \text{ eq. cm}^{-2} \cdot \text{s}^{-1} \), could be predicted for a simultaneous change of \( P_L \), \( P_T \), and \( \pi \), respectively. The total effect of all physical forces would amount to \(-16 \times 10^{-12} \text{ eq. cm}^{-2} \cdot \text{s}^{-1} \), representing a drop of \( J_{Na} \) of \(-26\% \), when referred to the control value of the perfused kidney (Table III). The combination of all physical forces simulating the pressure conditions of saline loading in vitro has thus a smaller relative effect than that observed in vivo, where \( J_{Na} \), as compared to antidiuretic conditions, dropped by 45% (4).

**Table III**

| Effect of Colloid Osmotic Pressure Change on Net Proximal Sodium Reabsorption |
|---------------------------------|------------------|----------------|
| **Pressure, mm H\(_2\)O**       | **Experimental \((E_i)\)** | **Experimental \((E_i)\)** |
| \( \pi \)                       | 120              | 0               |
| Stop-flow \( P_L \)             | 44.3±1.2 (21)    | 53.1±3.1 (11)   |
| \( P_T \)                       | 19.8±1.7 (4)     | 19.6±2.9 (5)    |
| Sodium flux, \( 10^{-12} \text{ eq cm}^{-2} \cdot \text{s}^{-1} \) | 62±7 (25)    | 37±4 (24)       |
| Net flux, \( 10^{-6} \text{ liter cm}^{-2} \cdot \text{s}^{-1} \) | 0.62±0.07 (25) | 0.37±0.04 (24)   |
| Rate constant, \( 10^{-3} \text{ min}^{-1} \) | 15.6±2.3 (25) | 7.8±0.9 (24)    |
| \( t_{1/2} \)                   | 65.7±6.8 (25)    | 147.0±33.9 (24) |
| Tubular diameter, \( 10^{-4} \text{ cm} \) | 110.0±3.7 (25) | 116.9±4.7 (24)   |
| **Control (C)**                 | 20 g/liter PVP   | 40 g/liter PVP  |
| \( P(E_i, C) \)                 | \( P(E_i, C) \)  | \( P(E_i, C) \)  |

Values are means ±SEM, and \( (n) \). For abbreviations, see Table I.

**Figure 3** Resistance changes produced by an alteration in peritubular capillary colloid osmotic pressure. A indicates the resistance change, mainly reflecting the transcellular permeation path. B and C estimate the variations in resistance of the paracellular shunt.

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### Table IV

**Effect of Colloid Osmotic Pressure Change on Permeability Characteristics of Proximal Tubule**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (C), 20 g/liter PVP</th>
<th>Experimental (E), 0 g/liter PVP (1st row)</th>
<th>P(E, C) (1st row)</th>
<th>Experimental (E), 40 g/liter PVP (2nd row)</th>
<th>P(E, C) (second row)</th>
<th>P(E, E)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peritubular membrane potential difference, 10^{-2} V</td>
<td>-46.5±2.7 (33)</td>
<td>-36.4±1.6 (25)</td>
<td>&lt;0.005</td>
<td></td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Cellular input resistance, 10^4 Ω</td>
<td>2.54±0.20 (19)</td>
<td>2.46±0.19 (25)</td>
<td>NS</td>
<td></td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Transepithelial potential difference, 10^{-4} V</td>
<td>-12.0±0.7 (60)</td>
<td>-10.6±0.6 (61)</td>
<td>NS</td>
<td></td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Transepithelial input resistance, 10^4 Ω</td>
<td>1.26±0.07 (58)</td>
<td>0.79±0.04 (60)</td>
<td>&lt;0.001</td>
<td></td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Transepithelial specific resistance, Ω cm²</td>
<td>428.6±89.7 (23)</td>
<td>262.8±56.2 (22)</td>
<td>NS</td>
<td></td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Length constant, 10^{-4} cm</td>
<td>929.3±101.4 (23)</td>
<td>724.5±86.3 (22)</td>
<td>NS</td>
<td></td>
<td>&lt;0.005</td>
<td></td>
</tr>
<tr>
<td>Tubular diameter, 10^{-4} cm</td>
<td>99.2±2.5 (23)</td>
<td>102.3±2.2 (22)</td>
<td>NS</td>
<td></td>
<td>NS</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Raffinose split drop</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b, descending phase, 10^{-4} min⁻¹</td>
<td>105.8±26.4 (18)</td>
<td>115.2±21.8 (17)</td>
<td>NS</td>
<td></td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>b', ascending phase, 10^{-4} min</td>
<td>282.1±24.8 (18)</td>
<td>369.0±44.5 (17)</td>
<td>NS</td>
<td></td>
<td>&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>P raffinose, 10^{-4} cm s⁻¹</td>
<td>0.67±0.15 (18)</td>
<td>1.05±0.21 (17)</td>
<td>NS</td>
<td></td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>P NaCl, 10^{-4} cm s⁻¹</td>
<td>2.47±0.23 (18)</td>
<td>3.29±0.32 (17)</td>
<td>&lt;0.05</td>
<td></td>
<td>&lt;0.02</td>
<td></td>
</tr>
<tr>
<td>Tubular diameter, 10^{-4} cm</td>
<td>91.2±3.4 (18)</td>
<td>99.2±5.2 (17)</td>
<td>NS</td>
<td></td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean, ±SEM, and (n).

