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Research Article

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Lack of Effect of Peritubular Protein on Passive NaCl Transport in the Rabbit Proximal Tubule

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ABSTRACT The effect of peritubular protein removal on passive NaCl transport was examined in the isolated rabbit proximal convoluted tubule (PCT). Three modes of passive NaCl transport were tested: (a) paracellular backflux of NaCl, (b) convective flow of NaCl through junctional complexes, and (c) anion gradient-dependent NaCl transport. The effect of peritubular protein removal on the paracellular permeability to NaCl was examined using transepithelial specific resistance. Eight PCT were perfused with ultrafiltrate (UF) and bathed in either serum or UF. Transepithelial specific resistance averaged 14.5±1.9 in the presence and $13.7\pm1.7 \ \Omega \ cm^2$ in the absence of peritubular protein. The effect of peritubular protein removal on the convective flow of a NaCl solution across junctional complexes was examined in the absence of active transport by using colloid osmotic pressure (COP) gradients. 12 PCT were perfused with simple salt solutions in Donnan equilibrium with and without protein at 20°C. A COP gradient of 60.1 and -60.1 mmHg drove only 0.06 and -0.23 nl/min, respectively. These values are $\sim 10\%$ of the value predicted for an effect of peritubular protein on NaCl solution flow (1.98 nl/min) and are approximately equal to the value predicted for pure water equilibration for the small osmotic pressure difference between solutions in Donnan equilibrium (0.17-0.18 nl/min). The effect of peritubular protein removal on the passive absorption of NaCl driven by anion concentration gradients was examined in seven PCT perfused with a high chloride solution simulating late proximal tubular fluid and bathed in either serum or UF at 20°C. Volume absorption averaged 0.34±0.20 in the presence and 0.39±0.20 nl/mm min in the absence of peritubular protein. In conclusion, peritubular protein removal did not significantly affect any of the three distinct modes of passive NaCl transport tested. The

lack of effect of peritubular protein removal on passive paracellular NaCl transport suggests that protein modulates an active transcellular NaCl transport process.

INTRODUCTION

We recently reported that in isolated perfused proximal convoluted tubules (PCT), removal of peritubular protein specifically inhibits NaCl absorption without affecting glucose and bicarbonate absorption (1). It was not possible from these studies to determine whether protein removal reduced volume absorption by inhibiting active transcellular or passive paracellular NaCl transport. The present studies investigate the effect of peritubular protein on passive NaCl transport in the in vitro perfused rabbit PCT.

Three distinct models have been proposed to account for an effect of peritubular protein on passive NaCl transport. First, and most widely accepted, is the paracellular backflux hypothesis. Changes in peritubular protein concentration are suggested to alter the passive backleak of sodium through the paracellular pathway without affecting active transcellular transport (2, 3, 4). This view has been supported by the observation that transepithelial specific resistance (R_m) , a property of the paracellular pathway (3, 4), decreases after a reduction in peritubular protein in the in vivo PCT (3, 4, 5). However, we have argued indirectly that resistance and the relative contribution of junctional complexes and lateral intercellular spaces to paracellular resistance are not affected by alterations in peritubular protein in vitro (6). Second, is the solvent drag hypothesis. According to this concept, the colloid osmotic pressure (COP) of peritubular protein provides a driving force for NaCl and water movement across junctional complexes in the same manner as it does across capillaries. Data relating to this hypothesis have been conflicting. Some studies report that protein added to the luminal perfusate inhibits spontaneous volume absorption (7, 8), whereas other studies have failed to show an effect of luminal protein (9, 10).

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Third, is the hypothesis that in some manner peritubular protein is regulating the passive reabsorption of NaCl that is driven by anion gradients. This view has not been tested. However, the passive component of NaCl transport accounts for 30–60% of the NaCl absorption observed when PCT are perfused with solutions simulating late proximal tubular fluid and bathed in serum (11). This hypothesis would require that peritubular protein influence the diffusive and/or the convective movement of NaCl.

The purpose of the present in vitro microperfusion study was to examine directly each of the three proposed models for the effect of peritubular protein on passive NaCl transport. Three groups of experiments were performed. In the first group, the effect of peritubular protein removal on R_m was examined in PCT perfused with protein-free ultrafiltrates (UF) of rabbit serum. In the second group, the direct effect of COP gradients from bath to lumen and from lumen to bath on volume absorption was measured in PCT perfused with simple NaCl solutions in the absence of active transport. In the third group, the direct effect of peritubular protein removal on the passive component of anion gradient-dependent NaCl transport was measured in PCT perfused at 20°C with a solution stimulating late proximal tubular fluid. The results show that peritubular protein removal does not affect R_m or the passive component of anion gradient-dependent NaCl transport. Furthermore, COP gradients acting across junctional complexes cannot account for the effect of protein on volume reabsorption. In conclusion, the lack of effect of peritubular protein removal on passive NaCl transport suggests that protein modulates active transcellular NaCl transport.

GLOSSARY

ΔAAR	change in absolute absorptive rate
b	bath

- D_{0} optical diameter
- I_{\circ} injected current
- J_v net volume absorptive rate
- λ length constant
- l lumen
- L tubular length
- MPD mean paired difference
- Δosm osmotic gradient
- $osm_{\rm B}$ bath osmolality
- osm_P perfusate osmolality
- P_{Na} sodium permeability

- P_i ion permeability
- PCT proximal convoluted tubule
- PD potential difference
- PD_L PD at collection end
- PD_o PD at perfusion end
- $R_{\rm c}$ core resistance
- R_i input resistance
- R_m transepithelial specific resistance
- $R_{\rm T}$ transepithelial resistance
- specific resistivity
- UF ultrafiltrate
- V potential difference
- $V_{H_{2O}}$ absolute pure water absorptive rate
- V_L collection rate
- V_o perfusion rate

METHODS

General. Isolated segments of rabbit PCT were dissected and perfused as previously described (6, 12, 13), except that the collection end of the tubule was cannulated as described by Stoner and Roch-Ramel (14). Briefly, kidneys from female New Zealand white rabbits were cut into slices in a plane perpendicular to the surface. Individual PCT were dissected in a UF-like solution that contained 104 mM NaCl, 25 mM NaHCO₃, 4 mM Na₂HPO₄, 7.5 mM NaAcetate, 1 mM CaCl₂, 1 mM MgSO₄, 5 mM KCl, 5 mM glucose, 5 mM alanine, and 5 mM urea. PCT were identified as juxtamedullary if obtained from immediately above the corticomedullary junction, and as superficial if obtained from the remaining cortex. Early PCT attached to glomeruli were avoided. All tubules were perfused at 39 and/or 20°C in a 1.2-ml temperature-controlled bath.

Three protocols were performed: (a) determination of the effect of peritubular protein removal on resistance, (b) examination of the direct effect of COP on NaCl solution flow, and (c) investigation of the effect of peritubular protein removal on the passive component of anion gradient-dependent NaCl absorption. Each protocol consisted of three or four experimental periods. The first period began after an equilibration time of 30 min. Subsequent periods were separated by an equilibration time of 20 min.

