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**Research Article**

Micropuncture studies have indicated that variation in peritubular oncotic pressure influences net transport of fluid out of the proximal tubule. The present in vitro studies on isolated perfused rabbit proximal convoluted tubules were designed to examine whether protein concentration gradient must act across the peritubular capillary membrane to influence reabsorption, or whether it can exert a direct effect across the tubular basement membrane 71 proximal tubules were perfused with ultrafiltrate made isosmolal to bathing fluids, the latter having identical electrolyte composition as the perfusing ultrafiltrate, but adjusted to three oncotic pressures: hypooncotic, protein 0.0 g/100 ml; control isooncotic serum, protein 6.4 g/100 ml; and hyperoncotic, protein 12.5 g/100 ml. Net volume flux (nl/mm per min), net Na flux (nEq/mm per min), unidirectional Na flux from bath to lumen (nEq/mm per min), and passive permeability coefficient ($\times 10^{-5}$ cm/sec) for Na ($P_{Na}$), urea ($P_{urea}$), and sucrose ($P_{sucrose}$) were determined using isotopic techniques. When the bath was hypooncotic, there was (as compared with isooncotic serum) a significant decrease in net volume (38%) and net sodium (40%) flux, but no change in $P_{Na}$, $P_{urea}$, or transtubular potential; however, $P_{sucrose}$ increased significantly (78%). In experiments in which hyperoncotic bath was used, there was (compared with isooncotic serum) an increase in net volume (28%) and net sodium (30%) flux, but transtubular potential difference did not […]



# Effect of Peritubular Protein Concentration on Reabsorption of Sodium and Water in Isolated Perfused Proximal Tubules

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ABSTRACT Micropuncture studies have indicated that variation in peritubular oncotic pressure influences net transport of fluid out of the proximal tubule. The present in vitro studies on isolated perfused rabbit proximal convoluted tubules were designed to examine whether protein concentration gradient must act across the peritubular capillary membrane to influence reabsorption, or whether it can exert a direct effect across the tubular basement membrane. 71 proximal tubules were perfused with ultrafiltrate made isosmolal to bathing fluids, the latter having identical electrolyte composition as the perfusing ultrafiltrate, but adjusted to three oncotic pressures: hypooncotic, protein 0.0 g/100 ml; control isooncotic serum, protein 6.4 g/100 ml; and hyperoncotic, protein 12.5 g/100 ml. Net volume flux (nl/mm per min), net Na flux (nEq/mm per min), unidirectional Na flux from bath to lumen (nEq/mm per min), and passive permeability coefficient ($\times 10^{-5}$ cm/sec) for Na ($P_{Na}$), urea ($P_{urea}$), and sucrose ($P_{sucrose}$) were determined using isotopic techniques. When the bath was hypooncotic, there was (as compared with isooncotic serum) a significant decrease in net volume (38%) and net sodium (40%) flux, but no change in $P_{Na}$, $P_{urea}$, or transtubular potential; however, $P_{sucrose}$ increased significantly (78%). In experiments in which hyperoncotic bath was used, there was (compared with isooncotic serum) an increase in net volume (28%) and net sodium (30%) flux, but transtubular potential difference did not change significantly.

These data demonstrated that changes in the ambient protein concentration gradient exert direct effects upon proximal tubular reabsorption. Because penetration of sucrose (an index of intercellular movement) but not urea (an index of transcellular movement) varied with changes in tubular reabsorption, it is suggested that oncotic pressure acts by altering the rate of back-leak of reabsorbate through extracellular pathways between tubular cells. It is concluded that an effect of protein concentration on reabsorption can be exerted directly across the basement membrane, without necessary interposition of the capillary bed.

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## INTRODUCTION

A number of studies have shown that alteration of plasma oncotic pressure may produce reciprocal changes in urinary excretion of sodium in animals and man (1–18). Infusions of oncotically active substances into the renal artery of dog (13, 14), into isolated kidney of dog (6, 7) and rabbit (17), and into the renal portal vein of frog (5) and bird (18) exert the effect exclusively on the infused side without effect on the contralateral kidney. It has been concluded from these types of studies that changes in oncotic pressure exert their effects directly on the kidney rather than through some extrarenal mechanism.

In 1956 Bresler (19) suggested that peritubular oncotic pressure exerted an important regulatory effect on tubular reabsorption of sodium, and that changes in peritubular oncotic pressure would occur not only as a result in alteration in arterial plasma protein concentration but also by changes in filtration fraction. Recent micropuncture studies have provided direct evidence that net reabsorption out of the proximal convoluted tubule is influenced by filtration fraction (20) and by peritubular oncotic pressure (21–25).

The exact mechanism by which changes in peritubular oncotic pressure modifies reabsorption has not been

established. Bresler (19) suggested the possibility that the oncotic forces acted directly across the tubular epithelium influencing the passive reabsorption of salt and water. Earley, Martino, and Friedler (15) suggested that the net removal rate of the reabsorbate from the proximal tubule determined by Starling forces may influence the proximal reabsorption by changing the renal interstitial volume. Lewy and Windhager (20) extended this hypothesis, advancing a model in which changes in the balance between the hydrostatic pressure within the intercellular spaces and the oncotic pressure of the peritubular capillary modify the geometry of the lateral interspaces or the basilar epithelial invaginations in such a way that net reabsorption of salt and water is altered.

The purpose of the present investigation was twofold: first, to examine whether changes in protein concentration gradient across the isolated perfused proximal convoluted tubule basement membrane can influence net water and sodium transport in the in vitro preparation without necessary interposition of the capillary bed; second, to ascertain whether protein concentration gradient exerts its effect on the transcellular or extracellular pathways of the tubular cells.

## METHODS

Isolated segments of proximal convoluted tubules (PCT)[1] were perfused in vitro by general techniques previously described (26, 27). Female New Zealand white rabbits weighing 1.5–2 kg were used in all experiments. Rabbits were sacrificed by guillotine decapitation without prior anesthesia or administration of any drugs. Immediately after sacrifice, the left kidney was removed, and a 1 mm slice removed and transferred into a chilled dish of rabbit serum where a single segment of PCT was dissected free without use of collagenase or other enzymatic agents. The pH of both the dissection serum and the subsequent bathing media were kept at 7.4 by continuous bubbling with 95% $O_2$–5% $CO_2$. After dissection, the tubule was transferred into the perfusion dish and, in all cases, perfused with ultrafiltrate of commercially available rabbit serum. In each study the perfusion fluid was isosmolar to the bathing media. The following three baths were used: (*a*) commercially available regular rabbit serum (normal bath), (*b*) ultrafiltrate of rabbit serum (hypooncotic bath), and (*c*) serum remnant of ultrafiltration (hyperoncotic bath). The compositions of these solutions are shown in Table I. Minor differences in osmolality among these solutions were adjusted by adding appropriate amounts of water.