b, b', rate constants of exponential function to a decimal base.
P_{\text{transep}}, P_{\text{NaCl}}: transepithelial permeability coefficient for raffinose and NaCl, respectively.

NS, difference not significant to the level of \( P = 0.05 \).

In the peritubular membrane potential difference, the resistance is in \( \pi \). Thus, no change could be detected in the resistance of the two tubular cell membranes in series that dominate the conductance of the transcellular pathway. In contrast, as shown in Fig. 3, both the transepithelial input resistance, as well as the specific transverse resistance, increased significantly with increasing colloid osmotic pressure in the capillaries. Since the cell membrane resistance is two orders of magnitude larger than the resistance of the paracellular pathway, the transepithelial resistance reflects mainly a resistance of the paracellular shunt resistor (15). Consequently, the observed changes in transepithelial resistance indicate a significant alteration of the ionic conductance of the paracellular pathway. The absence of any modification of the cellular membrane resistors further supports the paracellular location of the overall transepithelial conductance change. Inspection of the relationship between paracellular resistance and pressure \( \pi \) reveals that increments of oncotic pressure above normal (120 mm Hg) are less effective than decreases. This nonlinear behavior is in the same direction as that noted earlier for the relation between \( J_{\text{Na}} \) and \( \pi \) (Fig. 2).

The absolute values for the specific transepithelial resistance \( (R_{\text{as}}) \) in control conditions of the perfused kidney (20 g/liter PVP) averaged 429 \( \Omega \) cm², a value appreciably higher than the corresponding in vivo value (4). It is difficult to compare the previous study with the present data, because of slight differences in segmental localization along the tubule, different conditions of tubular flow, and possibly different physical param-
eters at the basal side of the epithelium. In the in vivo study, mainly early convoluted portions of the proximal tubule were used, and data from free-flow and stop-flow conditions were pooled. In the present series of experiments, systematically straight segments in the more distal portion of the proximal tubule were used, and only stop-flow conditions were used. Since the basolateral interspaces contribute to a large extent to the paracellular resistance (15), it is likely that changes in either ionic concentration within, or geometry of, the interspaces account for the difference in transepithelial resistance. Consistent with this view is the finding that net sodium reabsorption rates are higher in the intact than in the perfused Necturus kidney (4). Morphological studies of each segment of the proximal tubule under controlled pressure conditions, controlled intratubular flow rate, and diameter, with adequate determination of its net transport rate, will be required to further elucidate the present discrepancy in transepithelial resistance.

Table IV summarizes data on peritubular membrane and transepithelial potential measurements. The absolute values of peritubular transmembrane potential, somewhat lower than previous measurements (16), might be due to some degree of cell damage provoked in the present series by the cell impalement with double-barreled microelectrodes. In all three experimental conditions, the transepithelial potential difference was found to be lumen negative. Peritubular membrane potential differences were found to be significantly modified as a function of the PVP content of the perfusate, the potential difference being lower when low concentrations of colloids were present in the perfusion. A comparable dependency on plasma proteins was noted for rat skeletal muscle (17).