The effect of protein on passive NaCl absorption was examined by using rabbit serum (Irvine Scientific, Santa Ana, CA) and a protein-free UF of rabbit serum. The UF was prepared using low pressure with an ultrafiltration membrane (UM-10 Amicon Corp., Scientific Sys. Div., Lexington, MA) at 5°C. The composition of UF and rabbit serum are given in Table I.

Net volume absorption $(J_v, \text{nanoliters per millimeter min$ ute) was measured as the difference between the perfusionand collection rates (nanoliter per minute), normalized permillimeter of tubule length. Exhaustively dialyzed [methoxy-³H]inulin (New England Nuclear, Boston, MA) was $added to the perfusate at a concentration of 50 <math>\mu$ Ci/ml so that perfusion rate could be calculated. The collection rate was measured with a 50-nl constant-volume pipette. The length (L, in millimeters) and the diameter of the tubule (optical diameter [D_o], in micrometers) were measured with an evepiece micrometer.

The transepithelial potential difference (PD in millivolts) due to active ion transport was measured by using the perfusion pipette as the bridge into the tubular lumen. The

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TABLE I Composition of UF and Serum[•]

	Na ⁺	K+	Cl⁻	Osmolality	Protein			
		meq/liter		mosmol/kg	g/dl			
UF (10)	138.3±0.8	4.83±0.03	108.9 ± 0.65	293.8 ± 1.5	_			
Serum (10)	137.3 ± 0.5	4.81±0.1	100.2 ± 1.2	293.5 ± 1.4	5.5 ± 0.07			

• The bicarbonate concentration was adjusted to 25 meq/liter. Solutions were bubbled with a CO_2 - O_2 gas mixture to give an averge p CO_2 of 40 mmHg.

perfusion and bath solutions were connected to the recording and reference calomel half-cells, respectively, via a bridge containing perfusion or bath solution in series with a 3.6 M KCl/0.9 M KNO₃ agarose bridge. This arrangement avoids direct contact of the KCl/KNO₃ agarose bridges with solutions bathing PCT. When the bath was a protein-containing solution, a protein-free UF replaced the protein-containing solution in the bridge. This arrangement eliminates the measurement of the Donnan potential when the bath solution contains protein (1). The recording and reference calomel half-cells were connected to the high and low impedence side, respectively, of a WPI Dual Electrometer (model F-223, W-P Instruments, Inc., New Haven, CT). The voltage output was displayed on an Electronik 194 Lab Recorder (Honeywell, Inc., Fort Washington, PA).

I. R_m . In physiologic experiments designed to measure $R_{\rm m}$, the perfusion solution was UF and the bath solution was either UF or rabbit serum (Table I). In other experiments $R_{\rm m}$ was measured by using either a simple sodium chloride or choline chloride solution as the perfusate and bath. The simple salt solutions contained 160 mM NaCl or Choline Cl, 4 mM Na₂HPO₄, 1.0 mM CaCl₂, and 1.0 mM MgSO₄. The PD was measured at both the perfusion and collection ends of the tubule. The measurement of PD at the collection end (PD_L) was identical to that described for the perfusion end (PD_o) except that the KCl/KNO₃ agarose bridge made direct contact with the collected fluid. As the perfusion rates were >20 nl/min and the PCT were short, there should be no solute concentration-gradients along the length of the tubule, and the liquid junctions at the perfusion and collection end KCl/KNO₃ bridges should be symmetrical.

The R_m was determined by using cable analysis of the voltage attenuation after point source current injection (15) as shown in Fig. 1. A constant-current generator (CVRI Electronics, San Francisco, CA) injected a pair of 1-sec pulses of 50-100 nA of alternating current into the tubular lumen through a current-passing pipette that was concentrically arranged inside the tubule perfusion pipette and filled with 0.16 M NaCl. The constant-current generator was connected to the current-passing pipette and to the bath solution via Ag-AgCl electrodes. The resistance of the current-passing pipette was between 15 and $25 \times 10^6 \Omega$. During an experiment, the relative position of the current-passing pipette and the perfusion pipette was kept constant, with the current-passing pipette extending beyond the perfusion pipette by at least 10 µm. The coupling resistance between the current-passing pipette and the voltage-recording pipette at the perfusion end was $41.8 \pm 4.5 \ 10^3 \Omega$ (n = 13). This means that injection of 100 nA through the current-passing pipette in the absence of a tubule resulted in a voltage deflection of 4.2 mV at the perfusion end. This voltage was subtracted from the total voltage deflection observed in the presence of a tubule.

Cable analysis for a truncated cable (15) was used to calculate the length constant (λ , centimeters), the input resistance (R_i , ohms), the transepithelial resistance (R_T , Ω centimeter), the core resistance (R_e , ohms per centimeter), the electrical diameter (D_e , micrometers), and the R_m (ohms centimeter squared) according to the equation:

$$\lambda = L/\cosh^{-1} \left(\Delta P D_{\rm o} / \Delta P D_{\rm L} \right), \tag{1}$$



FIGURE 1 Schematic arrangement for measuring R_m . Square wave current pulses of 50-100 nA and 1-s duration are injected into the tubule lumen through a current-passing pipette inserted through the center of the perfusion pipette. Specific resistance is calculated from the induced voltage deflections at both the perfusion and the collection ends.

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where ΔPD_o and ΔPD_L are the voltage deflections induced by the injected current (I_o) after correction for the voltage deflection due to coupling resistance.

$$R_{\rm i} = \Delta P D_{\rm o} / I_{\rm o} \tag{2}$$

$$R_{\rm T} = \frac{(\Delta PD_{\rm o})(\lambda)(\tanh L/\lambda)}{I_{\rm o}}$$
(3)

$$R_{\rm c} = \frac{(\Delta P D_{\rm o})(\tanh L/\lambda)}{I_{\rm o}} \tag{4}$$

$$D_e = (4\rho/\pi R_c)^{1/2},$$
 (5)

where ρ is the specific resistivity of the perfusion solution, 55 Ω cm (15) for UF, 63.2 Ω cm for sodium chloride, and 69.3 Ω cm for choline chloride. The latter were measured with a conductivity meter (Western, model 90).

$$R_{\rm m} = 2(\pi \rho \lambda^3 R_{\rm i})^{1/2} (\tanh L/\lambda)^{1/2}.$$
 (6)

Both transepithelial PD and specific resistance were measured by using tightly fitting tubule holding and cannulating pipettes as electrical seals at the perfusion and the collection ends. Recently, Sakin and Boulpaep (16) have shown that the use of an outermost pipette containing liquid Sylgard 184 did not enhance electrical insulation during measurements of R_m when both ends of the tubule are cannulated. In order to examine this issue transepithelial PD and R_i (Eq. 2) were measured in the presence and in the absence of an outermost pipette containing liquid Sylgard 184 as described by Lutz et al. (15). No significant difference in either parameter was observed. Transepithelial PD averaged -2.49 mV with Sylgard 184 and -2.52 mV without Sylgard 184 with a mean paired difference (MPD) of -0.03 ± 0.09 mV (n = 5). R_i averaged $158 \times 10^3 \Omega$ with Sylgard 184 and 146 $\times 10^3 \Omega$ without Sylgard 184 with an MPD of 0.12±0.06 $\times 10^3 \Omega$ (n = 5). The increase in R_i observed with Sylgard 184, although not significant, is probably due to termination of the cable at a point closer to the site of current injection. These observations agree with the conclusions of Sakin and Boulpaep (16) and suggest that valid measurements of PD and resistance can be obtained in the absence of an outermost pipette containing Sylgard 184.