To minimize the effects of minor electrolyte differences in the perfusion fluid and each of the bathing media, equilibrium dialysis was performed among these solutions by the following technique. Approximately 20-ml aliquots each of ultrafiltrate, control serum, and hyperoncotic serum were placed in cellophane bags and dialyzed at 4°C against a liter of rabbit serum for 72 hr. All solutions were bubbled with 95% $O_2$–5% $CO_2$ for at least 10 min immediately before use. The bath was kept at 37°C and also bubbled continuously with the above mentioned gas mixture.

TABLE I

*Protein Concentration and Osmolality of the Bathing Fluids**

| | No. of determinations | Protein concentration | Osmolality |
|---|---|---|---|
| | | *g/100 ml* | *mOsm/kg water* |
| Hypooncotic bath | 37 | 0.0 ±0.0 | 305 ±1 |
| Normal bath | 55 | 6.4 ±0.1 | 307 ±1 |
| Hyperoncotic bath | 18 | 12.5 ±0.1 | 302 ±2 |

* Numbers are given as mean ±SE.

After initial hook-up, the tubule was allowed to stabilize its function for 30 min before collection of control periods. Three 10-min control periods were then obtained. Bath was then changed to vary its oncotic pressure, and another 10 min equilibration period started. After this, an additional three experimental periods were obtained. To minimize any systematic bias, the order in which oncotic pressure was varied in the bath was randomized. The experiments were divided into three groups according to the oncotic pressure of the bath used in the experimental periods: group I, hypooncotic bath; group II, normal control rabbit serum; and group III, hyperoncotic bath.

In order to measure net fluid reabsorption $^{125}$I-labeled human serum albumin (E. R. Squibb & Sons, New York) was added to the perfusate, and the concentrations of isotope were measured in both perfusate and collected fluid. Frequently it was noted that recovery of the volume marker, albumin-$^{125}$I, was less than 100% when perfusion fluid was placed directly into the collecting pipette without first perfusing the tubule. For this reason a great care was exercised when the concentration of albumin-$^{125}$I in the perfusate was measured. In order to minimize the error in the calculation of perfusion rate, the perfusate was put into the collecting pipette through a perfusion pipette at the end of each experiment. Then known values of perfusate were removed from the collected pipette by use of a calibrated, constant bore pipette. Specific activities of the perfusate could be calculated in exact manner using this procedure. It is felt that the reason for a lower specific activity of the perfusate obtained in the described manner, as contrasted to calculation directly from stock solution, lies in the fact that albumin-$^{125}$I has a tendency to adhere to glass. Because of the difficulties associated with albumin-$^{125}$I, a search was made for a more suitable volume marker. Iothalamate-$^{125}$I (Glofil-125, Abbot Laboratories, North Chicago, Ill.) has proved to be more ideal than albumin-$^{125}$I, and accordingly, this was used as a volume marker in the sucrose permeability experiments. Iothalamate-$^{125}$I does not seem to adhere to glass nor be absorbed into the oil in the collecting pipette so that 100% recoveries were uniformly obtained. In addition, the variation in net reabsorption between consecutive collection periods was significantly less than when albumin-$^{125}$I was used as a volume marker.[2]

In order to determine passive permeability coefficient of the tubule for sodium, for urea, and for sucrose, approxi-

[1] *Abbreviation used in this paper:* PCT, proximal convoluted tubules.

[2] Note added in proof: Since submission of this manuscript we have evaluated polyvinylpyrrolidone-$^{125}$I (Amersham/Searle Corp., Arlington Heights, Ill.) and find this volume marker much more ideal than either albumin-$^{125}$I or iothalamate-$^{125}$I.

TABLE II

*Summary of the Effects of Changing Bath Oncotic Pressure on Net Water Flux, Net Sodium Flux, Unidirectional Sodium Flux from Bath to Lumen, and Permeability Coefficient for Sodium, for Urea, and for Sucrose**

| | Hypooncotic experiment | | | | | Normal experiment | | | | Hyperoncotic experiment | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | (n) | $C_1$ | E | $C_2$ | | (n) | $C_1$ | $C_2$ | | (n) | $C_1$ | E | $C_2$ | |
| $J_V$‡ (*nl mm$^{-1}$ min$^{-1}$*) | (23) | 0.99 ±0.10 | 0.51 ±0.09 | | $P < 0.001$ | (13) | 1.18 ±0.12 | 1.13 ±0.10 | $P > 0.5$ | (9) | 0.97 ±0.17 | 1.38 ±0.15 | | $P < 0.05$ |
| | (17) | | 0.72 ±0.09 | 0.94 ±0.10 | $P < 0.005$ | | | | | (9) | | 1.27 ±0.18 | 1.07 ±0.20 | $P > 0.2$ |
| $\Phi_n^{Na}$ (*nEq mm$^{-1}$ min$^{-1}$*) | (21) | 0.157 ±0.018 | 0.080 ±0.014 | | $P < 0.001$ | (7) | 0.201 ±0.028 | 0.179 ±0.020 | $P > 0.2$ | (9) | 0.152 ±0.026 | 0.215 ±0.023 | | $P < 0.05$ |
| | (16) | | 0.106 ±0.014 | 0.150 ±0.016 | $P < 0.005$ | | | | | (9) | | 0.196 ±0.028 | 0.167 ±0.031 | $P > 0.2$ |
| $\Phi_{bl}^{Na}$ (*nEq mm$^{-1}$ min$^{-1}$*) | (7) | 0.501 ±0.094 | 0.485 ±0.085 | | $P > 0.5$ | (7) | 0.692 ±0.074 | 0.585 ±0.067 | $P > 0.2$ | | | | | |
| | (6) | | 0.366 ±0.083 | 0.344 ±0.058 | $P > 0.5$ | | | | | | | | | |
| $P_{Na}$ (*$10^{-5}$ cm sec$^{-1}$*) | (7) | 8.7 ±1.6 | 8.9 ±1.6 | | $P > 0.5$ | (7) | 11.9 ±1.3 | 10.2 ±1.2 | $P > 0.1$ | | | | | |
| | (6) | | 6.6 +1.5 | 6.1 ±1.0 | $P > 0.5$ | | | | | | | | | |
| $P_{urea}$ (*$10^{-5}$ cm sec$^{-1}$*) | (7) | 6.3 ±1.6 | 6.6 ±1.4 | | $P > 0.5$ | | | | | | | | | |
| | (6) | | 7.6 ±1.6 | 7.0 ±1.0 | $P > 0.5$ | | | | | | | | | |
| $P_{sucrose}$ (*$10^{-5}$ cm sec$^{-1}$*) | (12) | 0.69 ±0.17 | 1.23 ±0.28 | | $P < 0.05$ | | | | | | | | | |

* Numbers are given as mean ±SE.

