Influence of peritubular protein on solute absorption in the rabbit proximal tubule. A specific effect on NaCl transport.

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Research Article

The effect of removal of peritubular protein on the reabsorption of various solutes and water was examined in isolated rabbit proximal convoluted tubules (PCT) perfused in vitro. In 22 PCT perfused with ultrafiltrate (UF) and bathed in serum, volume absorption (Jv) was 1.44 nl/mm per min and potential difference (PD) was -3.6 mV. When these same PCT were bathed in a protein-free UF, Jv was reduced 38% without a change in PD. Simultaneous measurements of total CO2 net flux (JTCO2) and glucose efflux (JG) showed that less than 2% of the decrease in JV could be accounted for by a reduction in JTCO2 and JG, suggesting that removal of peritubular protein inhibited sodium chloride transport (JNaCl). Therefore, in eight additional PCT, JNaCl was measured, in addition to PD, Jv, JG, and JTCO2. In these PCT, the decrease in total solute transport induced by removal of bath protein was 201.7 +/- 37.5 posmol/mm per min. JG decreased slightly (9.1 +/- 3.9 posmol/mm per min); NaHCO3 transport did not change (9.2 +/- 6.6 posmol/mm per min); but JNaCl decreased markedly (160.6 +/- 35.7 posmol/mm per min). 80% of the decrease in Jv could be accounted for by a decrease in JNaCl. In 13 additional PCT perfused with simple NaCl solutions, a comparable decrease in Jv and JNaCl was observed when peritubular protein […]

Influence of Peritubular Protein on Solute Absorption in the Rabbit Proximal Tubule

A SPECIFIC EFFECT ON NaCl TRANSPORT

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ABSTRACT The effect of removal of peritubular protein on the reabsorption of various solutes and water was examined in isolated rabbit proximal convoluted tubules (PCT) perfused in vitro. In 22 PCT perfused with ultrafiltrate (UF) and bathed in serum, volume absorption (J_v) was 1.44 nl/mm per min and potential difference (PD) was −3.6 mV. When these same PCT were bathed in a protein-free UF, J_v was reduced 38% without a change in PD. Simultaneous measurements of total CO_2 net flux (J_{TCO_2}) and glucose efflux (J_G) showed that <2% of the decrease in J_v could be accounted for by a reduction in J_{TCO_2} and J_G, suggesting that removal of peritubular protein inhibited sodium chloride transport (J_{NaCl}). Therefore, in eight additional PCT, J_{NaCl} was measured, in addition to PD, J_v, J_G, and J_{TCO_2}. In these PCT, the decrease in total solute transport induced by removal of bath protein was 201.7±37.5 posmol/mm per min. J_G decreased slightly (9.1±3.9 posmol/mm per min); $NaHCO_3$ transport did not change (9.2±6.6 posmol/mm per min); but J_{NaCl} decreased markedly (160.6±35.7 posmol/mm per min). 80% of the decrease in J_v could be accounted for by a decrease in J_{NaCl}. In 13 additional PCT perfused with simple NaCl solutions, a comparable decrease in J_v and J_{NaCl} was observed when peritubular protein was removed without an increase in TCO_2 backleak.

In summary, removal of peritubular protein reduced J_v and J_{NaCl}, but did not significantly alter PD, J_G, J_{TCO_2}, or TCO_2 backleak. The failure to inhibit J_G and J_{TCO_2}, known sodium-coupled transport processes, indicates that protein removal does not primarily affect the Na-K ATPase pump system. Furthermore, since PD and TCO_2 backleak were not influenced, it is unlikely that protein removal increased the permeability of the paracellular pathway. We conclude that protein removal specifically inhibits active transcellular or passive paracellular NaCl transport.

INTRODUCTION

Numerous investigators have observed that lowering the protein oncotic pressure of the peritubular capillary blood in vivo or of the bath solution in vitro reduces net volume absorption from proximal convoluted tubules (PCT)[1] (1–9). Volume absorption from the glomerular ultrafiltrate (UF) however, involves the transport of a variety of solutes. Proximal reabsorption occurs in two phases (10, 11). In the first phase, neutral organic solutes, such as glucose, and non-chloride sodium salts, such as sodium bicarbonate, are almost completely removed by specific active transport processes. In the second phase, an essentially pure sodium chloride solution is removed. Since most previous studies on the effect of reduction of peritubular protein only measured the change in the concentration of the volume marker, inulin, the precise nature of the transported solute(s) affected by peritubular protein has been uncertain.

Several mechanisms whereby peritubular protein mediates the depression in fluid absorption have been postulated (12–14), but the most widely accepted view is the so-called Paracellular Backflux Hypothesis (1, 4, 13). According to this view, the regulatory effect of

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[1] *Abbreviations used in this manuscript:* PCT, proximal convoluted tubule; ECF, extracellular fluid; UF, ultrafiltrate; PD, potential difference; J_G, glucose efflux; J_v, volume absorption; J_{TCO_2}, total CO_2 net flux; J_{Cl}, chloride net flux; J_{NaCl}, sodium chloride net flux; J_{osm}, osmolar net flux; J_{NaHCO_3}, sodium bicarbonate net flux.

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protein operates by changing the generalized passive backleak of solutes through the paracellular pathway of the proximal tubule without affecting active transcellular transport. The Paracellular Backflux Hypothesis predicts that reductions in peritubular protein result in an ubiquitous and nonspecific inhibition of proximal reabsorption (15). In separate clearance studies, reductions in peritubular protein concentration by extracellular fluid (ECF) expansion reduce the reabsorption of bicarbonate (16, 17), glucose (18–20), and Ca^{++} and Mg^{++} (21). Although inhibition of proximal transport is assumed, these clearance studies do not define the nephron segment affected. In fact, in a recent micropuncture study which directly assessed proximal function, no inhibitory effect of ECF expansion on proximal bicarbonate reabsorption could be discerned (22).

