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### Research Article

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# Characteristics of NaCl and Water Transport in the Renal Proximal Tubule

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**ABSTRACT** Renal proximal tubular transport of salt and water has been examined using isolated perfused rabbit tubules. In this method direct measurements can be made under controlled conditions not readily achieved *in vivo*. The results are in general agreement with previous micropuncture studies in other species, supporting the validity of both sets of measurements.

In the present studies, absorption of sodium salts and water occurred without change in the concentration of Na in the lumen except when a poorly reabsorbed solute (raffinose) was present, in which case, mean concentration of Na in the lumen reached a steady-state value 33–35 mEq liter<sup>-1</sup> less than in the bath. The tubule is very permeable to sodium salts (sodium permeability =  $9.3 \times 10^{-5}$  cm sec<sup>-1</sup>,  $\sigma_{\text{NaCl}} = 0.68$ –(0.71) and to water (hydraulic conductivity [ $L_p$ ] = 2.9 to  $6.3 \times 10^{-5}$  cm sec<sup>-1</sup> atm<sup>-1</sup>). Net reabsorptive flux of Na was only 20% of the unidirectional Na flux.

The steady-state concentration difference for Na in the presence of raffinose and  $\sigma_{\text{NaCl}}$  in the present studies was the same as previously found by micropuncture in the rat. On the other hand Na permeability, net Na transport rate, and  $L_p$  were all from one-half to one-third as great in the isolated rabbit tubule as in the rat *in vivo*. Apparently, although the transport mechanisms appear to be basically the same in the two species, there are fewer transport units and passive permeability paths per unit length in the rabbit tubule than in the rat.

## INTRODUCTION

Walker, Bott, Oliver, and MacDowell (1) were the first to report that the glomerular filtrate is reduced in volume in the proximal convoluted tubule of rat and

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guinea pig without a change in the osmolality of the tubule fluid. Their micropuncture results have been repeatedly confirmed and extended to other species (2). In addition it has been established that the concentration of Na in tubule fluid remains equal to that of plasma during the reabsorptive process unless osmotic flow is restricted by inclusion of a poorly absorbed solute in the luminal fluid (2).

The present experiments were designed to re-examine the reabsorptive mechanism for Na and water in the isolated perfused rabbit proximal convoluted tubule. The system employed permits direct estimates, under controlled conditions not readily achieved in the *in vivo* preparation, of a number of phenomenological coefficients necessary for characterization of the tubule epithelium. It has been possible to measure the passive permeability of the epithelium to Na, the rate of active Na transport, the hydraulic conductivity of the epithelium to water, and the reflection coefficient for NaCl. The results are in large part similar to those obtained *in vivo* and have been interpreted within the framework of current views of the mechanisms of fluid reabsorption by the proximal tubule of the mammal.

## METHODS

Rabbit proximal convoluted tubules were dissected and perfused *in vitro* using a modification of a method previously described (3, 4), as summarized below.

New Zealand white female rabbits were injected intravenously with 10 cc of 15% solution of mannitol<sup>1</sup> and were killed by decapitation 10 min later. A piece of the right kidney was removed and placed in chilled rabbit serum for dissection. A superficial proximal convoluted tubule was dissected free and transferred to a separate dish where it was perfused while

<sup>1</sup> The purpose of mannitol was to slow collapse of the tubule thus facilitating subsequent perfusion. It was later found that perfusion could be successfully initiated even in fully collapsed tubules. Consequently the use of mannitol was discontinued midway through the experiments without any apparent change in the results.

TABLE I  
Sodium Flux and Permeability of Isolated Perfused Proximal Convoluted Tubules