II. COP. In experiments designed to examine the effect of COP (mmHg) on volume absorption in the absence of active transport, simple salt solutions were designed. These solutions did not contain preferentially reabsorbed solutes, such as glucose and sodium bicarbonate, or high molecular weight solutes that might be sieved, such as raffinose. The simple salt solution without protein (Salt) contained 160 mM NaCl, 4 mM Na₂HPO₄, 5 mM KCl, 1 mM CaCl₂, and 1 mM MgSO₄. The simple salt solution with protein (Protein) was prepared by addition of ~ 12 g/dl dialyzed albumin to the Salt solution at 5°C. The composition of the Salt and the Protein solution are given in Table II. These solutions were not osmotically adjusted, in order to maintain Donnan equilibrium.

The COP of rabbit serum and of the protein solution were calculated from the Landis-Pappenheimer equations for serum and albumin, Eqs. 7 and 8, respectively (17).

$$COP = 2.1C + 0.16C^2 + 0.009C^3, \tag{7}$$

$$COP = 2.8C + 0.18C^2 + 0.012C^3, \tag{8}$$

where C denotes concentration.

The absolute pure water absorption (V_{HgO} , nanoliters per minute) due to osmotic disequilibrium between the perfu-

 TABLE II

 Composition of Salt and Protein Solution*

	Na ⁺	К+	Osmolality	Protein
	meq	/liter	mosmol/kg	g/dl
Salt (14)	160.2 ± 1.2	5.01±0.02	306.3 ± 2.2	
Protein (14)	163.8 ± 1.5	5.11±0.06	311.7±2.6	10.3±0.3

* Solutions were bubbled with 100% O2 gas; pH was 7.4.

sion and bathing solutions can be calculated from the following equation: 1

$$V_{\rm HgO} = V_{\rm o}(\Delta osm/osm_{\rm B}), \tag{9}$$

where V_o is the perfusion rate in nanoliters per minute, osm_B is the osmolality of the bath in milliosmoles per kilogram, and Δosm is the osmotic gradient in milliosmoles per kilogram.

III. Anion gradient-dependent NaCl absorption. In experiments designed to examine the effect of protein on the passive component of anion gradient-dependent NaCl absorption, tubules were perfused at 39 and 20°C with a high chloride solution simulating late proximal tubular fluid. The high chloride solution (High Cl^-) contained 146.5 mM NaCl, 5 mM NaHCO₃, 4 mM Na₂HPO₄, 1 mM CaCl₂, 1 mM MgSO₄, 5 mM KCl, and 5 mM urea. This solution was bubbled with a CO₂-O₂ gas mixture to give an average PCO₂ of 40 mmHg and was osmotically adjusted to the serum and UF bathing solutions.

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 \pm SEM. *P* values for the MPD were computed by using the paired two-tailed *t* test. For comparison of R_m during sodium and choline chloride perfusions, a one-tailed Spearman's Rank Correlation Test (18) was used. Choline is an impermeant cation (19). Substitution of choline for sodium must therefore increase R_m and justifies the use of a one-tailed test. Significance was accepted at the 0.05 level.

RESULTS

I. Effect of peritubular protein removal on (R_m) . The effect of peritubular protein removal on R_m was examined in five superficial and three juxtamedullary PCT whose lengths ranged from 270 to 700 μ m and averaged 420±41 μ m (n = 10). In all tubules the perfusate was UF and three experimental periods were performed. In the first period, the control, the bath was rabbit serum; in the second period, the experimental, the bath was changed to UF; and in the third period, the recovery, the bath was changed back to rabbit serum. As seen in Fig. 2, replacement of the

¹ Eq. 9 can be derived from mass balance considerations, i.e., the amount of solute in is equal to the amount of solute out. $V_{L} = V_0 - V_{IIgO}$, $\Delta osm = osm_B - osm_P$, $V_0 osm_P = (V_0 - V_{IIgO}) osm_B$, $V_0 osm_P = V_0 osm_B - V_{IIgO} osm_B$, $V_{IIgO} osm_B = V_0 osm_B - V_{IIgO} osm_B$.



FIGURE 2 Effect of protein on R_m . The effect of peritubular protein removal on R_m was examined in five superficial (open circles) and three juxtamedullary (closed circles) PCT. Data and statistics are given below.

serum bath solution with protein-free UF did not significantly affect R_m. R_m was 15.1±2.2, 13.7±1.7, and $13.8\pm1.6 \ \Omega \ cm^2$ in the control, experimental, and recovery periods, respectively. There is a tendency for $R_{\rm m}$ to decrease from the control to the recovery period; however, the MPD was not significant, $1.3\pm0.9 \ \Omega \ cm^2$ (n = 8). Nonetheless, to control for this tendency for $R_{\rm m}$ to decline, two additional PCT were bathed with UF in the control and recovery periods and with serum in the experimental period: R_m was 13.7±3.4, 13.0±3.4, and $12.4\pm3.4 \ \Omega \ cm^2$, respectively. Table III shows that this tendency to decrease with time was also observed with the PD_0 and the PD_L of the tubule. In previous experiments, PD_o tended to increase between the control and the recovery periods (1). Thus, the tendency toward a time-dependent decrease in R_m , PD_o , and PD_L is probably related to the injection of current.

In a separate group of five tubules, R_m was measured during perfusion at 20°C with simple sodium and choline chloride solutions in the perfusate and bath. The purpose of these experiments was to show that the present measurements can detect changes in R_m . During perfusion with simple sodium chloride, R_m was $12.06\pm1.6 \ \Omega \ cm^2 \ (n=5)$. During perfusion with simple choline chloride solutions, R_m increased in every tubule, averaging $19.8\pm5.0 \ \Omega \ cm^2 \ (n=5)$. These data are significantly different at the 0.05 level using a onetailed Spearman's Rank Correlation test (18).