Permeability coefficients of sodium chloride and raffinose. Transepithelial solute permeability was also measured by means of the biphasic volume change of isotonic raffinose split-drops. This technique allows an estimate of the permeability coefficient for sodium chloride (PNaCl), and for raffinose (Praffinose). The results are shown in Table IV and Fig. 4. It is apparent that a decrease of PNaCl and Praffinose occurs with rising colloid osmotic pressures. Like the effect of changes of \( \pi \) on net sodium transport and transepithelial resistance, the changes in both permeability coefficients were more marked for a pressure step below than above the control level. The PNaCl values at 0 and 272 mm H2O were statistically different. Although the change in Praffinose parallels that of PNaCl, it remained below the significance level of \( P = 0.05 \). It should be noted that in the analysis of net volume and sodium flow to the lumen in expanding raffinose droplets, the permeability coefficient was calculated on the basis of the initial transepithelial sodium gradient. However, as the sodium concentration rises with time in such an experiment, theoretically a correction should have been used, leading to higher estimates of PNaCl. The permeability coefficient for sodium could therefore have depended even more on changes in capillary oncotic pressure than is suggested by the present data.

Estimates of transepithelial sodium flux components. The results obtained in these experiments provide adequate data for the calculation of different transepithelial components of sodium transport. The following transport parameters were used: (a) the transepithelial electrochemical potential difference of Na, (overall chemical gradient is zero, and the electrical potential difference is that given in Table IV), (b) the permeability coefficient of sodium PNa, which is taken to be approximated (4) by PNaCl of Table IV, (c) the net sodium flux, JNa, given in Table III. Using parameters (a) and (b), one can calculate the passive unidirectional fluxes for sodium by means of the Goldman-Hodgkin-Katz equations (18), assuming the particular conditions of the constant field. Specifically, although PNaCl was determined under a sizeable concentration difference, the assumption is made that PNa is independent of the electrochemical potential difference. The unidirectional passive influx, J\(_{\text{in}}\), of sodium from capillary to lumen, and efflux J\(_{\text{out}}\), are given by:

\[
J_{\text{in}} = \frac{V F \Delta N_{\text{Na}}}{RT \left(1 - e^{\psi_1}F/RTight)} \geq 0
\]

\[
J_{\text{out}} = \frac{V F \Delta N_{\text{Na}} e^{\psi_1 F/RT}}{RT \left(1 - e^{\psi_1}F/RTight)} \leq 0
\]

where \( V \) is the transepithelial potential difference \( (\psi_1 - \psi_2) \), and \( N_{\text{Na}} \) and \( N_{\text{Na}}^* \) the sodium concentration in the lumen and capillary, respectively, and \( RT \) and \( F \) have their usual meaning. Another flux component is the passive net flux, \( J_{\text{pass}} = J_{\text{in}} - J_{\text{out}} \), and finally, the net flux, \( J_{\text{Na}}^* \), is itself composed of the sum.
of the passive components $J_{Na}^a$ and the active component $J_{Na}^s$. The term $J_{Na}^s$ symbolizes all components of solute flow not accounted for by the electrochemical gradient, rather than strictly the flow through an active sodium pump. The results of the calculation of $J_{Na}^s$, $J_{Na}s^a$, $J_{Na}s^r$, and $J_{Na}$ are summarized in Fig. 5, together with the net flux $J_{Na}$. As can be seen, a progressive increase in peritubular colloid osmotic pressure from left to right in Fig. 5 results in:

(a) An increased $J_{Na}$, as illustrated by the increasing length of the thin arrow on the top of the figure. With the colloid osmotic pressure increase from 0 to 272 mm H$_2$O, $J_{Na}$ flux rises from 37 to 79 10^{-12} \text{ eq} \cdot \text{cm}^{-2} \cdot \text{s}^{-1}.

(b) A fall in the magnitude of the two unidirectional passive fluxes, represented in the figure by the dark arrows.

(c) Resulting from the asymmetry of the two unidirectional fluxes (mainly a consequence of the negative transepithelial potential) a value of 139 10^{-12} \text{ eq} \cdot \text{cm}^{-2} \cdot \text{s}^{-1} obtains for passive net Na backflux $J_{Na}^a$ at $\pi = 0$ mm H$_2$O. It decreases to 48 10^{-12} \text{ eq} \cdot \text{cm}^{-2} \cdot \text{s}^{-1}$, when $\pi$ is 272 mm H$_2$O.