The purpose of the present in vitro microperfusion study was to examine the effect of lowering peritubular oncotic pressure by removal of bath protein on the transport of volume, glucose, sodium bicarbonate, and sodium chloride in PCT. Using this technique, the tubule segment is clearly defined and the luminal flow rate can be controlled. Our results showed that removal of peritubular protein reduced volume absorption (J_v) from a UF solution by 38% and from a NaCl solution by 60%. Protein removal did not alter potential difference (PD) or the net reabsorption or the passive backleak of total CO_2 (TCO_2); it only slightly reduced the lumen to bath flux of glucose, but it dramatically inhibited the net reabsorption of NaCl. The change in NaCl flux can account for 80% of the change in J_v from a UF solution and ~93% of the change from a NaCl solution. The failure of protein removal to significantly inhibit TCO_2 and glucose absorption, known sodium-coupled transport processes, suggests that this maneuver does not specifically inhibit sodium transport across the basolateral membrane. Moreover, the failure of protein removal to reduce PD or to increase TCO_2 backleak suggests that this maneuver does not increase the permeability of the paracellular pathway. We conclude that removal of peritubular protein specifically decreases NaCl transport without primarily inhibiting the Na-K ATPase pump system or altering the permeability of the paracellular pathway.

METHODS

Isolated segments of rabbit PCT were dissected and perfused as previously described (23–25). Briefly, kidneys from female New Zealand white rabbits were cut into slices in a plane perpendicular to the surface. Individual PCT were dissected in UF-like perfusion solution (complete solution, Table I, reference 24) to which 5% vol/vol bovine calf serum was added. PCT were identified as juxtamedullary if obtained from immediately above the corticomedullary junction, and as superficial if obtained from the remaining cortex. All tubules were perfused at 39°C in a 1.2-ml temperature controlled bath.

Experimental protocol. All tubules were studied during three periods: control, experimental, and recovery. In all periods, the perfusate was either UF or a NaCl solution. In the control and the recovery periods, the bath was protein-containing rabbit serum. In the experimental period, the bath was protein-free UF. In each period the PD and J_v were measured in addition to one or all of the following: glucose efflux (J_G), total CO_2 net flux (J_{TCO_2}), and chloride net flux (J_{Cl}). The control period began after a 30-min equilibration period at 39°C. Experimental and recovery periods were also preceeded by 30-min equilibration periods. At least two samples of ~100 nl each were obtained during each period and were transferred to a dish covered with water-equilibrated mineral oil. Using this technique, measurements of J_v, J_G, J_{TCO_2}, and J_{Cl} could be calculated from measurements in the same sample.

Composition of solutions. The bath solution used in all experiments was either rabbit serum (Irvine Scientific, Santa Ana, Calif.) or a protein-free UF of rabbit serum. The UF was prepared using low pressure and an ultrafiltration membrane (UM-10 Amicon Corp. Scientific Sys. Div., Lexington, Mass.) at 5°C. The composition of UF and serum are shown in Table I. The perfusion solutions used in all experiments were either UF or an essentially NaCl solution. High Cl^--low HCO_3^- perfusate (for composition of solution see Table II) resembles late PCT fluid and was used to examine the effect of protein on NaCl absorption alone. High Cl^--no HCO_3^- perfusate (for composition of solution, see Table II) was used to examine the effect of protein on bicarbonate backleak. In all experiments the osmolality of each perfusate and bath solution was identical.

Measurements of PD, J_v, J_{TCO_2}, J_G and J_{Cl}. The transepithelial potential difference due to active ion transport (PD, mV) was measured using the perfusion pipette as a bridge into the tubular lumen as previously described (25). The perfusion and bath solutions were connected to their respective calomel electrodes via a bridge containing a protein-free UF of rabbit serum in series with a 3.6 M KCl/0.9 M KNO_3 agarose bridge. The interposition of bridges containing a protein-free UF of rabbit serum has two purposes: (*a*) it avoided direct contact of KCl/KNO_3 agarose bridges with solutions bathing PCT and (*b*) it eliminated the measurement of the Donnan potential when the bath solution was rabbit serum. Elimination of the measurement of the Donnan potential is essential for the interpretation that the present measurements of PD reflect active ion transport. When the perfusion and bath solutions are a protein-free UF of serum, the liquid junction potential at the bath UF-KCl/KNO_3 agarose bridge is equal and opposite to the liquid junction potential at the perfusate-KCl/KNO_3 agarose bridge, and it is clear that the PD measured is due only to active ion transport. On the other hand, when the bath solution is protein-containing rabbit serum, the measured PD is the sum of the PD due to active ion transport, the Donnan potential across the PCT, and the liquid junction potential between the protein-containing rabbit serum and the protein-free UF bridge. We have previously (24, footnote 1) calculated that the Donnan potential and this liquid junction potential are +1.49 and −1.42 mV. Thus, when the PCT is bathed in rabbit serum and the protein-free UF bridge is interposed between the serum and the KCl/KNO_3 agarose bridge, the Donnan potential is essentially cancelled by the liquid junction potential at the serum-UF interface and the measured PD accurately reflects the true active transport PD.

Net volume absorption (J_v, nl/mm per min) was measured as the difference between the perfusion (V_o, nl/min) and collection (V_L, nl/min) rates, normalized per millimeter of tubule length. Exhaustively dialyzed (22) [*methoxy*-^{3}H]inulin (New England Nuclear, Boston, Mass.) was added to the perfusate

at a concentration of ~50 μCi/ml so that V_0 could be calculated. The collection rate was measured using an ~100-nl constant volume pipette. Collection rate was maintained approximately constant; therefore, small variations in perfusion rate are expected when volume absorption changes. The length (L, mm) of the tubule perfused was measured by eyepiece micrometer.