A test of the adequacy of cable analysis is a comparison between the D_e and D_o of the core of the cable. The diameter of the core calculated from electrical measurements (Eq. 5) was $28.1\pm2.8 \ \mu m$ (n = 10), with UF perfusate, $27.7\pm0.5 \ \mu m \ (n = 5)$ with sodium chloride perfusate, and 22.3 \pm 2.0 μ m (n = 5) with choline chloride perfusate. The diameter of the core measured optically was 20.0±1.6 μ m (n = 10) with UF and was 21.2 \pm 0.8 µm (n = 5) with simple salt solutions. The mean D_e was usually greater than the mean D_o . A comparable discrepancy has been observed by most investigators who have used cable analysis to measure $R_{\rm m}$ (Table IV). As pointed out by Lutz et al. (15), its cause probably is due to the fact that optical measurements include the microvilli of the brush border, \sim 3 μ m in perfused PCT (20). Accordingly, the barrier to current passage would approximate the level of the junctional complex.

The average R_m observed in the presence and absence of peritubular protein was $14.04\pm0.94 \ \Omega \ cm^2$ (n = 30), at least twice the value reported by other investigators (Table IV). Intranephron heterogeneity, in which R_m is higher in early than in late PCT (21), is not the cause of this discrepancy, because PCT attached to their glomerulus were excluded at dissection. The value calculated for R_m is most sensitive to variation in ΔPD_L . Therefore, it is likely that the higher R_m reported in Table III and Fig. 1 is due to cannulation of the PCT at the collection end.

II. Effect of COP gradients on volume absorption in the absence of active transport. Numerous investigators have shown that in the isolated PCT perfused with UF at 39°C, removal of peritubular protein reduced spontaneous volume absorption coupled to active transport (1, 10, 22-24). The present experiments

·	Effect of Perstubular Protein Removal on PD and Cable Analysis Parameters									
	PD.	PDL	ΔPD。	ΔPD_{L}	λ	Rı	R _T	R _e	R _m	
	mV			μm	10 ³ Ω	$10^{3}\Omega \ cm$	10 ⁶ Ω/cm	Ω cm²		
Protein (8)	-3.2 ± 0.3	-3.3 ± 0.6	13.9±1.3	1.3 ± 0.3	139.3 ± 12	127±12	1.65 ± 0.2	9.7±1.4	15.1 ± 2.2	
UF (8)	-3.3 ± 0.3	-3.9 ± 0.6	13.4 ± 2.8	1.3 ± 0.3	135.9 ± 8.7	132 ± 15	1.49 ± 0.1	9.6±1.4	13.7±1.7	
Protein (8)	-3.0 ± 0.3	-3.1±0.6	13.5 ± 1.3	1.1 ± 0.2	132.3 ± 14	119±13	1.47 ± 0.2	9.9±1.3	13.8±1.6	

 TABLE III
 Effect of Peritubular Protein Removal on PD and Cable Analysis Parameters

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TABLE IV Comparison of D_e and R_m in Mammalian PCT

Species	D.	R _m	Ref.
	μm	Ω cm²	
Rabbit	29.6	6.96	15
	28.1	14.04	Fig. 1
Rat	26-30	4.9-5.7	50
	32.8	5.5	5
Early PCT	32	11.6	21
Late PCT	37	5.6	21
Dog	34.6	5.6	51

The measured D_o was 20-24 μ m in the rat and rabbit PCT and 35 μ m in the dog PCT.

were designed to examine directly whether COP gradients exert their effect on volume absorption by inducing the convective flow of a NaCl solution across junctional complexes. To avoid spontaneous volume absorption coupled to active transport and the generation of reverse osmotic gradients due to sieving of solutes other than NaCl, PCT were perfused with simple salt solutions lacking preferentially reabsorbed solutes, such as glucose, alanine, and NaHCO₃, and high molecular weight solutes, such as raffinose. To avoid the diffusive movement of NaCl, the simple salt solution with protein (Protein) and the simple salt solution without protein (Salt) were in Donnan equilibrium. Inevitably, solutions in Donnan equilibrium have small osmotic pressure differences; the osmotic pressure difference between the Salt and the Protein solution was 5.4 mosmol/kg (Table II). In summary, these experiments were designed to examine solely the effect of COP gradients on convective NaCl transport.

According to the solvent drag hypothesis (7), COP gradients are directly and linearly related to volume flow.

Table V presents an analysis of the effect of COP gradients on the change in volume absorption. In the first row our previous data is depicted; in the second row the present data is represented. In our previous experiments we found that removal of 6 g/dl peritubular protein reduced spontaneous volume absorption coupled to active transport by $0.55 \text{ nl/mm} \cdot \text{min}$ (Table III of ref. 1). The PCT averaged 1.2 mm; therefore, the inhibition of absolute volume absorption was 0.66 nl/min. According to Eq. 7, the COP of 6 g/dl serum protein is 20.3 mmHg. If it is assumed that in these experiments peritubular protein removal eliminated the component of volume absorption attributable to the convective flow of a NaCl solution through junctional complexes, then it can be calculated that COP gradients directly drive 0.66 nl/min per 20.3 mmHg, or 0.033 nl/min mmHg.

In the present experiments active transport was absent and a 10.3 g/dl albumin solution was used to examine the direct effect of COP gradients. According to Eq. 8, the COP of a 10.3 g/dl albumin solution is 60.1 mmHg. If COP gradients drive volume absorption at a rate of 0.033 nl/min mmHg, then a COP gradient of 60.1 mmHg would be expected to drive 1.98 nl/ min. This is the expected change in absolute volume absorptive rate, predicted ΔJ_v . On the other hand, if COP gradients do not induce the flow of a NaCl solution across junctional complexes, then the small osmolality difference between the Salt and the Protein solutions, due to their being in Donnan equilibrium, would be expected to drive a small amount of volume absorption due to pure water equilibration. The amount can be calculated from Eq. 9. In the present experiments, V_0 averaged 10.0±0.34 nl/min (n = 12), osm_B

							Δ AAR (nl/min)	
		Con	dition				Pre	dicted
Active	Per	iod 1	Per	iod 2	COP	Observed	Δ <i>J</i> .•	ΔV _{He} oț
					mmHg			
Present	UF	Serum	UF	UF	-20.3	-0.66	_	_
Absent	Salt Protein	Salt Protein	Salt Protein	Protein Salt	+60.1 -60.1	+0.06 -0.23	1.98 -1.98	0.17 -0.18

TABLE V Analysis of the Effect of COP Gradients on the Change in Absolute Absorptive Rate (Δ AAR)

• The ΔJ_{ν} was calculated assuming that the effect of peritubular protein removal on J_{ν} observed in the presence of active transport is due to COP; (-0.66 nl/min)/(20.3 mmHg) = 0.033 nl/min mmHg.

 \ddagger The ΔV_{HgO} was calculated assuming pure water equilibration according to Eq. 9.

averaged 311.7 and 306.3 mosmol/kg (Table II), and Δ osm averaged 5.4 mosmol/kg (Table II). According to Eq. 9, the predicted $V_{\rm HgO}$ was 0.17 nl/min when the bath solution was Protein and -0.18 nl/min when the bath solution was Salt. These are the expected changes in $V_{\rm HgO}$, Predicted Δ $V_{\rm HgO}$.