Bath	Perfusate	Perfu- sion rate (V <sub>o</sub> )	Collec- tion rate (V <sub>L</sub> )	Lumi- nal area (A)	Na concentration			Net vol flux (J <sub>v</sub> )	Net Na flux (MN <sub>Na</sub> )	Na flux to bath (MN <sub>Na,b</sub> )	Na permea- bility bath to lumen (PN <sub>Na,b-l</sub> )	
					Bath [Na] <sub>b</sub>	Per- fused [Na] <sub>o</sub>	Col- lected [Na] <sub>L</sub>					[Na <sup>22</sup> ] <sub>o</sub> [Na <sup>22</sup> ] <sub>L</sub>
		cm <sup>3</sup> sec <sup>-1</sup> × 10 <sup>-3</sup>	cm <sup>3</sup> sec <sup>-1</sup> × 10 <sup>-3</sup>	cm <sup>2</sup> × 10 <sup>-4</sup>	Eq cm <sup>-3</sup> × 10 <sup>-6</sup>			cm sec <sup>-1</sup> × 10 <sup>-5</sup>	Eq cm <sup>-2</sup> sec <sup>-1</sup> × 10 <sup>-9</sup>	Eq cm <sup>-2</sup> sec <sup>-1</sup> × 10 <sup>-9</sup>	cm sec <sup>-1</sup> × 10 <sup>-5</sup>	
Serum	Ultrafiltrate	417	385	5.34	155	145	150	1.13	5.99	5.08	22.5	11.2
		422	405	5.02	154	145	147	1.16	3.39	3.30	22.8	12.7
		332	326	7.22	146	147	142	1.13	0.83	3.47	9.3	4.0
		212	190	7.54	148	147	140	1.53	2.91	6.04	20.3	9.6
		342	313	7.40	155	145	148	1.35	3.92	4.42	25.1	13.3
	Mean				152	146	145		3.41	4.46	20.0	10.2
Serum	Ultrafiltrate +raffinose (isosmotic)	208	207	5.15	143	114	110	1.48	0.19	1.82	17.9	11.2
		320	280	5.65	144	114	120	1.28	7.07	5.10	23.6	12.8
		348	371	6.15	150	114	109	1.22	-3.74	-1.24	8.8	6.7
		275	294	6.59	147	114	108	1.59	-2.88	-0.61	18.9	13.3
		233	249	5.65	143	116	106	1.35	-2.83	1.12	11.2	7.0
	Mean				145	116	107	1.21	2.71	8.32	15.5	5.0
Serum +NaCl	Raffinose	172	172	5.34	205				0	-15.5		9.2
		133	112	5.15	201			0.234	4.08	-7.18		5.2
		255	240	8.80	205			0.180	1.70	-10.1		6.4
		178	168	5.65	211			0.190	1.77	-11.9		7.3
		184	187	10.1	206			0.230	-0.30	-8.89		6.0
	Mean				211			0.322	-1.43	-11.9		8.0
	Mean				207				0.97	-10.9		7.0

observed with either an inverted microscope at a magnification of 50–400 or a dissecting microscope at a magnification of 6–100. During perfusion the tubules were bathed in rabbit serum bubbled with 95% O<sub>2</sub>-5% CO<sub>2</sub> at 37°C. The following three perfusion solutions were used: (a) an ultrafiltrate of the same lot of serum as that used in the bath (prepared with Amicon PM-10 membranes), (b) a raffinose solution containing 0.001 M MgSO<sub>4</sub>, 0.001 CaCl<sub>2</sub>, and 0.0025 K<sub>2</sub>HPO<sub>4</sub>, and (c) a mixture of (a) and (b). Solutions containing raffinose were adjusted to pH 7.4 and to the same osmolality as the bath. The osmolality of the serum was lowered to equal that of the ultrafiltrate by addition of an appropriate amount of water. No adjustment was made to equalize the concentration of Na in plasma and ultrafiltrate, the former being higher (Table I) owing to the presence of protein. The bathing solution was exchanged with fresh medium every 10–20 min in all experiments with the exception of those in which <sup>24</sup>Na was included in the bath. In these water was added to the bath every 10 min in amounts sufficient to correct for evaporative loss.

The fluid in the collecting pipet holding the tubule was covered with mineral oil to prevent evaporation. In addition when the Na<sup>+</sup> permeability of the tubule was measured, the tubule was sealed into the collecting pipet with Sylgard 184 (5). This was done to avoid any isotopic exchange of Na in collected fluid with that in the bath by diffusion through a potential space between the outer surface of the tubule and the inner surface of the pipet. The collected fluid was removed at timed intervals, and its volume was measured in a calibrated glass capillary.