The net total CO_2 flux (J_{TCO_2}, pmol/mm per min) was calculated as the difference between the amount of TCO_2 delivered and the amount collected according to the balance equation:

$$J_{TCO_2} = \frac{V_0C_0 - V_LC_L}{L} \quad (1)$$

where C_0 and C_L (mmol/liter) represent the concentrations of total CO_2 in the perfused and the collected fluid, respectively. CO_2 measurements of perfused and collected fluid were performed by microcalorimetry (picapnotherm), as described (26). Within the physiologic pH range, the TCO_2 in a sample represents bicarbonate plus dissolved CO_2.

The glucose transport rate from lumen to bath (J_G, pmol/mm per min) was calculated as the difference between the amount of glucose delivered and the amount collected according to the balance equation:

$$J_G = \frac{V_0C_0^* - V_LC_L^*}{L} \frac{[G_0]}{C_0^*} \quad (2)$$

where C_0^* and C_L^* (cpm/nl) represent the concentrations of [^{14}C]glucose (Amersham Corp., Arlington, Ill.) in the perfused and collected fluid, respectively, which was added at a concentration of ~50 μCi/ml and $[G_0]$ (mmol/liter) is the chemical concentration of glucose in the perfusion fluid. $[G_0]$ was measured using a modification of the enzymatic technique of Barthelmai and Czok (27) (Calbiochem Glucose Stat-Pack, Calbiochem-Behring Corp., American Hoechst Corp., San Diego, Calif). Eq. 2 assumes that no significant changes occur in the specific activities of the isotopic glucose between the perfused and collected solutions. This assumption is supported by the observations of Dennis and Brazy (28) who found that the glucose backflux from bath to lumen is small, ~10% of the lumen to bath.

The net chloride flux (J_{Cl}, peq/mm per min) was calculated as the difference between the amount of chloride delivered and the amount collected according to the balance equation:

$$J_{Cl} = \frac{V_0C_0 - V_LC_L}{L} \quad (3)$$

where C_0 and C_L (meq/liter) represent the concentration of chloride in the perfused and collected fluid, respectively. Chloride measurements of perfused and collected fluid were performed by the electrometric titration technique of Ramsay (29).

Statistics. There were two to three measurements of each parameter in a given period for a given tubule. The mean values for individual periods in an individual tubule were used to calculate the mean value for that period. Data are expressed as mean±SEM. Control and experimental observations were always made in the same tubule; therefore, *P* values for the paired differences were computed and compared with zero using the paired *t* test. Significance was accepted at the 0.05 level.

RESULTS

Effect of peritubular protein removal of J_v and PD in PCT perfused with UF. The effect of removal of peritubular protein on J_v was examined in 13 superficial PCT and in 9 juxtamedullary PCT. As seen in Fig. 1, replacement of the serum bath solution with protein-free UF resulted in a marked decrease in J_v in all PCT studied. Fig. 1 also shows that there was no obvious difference between superficial and juxtamedullary PCT in their response to removal of bath protein. Therefore, all subsequent data are reported without distinguishing between the two nephron populations. Table III, row 2 shows that J_v was reduced from 1.41 nl/mm per min in the control period to 0.89 nl/mm per min in the experimental period. In the recovery period, protein was returned to the bath solution and J_v returned to 1.46 nl/mm per min, indicating that the effect of protein of J_v was reversible. The average J_v in the presence of bath protein was 1.44 nl/mm per min. Thus, removal of bath protein inhibited J_v by 38%. This degree of inhibition agrees well with results of previous investigators who have reported inhibition of 30–50% (5, 6, 8, 9).

Data for PD for these same PCT are given in Table III, row 1. In agreement with our previous results (24)

TABLE I
Composition of Ultrafiltrate and Serum

	Na^+	K^+	Cl^-	TCO_2*	Glucose	Osmolality
	meq/liter			*mmol/liter*		*mosmol/kg*
UltraFiltrate	142.4±1.2 (9)	4.5±0.2 (6)	110.6±1.2 (14)	26.3±1.1 (12)	6.6±0.3 (25)	305.9±1.6 (22)
Serum	142.2±1.2 (9)	5.0±0.2 (6)	100.6±1.2 (14)	22.9±0.2 (5)	—	304.8±1.7 (31)
Plasma H_2O‡	151.4	5.3	106.6	24.4	—	—

* Solutions bubbled with a CO_2-O_2 gas mixture to give an average pCO_2 of 40 mm Hg.
‡ Calculated using an average serum protein concentration of 6.1 g/dl.

TABLE II
*Composition of Artificial Perfusion Solutions**

	High Cl^- low HCO_3^-‡	High Cl^- no HCO_3^-§
	mM	*mM*
Salts		
NaCl	146.5	155.5
$NaHCO_3$	5	—
Na_2HPO_4	4	2
$CaCl_2$	1	1
$MgSO_4$	1	1
KCl	5	5
Organic solutes		
Urea	5	5
Other		
Acetazolamide	—	0.1

* All solutions bubbled with a CO_2-O_2 gas mixture to give an average bath pCO_2 of 40 mm Hg.
‡ The measured perfused concentration of Cl^- was 148.2±1.2 meq/liter (8); the measured perfused concentration of TCO_2 was 6±0.6 mmol/liter (5).
§ The measured perfused concentration of Cl^- was 156.7±3.5 meq/liter (5); the measured perfused concentration of TCO_2 was 0.6±0.1 mmol/liter (5).