‡ Abbreviations: $J_V$ = net water flux; $\Phi_n^{Na}$ = net sodium flux; $\Phi_{bl}^{Na}$ = unidirectional sodium flux from bath to lumen; $P_{Na}$ = permeability coefficient for sodium; $P_{urea}$ = permeability coefficient for urea; $P_{sucrose}$ = permeability coefficient for sucrose; n = number of experiments; $C_1$, E, $C_2$ = preexperimental control, experimental, postexperimental control period.

mately 10 $\mu$Ci of $^{22}$Na (International Chemical Nuclear Corporation, Irvine, Calif.), 20 $\mu$Ci of urea-$^{14}$C (International Chemical Nuclear Corporation), and 30–100 $\mu$Ci of sucrose-$^{14}$C (New England Nuclear Corp., Boston, Mass.) were added to the bath, respectively. Concentrations of the isotopes in the bath were corrected for plasma water. Radioactivity of $^{125}$I and $^{22}$Na were determined by three-channel well scintillation counters (Packard 3003, Packard 3365). $^{14}$C was counted by a liquid scintillation counter (Packard 2420). The concentrations of sodium of the perfusate and the bathing solution were measured by an IL flame photometer (Instrumentation Laboratory Inc., Watertown, Mass.), and osmolality with a wide-range osmometer (Advanced Instruments Inc., Newton Highlands, Mass.).

The transtubular potential difference was measured by connecting the bath and perfusion pipette via equivalent bridges (300 mOsm liter$^{-1}$ Ringer's in 4% agar) to saturated KCl solution which contained Beckman (Beckman Instruments, Fullerton, Calif.) calomel half-cells. The circuit was completed by placing a voltage reference source and a battery-operated Keithley Model 602 (Keithley Instruments Inc., Cleveland, Ohio) electrometer in the circuit. The stability of this system was excellent with base line voltage drift of less than $\pm$ 0.3 mv for the duration of an experiment. Electrical seal was obtained at the tissue/glass junction of the perfusion end with Sylgard 184 (Dow Corning Corp., Midland, Mich.) by techniques previously described by Burg and Orloff (28).

*Calculations.* Net water flux ($J_v$, nl mm$^{-1}$ min$^{-1}$) was calculated according to the following formula:

$$J_v = \frac{V_i - V_0}{L} = \frac{V_0}{L}\left(\frac{[^{125}I]_0}{[^{125}I]_i} - 1\right), \quad (1)$$

where $V_i$ is perfusion rate (nl min$^{-1}$); $V_o$, collection rate (nl min$^{-1}$); L, length of the tubule (mm); and $[^{125}I]_i$ and $[^{125}I]_o$, concentration of the perfusate and collected fluid, respectively.

Net sodium flux ($\Phi_n^{Na}$, nEq mm$^{-1}$ min$^{-1}$) was expressed as:

$$\Phi_n^{Na} = [Na]_i J_v, \quad (2)$$

where $[Na]_i$ is concentration of sodium in the perfusate (nEq nl$^{-1}$).

Passive permeability coefficient for sodium ($P_{Na}$), for urea ($P_{urea}$), and for sucrose ($P_{sucrose}$) was expressed as follows (cm sec$^{-1}$):

$$P_{Na,\ urea,\ sucrose} = \frac{V_0'[R_i]}{A[\overline{R_b - R_i}]}, \quad (3)$$

where A(cm$^2$) is luminal surface area assuming I.D. = 20 $\mu$; $V_o'$, collected volume expressed as ml sec$^{-1}$; and $[R]_b$ and $[R]_i$ are concentration of the corresponding isotopes in the bath and collected fluids, respectively. $[\overline{R_b - R_i}]$ is the logarithmic mean concentration gradient between the bath and collected fluid $^{22}$Na concentration. Equation 3 has two sources of error. First, it does not account for transmembrane potential differences, and second, a correction for active outward transport of $^{22}$Na which has diffused into the lumen is not included. The latter objection is of minor significance since net transport of fluid out of the PCT in these experiments was roughly 10%, or less, of the perfusion rate. However, a transmembrane potential difference of 6 mv with lumen negative would induce such a force that our calculated permeability coefficient for $Na^+$ would be 25% higher. As presented elsewhere in results, there was no significant difference in transmembrane potential difference when ambient oncotic pressure was varied so no systematic error is introduced from this source when passive permeabilities of Na are compared in baths of differing protein concentration. In this study we were more concerned with relative permeabilities between control and experimental periods than with absolute values so we feel no serious difficulties are associated with equation 3 when applied in this context.

Unidirectional sodium flux from bath to lumen ($\Phi_{bl}^{Na}$, nEq mm$^{-1}$ min$^{-1}$) was calculated by the equation

$$\Phi_{bl}^{Na} = P_{Na}[Na]_b, \quad (4)$$

where $[Na]_b$ is equal to the concentration of sodium in the bath.

The length of the tubule was measured with an ocular micrometer. Luminal surface area was calculated from the approximate tubular diameter of 20 $\mu$. All the data presented were derived from mean of three periods except for experiments determining permeability coefficient for sucrose, in which samples of three periods were combined to obtain statistically sufficient counts for sucrose-$^{14}$C. Results are expressed as mean $\pm$standard error. The statistics were performed using paired $t$ test analysis comparing the measured absolute values of the parameters between the control and experimental periods.