In order to permit calculation of net fluid transport either albumin <sup>125</sup>I or <sup>131</sup>I was added to the perfusate, and the concentration of isotope was measured in both the perfusate and the collected fluid. In studies designed to determine the Na concentration of the collected fluid and the permeability of the tubule to Na, <sup>22</sup>Na was added to the perfusate and <sup>24</sup>Na to the bath. The Na concentration in the perfusate ([Na]<sub>o</sub>) and bath ([Na]<sub>b</sub>) and the radioactivity of the perfusate (<sup>22</sup>Na]<sub>o</sub>) and bath (<sup>24</sup>Na]<sub>b</sub>) were measured, as well as the radioactivity (<sup>22</sup>Na]<sub>L</sub> and <sup>24</sup>Na]<sub>L</sub>) in the collected perfusion fluid. The Na concentration in the collected fluid, [Na]<sub>L</sub>, is the sum of that Na originating from the perfusion fluid, [Na']<sub>L</sub>, and from the bath [Na'']<sub>L</sub> (*vide infra*). Thus, [Na]<sub>L</sub> = [Na']<sub>L</sub> + [Na'']<sub>L</sub>. Assuming that <sup>24</sup>Na and <sup>22</sup>Na are perfect tracers,

$$\frac{[Na']_L}{[^{22}Na]_L} = \frac{[Na]_o}{[^{22}Na]_o}$$

and

$$\frac{[Na'']_L}{[^{24}Na]_L} = \frac{[Na]_b}{[^{24}Na]_b}$$

Combining these equations and rearranging,

$$[Na]_L = \frac{[^{22}Na]_L [Na]_o}{[^{22}Na]_o} + \frac{[^{24}Na]_L [Na]_b}{[^{24}Na]_b}$$

The above method for the determination of the concentration of Na would yield erroneous results were the specific activity of the tubule fluid appreciably affected by unlabeled Na originating in the cells. This is not a source of error in the

current studies since little Na (relative to that in lumen and bath) is present in tubule cells, and in addition cellular exchange is so rapid (6) that intracellular Na is completely labeled by the time the experimental collections are begun (i.e. more than 5 min after placement of the tubule in the radioactive solutions). The radioactivity of each isotope was determined in a three-channel well scintillation counter (Packard 3003).  $^{24}\text{Na}$  was counted immediately at the completion of the study and again 14 days later at which time it had decayed.  $^{22}\text{Na}$  and  $^{125}\text{I}$  were determined only on the 14 day sample. The osmolality of the collected perfusion fluids was measured using the method of Ramsay and Brown (7). Perfusate and bath osmolality were measured with a Bowman-Aminco osmometer. The concentrations of Na in the perfusate and bathing solution were measured with an IL flame photometer (Instrumentation Laboratory, Inc., Watertown, Mass.) and pH with a Beckman expanded scale pH meter.

The sources of radioisotopes were:  $^{125}\text{I}$ , E. R. Squibb & Sons, New York;  $^{24}\text{Na}$ , International Nuclear Corporation;  $^{22}\text{Na}$ , New England Nuclear Corp., Boston, Mass.;  $^{131}\text{I}$ , Abbott Laboratories, North Chicago, Ill.

*Calculation of fluxes and permeabilities.* Net Na flux ( $M_{\text{Na}}$ ) was calculated from the relationship

$$M_{\text{Na}} = \frac{V_0[\text{Na}]_0 - V_L[\text{Na}]_L}{A}$$

where  $V_0$  is the perfusion rate,  $V_L$  the rate of flow into the collecting pipet, and  $A$  the surface area of the tubule.

Unidirectional Na flux from lumen to bath ( $M_{\text{Na}_{1 \rightarrow b}}$ ) was calculated from the relationship  $M_{\text{Na}_{1 \rightarrow b}} = P_{\text{Na}_{1 \rightarrow b}}([\text{Na}]_0 + [\text{Na}]_L)/2$ , where  $P_{\text{Na}_{1 \rightarrow b}}$  is the Na permeability from lumen to bath and  $([\text{Na}]_0 + [\text{Na}]_L)/2$  is the mean concentration of Na in the tubule lumen.  $P_{\text{Na}_{1 \rightarrow b}}$  was determined as previously described (8) where

$$P_{\text{Na}_{1 \rightarrow b}} = \frac{V_0 - V_L}{A} \left[ \frac{\ln([\text{Na}]_0/[\text{Na}]_L)}{\ln(V_0/V_L)} + 1 \right]$$

In the experiments in which these calculations were used (Table I, top and middle)  $[\text{Na}]_0$  was similar to  $[\text{Na}]_L$ , so that  $([\text{Na}]_0 + [\text{Na}]_L)/2$  is an adequate approximation of the mean concentration of Na in the tubule lumen.