(d) The active flux component of Na ($J_{Na}^s$), obtained by difference, as shown by the open arrows of Fig. 5, was not found to increase with rising colloid osmotic pressure.

This analysis of the flux components of sodium movement allows us to interpret the change in net sodium transport with rising colloid osmotic pressure as the consequence of a preferential decrease in net passive sodium ion back diffusion.

**DISCUSSION**

The natriuresis accompanying volume expansion has been extensively used as a model for the regulation of sodium balance at the proximal tubular level. The present experiments have analysed and evaluated separately the role of the different physical forces that could influence proximal tubular Na reabsorption during the state of diuresis that results from saline infusions.

The results of the present study indicate that, like the mammalian kidney (19, 20), intravenous saline loading in *Necturus* is associated with an increase in luminal and peritubular hydrostatic pressure and a fall in capillary oncotic pressure. The results clearly show that each individual pressure factor per se can produce significant changes in sodium flux, when examined separately in the perfused kidney.

Without assuming any direct influence of these pressure factors on sodium reabsorption, altered fluid reabsorption could be interpreted as the consequence of bulk fluid ultrafiltration. Because a given pressure change was sometimes accompanied by a slight modification of another pressure parameter, the fluid reabsorption data of Tables II and III have been represented in Fig. 6 on a single diagram as a function of total transepithelial driving force, $P_{L} - (P_{RT-E})$.
The effect of variation in $P_L$ (Fig. 6 and Table II, group A) on the mean fluid reabsorptive rates allows an estimate of the apparent hydraulic permeability, ($L_{app}$) of the *Necturus* proximal tubule of $5.2 \times 10^{-4}$ cm s$^{-1}$ (cm H$_2$O)$^{-1}$ in a pressure range about stop-flow values. Smaller pressure effects could occur over the free-flow pressure range. In the mammalian tubule, conflicting evidence is available regarding the effect of variation of $P_L$ on net fluid absorption, with apparent hydrostatic conductivity coefficients ranging for the isolated rabbit convoluted proximal tubule from $12 \times 10^{-8}$ cm s$^{-1}$ (cm H$_2$O)$^{-1}$ (10) to values not significantly different from zero (9).

When hydrostatic pressure was altered at the peritubular border of the epithelium, transepithelial apparent hydraulic flow does not remain a linear function of pressure. The apparent hydraulic conductivity for the small peritubular hydrostatic pressure steps (Table II, group B) could be calculated as $9.2 \times 10^{-8}$ cm s$^{-1}$ (cm H$_2$O)$^{-1}$, similar to the $L_{app}$ observed when luminal pressure was changed. With further increments of $P_{TR}$, a sudden increase in transepithelial conductance was observed to $142 \times 10^{-8}$ cm s$^{-1}$ (cm H$_2$O)$^{-1}$ while reversed flow from capillary to lumen took place. This finding strongly suggests that dramatic geometrical changes of the cell size or interspaces caused gross alterations of the hydraulic permeability of the epithelium. Small pressure changes in the peritubular circulation exerted an effect commensurate with that reported in *Necturus* (8). The available data on the effect of peritubular pressure changes in mammalian kidneys do not lend themselves to comparison. Pressure or flux values were estimated only indirectly (10, 12, 13), and the effects of only small increments of $P_{TR}$ tested.

The general relationship between the concentration of plasma proteins (or other macromolecular substitutes) and proximal sodium reabsorption has been well established both in the mammalian kidney in vivo and in the isolated tubule, and is confirmed by the present observations (2, 9, 14, 21–23). However, a single report on the isolated rabbit tubule indicates that the colloid osmotic pressure gradient per se does not alter fluid absorption (24). The nonlinear effects of changes in colloid osmotic pressure are in keeping with previous observations (9, 14, 23). If fluid absorption were a consequence of the colloid osmotic driving force only, the hydraulic conductance as measured with a colloid osmotic gradient could be computed. Fig. 6 indicates osmotic $L_{app}$ values much smaller than those obtained from hydrostatic pressure gradients, 2.3 $10^{-8}$ cm s$^{-1}$ (cm H$_2$O)$^{-1}$, and 1.1 $10^{-8}$ cm s$^{-1}$ (cm H$_2$O)$^{-1}$, over pressure intervals of 0–120 and 120–272 mm H$_2$O, respectively.