## RESULTS

*Net water and net sodium fluxes.* The effects of oncotic pressure changes in the bath on net water and net sodium fluxes were determined in 71 tubules perfused with ultrafiltrate isoosmotic to the bath. The results are summarized in Table II, and the individual values for net water flux are shown in Fig. 1. In 13 control experiments in which the bath composition was kept unchanged and consisted of regular rabbit serum during the initial three collection periods and the subsequent three experimental collection periods, both net water and net sodium fluxes were not significantly different in the two periods (Fig. 1). Respective changes in these parameters were 1.6 $\pm$6.6% ($P > 0.5$) and $-$4.1 $\pm$10.3% ($P > 0.1$). These data indicate that the transport characteristics of the isolated tubule remain unaltered throughout the course of the experiment.

When the bath was changed from the regular serum to ultrafiltrate (hypooncotic experiments), both net water and net sodium fluxes decreased significantly, with mean per cent changes of $-$40.0 $\pm$13.5% ($P < 0.001$) and of $-$37.2 $\pm$13.6% ($P < 0.001$), respectively. As shown in Fig. 1, 19 of 23 tubules show apparent decreases in net water flux and only one experiment shows an opposite result. Conversely, when the bath was changed from ultrafiltrate to the regular serum, both net water and net sodium fluxes increased significantly. The mean per cent changes from post-experimental control were $-$23.2$\pm$4.8% ($P < 0.05$) and $-$28.3 $\pm$5.2% ($P < 0.05$), respectively.

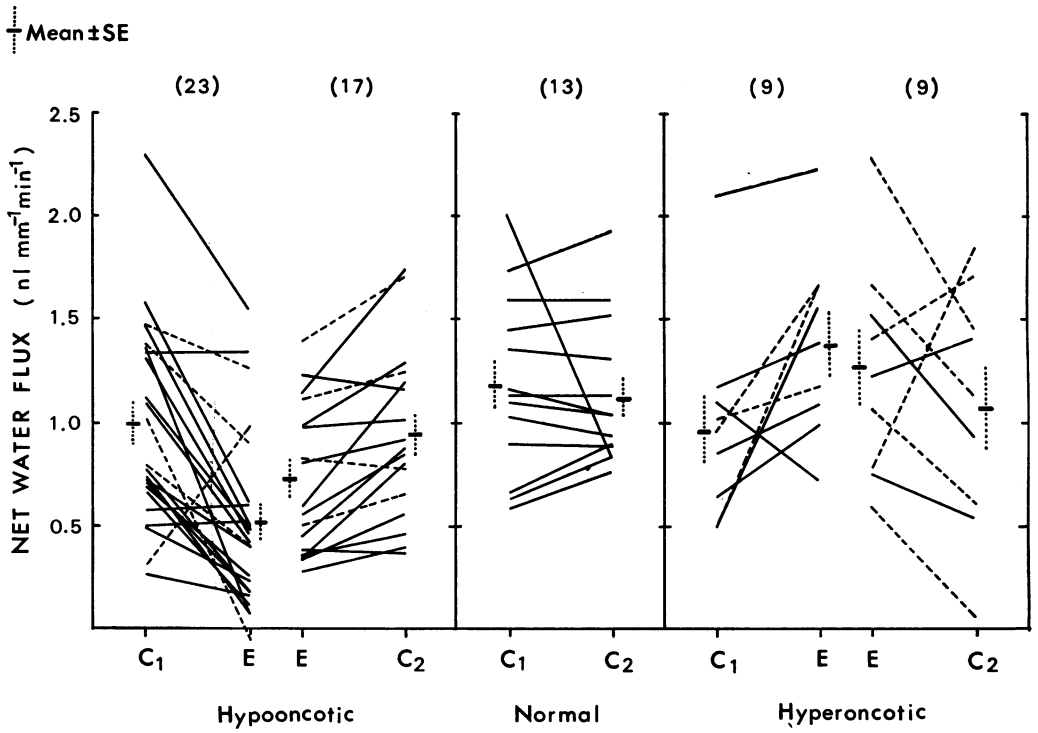


FIGURE 1 The effect of varying ambient oncotic pressure on net water flux of isolated perfused rabbit proximal convoluted tubules. $C_1$, E, and $C_2$ denote preexperimental control, experimental, and postexperimental control periods, respectively. Number of observations are indicated in the parenthesis. The broken lines indicate the data obtained by the experiments in which fluids dialyzed against each other were used for perfusate and bath.

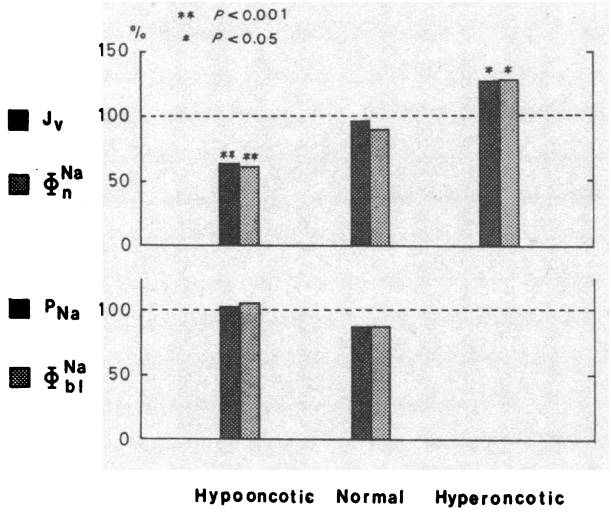


FIGURE 2 The effect of changing bath oncotic pressure on: net water ($J_v$) and net sodium ($\Phi_n^{Na}$) fluxes unidirectional sodium flux from bath to lumen ($\Phi_{bl}^{Na}$); and permeability coefficient for sodium ($P_{Na}$). Values are expressed as percentages of controls. $P$ values are calculated by paired $t$ analysis using controls and experimental period in each individual experiment. Both pre- and postexperimental controls are included.

When the bath was changed from regular serum to serum remnant of ultrafiltration (hyperoncotic experiments), both net water and net sodium fluxes increased in eight of nine experiments. The mean per cent increases were $+70.9 \pm 33.6\%$ and $+72.0 \pm 33.9\%$, respectively. These values are statistically different from controls ($P < 0.05$). When the bath was changed from hyperoncotic to normal control bath, per cent decreases from the postexperimental control were $-25.5 \pm 16.5\%$ for net water flux and $-24.8 \pm 16.2\%$ for net sodium flux. Although these changes were not statistically significant ($P > 0.3$), six of nine experiments showed a marked decrease in net water and sodium flux from the periods in which hyperoncotic bath was used as compared to baths in which isooncotic control rabbit serum was used.