Na permeability from bath to lumen ( $P_{\text{Na}_{b \rightarrow 1}}$ ) in experiments in which ultrafiltrate of serum or ultrafiltrate of serum

plus raffinose was perfused (Table I, top and middle) was calculated as:

$$P_{\text{Na}_{b \rightarrow 1}} = \frac{M_{\text{Na}_{1 \rightarrow b}} - M_{\text{Na}}}{[\text{Na}]_b}$$

and in experiments in which raffinose solution was perfused as

$$P_{\text{Na}_{b \rightarrow 1}} = \frac{[\text{Na}]_L}{[\text{Na}]_b} \times \frac{P_{\text{Na}_{1 \rightarrow b}}}{1 - \exp[-(P_{\text{Na}_{1 \rightarrow b}}A)/V]}$$

where  $V = (V_0 + V_L)/2$  and  $P_{\text{Na}_{1 \rightarrow b}}$  is assumed to equal  $14 \times 10^{-5} \text{ cm sec}^{-1}$ , the mean value obtained in experiments with Na containing perfusion fluids. Actually the precise value of  $P_{\text{Na}_{1 \rightarrow b}}$  employed in this calculation makes relatively little difference, since for the worst case (slowest perfusion rate) a 50% underestimate of  $P_{\text{Na}_{1 \rightarrow b}}$  yields only a 12% overestimate of  $P_{\text{Na}_{b \rightarrow 1}}$ .

The length of the tubule ( $L$ ) was measured with an ocular micrometer. Luminal area ( $A$ ) was calculated from  $L$ , assuming a diameter of  $20 \mu$ . The arbitrary diameter of  $20 \mu$  was chosen in order to facilitate comparison with other tubule studies in which this figure has generally been used.

Results are expressed as mean  $\pm$ SEM (number of tubules studied). The data for each tubule are the mean of the results of two or three collection periods under a given set of conditions.

## RESULTS

An approximation of the conditions which exist in vivo was obtained by bathing the proximal tubule in rabbit serum and perfusing it with an ultrafiltrate of the same serum. Net fluid absorption was measured under these circumstances in two sets of studies. In the first (five experiments were done) (Table I, upper set)  $1.24 \pm 0.30 \text{ nl mm}^{-1} \text{ tubule length min}^{-1}$  ( $3.41 \times 10^{-5} \text{ cm}^3 \text{ cm}^{-2}$  luminal area  $\text{sec}^{-1}$ ) was absorbed, and in the second group (Table II)  $1.59 \text{ nl mm}^{-1} \text{ min}^{-1}$ . These results are in reasonable agreement with those published previously from this laboratory (4). In addition, as in rat (1), dog (9), and monkey (10) in vivo, Na is reabsorbed isototically, i.e. without a change in the concentration of  $[\text{Na}]$  in the luminal fluid. As can be seen in Table I (upper set), the concentration of Na in collected fluid was  $145 \text{ mmoles liter}^{-1}$ , that in the ultrafiltrate per-

TABLE II  
*Measurement of Reflection Coefficient for NaCl ( $\sigma_{\text{NaCl}}$ ) in Isolated Perfused Rabbit Proximal Convuluted Tubules*

Method	Perfusate	Bath	Volume flow (Jv) <i>nl mm<sup>-1</sup> min<sup>-1</sup></i>	Concentration difference ( $\Delta C$ ) <i>mOsm</i>	$\sigma_{\text{NaCl}}$
Equal concentration	Ultrafiltrate	Serum	+1.59	0	0.71 $\pm$ 0.06 (6)
		Serum + NaCl	+3.72	+120	
		Serum + Raffinose	+4.61	+118	
		Serum + H <sub>2</sub> O	-0.68	- 55	
Zero flow	Raffinose	Serum	-2.89	0	0.68 $\pm$ 0.06 (6)
		Serum + NaCl	+0.90	+178	