Fig. 3 shows that the MPD in volume absorption induced by COP gradients of +60.1 mmHg and -60.1 mmHg were 0.05 ± 0.07 and -0.19 ± 0.06 nl/mm · min in the absorptive and secretory directions, respectively. Because the tubular length averaged 1.2 mm, the observed Δ AAR are 0.06 and -0.23 nl/min. The observed Δ AAR and those predicted for ΔJ_v and ΔV_{HsO} are in the last three columns of the second row in Table V. Comparison shows that the observed Δ AAR are consistent with pure water equilibration due to the small osmotic gradient (Predicted ΔV_{HsO}), but not with the induction of a convective flow of a NaCl solution by COP gradients (Predicted ΔJ_v).

III. Effect of peritubular protein removal on the passive component of anion gradient-dependent NaCl absorption. The effect of peritubular protein removal on the passive component of NaCl absorption and transepithelial PD was examined in four superficial and three juxtamedullary PCT. In all tubules the perfusate was a high chloride solution simulating late proximal tubular fluid (High Cl⁻). Four experimental periods were performed. In the first period, the bath was rabbit serum and the temperature was 39° C. In this period, active plus passive NaCl absorption was measured. In the temperature was rabbit serum and the temperature was rabbit serum and the temperature is the second period, the bath was rabbit serum and the temperature was reduced to 20°C to inhibit active transport. In this period, passive NaCl absorption in the presence of peritubular protein was measured. In

the third period, the bath was a protein-free UF and the temperature was 20°C. In this period, passive NaCl absorption in the absence of peritubular protein was measured. In the fourth period, the bath was High Cl⁻ and the temperature was 20°C. In this period, baseline NaCl absorption in the absence of anion concentration gradients and active transport was measured. Superficial and juxtamedullary PCT behaved similarly with respect to their response to protein removal in the absence of active transport; therefore, the data were pooled. The tubular lengths averaged 1.3 ± 0.1 mm (n = 7).

As seen in the first and second periods in Fig. 4, active plus passive volume absorption was 0.90 ± 0.13 nl/mm \cdot min and was reduced by ~60% to 0.34 ± 0.2 nl/mm \cdot min when active transport was inhibited. No further effect on passive volume absorption was observed after removal of peritubular protein. The MPD between periods two and three was -0.06 ± 0.08 nl/ mm \cdot min. However, as seen in the fourth period, volume and NaCl absorption were reduced to zero in the absence of active transport, when anion concentration gradients were eliminated, 0.00 ± 0.07 nl/mm \cdot min.

Table VI shows the effect of bath composition and temperature on transepithelial PD. Neither inhibition of active transport by cooling nor removal of peritubular protein in the presence of inhibition of active transport significantly affected the PD. However, replacement of the bath solution with a high chloride solution identical to the perfusate eliminated the PD. These data suggest that the PD in PCT perfused with

PERFUSATE	SALT	SALT	PROTEIN	PROTEIN	
BATH	SALT	PROTEIN	PROTEIN	SALT	
0.4 Jv_o (nl/mmmin) −0.4	-			M	
MEAN (9)	0.00 ± 0.03	+0.08 ± 0.07	-0.04 ± 0.03	-0.22 ± 0.06	
MPD	+ 0.05 ± 0.07		-0.19 ± 0.06		
Р	1	NS	< 0.025		

FIGURE 3 Effect of COP gradients on volume flow (J_v) in the absence of active transport. The protein solution had a COP of 60.1 mmHg. The effect of COP gradients of 60.1 and -60.1 mmHg were examined in seven superficial (O) and five juxtamedullary (\bullet) PCT at 20°C. Data and statistics are given below. Analysis of data is given in Table V.



FIGURE 4 Effect of protein on anion gradient-dependent NaCl absorption in the absence of active transport. In the first period, active plus passive NaCl transport was measured at 39°C in PCT perfused with high chloride solution (High Cl^-) and bathed in serum. In the second and third periods, the effect of protein on the passive component of anion gradient-dependent NaCl absorption was examined in PCT perfused at 20°C. In the fourth period, base-line transport was measured at 20°C in the absence of anion gradients. Data and statistics are given below.

TABLE VI Effect of Bath Composition and Temperature on PD in Tubules Perfused with a High Chloride Solution (High Cl ⁻)								
Bath	Serum	Serum	UF	High Cl ⁻				
Temperature, °C	39	20	20	20				
PD, mV	+1.7±0.7	+1.8±0.7	+1.7±0.7	0.0±0.7				
MPD	0.1-	±0.16 0.1±	:0.05 1.7±0).68*				

* Significantly different at the 0.05 level.

a high chloride solution is due entirely to the chloridebicarbonate concentration gradients and that active NaCl absorption from a high chloride perfusate proceeds without generating an electrical PD.

These data show that inhibition of active transport by cooling inhibits volume and NaCl absorption by 60% but does not alter the transepithelial PD. Approximately 40% of volume and NaCl absorption in the presence of anion concentration gradients could be attributed to passive processes.² More importantly, removal of peritubular protein in the absence of active transport had no effect on passive volume and NaCl absorption. From these observations it appears that removal of peritubular protein inhibits active, rather than passive NaCl absorption.

DISCUSSION

Numerous investigators have observed that reducing the peritubular protein concentration in vivo by extracellular fluid volume expansion or in vivo by removal of peritubular protein, inhibits J_v in the PCT (1-3, 9, 10, 22-24, 26, 27). This effect can be reversed by replacement of protein with colloids such as polyvinylpyrrolidone (10) and dextran (28, 29), suggesting that COP is the active factor supporting reabsorption. The most widely accepted hypothesis of the mechanism whereby COP exerts its effect on proximal volume absorption is that COP modulates the passive backleak of solutes and water through the paracellular pathway (2-4).

Our recent studies on the effect of peritubular protein removal on solute transport and on paracellular permeability properties in the in vitro PCT, however, have added new information that is difficult to integrate into the passive backleak model of the mechanism of action of peritubular protein. First, we observed that peritubular protein removal did not alter two indices of paracellular permeability: PD and relative sodium-to-chloride permeability (6). Second, we identified NaCl as the solute specifically inhibited by peritubular protein removal (1). Peritubular protein removal had no effect on glucose or bicarbonate absorption (1). These studies, however, could not identify whether an active or a passive component of NaCl absorption was modulated by peritubular protein. Thus, the purpose of the present studies was to examine directly the effect of peritubular protein removal on three distinct modes of passive NaCl absorption: (a) paracellular backflux of NaCl, (b) convective flow of a NaCl solution through junctional complexes, and (c)anion gradient-dependent NaCl absorption. The following discussion will consider each of these three modes separately, and will compare the present in vitro data on protein removal to the in vivo effects of extracellular fluid volume expansion.