To exclude the possibility that minor differences in ionic compositions of the baths may influence net reabsorption, fluids dialyzed against each other were used for perfusate and baths in 18 experiments. These experiments are depicted by broken lines in Fig. 1. These results when analyzed separately do not differ from others and therefore data presented included means from all studies performed.

Fig. 2 summarizes the data when the two groups, experimental bath preceding control and control bath preceding experimental, are pooled. When the results of the hypooncotic baths are compared to control, there was a 38% decrease in net water flux and 40% decrease in net sodium flux. These values are significantly different from controls ($P < 0.001$). Similarly, when all the hyperoncotic experiments are compared to controls, there was a mean increase in net water flux of 28% and an increase of 30% in net sodium flux. These values also were statistically different from controls ($P < 0.05$).

It is conceivable that in the process of making ultrafiltrate by pressure dialysis using Aminco PM-30 membranes (American Instrument Co., Inc., Silver Springs, Md.) some unrecognized factor responsible for supporting transport is dialyzed out. Evidence against this hypothesis are the experiments in which commercially available bovine albumin (Pentex Biochemical, Kankakee, Ill.) was added to ultrafiltrate in such a manner that the final protein concentration was 6.6 ±0.1 g/100 ml as compared to the rabbit serum used in this series of 6.5 g/100 ml. The primary purpose of these experiments was to determine whether the reconstituted ultrafiltrate with added protein could maintain net transport at the same level as the control serum when each of these were used as the bathing fluid. Also in this series, the same ultrafiltrate without additional protein was used as a bath. In each instance the reconstituted protein containing ultrafiltrate was placed in a cellophane bag and dialyzed against the same solution which was later used as the bath. In five experiments the reconstituted ultrafiltrate was dialyzed against serum, while in three studies it was dialyzed against protein-free ultrafiltrate. The technique of dialysis was the same as previously described. The order in which these bathing fluids were used was the following: (*a*) control rabbit serum, (*b*) reconstituted ultrafiltrate with added protein, and (*c*) protein-free ultrafiltrate. In these eight consecutive experiments the mean reabsorption was 0.75 ±0.07 nl/min during the first three periods when rabbit serum was used as a bath, it increased to 1.02 ±0.10 nl/min during the second three periods when bath was changed to ultrafiltrate to which the protein was added ($P < 0.02$), and decreased to 0.28 ±0.15 nl/min when ultrafiltrate without protein was used as the bath ($P < 0.01$). These results clearly indicate that the effects described in these studies are due to the presence of protein, and not secondary to some unrecognized dialyzable factor. It is interesting to note that although the total protein concentration of the control bath and the ultrafiltrate with the added albumin were the same, the latter had a somewhat higher absolute reabsorption. This probably is due to the in-

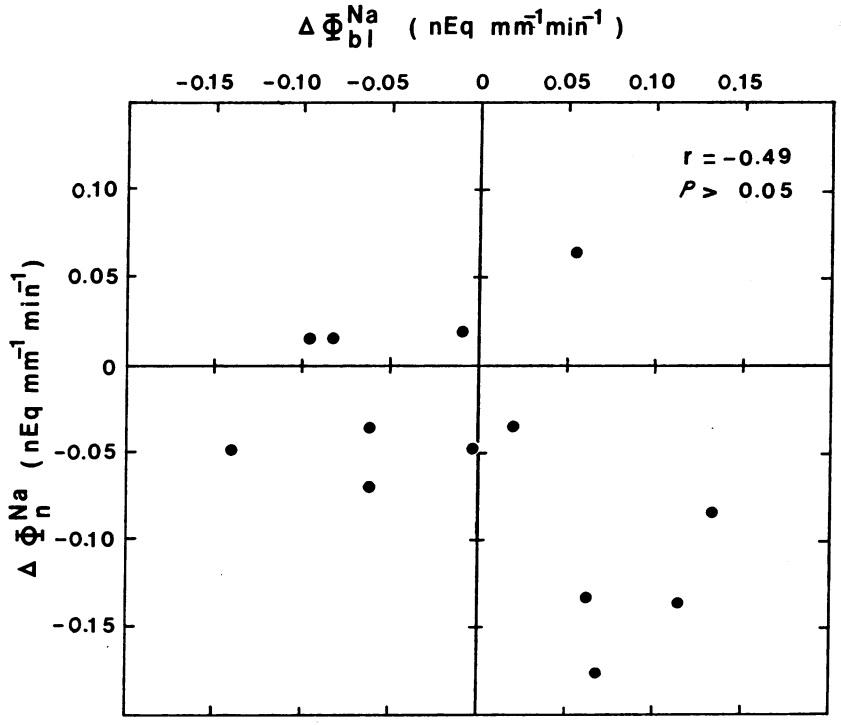


FIGURE 3 The relationship between changes in net sodium flux ($\Phi_n^{Na}$) and changes in unidirectional sodium flux ($\Phi_{bl}^{Na}$) in hypooncotic experiments.