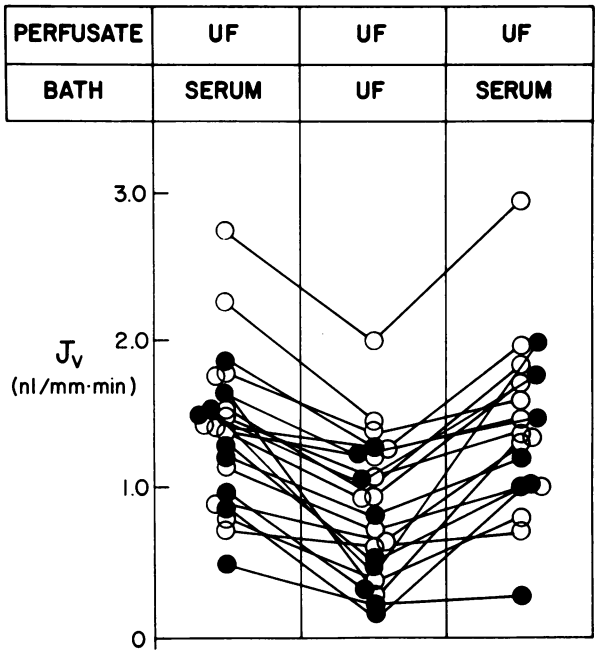

FIGURE 1 Effect of bath protein on volume absorption. Volume reabsorptive rates (J_v) for individual superficial (open circles) and juxtamedullary (filled circles) PCT are plotted during the control, experimental, and recovery periods. Mean data are given in Table III.

and those of other investigators (6, 8, 9) we did not observe a significant change in PD upon removal of peritubular protein. PD was −3.3 mV in the control period and −3.7 mV in the experimental period. These data are not significantly different. In fact, PD continued to increase in the recovery period to −3.9 mV, suggesting a time-dependent increase in PD rather than an effect of protein. The average PD was −3.6 mV in the presence of protein and −3.7 mV in the absence of protein.

For the 22 tubules in this series the mean tubule length was 1.2±0.1 mm; and the mean perfusion rates were 16.7±1.0, 16.0±0.9 and 16.3±1.0 nl/mm during the control, experimental, and recovery periods, respectively.

Effect of peritubular protein removal on J_G and J_{TCO_2} in PCT perfused with UF. Removal of peritubular protein reduced J_v without changing PD (Table III, 6, 8, 9). In the initial portions of a PCT perfused with UF there is preferential reabsorption of organic solutes, such as glucose, and of nonchloride sodium salts, such as sodium bicarbonate, by specific sodium-coupled active transport processes (10, 11). To evaluate whether protein removal influenced these processes, we measured glucose efflux and J_{TCO_2} in the same PCT that we measured J_v and PD. Data for J_G and J_{TCO_2} are given in Table III, rows 3 and 4.

Glucose efflux was 72.9 pmol/mm per min in the initial control period and decreased significantly to 64.2 pmol/mm per min during the experimental period. However, the value during the experimental and recovery periods was not significantly different ($P < 0.05$). It is not clear from these results whether the slight decrease from the initial control to the experimental period is the result of a time-dependent change in J_G, or an effect of protein removal. In any case, J_G averaged 70 pmol/mm per min in the presence of protein and 64.2 pmol/mm per min in the absence of protein. The inhibition of glucose reabsorption could account for only 2% of the inhibition of J_v observed in these same tubules. The control rates of glucose transport from lumen to bath agree well with those previously published, ~80 pmol/mm per min (28, 30).[2]

J_{TCO_2} was not significantly influenced by removal of peritubular protein. J_{TCO_2} averaged 83.6 pmol/mm per min in the presence of protein and 79.4 pmol/mm per min in the absence of protein (Table III). The control rates of net T_{CO_2} transport agree well with those previously published, ~80 pmol/mm per min (31, 32). We, however, did not observe a difference between superficial and juxtamedullary PCT as Jacobson (32) did.[3]

[2] We recognize that J_G is a unidirectional flux and thus does not represent net solute transport. However, Dennis and Brazy (28) have recently measured the bath to lumen flux of glucose and found it to be ~10% of the lumen to bath flux. This small amount can be considered negligible.

[3] Jacobson's superficial PCT had a J_{TCO_2} of 45 pmol/mm per min (32). We attribute the lower J_{TCO_2} in his studies to the fact that his PCT were obtained from the first 1–1.5 mm of the

TABLE III
Effect of Bath Protein on PD, J_v, J_G, and J_{TCO_2} in PCT Perfused with an Ultrafiltrate of Serum

	Control	Experimental	Recovery
PD (mV) (21)	−3.3±0.2	−3.7±0.3	−3.9±0.4
J_v (nl/mm per min) (22)	1.41±0.1	0.89±0.1‡	1.46±0.1
J_G (pmol/mm per min) (22)	72.9±6	64.2±4*	66.6±5
J_{TCO_2} (pmol/mm per min) (17)	81.1±8	79.4±7	86.0±8

* Mean paired difference from control significant at the 0.01 level.
‡ Mean paired difference from control significant at the 0.001 level.

Effect of peritubular protein removal on simultaneously measured J_v, J_G, J_{TCO_2}, and J_{Cl} in PCT perfused with UF. Removal of protein from the bath solution reduced volume absorption by 38% in PCT, slightly but significantly inhibited glucose efflux, but did not significantly inhibit J_{TCO_2} (Table III). The slight inhibition of glucose efflux could account for only 2% of the inhibition of volume absorption. These observations suggested that protein might be inhibiting NaCl reabsorption in PCT perfused with UF. Therefore, we measured J_v, J_G, J_{TCO_2}, and J_{Cl} in the same PCT.[4] Five superficial and three juxtamedullary PCT were examined, and as previously observed (Fig. 1), there were no differences between these two nephron populations. For the eight tubules in this series the mean length was 1.2±0.1 mm; the mean perfusion rate was 13.5±0.7, 12.8±0.8, and 13.9±0.9 nl/min during the control, experimental, and recovery periods, respectively.