I. Paracellular backflux of sodium. The paracellular backflux hypothesis suggests that active sodium transport is constant and that the modulation of I_{ν} induced by changes in peritubular protein is due to variations in the passive backflux of sodium through the paracellular pathway. This view is best supported by data obtained from in vivo preparations showing that R_m and PD decrease after extracellular fluid volume expansion. In the Necturus PCT, Boulpaep (3) observed a decrease in R_m from 70 to 22 Ω cm² and in PD from -15.4 to -9.75 mV after volume expansion. In the late proximal tubule of the rat, Seely (5) found that decreasing peritubular capillary COP decreased R_m from 5.5 to 5.4 Ω cm², this difference was significant when expressed as total resistance and calculated on a paired basis. The decreased paracellular resistance has been proposed to enhance the backflux of sodium from the lateral intercellular space to the tubular lumen across junctional complexes. The driving force for enhanced sodium backflux was believed to be either intercellular space hypertonicity or the lumen-negative PD (3, 4). Recent evidence, however,

² Jacobson (25) found that $\sim 60\%$ of volume absorption in the presence of anion concentration gradients could be attributed to passive processes in superficial rabbit PCT; whereas none of it was passive in juxtamedullary PCT. In the present experiments, the passive component of volume absorption was 100% in one superficial PCT and zero in one juxtamedullary PCT. The remaining PCT were intermediate regardless of the region of the cortex from which they were dissected. It is, however, interesting to note that these differences may be related to the relative sodium-to-chloride permeability ratios of the PCT. The one superficial PCT was highly chloride selective; the one juxtamedullary PCT was highly sodium selective, and the remaining PCT were intermediate in their ion selectivity. The difference between Jacobson's and the present experiments may lie in the composition of the high chloride solution, the manner of inhibition of active transport, or in the control volume reabsorptive rates. Jacobson's high chloride solution contained 10 mM sodium cyclamate, ouabain was used rather than cooling to inhibit active transport, and he obtained a control volume reabsorptive rate of only 0.47 and 0.49 nl/mm · min in superficial and juxtamedullary PCT, respectively.

indicates that lateral intercellular spaces are not hypertonic (6, 30) and that the PD along all but the initial mammalian superficial proximal tubule is lumen-positive (30, 31). In the absence of lateral intercellular space hypertonicity and in the presence of a lumenpositive PD, a decrease in paracellular resistance would enhance the passive reabsorption of sodium, rather than the passive backflux of sodium. These theoretical considerations question the paracellular backflux hypothesis.

Recently, three observations in the in vitro PCT have suggested indirectly that paracellular resistance is not altered by protein removal. First, in rabbit PCT perfused with UF, volume absorption is reduced but transepithelial PD is not affected by removal of peritubular protein (1, 6, 10, 22, 23). Second, in rabbit PCT the relative sodium-to-chloride permeability ratio is not altered by removal of peritubular protein (6). This observation suggests that removal of peritubular protein does not alter either the ion selectivity of the junctional complexes or the relative contribution of the junctional complexes and lateral intercellular spaces to the paracellular resistance (6). Third, in amphibian PCT, volume absorption is inhibited, but neither transepithelial nor basolateral membrane PD are reduced by removal of bath colloid (16).

In the present study, R_m was measured directly in the rabbit PCT and found to be unaltered by removal of peritubular protein (Fig. 2). At this point it is worthwhile to estimate the decrease in R_m that would have been required to account for our previously observed 40% decrease in J_{v} (1) according to the paracellular backflux hypothesis. The paracellular backflux hypothesis proposes that the rate of active sodium absorption at the basolateral membrane is constant and that $I_{\rm v}$ is decreased after peritubular protein removal because of an increase in the net passive sodium backflux. The net passive sodium backflux is increased because of an increase in paracellular sodium permeability. The increase in sodium permeability required to account for a 40% decrease in net sodium absorption can be calculated with the data shown in Table VII and the equations for unidirectional passive sodium fluxes (32).

Passive influx:

$$J_{Na}^{P(b-l)} = -P_{Na} \frac{VF}{RT} \frac{[Na]_{l}e^{VF/RT}}{1 - e^{VF/RT}}$$
(10)

Passive efflux:

$$J_{Na}^{P(1-b)} = P_{Na} \frac{VF}{RT} \frac{[Na]_{b}}{1 - e^{VF/RT}},$$
 (11)

where b is the bath, l is the lumen, V is potential

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TABLE VII Data Used for Calculation of Unidirectional Passive Sodium Fluxes*

Bath Protein	Present	Absent
J _v , nl/mm∙min	1.4	0.84
J_{Na}^{Net} , pmol/mm · mint	210	126
PD, mV	-3	-3
$P_{\rm Na}, cm/s$	10-4	_
$[Na]_1 = [Na]_b, mM$	150	150
Tubule diameter, μm	20	20

• All data are approximated from Ref. 1 except for P_{Na} in the presence of bath protein which was obtained from Ref. 35. ‡ Calculated assuming J_v is isonatric.

difference, P_{Na} is the sodium permeability and R, T, and F have their usual meanings.

Fig. 5 shows the calculated unidirectional passive sodium fluxes in the presence and absence of bath protein. In the presence of bath protein, the net sodium flux of 210 peq/mm \cdot min is $\sim 20\%$ of the unidirectional passive sodium fluxes. But because the PD is lumen-negative, passive sodium influx exceeds passive sodium efflux by 140 $peq/mm \cdot min$ and the calculated rate of active sodium absorption is $350 \text{ peg/mm} \cdot \text{min}$. In the absence of bath protein, the net sodium flux is 126 $peq/mm \cdot min$, and according to the paracellular backflux hypothesis the rate of active sodium absorption is constant at 350 peg/mm \cdot min. Thus, in the absence of bath protein, the net passive backflux of sodium must be 224 peq/mm \cdot min. The P_{Na} required to give a net passive sodium efflux of 224 peq/mm. min can be calculated from solution of Eq. 12.

Eq.
$$10 + \text{Eq. } 11 = -224 \text{ peq/mm} \cdot \text{min.}$$
 (12)

As shown at the bottom right of Fig. 5, P_{Na} in the absence of bath protein must be increased to 1.6×10^{-4} cm/s. This calculation shows that a 60% increase in P_{Na} is required to account for a 40% decrease in net sodium transport. The increase in P_{Na} will be inversely related to the decrease in R_m according to Eq. 13 (19).

$$\Sigma P_{\rm i} \bar{C}_{\rm i} = RT/F^2 (1/R_{\rm m}), \tag{13}$$

where P_i is the ion permeability, \bar{C}_i is the mean ion concentration, and R, T, and F have their usual meanings.