TABLE III

*Effect of Hypooncotic Bath on Permeability Coefficient for Sucrose**

| Exp. No. | $V_i$‡ | $J_v$ | Change | Counts of sucrose-$^{14}C$ | | $P_{sucrose}$ | Change | $C_{sucrose}$ |
|---|---|---|---|---|---|---|---|---|
| | | | | Collected fluid | Bath | | | |
| | *nl min*$^{-1}$ | *nl mm*$^{-1}$ *min*$^{-1}$ | % | *cpm nl*$^{-1}$ | *cpm nl*$^{-1}$ | $10^{-5}$ *cm sec*$^{-1}$ | % | *nl mm*$^{-1}$ *min*$^{-1}$ |
| 1 C | 16.37 | 0.59 | | 0.09 | 12.8 | 0.21 | | 0.08 |
| E | 15.82 | 0.22 | −62.7 | 0.14 | 12.6 | 0.32 | +52.4 | 0.12 |
| 2 C | 7.47 | 0.76 | | 3.64 | 36.8 | 1.46 | | 0.55 |
| E | 9.92 | 0.20 | −73.7 | 3.66 | 37.4 | 2.16 | +47.9 | 0.81 |
| 3 C | 14.62 | 0.77 | | 0.12 | 30.2 | 0.13 | | 0.05 |
| E | 11.48 | 0.44 | −42.9 | 0.35 | 28.6 | 0.32 | +46.2 | 0.12 |
| 4 C | 13.92 | 1.37 | | 1.17 | 36.1 | 0.81 | | 0.31 |
| E | 13.82 | 1.34 | −2.2 | 0.67 | 35.2 | 0.49 | −39.5 | 0.18 |
| 5 C | 11.36 | 1.18 | | 3.53 | 47.8 | 2.17 | | 0.82 |
| E | 11.51 | 0.18 | −84.7 | 3.79 | 43.6 | 2.83 | +30.4 | 1.07 |
| 6 C | 13.15 | 1.14 | | 0.25 | 44.3 | 0.17 | | 0.06 |
| E | 12.80 | 0.32 | −63.5 | 0.30 | 45.2 | 0.21 | +23.5 | 0.08 |
| 7 C | 7.21 | 0.75 | | 2.07 | 56.6 | 0.50 | | 0.19 |
| E | 9.78 | 0.41 | −45.3 | 1.29 | 44.8 | 0.58 | +16.0 | 0.22 |
| 8 C | 10.44 | 0.88 | | 1.33 | 40.3 | 0.64 | | 0.24 |
| E | 11.63 | 0.77 | −12.5 | 1.16 | 45.4 | 0.56 | −12.5 | 0.21 |
| 9 C | 8.07 | 1.27 | | 1.02 | 41.8 | 0.40 | | 0.15 |
| E | 7.86 | −0.15 | −111.8 | 4.90 | 50.2 | 1.99 | +397.5 | 0.75 |
| 10 C | 8.12 | 1.38 | | 1.66 | 49.1 | 0.65 | | 0.24 |
| E | 10.12 | 0.15 | −89.1 | 3.86 | 44.4 | 2.51 | +286.2 | 0.95 |
| 11 C | 6.44 | 0.73 | | 0.20 | 7.5 | 0.35 | | 0.13 |
| E | 7.93 | 0.38 | −47.9 | 0.82 | 6.7 | 1.86 | +431.4 | 0.70 |
| 12 C | 8.52 | 1.00 | | 0.29 | 8.5 | 0.74 | | 0.28 |
| E | 9.12 | 0.64 | −36.0 | 0.21 | 6.1 | 0.83 | +12.2 | 0.31 |

* Experimental data of individual experiments are given.

‡ Abbreviations: $V_i$ = perfusion rate; $J_v$ = net water flux; $P_{sucrose}$ = permeability coefficient for sucrose; $C_{sucrose}$ = clearance of sucrose; C, E = control and experimental period.

creased number of oncotically active particles contained in the ultrafiltrate solution reconstituted by addition of only albumin when compared to the control serum where the total concentration of protein must be made up of a smaller number of oncotically active particles due to the presence of high molecular weight globulins.

*Unidirectional sodium flux from bath to lumen and passive permeability coefficient for sodium.* Passive permeability coefficient for $^{22}Na$ and unidirectional influx of Na were determined in 20 tubules to examine whether changes in these parameters were responsible for the observed changes in net water and net sodium fluxes when varying oncotic pressures were used in the bath (Table II, Fig. 2). In seven control experiments these variables were unchanged throughout the experiment. The control permeability coefficient for $^{22}Na$ obtained here are comparable to ones previously reported by Kokko, Burg, and Orloff (27). In these studies the positively charged $^{22}Na$ was added to the bath, while in the earlier studies (27) $^{22}Na$ was added to the perfusion fluid. In hypooncotic experiments, neither unidirectional sodium flux nor permeability for sodium was changed significantly in spite of the changes in net water and net sodium fluxes. As shown in Fig. 2, the per cent changes when compared to control baths in back flux of sodium were 0% while the per cent changes in permeability coefficients for Na were + 5%. These values are not significantly different from unity.

In Fig. 3 the changes in net sodium fluxes are plotted against the changes in unidirectional sodium fluxes from bath to lumen in hypooncotic experiments. Although there is a tendency of a negative correlation

between these variables, the correlation coefficient of $-0.49$ is not statistically significant ($P > 0.05$).

*Permeability coefficient for urea.* Permeability of the proximal convoluted tubule for urea was determined in 13 experiments in which isooncotic and hypooncotic baths were used. The results are shown in Table II. In the control period permeability for urea was $6.6 \times 10^{-5}$ cm $sec^{-1}$. This value is approximately one half of the permeability coefficient for sodium and comparable to the value obtained when urea-$^{14}C$ was added to equilibrium solution which was used as the perfusion fluid (29). The value of $7.1 \times 10^{-5}$ cm $sec^{-1}$ in post-experimental control is not different from the preexperimental control value when analyzed by the paired $t$ test. When the bath was changed from regular serum to hypooncotic fluid or vice versa, the permeability coefficient for urea did not change.

*Permeability coefficient for sucrose.* In order to evaluate whether changes in oncotic pressure of the bath influence the permeability for a substance which is supposed to penetrate primarily via an extracellular route, sucrose-$^{14}C$ was added to the bath in a sufficient amount to obtain statistically significant counts in the collected tubular fluid. In order to assure sufficient $^{14}C$ counts, the samples from three collection periods were combined and put into a single counting vial. 350 nl of fluid was collected on average, containing a mean of 567 cpm. The observations were made using 12 tubules with regular isooncotic rabbit serum as control with subsequent changes to hypooncotic bath during experimental period. The results are summarized in Table II and individual values are shown in Table III.

In this series of experiments, net water flux decreased from $0.99 \pm 0.08$ to $0.41 \pm 0.11$ nl $mm^{-1}$ $min^{-1}$ when the bath was made hypooncotic, while permeability coefficient for sucrose increased from $0.69 \pm 0.17$ to $1.23 \pm 0.28 \times 10^{-5}$ cm $sec^{-1}$. When each tubule is used as its own control, the per cent change from the control was $116.7 \pm 47.1\%$ ($P < 0.05$). The permeability coefficient for sucrose indicates that the PCT is approximately 20 times more permeable to sodium and 10 times more permeable for urea than for sucrose when normal isooncotic bathing media is used. Fig. 4 shows relationship between changes in net water flux and changes in