Fig. 2 and Table IV show that, in accord with our previous results (Table III), removal of protein resulted in a marked depression in total solute osmolal flux (J_{osm})[4] without comparable changes in either glucose or $NaHCO_3$ osmolar fluxes. Total solute osmolar flux averaged 574.9 posmol/mm per min in the presence of peritubular protein and decreased markedly to 372.9 posmol/mm per min after removal of peritubular protein. Glucose osmolar flux decreased slightly, but significantly, from 68.6 to 60.5 posmol/mm per min. For the eight tubules shown in Fig. 3 the glucose osmolar flux during the experimental and recovery periods were significantly different ($P < 0.05$)[5] $NaHCO_3$ osmolar flux was not significantly affected by peritubular protein; it averaged 157.4 posmol/mm per min in the presence and 151.6 posmol/mm per min in the absence of protein. The reduction in J_{osm}, however, was mirrored by a substantial reduction in NaCl osmolar flux; it averaged 214.7 posmol/mm per min in the presence and 54.2 posmol/mm per min in the absence of protein.

Fig. 3 presents these data in terms of the average change in the osmolar transport rates (ΔJ_{osm}). Removal of peritubular protein reduced total osmolar absorption by 201.7±37.5 posmol/mm per min. The ΔJ_{osm} for glucose and $NaHCO_3$, were 9.1±3.8 and 9.2±6.6 pos-

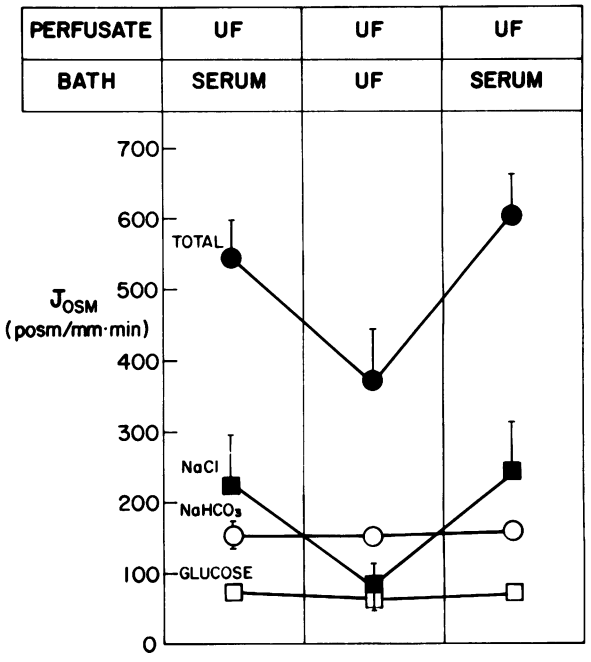

FIGURE 2 Effect of bath protein on solute osmolar fluxes. Mean osmolar fluxes (J_{osm}) for total solute (filled circles), for NaCl (filled squares), for $NaHCO_3$ (open squares), and for glucose (open circles) are plotted for the control, experimental, and recovery periods. These data and statistics are given in Table IV.

postglomerular nephron, whereas our PCT were beyond the first millimeter. Visual observation indicates that the first millimeter of superficial PCT have smaller luminal diameters and thus lower surface areas.

[4] To compare glucose, sodium bicarbonate, and J_{NaCl} the total solute flux absorbed, the data for this series of tubules is presented in terms of osmolal fluxes. For the purpose of comparison the osmotic coefficient for $NaHCO_3$ and NaCl is taken to be 2.0, and J_{TCO_2} is taken to be equal to the net absorption of bicarbonate. The mean osmolality of the perfusion solution for the eight tubules was 306.6±3.8 mosmol/kg H_2O. The total osmolar flux is calculated assuming isosmotic volume reabsorption.

[5] The recovery of J_G in the eight tubules is in contrast to the failure of recovery observed in the 22 tubules shown in Table III. The cause of the difference is unclear. When glucose effluxes in all tubules in the present manuscript are evaluated, the mean paired difference in J_G is significant ($P < 0.005$) between control and experimental periods, but not between experimental and recovery periods ($P > 0.05$). Therefore, a time-dependent decrease in J_G cannot be differentiated from a slight decrease in J_G induced by removal of peritubular protein.

TABLE IV
Effect of Protein on the Osmolar Flux of Total Solute, Glucose, $NaHCO_3$, and NaCl in PCT Perfused with an Ultrafiltrate of Serum

	Control	Experimental	Recovery
Total solute (posmol/mm per min) (8)	545.9±57	372.9±69§	603.8±58*
J_G (posmol/mm per min) (8)	68.6±7	60.5±6‡	67.4±7
J_{NaHCO_3} (posmol/mm per min) (8)	155.1±19	151.6±18	159.7±15
J_{NaCl} (posmol/mm per min) (8)	181.6±52	54.2±26§	247.8±54*

* Mean paired difference from control significant at the 0.05 level.
‡ Mean paired difference from control significant at the 0.01 level.
§ Mean paired difference from control significant at the 0.001 level.

mol/mm per min, respectively. The ΔJ_{osm} for glucose was significant ($P < 0.05$), whereas the ΔJ_{osm} for $NaHCO_3$ was not significant. On the other hand, fully 80% of this reduction in total solute transport can be accounted for by the ΔJ_{osm} for NaCl of 160.6±35.7 posmol/mm per min. These findings demonstrate that removal of peritubular protein specifically inhibits NaCl absorption.