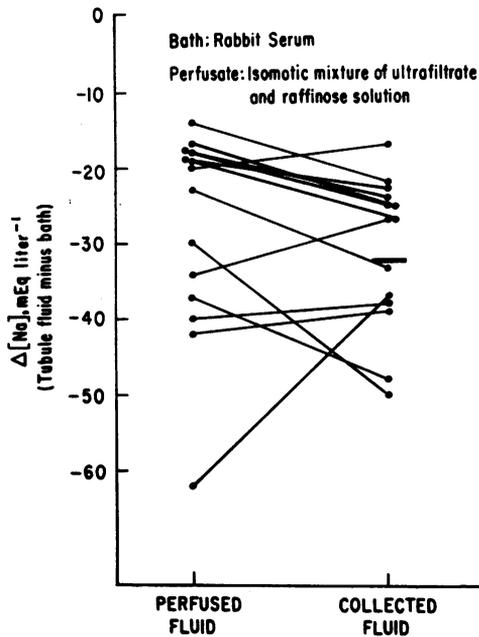


FIGURE 1 Steady-state concentration difference for Na in isolated perfused proximal convoluted tubules. Bath was rabbit serum; perfusate was an isosmotic mixture of ultrafiltrate and raffinose solution. Mean concentration difference in collected fluid ( $33 \text{ mEq liter}^{-1}$ ) is assumed to be equal to the mean steady-state value under these conditions.

fused  $146 \text{ mmoles liter}^{-1}$ . The mean net flux of Na in the same studies (five experiments) was  $4.46 \times 10^{-9} \pm 0.47 \text{ Eq cm}^{-2} \text{ sec}^{-1}$ .

Reabsorption of NaCl without its complement of water occurs in vivo when a poorly absorbable solute such as mannitol is added in isosmotic proportions to the proximal fluid (11). The osmotically active solute restricts the efflux of water which in the absence of mannitol, for example, is coupled osmotically with net NaCl absorption. Continuing net NaCl absorption results in a fall in the concentration of Na in the luminal fluid until a limiting gradient across the cell is reached, at which time net movement of both water and Na virtually ceases. In the rat limiting or steady-state concentration of Na in the lumen has been found to be  $35 \text{ mEq liter}^{-1}$  less than that in plasma (12, 13).

The steady-state concentration difference for Na across the isolated perfused rabbit proximal tubule was determined in a series of studies in which the tubules were perfused at slow rates ( $1\text{--}5 \text{ nl min}^{-1}$ ) with isosmotic solutions composed of an ultrafiltrate of serum mixed in varying proportions with an isosmolar raffinose solution. The results are illustrated in Fig. 1. It is apparent that despite differences in the initial Na concentration of the perfusate which varied between 14 and  $62 \text{ mEq liter}^{-1}$  less than in the bath, the mean concentration of Na in the collected perfusion fluid was  $33 \text{ mEq liter}^{-1}$

less than in the bath. It is reasonable to conclude that the latter figure represents the mean limiting or steady-state concentration difference since in eight of nine tubules in which the initial concentration difference of Na was less than  $33 \text{ mEq liter}^{-1}$  it increased, and in four of five in which the initial difference was greater it decreased. In another series of studies performed at more rapid perfusion rates (Table I, middle set) the Na concentration of the perfusate was  $31 \text{ mEq liter}^{-1}$  less than that of the bath, and the mean difference between collected tubule fluid and bath was  $35 \text{ mEq liter}^{-1}$ .