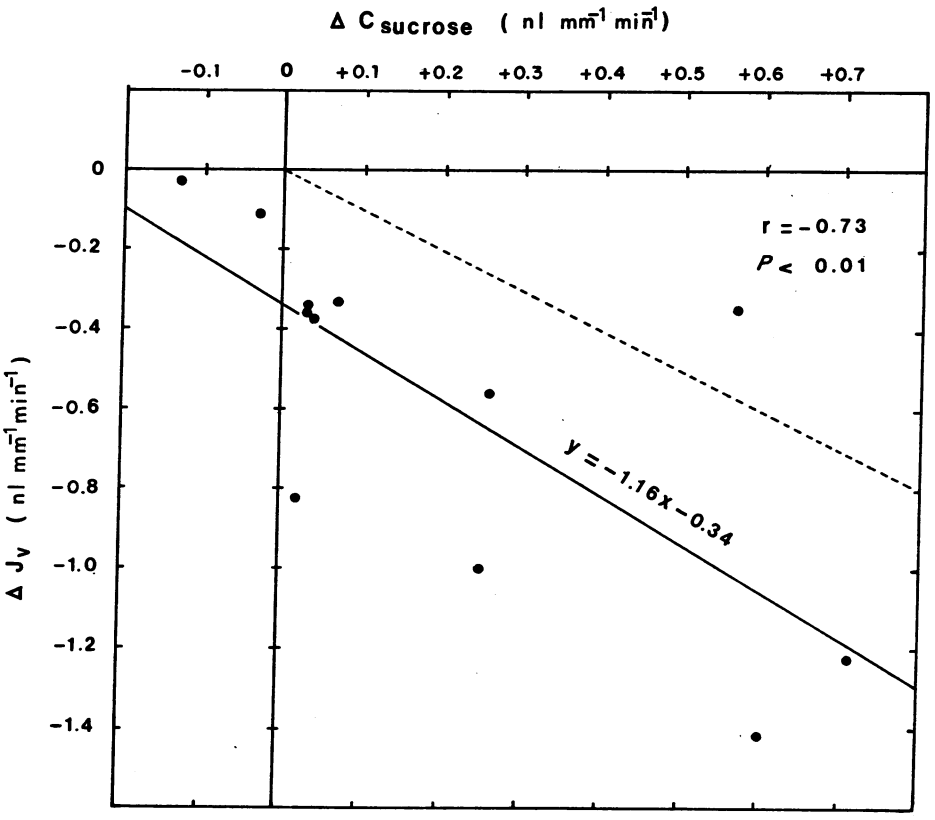


FIGURE 4 Significant correlation between changes in net water flux ($J_v$) and changes in permeability for sucrose expressed as clearance from bath to lumen ($C_{sucrose}$). The regression line was calculated by least square methods. The broken line indicates a line of identity showing the changes in net flux are completely identical with changes in sucrose clearance.

permeability for sucrose. There is correlation between these variables. The correlation coefficient of $-0.73$ is statistically significant at 0.01 level. In this series we have also expressed permeability characteristics for sucrose in terms of clearance from bath to lumen as nl $mm^{-1}$ $min^{-1}$ for the convenience of the comparison with the magnitude of the changes in net water flux. The regression line has a slope of $-1.16$ and intercepts $y$ axis at $-0.34$ nl $mm^{-1}$ min. The broken line in Fig. 4 shows the line of identity indicating changes in net water flux completely depend on changes in permeability for sucrose. The regression line was significantly different from this line.

*Electrical potential difference.* The transtubular potential under control conditions (perfused with isosmolal ultrafiltrate and bathed with isooncotic rabbit serum), ranged in six consecutive experiments from 3.7 to 7.1 mv with a mean of 5.1 mv with the lumen negative. When the bath was changed to hypo- or hyperoncotic media, the maximum unidirectional changes in transtubular potential differences was 0.6 mv, but no statistical difference was noted in the mean potential differences in these groups. At the end of these experiments, $10^{-5}$M ouabain was added to the bath and a prompt decrease to near zero was noted in the transtubular potential difference.

## DISCUSSION

A variety of studies suggests that peritubular oncotic pressure plays an important regulatory role in proximal reabsorption of salt and water (20–25). It has been hypothesized that these oncotic forces exert their effect across the capillary basement membrane and influence the uptake of reabsorbate from the renal interstitium; the latter, by influencing interstitial hydrostatic pressure, modulates reabsorption from the tubular lumen (20).

The present in vitro perfusion studies indicate that variations in the protein concentration of the bathing media significantly affect the rate of reabsorption from the proximal tubule without interposition of the capillary basement membrane (Table II). Changing the bath from isooncotic to hypooncotic media inhibits reabsorption by 38%, whereas raising the bath protein concentration to 12.4 g/100 ml increases reabsorption by 28% when compared to isooncotic bath with a protein concentration of 6.5/100 ml. It is of interest that protein-free bath inhibited reabsorption to the same extent as observed by Spitzer and Windhager (24) when they perfused peritubular capillaries with protein-free solutions.

There are several possible mechanisms whereby changes in oncotic pressure can influence tubular reabsorption in the absence of a capillary network. Bressler (19) suggested the possibility that the oncotic forces acted directly across the tubular epithelium influencing the passive reabsorption of salt and water. Giebisch, Klose, Malnic, Sullivan, and Windhager (30) and Kashgarian, Mitchel, and Epstein (31) could not demonstrate any effect of the addition of protein to the intratubular fluid on the rate of reabsorption. These studies constitute evidence against Bressler's hypothesis. However, it is conceivable that under these experimental protocols small changes in reabsorption might not be detectable by the split-drop techniques.

An alternative hypothesis is that the protein in the bathing medium exerts its oncotic pressure across the tubular basement membrane, and consequently influences the volume of the basal labyrinth and intercellular compartments. A hypooncotic bath would retard the transfer of reabsorbate out of these areas causing dilatation of these spaces, while a hyperoncotic bath would enhance reabsorption and cause further collapse of these spaces. In order for the system to behave in this fashion, the tubular basement membrane would have to have the following properties: (*a*) a high hydraulic conductivity so that the movement of water can be driven by small hydrostatic and oncotic pressure differences, (*b*) essentially zero reflection coefficient for dissolved crystaloids so that small concentration gradients of these substances do not nullify the hydrostatic and oncotic pressure gradients, and (*c*) a finite reflection coefficient for plasma proteins so that their presence in the outside fluid will generate an effective osmotic pressure. Recent studies of Welling and Grantham (32) indicate that isolated tubular basement membrane when stripped of its epithelial cells indeed does retard the penetration of albumin.