It is virtually certain that the discrepancy between the transepithelial hydraulic conductances as measured by hydrostatic or oncotic pressure changes cannot be explained by an overestimate of the colloid osmotic pressure in vitro. For the colloid osmotic pressure measurements, the reflection coefficient of the epithelium for macromolecules has been assumed identical to that of a diaflo membrane. Reflection coefficients of 0.1 or less would be necessary to match the slope of the effect of $P_{TR}$ to that of $\pi$. Indeed, it has been pointed out that an important fraction of plasmatic proteins or substitutes leaks across the capillary wall (25). This raises uncertainties about the barrier across which the oncotic pressure gradient is exerted or what its actual magnitude across each barrier is. Differences in absolute values of oncotic and hydrostatic permeability coefficients have been reported for single and complex membrane systems (26, 2). The observed scatter of values for $L_{app}$ by two orders of magnitude questions the validity of such measurements, whenever structural changes induced by the applied force are not excluded, or whenever prolonged exposures to a given force or large changes in driving force are applied to assess a measurable variation in flux. Moreover, in order to avoid unstirred layer artifacts, slope hydraulic conductivities should be derived from instantaneous flow measurements, an experimental condition not yet realized in micropunc-

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**Figure 6** Transepithelial volume fluxes plotted against net driving force (sum of luminal hydrostatic, peritubular hydrostatic, and colloid osmotic pressure difference). Crosses represent experiments in which $P_L$ was selectively altered. Open circles represent experiments in which $P_{TR}$ was selectively modified. Triangles represent experiments in which peritubular $\pi$ was changed.

$J_V$  
$10^{-6}$ liter cm$^{-2}$ s$^{-1}$  
$P_L - (P_{TR} - \pi)$ mm H$_2$O
ture experiments. At any rate, overall transepithelial $L_{RT}$ values treat the epithelium as a single hydraulic resistance and do not assess the actual water conductivities of the various barriers in series and parallel composing the epithelium.

The single effect on $J_{w}$ of each of the three pressure parameters studied in this work, $P_{c}$, $P_{cr}$, and $r$, was combined to predict the total contribution of all three physical forces to the natriuretic effect of volume expansion. Pressure changes of the same magnitude as that observed in volume expansion, when combined, account for a total drop of 26% from control reabsorptive rate, while a 45% drop was actually observed in saline loading. Thus, only a major part of the total effect of volume expansion is directly controlled by pressure changes. A larger proportion could possibly be mediated by various pressure changes, if some of the single pressure parameters potentiated the effect of others. In rat kidney, it was shown that selective elevation of the capillary oncotic pressure during saline diuresis to its antidiuretic level reversed the inhibition of proximal sodium reabsorption incompletely (20). As in the mammalian kidney, the present data leave a fraction of the effect to be mediated by other natriuretic mechanisms.

The electrical measurements of epithelial permeability indicate that changes in capillary oncotic pressure do not affect the electrical resistance of single cell membranes of the proximal tubule. On the other hand, total specific transverse resistance is directly related to changes in capillary oncotic pressure. A large body of evidence (4, 15, 28) emphasizes that cellular resistances exceed transepithelial resistances by about two orders of magnitude. This finding has been interpreted to indicate the existence of an important paracellular conductance bypassing the cells in parallel. Therefore, the changes of total transepithelial resistance found in the present study definitely reflect alterations in the electrical conductance of the paracellular pathway. More specifically, an increase in the total parallel conductance points to an increase of ion conductance, presumably for the predominant extracellular ions such as Na and Cl. Treating the paracellular current pathway as a single barrier for ion diffusion, an increased ionic conductance can be due either to an alteration of the concentration gradients for these ions or to changes in their respective permeability coefficients. Independent direct measurements of the permeability coefficient of NaCl in this study provide additional evidence that increasing capillary oncotic pressure causes a drop in permeability coefficient for NaCl. The similar direction of changes in permeability coefficient for raffinose, although not significant, is consistent with the interpretation that changes in the overall epithelial permeability pattern occurred. Furthermore, since the volume distribution of several carbohydrates (mannitol, sucrose, raffinose and inulin) does not appear to be different (29, 30), raffinose molecules can be considered to be distributed only in the extracellular space. Thus, a change in the permeability for such a substance should therefore be an indicator of the leakiness of extracellular transepithelial diffusion channels. In view of the electrical and micropuncture evidence, we propose that the decrease in permeability coefficient of NaCl is caused by a change in the permeability of the paracellular shunt path. The three effects of increased capillary oncotic pressure, i.e., a decrease in paracellular electrical conductance, a fall in the permeability coefficient of NaCl, and an apparent drop in the permeability coefficient for an extracellular marker like raffinose, constitute strong evidence that the capillary colloid osmotic pressure determines paracellular ion permeability, $P_{v}$ in particular, rather than the ion content of the paracellular path.