Net Na flux ( $M_{Na}$ ), unidirectional Na flux from lumen to bath ( $M_{Na1 \rightarrow b}$ ) and the permeability of Na of the tubule from bath to lumen ( $P_{Na b \rightarrow 1}$ ) were measured under the following three sets of experimental conditions: (a) during perfusion of tubules bathed in rabbit serum with an ultrafiltrate of serum, the condition which most closely resembles the in vivo physiologic state; (b) during perfusion with an isosmolar mixture of raffinose and ultrafiltrate of serum of tubules bathed in serum (the Na concentration difference between the perfusion solution and the bath approximated the steady-state concentration difference obtained in the studies described above [Fig. 2]); and (c) during perfusion with a Na-free raffinose solution (isosmotic to normal serum) of tubules bathed in rabbit serum to which sufficient NaCl was added to minimize osmotic flow of water into the lumen (*v.i.*). Under these circumstances Na which enters the lumen from the bath must be accompanied by anion, presumably chloride so that the permeability measured is that of the tubule to the Na salt (principally NaCl), rather than to Na alone. Any contribution of either exchange diffusion or forced exchange of Na with another cation is excluded.

In the first series of studies (Table I, upper set), as indicated above, net fluid absorption occurred without

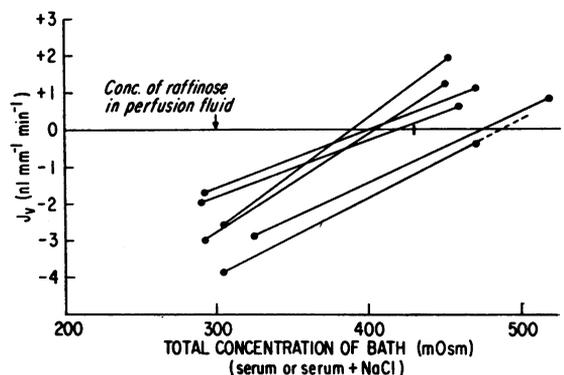


FIGURE 2 Measurement of reflection coefficient for NaCl ( $\sigma_{NaCl}$ ) in isolated perfused rabbit proximal convoluted tubule by the zero flow method. Perfusate was raffinose solution whose mean concentration ( $298 \text{ mOsm}$ ) is indicated by the arrow. Mean concentration of bath for zero flow ( $J_v = 0$ ) was  $435 \text{ mOsm}$ . Thus,  $\sigma_{NaCl} = 298/435 = 0.68$ .

a change in the Na concentration of the perfusate. The mean net flux,  $M_{Na}$ , was  $4.46 \times 10^{-9}$  Eq  $\text{cm}^{-2} \text{sec}^{-1}$ . This was approximately 20% of the unidirectional flux of Na from lumen to bath ( $M_{Na1 \rightarrow b} = 20.0 \times 10^{-9}$  Eq  $\text{cm}^{-2} \text{sec}^{-1}$ ). The mean permeability of the tubule to Na from bath to lumen was  $10.2 \times 10^{-5}$  cm  $\text{sec}^{-1}$ .

The second set of studies (Table I, middle set) was designed to measure the unidirectional Na flux from lumen to bath ( $M_{Na1 \rightarrow b}$ ) and the permeability to Na from bath to lumen ( $P_{Na b \rightarrow 1}$ ) in the steady state, i.e. in the absence of net Na and H<sub>2</sub>O movement, in order to minimize the effect of solute and water interactions on the fluxes. Although on the average this state was approached, significant net fluxes occurred in individual studies. It is uncertain whether this represents functional variation between tubules or is a reflection of the difficulty of measuring with precision net water flux at high rates of perfusion.<sup>2</sup> The mean Na permeability from bath to lumen was  $9.3 \times 10^{-5}$  cm  $\text{sec}^{-1}$  which is not significantly different from the result obtained in the experiments in which the perfusate was ultrafiltrate alone.

In the third series of experiments (Table I, lower set) net flux of water into the 312 milliosmolar raffinose perfusion solution was virtually eliminated (on the average) by increasing the osmolality of serum in the bath to 439 mOsm by the addition of NaCl. Under these conditions the mean Na permeability from bath to lumen (which must equal the permeability to NaCl, *v.s.*) was  $7.0 \times 10^{-5}$  cm  $\text{sec}^{-1}$ . This result cannot be interpreted with certainty without knowledge of the electrical potential difference. The relatively small difference in the Na permeability with and without Na salts in the lumen however is consistent with lack of important interaction between the Na fluxes in the two directions such as would occur were exchange diffusion or single file diffusion a major mechanism for Na permeation in the tubule. Baumann, Frömter, and Ullrich (14) obtained a different result (when comparing Na to NaCl flux) in rat proximal convoluted tubules perfused *in vivo*. The permeability measured from the appearance of Na in raffinose solution perfused through the tubule lumen ( $36\text{--}89 \times 10^{-5}$  cm  $\text{sec}^{-1}$ ) was greater than the isotonic Na permeability ( $25 \times 10^{-5}$  cm  $\text{sec}^{-1}$ ) measured with NaCl in the lumen rather than less, as in the present studies. The reason for this difference is unclear at present.