Lewy and Windhager (20) have suggested that the mechanism of isotonic proximal reabsorption is similar to the model proposed by Curran and MacIntosh (33) and extended to the gall bladder by Diamond and Bossert (34). According to this model, solute is transported by the tubular epithelium into the poorly mixed third compartment and generates a local osmotic gradient which promotes bulk flow of water. The solution of salt and water is then forced out of this interspace across the tubular basement membrane by a small hydrostatic pressure gradient. Lowering the protein in the outside solution, for example, would exert its regulatory effect primarily by retarding the transfer process across the basement membrane. As a secondary consequence, the volume of the intercellular space and basilar labyrinth are increased. Lewy and Windhager (20) have suggested that expansion of these interspaces might reduce reabsorption by one of three mechanisms: (*a*) inhibition of the active sodium pump, (*b*) increased sodium permeability at the site

of the active pump, thereby increasing the back-leak, and (*c*) increased hydrostatic pressure augmenting the passive back-flux of solute and water through the junction of the intercellular space.

The present studies provide some insight as to which of these mechanisms might be operative. Although the possibility of a direct effect on an active sodium pump was not examined in detail, we did observe that hypooncotic decreased and hyperoncotic increased reabsorption but had no effect on transmembrane potential difference. Studies of Burg and Orloff (28) and Kokko and Rector (35) indicate that the transmembrane potential difference in the isolated tubule is in fact a transport potential which is reversibly reduced by both ouabain and cooling (35). The failure of hypo- and hyperoncotic bath to have any detectable effect on the transmembrane potential differences is suggestive, but admittedly not conclusive, evidence against an effect on the active transport process.

To determine whether hypooncotic bath might alter passive sodium influx either by increasing the specific Na permeability of the membrane or opening channels for the back-leak of sodium and water, we examined permeability of sodium from bath to luminal fluid. The $P_{Na}$ determined by this manner was comparable to that obtained when the flux of $^{22}Na$ was measured from lumen to bath (Table II). Despite the relatively high permeability to Na and importance of interspace to the over-all process, alteration of bath oncotic pressure had no measurable effect on $P_{Na}$ (Table II). These results, therefore, fail to provide any direct evidence in support of the hypothesis that changes in oncotic pressure exert their effect on the back-leak of Na from interstitium into tubular lumen. However, they do not exclude this possibility, since the unidirectional back-flux of Na is 4–5 times greater than net Na reabsorption (Table II), so that small changes in the back-leak might produce significant changes in net reabsorption without detectably altering the unidirectional influx of $^{22}Na$.

Because of the high sodium permeability relative to the magnitude of net reabsorption, we examined the effect of alterations of oncotic pressure on the permeability of two less permanent solutes, urea and sucrose. Our studies revealed two important differences in the permeability of these solutes. First, $P_{urea}$ was approximately 1/2 $P_{Na}$, but approximately 10-fold greater than $P_{sucrose}$ (Table II). Second, lowering oncotic pressure of the bath had no effect on urea permeability, but markedly increased $P_{sucrose}$. If one considers that sucrose has a volume distribution limited to extracellular space (inulin space), then these marked differences between $P_{urea}$ and $P_{sucrose}$ might be attributed to different paths of permeation. The major path for sucrose is probably paracellular, through the tight junction, whereas urea is distributed throughout total tissue water and, therefore, its major pathway of transmembrane penetration is through the cells in addition to the paracellular pathways. The fact that only $P_{sucrose}$ was increased by the hypooncotic bath, suggests that variation in oncotic pressure influences net reabsorption by increasing and decreasing the backflow of reabsorbate from interstitial space to lumen via the tight junction. If the concentration of sucrose in bath media and interstitial space were the same, one would predict that with hypooncotic bath the increase in clearance of sucrose into the lumen, as shown in Fig. 4 and Table III, should equal the decrease in net reabsorption. This is clearly not the case; the decrease in net reabsorption was two times greater than the increase in the clearance of sucrose. This discrepancy might be explained in one of two ways. One possibility is that some process other than back-flow of reabsorbate via the tight junction was contributing to reduction in net reabsorption. An alternative, and more likely possibility, is that the sucrose concentration in the interstitium is lower than in the bath. This would result from the fact that the simultaneous process of reabsorption from lumen to interstitium through the paracellular space is exerting a convective force which prevents sucrose from achieving equilibrium between the paracellular space and the bath.

These studies therefore indicate that in perfused isolated tubules lowering the protein concentration in bath inhibits and raising protein concentration in bath enhances net reabsorption of salt and water without changing transmembrane potential differences or permeability coefficients for urea and sodium. The permeability of sucrose, which is used as an index of paracellular pathway permeability, is increased with hypooncotic bath. These data are best explained by the following model. Salt and water reabsorbed by tubular epithelium enter the intercellular space and are driven across the tubular basement membrane into the bath by a combination of hydrostatic and oncotic pressures acting across this membrane. Lowering the oncotic pressure in the bath reduces the driving force across the tubular basement membrane, causing expansion of the interspace and possibility a slight increase in its hydrostatic pressure. As a consequence of these changes a fraction of reabsorbate is driven back into the lumen through the tight junction. Raising the oncotic pressure in the bath produces the reverse sequence of these events.

The foregoing model might be criticized on the basis that previously determined osmotic water permeabilities for the proximal convoluted tubule (27, 32, 36) are so low that the protein concentration gradients cannot exert their effect simply by osmotic forces. Indeed, re-

cent studies of Welling and Grantham (32) have demonstrated that the basement membrane has a hydraulic conductivity several orders of magnitude greater than the intact tubular membrane. Thus it might be argued that fluid transported into the lateral intercellular spaces should simply reflux through the basement membrane with only a small fraction returning back into the lumen. Thus, for the presented model to be operative the hydraulic resistance of the "tight junction/lateral intercellular space complex" must decrease, presumably because of dilatation of the intercellular spaces. In support of this are the findings that sucrose permeability increases twofold when protein-free bath is used. In addition, this model is consistent with recent electrophysiological studies of Windhager, Boulpaep, and Giebisch (37) and Boulpaep (38) who were able to show the presence of a low-resistance pathway around the cells, and that the resistances of this pathway were further reduced in saline diuresis.

The present studies clearly indicate that protein concentration gradient can act across the tubular basement membrane without interposed capillary network. These studies, however, do not exclude the possibility that in the intact kidney the alteration of peritubular oncotic pressure exerts its effects primarily across the capillary basement membrane altering the interstitial pressure, and that changes in interstitial pressure might secondarily influence the transfer of fluid across the tubular membrane, the size of interspace, and back-flow across the tight junction.

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