The consequences of an increased paracellular ion permeability on ion fluxes can be predicted. Increased sodium permeability enhances the asymmetry of the two unidirectional passive sodium fluxes. Sodium ions, however, are also reabsorbed actively across the proximal tubule, and as in other epithelia (31), such transport takes place at the basal and lateral interspaces. The present study provides no evidence supporting the possibility that an active transport component of sodium movement is affected by colloid osmotic pressure in the same direction as the reabsorptive flux. A similar conclusion had been reached excluding an important role for changes of the active transport term during reduced proximal sodium transport after extracellular volume expansion (4). Since a considerable portion of renal oxygen consumption appears to be related to sodium reabsorption, oxygen consumption data could further establish this point. Data are lacking on the effects of isotonic volume expansion on renal oxygen consumption. However, during distal blockade of sodium transport, it was found that mannitol diuresis does not further alter oxygen consumption, despite a reduction in net proximal sodium reabsorption (32). In the case of saline diuresis, an unaltered active flux component would be compatible with the finding of a constant Na/Os stoichiometric ratio, if, as in the study on osmotic diuresis (32), the actual $J_{w}$ is used in this ratio.

The preceding analysis demonstrates that the distinction between possible changes affecting the paracellular or the transcellular pathway necessitates the measurement of cell membrane resistances. The experimental model of the Necturus tubule is unique in allowing a separate determination of cellular and paracellular ionic conductances. Several arguments support the notion that no fundamental differences exist between the Necturus and the mammalian kidney with respect to the control of sodium reabsorption at the proximal tubular level. Both
kidneys show an identical response to volume expansion (1, 4, 33). Also, both proximal epithelia are leaky to ions, as evidenced by their low specific resistance (15, 34). Finally, the chemical gradient of sodium ions is similar across the proximal tubular epithelium of the amphibian and mammalian kidney, and the lumen is electrically negative either throughout the length of the proximal tubule (5, 34, 35) or at least the first loops of the proximal segment (36, 37). On the basis of the present experiments, it is tempting to speculate that the changes in transepithelial resistance in the mammalian kidney induced by altered colloid osmotic pressure (38) are also due to modifications of the paracellular permeation path.

Several mechanisms have been proposed to visualize how physical forces regulate the rate of tubular net sodium reabsorption. Three different possibilities have been considered.

The first possibility involves the concept of transepithelial ultrafiltration across a single semipermeable barrier. Net fluid absorption would be a function of the balance of all hydrostatic and colloid osmotic pressure forces and the hydraulic permeability of the epithelium. Small filtration coefficients of the proximal tubular epithelium constituted the major objection to this theory. Measurements obtained from both luminal colloid osmotic pressures in the rat proximal tubule have recently challenged the accepted low water permeability values (39). However, in a series of experiments in which both peritubular and luminal colloid osmotic pressures were altered, such high water permeability values have not been confirmed (23, 40). The present experimental results suggest that transepithelial pressure differences per se may in part contribute to net fluid absorption. Unless accurate measurements are available for the hydraulic slope conductivity measured for minute pressure displacements around the physiological level, no final conclusion is warranted as to the importance of ultrafiltration in net reabsorption.