The increase in P_i will be equivalent for Na and Cl because the relative solution-to-chloride permeability ratio is not altered by peritubular protein removal (6). Accordingly, a 60% increase in P_i will be associated with a 40% decrease in R_m . This would have meant that R_m would have had to decrease from an average



FIGURE 5 Calculated unidirectional sodium fluxes in the presence and in the absence of bath protein. Active and passive sodium fluxes are calculated from equations 10 and 11 and the data in Table VII. In the absence of bath protein, the $P_{\rm Na}$ required to account for a 40% decrease in net sodium absorption according to the paracellular backflux hypothesis is 1.6×10^{-4} cm/s, a value 60% greater than observed in the presence of bath protein.

of 14 Ω cm² in the presence of bath protein to 8.4 Ω cm² in the absence of bath protein.³ The present data, therefore, indicate that the effect of removal of peritubular protein on proximal solute and water absorption cannot be accounted for by a change in $R_{\rm m}$ as suggested by the paracellular backflux hypothesis.

II. NaCl solution flow through junctional complexes. Carl Ludwig (34) first proposed that the COP of serum proteins could be responsible for tubular salt and water reabsorption in 1843. He envisioned that the tubular wall was freely permeable to small molecular weight solutes, but impermeable to serum proteins. As a result, COP provided the driving force for solute and water absorption, obviating the need for active solute transport. Because this mechanism cannot account for urine whose osmolality is greater than that of plasma, it was discarded. More recently, Schnermann (7) has revived Ludwig's theory to explain the effect of peritubular protein on isosmotic proximal tubular solute and water absorption, while maintaining the view that active solute transport is the principle driving force for water absorption. This modern Ludwig-like theory proposed that the junctional complexes are highly permeable to small molecular weight solutes and water, but impermeable to peritubular protein. The COP provided the driving force for the proteinsensitive component of proximal volume absorption.

This view is best supported by data showing that addition of luminal protein dramatically inhibited volume absorption in the rat PCT (7, 8). Other studies (9, 10) in both the rat and the rabbit PCT, however, find that luminal protein does not influence volume absorption.

In order to examine the effect of COP gradients on a component of proximal volume absorption not associated with active solute transport, the present studies measured volume absorption at 20°C. Fig. 3 and Table V show that COP gradients do not drive a component of volume absorption larger than can be attributed to pure water equilibration between perfusion and bathing solutions. These data indicate that there is not a highly NaCl- and water-permeable pathway in the PCT across which COP gradients operate. This conclusion is in accord with measured NaCl reflection coefficients in the PCT. The reflection coefficient for NaCl in the PCT has been measured to be between 0.7 and 0.9 (35, 36). In either case, COP gradients would cause significant sieving of luminal NaCl and eventual osmotic equilibration.

It might be argued that the permeability properties of the pathway across which protein exerts its direct colloid osmotic effect, presumably the junctional complexes, have been altered by inhibition of active transport. For instance, the lateral intercellular spaces have been observed to be collapsed in the absence of active transport (37). Their width, however, is still substantial, ~ 200 Å(38). Although such a constrained environment might restrict the diffusion of albumin by as much as one to two orders of magnitude, the albumin concentration at the contraluminal surface of the junc-

³ This calculation provides a minimum estimate of the required change in R_m because it assumes that the PD along the tubule is constant. PD becomes less negative and may become positive with length (31, 33), thus decreasing the driving force for sodium backflux.

tional complexes would be equal to that in the bath after the 20-min equilibration period and in the presence of negligible fluid velocities within the lateral intercellular spaces (39). Accordingly, a convective flow of a NaCl solution across junctional complexes should have occurred if the junctional complexes had a high water permeability and a low reflection coefficient for NaCl, irrespective of changes in paracellular geometry.

III. Passive component of anion gradient-dependent NaCl absorption. It is generally agreed that passive NaCl transport processes account for 30-60% of the NaCl transport in the late superficial PCT where anion gradients are present (11). The passive component of anion gradient-dependent NaCl transport has been attributed to both diffusive and convective transport mechanisms. A comparison of periods two and three in Fig. 4 shows that removal of peritubular protein did not influence the passive component of anion gradient-dependent NaCl transport; the MPD was -0.06 \pm 0.08 nl/mm·min. Although it is possible that the diffusive and convective components are changing reciprocally, it is more likely that neither the diffusive nor the convective component of NaCl transport are influenced by peritubular protein. The conclusion that the diffusive component of NaCl transport is constant is supported by Imai and Kokko's (23) observation that $P_{\rm Na}$ was not altered by protein and with our data that show that neither the relative sodium-to-chloride permeability (6) nor the R_m (Fig. 2) were altered by peritubular protein removal. If the diffusive component is constant, then it follows that the convective component is also constant, indicating that the reflection coefficients for sodium chloride, sodium bicarbonate, and organic solutes are unchanged by peritubular protein removal.

IV. Effect of peritubular protein removal in vitro. The present observations show that peritubular protein does not act on three distinct modes of passive NaCl transport. First, directly determined R_m is not altered by protein removal. Consequently, the inhibitory effect of protein removal on proximal volume absorption cannot be attributed to an increase in the backflux of sodium across junctional complexes. Second, COP gradients in the absence of active transport do not generate volume absorption over that that can be attributed to pure water equilibration between the luminal and peritubular solutions. Consequently, the inhibitory effect of protein removal on proximal volume absorption cannot be attributed to removal of a direct effect of protein on the flow of a NaCl solution across junctional complexes. Third, the directly determined passive component of anion gradient-dependent NaCl transport is not changed by peritubular protein removal. Consequently, the inhibitory effect of protein cannot be attributed to alterations in diffusive or convective NaCl transport.

The failure of peritubular protein removal to inhibit any of the three modes of passive NaCl transport examined suggests that protein modulates active NaCl transport. The magnitude of the effect of protein removal on active NaCl transport can be estimated from a comparison of the effect of protein removal in the presence of active transport and of inhibition of active transport on volume absorption from a high chloride solution. These comparisons are shown in Table VIII. The first column gives volume absorption in the presence of active transport and peritubular protein, 0.90 nl/mm · min; the second column gives volume absorption in the presence of active transport but in the absence of peritubular protein, 0.37 nl/mm · min; and the third column gives volume absorption in the absence of active transport and in the presence of peritubular protein, 0.34 nl/mm·min. Comparison of the first and second columns shows that removal of peritubular protein in the presence of active transport, reduced volume absorption by $\sim 60\%$. Comparison of the first and third columns shows that inhibition of active transport in the presence of peritubular protein also reduced volume absorption by $\sim 60\%$. The identity of the percentages of volume absorption inhibited by removal of peritubular protein in the presence of active transport and by inhibition of active transport suggests that peritubular protein removal inhibits virtually all active volume and NaCl absorption from a high chloride solution.