Certain solutes fail to exert the full osmotic effect predicted from the van't Hoff relationship ( $\pi = nRT\Delta C$ , where  $\pi$  = osmotic pressure and  $n$ ,  $R$ ,  $T$  have their usual meanings, and  $\Delta C$  = difference in concentration

<sup>2</sup> With a flow rate of 20 nl  $\text{min}^{-1}$  ( $333 \times 10^{-9}$  cm<sup>3</sup>  $\text{sec}^{-1}$ ) through a tubule 1 mm long a 5% error in the concentration of albumin results in difference of 1 nl  $\text{mm}^{-1} \text{min}^{-1}$  ( $2.66 \text{ cm} \text{sec}^{-1} \times 10^{-6}$ ) in the estimation of net fluid flux

across the membrane) on water flow across artificial and biological membranes. Staverman (15) correctly interpreted this to indicate penetration of the solute through the membrane and defined the fractional deviation from the van't Hoff pressure,  $\sigma$ , or reflection coefficient, as the ratio of the experimentally observed  $\pi$  to the calculated  $\pi$ . Subsequently the importance of the reflection coefficient for defining the properties of biological membranes has been emphasized (16). At least two experimental techniques, the "zero flow" and "equal concentration" methods, have been devised to measure  $\sigma$  (17). In both the effect of a test solute is compared with that of an impermeant reference solute. In the zero flow method the test solute is placed on one side of the membrane and the reference solute on the other. The ratio of the solute concentration (reference/test) at which zero net flow occurs is equal to  $\sigma$ . In the equal concentration method the increment in osmotic flow induced by equal concentrations of test and reference solutes across a membrane are compared. The reflection coefficient is the ratio of the increment in flow induced by the test solute to that induced by the reference solute. Precision in the determination of  $\sigma$  is limited in consequence of movement of the test solute or water across the membrane which thereby alters the osmotic gradient. Modifications of both methods have been used in the present studies to determine the reflection coefficient of the rabbit proximal tubule for NaCl ( $\sigma_{NaCl}$ ). The data are illustrated in Table II and Fig. 2.

The concentration of NaCl in the bath necessary to counterbalance the osmotic effect of 300 mOsm raffinose in the perfusion solution (zero flow method) was determined as follows. The tubule was first bathed in normal serum and perfused with a raffinose solution. As can be seen in Table II, a mean of 2.89 nl  $\text{mm}^{-1} \text{min}^{-1}$  of fluid moved into the tubule lumen. Following this, NaCl was added to the bathing solution to achieve a final osmolality approximately 178 mOsm greater than that of the perfusate. Under these circumstances the direction of flow reversed; the mean absorption from the lumen was 0.90 nl  $\text{mm}^{-1} \text{min}^{-1}$ . The sequence of the procedure was reversed in some studies, and the individual data are illustrated in Fig. 2. The osmolality of the bath (or the concentration of NaCl) at which no net flow occurred was determined by extrapolation, and  $\sigma_{NaCl}$  was calculated as above. The mean reflection coefficient was  $0.68 \pm 0.06$  (6).

The reflection coefficient of 0.68 is an imprecise estimate for two reasons. In the first place, NaCl entered the lumen in the course of the study, thereby reducing the concentration gradient below that used in the calculation. The mean concentration of Na in the collected fluid was 26 and 40 mEq liter<sup>-1</sup> when the tubule was bathed in isosmotic and hyperosmotic sera, respectively. Were this taken into account the  $\sigma$  would

be approximately 10% higher. In addition flow across biological membranes is not necessarily a linear function of the osmotic gradient, so that the linear extrapolation used in Fig. 2 may be in error. The error so introduced is probably of negligible significance since net flow approximated zero when the bath was hypertonic. Bauman, Frömter, and Ullrich (14) estimated  $\sigma_{\text{NaCl}}$  of the rat proximal convoluted tubule in vivo by determining the concentration of raffinose in the perfusate necessary to eliminate net water flow, and they obtained the same result (0.69) as did we.