The second theory on the effects of pressure on net sodium transport assumes a direct effect on the active extrusion mechanism proper. Such influence of hydrostatic pressure has been postulated in frog skin (41). The present flux analysis indicates that alterations of the active Na transport component are not required. A direct method of assessment of the rate of sodium extrusion after electrophoretic injection of Na ions into single cells of *Necturus* in vivo has been developed in this laboratory (42). By this method, it has been found that volume expansion does not affect the rate constant of active Na extrusion across the peritubular cell border.4

The third alternative considers the complex effects of pressure changes on the different fluid compartments within the epithelium. Lewy and Windhager (3) proposed that colloid osmotic pressure does not act as a transepithelial driving force across a single barrier, but determines the exchange of water and solutes at the level of the basal border of the epithelium, thereby modifying the rate of uptake of solute and water from the interspaces to the capillaries. Since the early formulation of this hypothesis, several interpretations have been given to this crucial link, both by the original proponents and subsequent investigators (2, 4, 9, 14, 21). All proposals have postulated that after a decreased uptake of fluid from basal labyrinth to peritubular capillaries, an increased backflux into the lumen would take place of either solvent and/or solutes.

The intricate mechanism of backflux deserves to be better defined:

(a) Backflux of bulk solvent and solutes was proposed by some investigators (2, 9, 14, 21). Increased bulk flow from blood to lumen would be obtained either by an increased hydrostatic pressure within the interspaces or a higher intercellular NaCl concentration. Alternatively, a modification in backflux could follow a change in water permeability, diffusional permeability for NaCl, reflection coefficient for the solute, or ion transference number of the appropriate barrier. Overall transepithelial transport coefficients are of little significance in predicting the mechanism responsible for a bulk backflow of solutes and water. It needs to be established via which route bulk flow to the lumen takes place, either by a transcellular or by a paracellular path. The first possibility, a transcellular bulk flow, has not received serious attention in the literature, since cell membranes in general have a low solute and water permeability. The second possibility, a bulk flow through the paracellular route, i.e. the tight junction, has been the object of extensive speculation (2, 9, 14, 21). The occurrence of an increased hydrostatic pressure or of an increased concentration of sodium within the interspaces during decreased net Na transport has not been experimentally shown and would not lead to a bulk flow preferentially directed from blood to tubular lumen. Therefore, asymmetrical properties of the tight junction have been postulated (9, 14). Our observed changes in conductance, and changes in $P_{s}=\text{net}$ and $P_{\text{ext,base}}$ are in line with a possible alteration of the solute permeability of the tight junction. With respect to the hydraulic filtration coefficient of the tight junction ($L_{p}$), the effects of serum proteins on overall $L_{p}$ have been tested in the isolated tubule (9). But since no variation in $L_{p}$ was detected for fluid movement from the lumen to the interspaces, a hydraulically rectifying tight junction has been suggested (9). Such conclusion can be questioned since overall $L_{p}$ does not necessarily assess the $L_{p}$ of the tight
junction. Data are lacking on possible changes in solute reflection coefficient and ion transference number of the tight junction.

(b) A different mechanism was proposed (2, 4) involving changes in net backflux of Na ions to the lumen. Sodium ions are presumably actively transported into the lateral interspaces. A fraction of this Na⁺ moiety leaks back to the lumen, following the electrochemical gradient for sodium. Enhanced backflux of sodium ions into the lumen could be caused by either an increased electrochemical sodium gradient, or changes in electrical conductance or transference number for the sodium ion.

As with the bulk flow hypothesis, again two different pathways for ionic back-diffusion exist. The first possibility, a transcellular route, sodium ions crossing the lateral membranes and diffusing back to the cell cytoplasm, has been suggested (2), but the present studies clearly contradict such a possibility. Cellular conductance remains unaltered and the membrane potential changes at the peritubular border in this study are opposite to that favorable for an ionic backflux into the cell interior. The second alternative, favoring ionic back diffusion through a paracellular route, has been proposed as a mechanism for impaired net sodium transport during volume expansion (4). In this case, passive ion back-diffusion is governed by the electrochemical gradient prevailing across the tight junction and the sodium ion permeability of that barrier. From earlier data obtained in Necturus kidney during volume expansion and as an interpretation of the present data, we choose the latter possibility as the most likely of all four types or routes of backflux. No direct evidence is available for changes in either chemical or electrical gradient across the junctional complex. However, the observed modification of backflux can be explained as an alteration in ionic permeability coefficient for sodium of the tight junction.

In conclusion, the present results demonstrate that physical forces at the peritubular border of the proximal epithelium control the extent of sodium ion backflux by modulating the permeability of a leaky tight junction. This proposal assigns an important regulatory role to the paracellular pathway in the homeostasis of proximal tubular sodium transport.

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