Effect of peritubular protein removal on PD, J_v, and collected $[Cl^-]$ and $[TCO_2]$ in PCT perfused with high Cl^--low HCO_3^- solutions. We have observed that removal of peritubular protein from PCT perfused with UF resulted in a marked inhibition of volume absorption which was perhaps exclusively associated with a decrease in NaCl transport. We, therefore, examined the effect of protein removal on PD, J_v, and the collected anion concentrations in PCT perfused with high Cl^--low HCO_3^- solutions (for composition of solution, see Table II). This high Cl^--low HCO_3^- solution simulates fluid that normally exists in the late PCT where predominately NaCl absorption takes place (11). For the eight PCT in this series the mean tubule length was 1.2±0.1 mm; the mean perfusion rate was 12.5±0.4, 12.0±0.3, and 12.9±0.6 nl/min during the control, experimental, and recovery periods, respectively.

Table V shows PD, J_v, and the collected concentrations of chloride and TCO_2. Neither PD nor collected $[Cl^-]$ or $[HCO_3^-]$ were affected by removal of peritubular protein, however, J_v was dramatically reduced. J_v averaged 0.90 nl/mm per min in the presence of bath protein and 0.37 nl/mm per min in the absence of bath protein. Removal of bath protein inhibited J_v by 60%. Since the collected fluid anion concentrations did not change, almost all (93%) of the inhibition of volume can be accounted for by inhibition of NaCl absorption.

Effect of peritubular protein removal on J_v and collected $[Cl^-]$ and $[TCO_2]$ in PCT perfused with high Cl^--no HCO_3^- solutions plus acetazolamide. The observation that net J_{TCO_2} in PCT perfused with UF was not influenced by removal of bath protein does not necessarily rule out the possibility that removal of bath protein did not increase the bath to lumen flux of TCO_2.

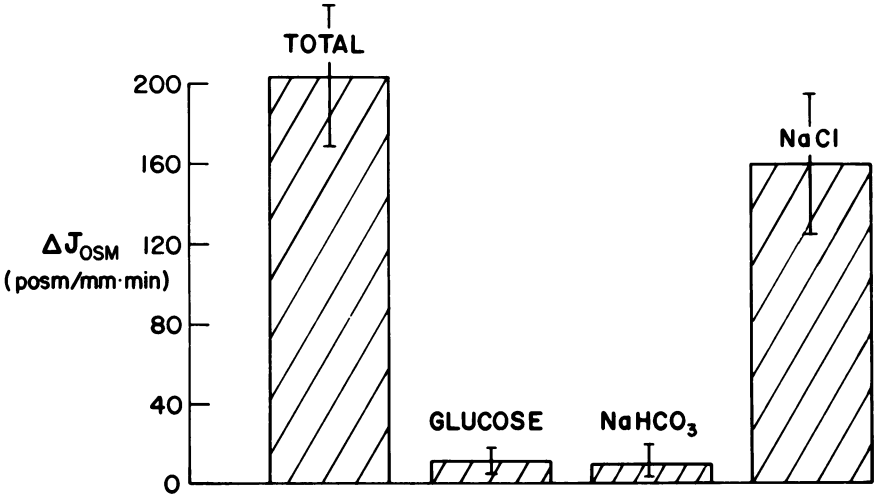

FIGURE 3 Change in solute fluxes induced by removal of bath protein. The change in the solute fluxes (ΔJ_{osm}) of glucose, $NaHCO_3$, and NaCl are compared with the total. At least 80% of the change in the total flux can be accounted for by the change in NaCl. These data and statistics are given in Table V.

TABLE V

Effect of Protein on PD, Volume Absorption, and Collected Concentrations of Chloride and T_{CO_2} in PCT Perfused with High Cl^--Low HCO_3^- Solutions

	Control	Experimental	Recovery
PD (mV) (8)	+1.6±0.6	+1.0+0.8	+1.4+0.7
J_v (nl/min per min) (8)	0.83±0.09	0.37±0.18*	0.96±0.16
Collected [Cl^-] (meq/liter) (8)	147.2±1.9	147.0±1.6	150.6±1.9
Collected [TCO_2] (mmol/liter) (8)	5.3±0.6	5.4±0.5	4.5±0.3

* Mean paired difference from control significant at the 0.01 level.

It is possible that increased lumen to bath active transport might have off-set an increased bath to lumen flux. To examine the possible effect of removal of bath protein on the bath to lumen flux of TCO_2, five PCT were perfused with a high Cl^--no HCO_3^- solution (Table II) and bathed in rabbit serum. 0.1 mM acetazolamide was added to both the perfusion and the bath solution to inhibit active absorption of TCO_2 (31), but not volume or chloride absorption. J_v and collected [Cl^-] and [TCO_2] were measured. In this series of tubules the mean length was 1.1±0.1 mm; the mean perfusion rate was 18.8±1.1, 17.8±1.1, and 20.3±1.7 nl/min during the control, experimental, and recovery periods, respectively.

Table VI shows that removal of protein significantly inhibited J_v, but not the collected [Cl^-] or [TCO_2]. The chloride net fluxes were 120.8±42.7, 60.9±50.6, and 161.1±40.4 peq/mm per min during the control, experimental, and recovery periods, respectively. The J_{Cl} in the absence of protein was significantly less ($P = 0.001$) than in the presence of protein. In contrast, there was no significant difference between periods in the TCO_2 backflux.