The actual mode of active NaCl transport inhibited by peritubular protein removal is unknown. Because peritubular protein addition or removal occurs adjacent to the basolateral cell membrane, it is likely that protein exerts its effect on the basolateral, rather than on the luminal cell membrane. In support of the view that protein is acting on the basolateral cell membrane are the recent observations in proximal straight tubules that protein addition depolarizes the basolateral PD

TABLE VIII Comparison of the Effect of Protein Removal and Inhibition of Active Transport on Volume Absorption (J_o) from a High Chloride Solution (High Cl⁻)

Perfusate	High Cl⁻	High Cl⁻	High Cl ⁻
Bath	Serum	UF	Serum
Temperature, °C	39	39	20
$J_v, nl/mm \cdot min$	0.90°‡	0.37°	0.34‡

Data are from Table V of Ref. 1. The J_v in the presence of protein and active transport was 0.90 nl/mm • min.
‡ These are data from Fig. 4.

(40). We have shown previously that peritubular protein removal does not inhibit substantially either glucose or sodium bicarbonate reabsorption in PCT perfused with UF (1). Failure to inhibit these sodium cotransport processes was interpreted as evidence against an effect of protein removal on the Na-K ATPase pump system located in the basolateral cell membrane. Table V of ref. 1 and Table VI of this paper show that removal of peritubular protein does not alter transepithelial PD at either 39 or 20°C, respectively. Thus, we conclude that peritubular protein removal specifically inhibits neutral NaCl transport⁴ across the basolateral cell membrane without inhibiting the Na-K ATPase pump system.

V. Comparison of the effects of peritubular protein removal in vitro to extracellular fluid volume expansion in vivo. The relevance of these in vitro studies to the in vivo circumstance where peritubular protein concentration is lowered by expansion of extracellular volume with noncolloidal salt solutions should be considered. Removal of peritubular protein in vitro and extracellular fluid volume expansion in vivo have similar effects on proximal transport processes and dissimilar effects on proximal paracellular permeability properties. On the one hand, both experimental models show a reduction in volume absorption with an inhibition of NaCl transport (1, 41, 42), but no effect on $NaHCO_3$ (1, 41, 42). On the other hand, the available in vivo data strongly support an increase in paracellular permeability properties (3, 4, 5); whereas in vitro data show no change in paracellular permeability properties (Fig. 2, ref. 1, 6, 23). We have previously suggested that the changes in paracellular permeability properties observed in vivo may be secondary to increases in interstitial hydrostatic pressure and are not the primary cause for the reduction in volume and NaCl transport (1). An argument in favor of this view is that an increase in paracellular permeability in vivo would stimulate, rather than inhibit, NaCl absorption: The chloride concentration is greater in the lumen than in the blood, favoring chloride absorption and the transepithelial PD, in the late proximal tubule where the bulk of NaCl is absorbed, is lumen-positive, favoring sodium absorption. Thus, two lines of evidence suggest that the present in vitro results on peritubular protein removal bear importantly on extracellular

fluid volume expansion in vivo. First, both models show NaCl specificity. Second, in both models a decrease in tubular resistance would stimulate, not inhibit, NaCl absorption. Ultimately, however, the relevance of the in vitro protein removal data to in vivo extracellular fluid volume expansion resides in the mechanism by which changes in peritubular capillary protein concentrations in vivo are transduced to the basolateral membrane of the proximal tubule.

Several investigators working in vivo in a variety of experimental models have attempted to correlate rates of absolute proximal absorption to the sum of the hydraulic and oncotic pressures across the peritubular capillary walls (43-48). In general, the correlations have been excellent, primarily due to changes in peritubular capillary Starling forces. Interstitial fluid space Starling forces, as estimated from renal lymph protein concentrations and subcapsular hydraulic pressure, have been small (43) and have correlated poorly, if at all, with absolute proximal reabsorptive rates (43, 46). The failure of absolute proximal reabsorption to parallel closely changes in interstitial hydraulic or oncotic pressures leaves unexplained the mechanism by which absolute proximal reabsorption is linked to peritubular capillary hydraulic and oncotic pressures. If the peritubular capillary, the interstitial fluid space, tubular basolateral membrane, and tubular cells are connected in series as shown in Fig. 6, panel A, then the tubular transport process influenced by peritubular protein must be sensitive to very small, experimentally undetectable changes in interstitial fluid space Starling forces. Alternatively, the renal lymph and the subcapsular interstitial space may not be representative of the Starling forces in the fluid compartment adjacent to the tubular basolateral membrane. Recent evidence strongly supports this view. Ultrastructural data in the rat obtained by Pederson et al. (49) suggests that the peritubular capillary wall is in tight apposition to the tubular basement membrane over $\sim 50\%$ of the tu-



FIGURE 6 Relationship between peritubular capillary (PC), interstitial fluid space (IFS), tubular basement membrane (TBM), and tubular cells. Panel A: PC, IFS, TBM, and cells are connected in series so that the IFS is interposed between PC and tubular cells. Panel B: PC and TBM are in tight apposition over 50% of cell basal surface area and 77% of the total fenestrated area of the capillary wall directly faces the TBM (49). Thus, there is direct communication between PC and tubular cells.

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⁴ Protein removal inhibits NaCl transport without changing the transepithelial PD (Table IV, ref. 1), suggesting that transcellular NaCl transport is neutral. The fact that transcellular NaCl transport is neutral does not define the mechanism of NaCl transport across the luminal or basolateral membranes. It is consistent with several modes of neutral transport and does imply direct one for one coupling via a neutral NaCl symporter.

bular basal surface. There appears to be little, if any, fluid in the intervening space (49). Furthermore, at least 77% of the fenestrations in the peritubular capillary are in the portion of peritubular capillary that abuts the tubular basement membrane (49). Accordingly, the relationship between the peritubular capillaries, the interstitial fluid space, the tubular basolateral membrane, and the tubular cells is probably as depicted in Fig. 6, panel B. In Fig. 6, panel B two distinctly different pathways for reabsorbate to pass from proximal tubule cells to peritubular capillary lumen are represented. First, there is a long and complex pathway that passes through the interstitial fluid space and enters the peritubular capillary where fenestrations or endothelial pores are sparse. Second, there is the short and direct pathway from tubule cell across the peritubular capillary surface with a large fraction of the endothelial pores. This second pathway might well represent the route by which changes in peritubular Starling forces are transduced to the proximal tubule cell. The mechanism for the transduction would be direct, without involving the interstitial fluid space, and as such, would be similar for in vitro protein removal and in vivo extracellular fluid volume expansion.

In summary, we have shown that peritubular protein removal in vitro specifically inhibits active neutral NaCl transport⁴ from proximal convoluted tubules. The relevance of this in vitro observation to the mechanism of extracellular fluid volume expansion in vivo remains to be explored. In view of the present in vitro data, however, it is possible that the effects of extracellular fluid volume expansion in vivo are twofold. First, there would be a specific effect of capillary protein concentration on volume and NaCl absorption. This effect would occur in vivo and in vitro and would be primary. Second, there would be an effect of interstitial hydrostatic pressure on paracellular permeability properties. This effect would be small and secondary.

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