Our observation that the collected TCO_2 averaged 1.2 mM in the presence of bath protein and 1.0 mM in the absence of bath protein is consistent with passive equilibration of dissolved CO_2, assuming a bath pCO_2 of 40 mm Hg, and with a negligible passive bicarbonate ion permeability coefficient. However, Holmberg et al. (33) have recently reported a bicarbonate ion permeability coefficient for the rabbit PCT of 2.2±0.3 10^{-5} cm/s. Such a coefficient would have predicted an additional increase in the collected concentration of TCO_2 of ~1 mM attributable to bicarbonate ion permeability. This difference in the apparent bicarbonate ion permeability coefficient might be real or it might be due to differences in experimental design. The present study utilized faster perfusion rates (19 nl/min) making the determination of J_{TCO_2} less accurate and the studies were performed in the presence of active sodium transport; whereas the studies of Holmberg et al. (33) utilized slower perfusion rates (7–10 nl/min) and inhibited active sodium transport. In any case, the data in Table VI indicate that the absolute magnitude of TCO_2 backleak in the rabbit PCT is small and unaffected by alterations in peritubular protein concentration.

DISCUSSION

In the present studies we have observed that removal of peritubular protein reduces J_v, does not change PD, TCO_2 absorption or TCO_2 backflux, slightly reduces J_G, and markedly inhibits chloride absorption. Approximately 80–90% of the reduction of J_v induced by removal of peritubular protein can be attributed to a specific inhibition of net NaCl absorption. These observations must be considered in the context of the currently accepted view of the mechanism whereby peritubular protein affects proximal J_v, the so-called Paracellular Backflux Hypothesis (1, 4). According to this hypothesis, active transport of solutes and water is constant and the modulation of net reabsorption induced by changes in peritubular protein is ascribed to variations in the nonspecific leak of the transported solutes back into the lumen. The following discussion will consider the present observations in relation to the effect of peritubular protein removal on active sodium transport and on paracellular permeability. The data obtained in vitro where peritubular protein removal is the experimental maneuver, are compared to that obtained in vivo, where ECF expansion is the experimental maneuver. Possible models for the mechanism of the effect of peritubular protein on volume and NaCl absorption are considered.

Effect of peritubular protein removal on active sodium transport. In proximal tubules perfused with

TABLE VI

Effect of Protein on Volume Absorption, and Collected Concentrations of Chloride and T_{CO_2} in PCT Perfused with No HCO_3^- Solutions plus Acetazolamide

	Control	Experimental	Recovery
J_v (nl/mm per min) (5)	0.81±0.2	0.16±0.2*	1.01±0.3
Collected [Cl^-] (peq/liter) (5)	154.8±2.9	154.4±2.4	156.8±3.3
Collected [TCO_2] (mmol/liter) (5)	1.6±0.7	1.0±0.4	0.8±0.4

* Mean paired difference from control significant at the 0.001 level.

UF there is preferential reabsorption of neutral organic solutes and sodium salts of bicarbonate, acetate, phosphate, citrate and lactate. Recent evidence indicates that virtually all of these preferentially reabsorbed solutes and anions are transported from the tubular lumen into the cell by specific sodium-coupled transport processes (34–38). The sodium-carrier complex derives the energy to cross the brush border membranes from the electrochemical gradient for sodium ions. The low intracellular sodium concentration is maintained by the Na-K ATPase pump system located in the basolateral cell membrane. Inhibition of Na-K ATPase pump system increases cell sodium and reduces sodium-coupled transport processes (31).

In the present studies we used glucose efflux and $NaHCO_3$ absorption as indices of sodium-coupled transport processes. We found that $NaHCO_3$ absorption was not significantly reduced by removal of peritubular protein and that although glucose efflux was slightly reduced, the effect could only account for 2% of the reduction in J_v. Furthermore, a time-dependent decrease in J_G could not be ruled out. The failure of peritubular protein removal to inhibit substantially J_G and J_{TCO_2} indicates that the reduction in volume absorption observed during this maneuver is probably not the consequence of a generalized increase in cellular sodium concentration and thus of a primary inhibition of the Na-K ATPase pump system. However, the data presented do not rule out some degree of inhibition of the Na-K ATPase pump system.

The effect of protein removal on the permeability of the paracellular pathway. The Paracellular Backflux Hypothesis proposes that the regulatory effect of protein operates by changing the passive backleak of solutes through the paracellular pathway without affecting active transcellular transport. Our observations that protein removal does not alter J_G or J_{TCO_2} are consistent with the view that active sodium transport is not primarily affected and, therefore, agree with one of the major tenets of the Paracellular Backflux Hypothesis. The other major tenet of the Paracellular Backflux Hypothesis predicts that decreased peritubular protein should increase paracellular permeability properties (4, 13).

Evidence supporting increased paracellular permeability induced by the lowering of peritubular protein concentration however, has not been universally observed. The available data from in vivo preparations, such as the rat and the *Necturus* PCT, and the rat intestine, where ECF expansion is the experimental maneuver, strongly support an increase in paracellular permeability properties. Many indices of paracellular permeability change with a reduction in peritubular protein in the direction expected. For instance, nonelectrolyte and sodium permeability increase (4, 39); PD and electrical resistance decrease (4, 13, 40), and lateral intercellular space width increases (41).

In contrast, most of the available data in the in vitro rabbit PCT, where removal of peritubular protein is the experimental maneuver, do not support an increase in paracellular permeability properties. For instance, sodium permeability (6), PD (Table III and references 6, 8, 13), and the relative sodium to chloride permeability ratio (24) do not change; sucrose permeability increases (6), but this has been attributed to solute-solvent interaction rather than to change in permeability (8); lateral intercellular space width actually decreases (14).

In the present studies we used PD and TCO_2 backleak as indices of paracellular permeability. We found that PD was not significantly reduced by protein removal and that TCO_2 backleak was not significantly increased. The observation that TCO_2 backleak was not altered is direct evidence that protein removal did not result in a nonspecific increase in paracellular permeability. Since PD in proximal tubules is the net effect of active transcellular sodium transport and paracellular resistance, the observations that both PD and active sodium transport are constant suggest that paracellular resistance is not affected by removal of peritubular protein. The results of the present study, suggesting no effect of protein on paracellular permeability properties, are in agreement with other studies on the in vitro rabbit PCT, but not with those on the in vivo PCT.

The discrepancy between the in vivo and the in vitro changes in paracellular permeability properties questions the role of the alterations in paracellular pathway in the decrease in J_v following reductions in peritubular protein concentration. A possible explanation for this discrepancy is that some factor present in vivo, but not in vitro, is responsible for the alteration of paracellular permeability properties observed in vivo, but that the observed changes in paracellular permeability are not responsible for the changes in J_v. The most likely candidate for this factor would be increased peritubular hydrostatic pressure. During free-flow in vivo, ECF expansion increases peritubular hydrostatic pressure (42) and lateral intercellular space width (41). Such changes in paracellular geometry could easily influence paracellular permeability (43, 44). In contrast, in the in vitro rabbit PCT there are no changes in peritubular hydrostatic pressure, and lateral intercellular space width actually decreases slightly following protein removal (14). However, the change in width is small, from 333±36 to 260±38 Å, and may not be sufficient to alter paracellular permeability. Whatever the cause of the discrepancy between in vivo and in vitro effects of protein removal on paracellular permeability, both experimental models show a reduction in volume absorption. The reduction of volume absorption in vitro,

however, does not appear to be due to an alteration in paracellular permeability.

Possible mechanism of action of peritubular protein. Removal of peritubular protein inhibited NaCl and water reabsorption without significantly influencing PD, J_G, J_{TCO_2}, and TCO_2, backleak (Tables III, IV, and Fig. 3). These observations suggest that peritubular protein removal does not primarily inhibit the Na-K ATPase pump system nor alter paracellular permeability properties. Other mechanisms by which peritubular protein modulates net NaCl and water absorption in the in vitro rabbit PCT must, therefore, be postulated.

The protein concentration in the basal infoldings and lateral intercellular spaces in the rabbit PCT probably reflects the bath protein concentration, since the basement membrane in the in vitro rabbit PCT is highly permeable to protein (45). Lateral intercellular space protein might affect NaCl and water absorption by modulating either a passive paracellular or an active transcellular NaCl transport mechanism.

There are two possible types of passive NaCl transport which might be affected by protein. First, protein might exert a direct effect on volume flow across the junctional complexes, as suggested in 1843 by Ludwig (46) and more recently by Bresler (47, 48) and Schnermann (12). For protein to affect the flow of a NaCl solution across junctional complexes it is necessary that the junctional complexes have a high hydraulic conductivity, a low reflection coefficient for NaCl, and a high reflection coefficient for other solutes. At present there is no data addressing any of these issues. The principle argument against a direct effect of protein across junctional complexes is the observation that addition of protein to the luminal solution does not influence J_v (7, 8, 49). Other studies (12, 50), however, find that luminal protein dramatically inhibits J_v. Second, protein might influence the passive component of anion gradient-dependent NaCl transport (10, 11). This mode of NaCl transport accounts for 30–60% (10, 11, 51) of the NaCl transport observed when PCT are perfused with a high Cl^--low HCO_3^- solution (Table II) and bathed in rabbit serum. The passive component of anion-gradient-dependent NaCl transport has been attributed to both diffusive and convective transport mechanisms. For protein to affect diffusive NaCl transport, either paracellular resistance or the relative sodium-to-chloride permeability ratio (P_{Na}/P_{Cl}) must be altered by protein. The present studies suggest that resistance is not affected by protein, and previous studies (24) show that P_{Na}/P_{Cl} is not affected. For protein to influence convective NaCl transport, the reflection coefficient for NaCl or $NaHCO_3$ must be altered. Although there are no data on the influence of protein on PCT reflection coefficients, the recent observation (52) that the reduction in volume and NaCl absorption induced by lowering of peritubular protein concentration in vivo can be observed when anion gradients are abolished suggests that anion-gradient NaCl transport is not the transport mechanism altered by peritubular protein removal.

In addition, there are two possible types of active transcellular NaCl transport which might be affected by protein. First, protein might exert its action on volume flow at the lateral cell membrane where cellular channels communicate with lateral intercellular spaces, as suggested by Imai and Kokko (8) and Tisher and Kokko (14). In order for protein to affect the flow of a NaCl solution across intracellular channels, there must be a specific NaCl transport process into these channels and other solutes must be excluded. Without protein, the channels would expand promoting a localized backleak of NaCl into the cell cytoplasm. In essence, this model would behave similarly to the Paracellular Backflux Hypothesis, except that the leak would be in an electrically silent pathway, since the channels are not transcellular, and the leak would not be accessible to luminal protein. There are no data bearing on this model. Second, protein might influence neutral transcellular NaCl transport (53). This mode of NaCl transport has been suggested to account for two-thirds of NaCl transport observed in tubules perfused with high Cl^--low HCO_3^- solutions (Table II) (53). For protein to influence neutral NaCl transport, a specific effect of protein on chloride transport from cell to bath is required. Again, no data on this hypothesis are available.

In summary, our present observations that removal of peritubular protein selectively inhibits NaCl absorption, but not PD, glucose or bicarbonate absorption, or bicarbonate backleak are not consistent with the Paracellular Backflux Hypothesis. We propose that the changes in paracellular permeability properties observed in vivo, supporting the Paracellular Backflux Hypothesis, may be secondary to increases in interstitial hydrostatic pressure rather than a primary cause of the reduction of J_v. The primary mechanism of the reduction in NaCl and J_v in the in vitro rabbit PCT might be an effect of protein on active transcellular NaCl transport or on passive paracellular NaCl transport.

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