The reflection coefficient was also determined in a second set of studies in which a modification of the equal concentration method was used. The data are presented in Table II. In these experiments in order to minimize changes in the NaCl concentration of the perfusate short segments of tubules (1.2 mm mean length) were perfused rapidly (25 nl min<sup>-1</sup>) with an ultrafiltrate of rabbit serum and the absorptive rate measured. The increment in net absorption induced by the addition of 120 mOsm of NaCl to the bathing serum was compared with that induced by 118 mOsm of raffinose and  $\sigma_{\text{NaCl}}$  calculated as discussed above. In each of the six studies two measurements of absorption with normal serum in the bath were made at the beginning and end of the experiment. Since there were no significant differences observed between the early and late periods, the data from the four periods were pooled to calculate the mean rate of fluid absorption ( $J_v$ ) without an osmotic gradient. The mean of three periods each with either raffinose or NaCl added to the bath was used to calculate the respective increments in absorptive rate (osmotic flows). The order of additions was varied appropriately. Using this procedure the mean reflection coefficient was  $0.71 \pm 0.06$  (6). This value is also subject to errors introduced by alteration in the composition of the perfusate which cannot be avoided. In this regard it is of some interest that we observed as did Ullrich, Rumrich, and Fuchs in the rat (18) that the osmolality of the collected raffinose solution (mean 335 mOsm) rises above that of bathing serum (mean 306 mOsm) despite net flow of fluid into the tubule lumen. Apparently the permeability of the proximal tubule to Na is so great that relatively more Na salts enter the lumen by diffusion and perhaps by solvent drag than does water by osmosis.

The hydraulic conductivity ( $L_p$ ) of the tubule to water was also estimated from the results of the studies in Table II. The estimated values and the experimental conditions are listed in Table III. Since these are the same data as were used to determine  $\sigma_{\text{NaCl}}$ , the calculated values of  $L_p$  are subject to the same uncertainties as are those for  $\sigma_{\text{NaCl}}$  and must be regarded as approximations.

TABLE III  
Measurement of Water Permeability (Hydraulic Conductivity,  $L_p$ ) in Rabbit Proximal Convoluted Tubule

Lumen	Bath	Concentration difference ( $\Delta C$ )	$L_p$
		mOsm	(cm <sup>3</sup> cm <sup>-2</sup> sec <sup>-1</sup> atm <sup>-1</sup> × 10 <sup>-5</sup> )
Ultrafiltrate	Serum + raffinose	+117	2.9 ± 0.2 (7)
Raffinose	Serum	0	3.7 ± 0.4 (6)
Ultrafiltrate	Serum + water	-55	6.3 ± 0.7 (7)

Three different values of  $L_p$  obtained under separate experimental conditions were calculated.

The first calculation was based on the results of the studies (Table III) in which the driving force for water movement was the osmotic pressure due to 117 mM raffinose added to the serum in the bath. Net flow of water was therefore out of the lumen. Under these circumstances

$$L_p = \frac{\Delta J_v}{RT C_{\text{raffinose}}}$$

where  $\Delta J_v$  = the increment in net flow of water per unit surface area per unit time induced by the increase in osmolality due to raffinose ( $C_{\text{raffinose}}$ ) and R and T have the usual significance. Raffinose, as in the studies described earlier, is assumed to have a  $\sigma$  of 1. The mean  $L_p = 2.9 \times 10^{-5}$  cm sec<sup>-1</sup> atm<sup>-1</sup>.

In the second series of studies with raffinose as the perfusion solution and serum in the bath net water flow is into the lumen.

$$L_p = \frac{J_v}{RT(C_{\text{raffinose}} - \sigma_{\text{NaCl}}[C_{\text{Na}_b} - C_{\text{Na}_L}/2])}$$

where  $J_v$  = the rate of net water flow per unit membrane area per unit time and  $[C_{\text{Na}_b} - C_{\text{Na}_L}/2]$  is the mean concentration difference in mOsm liter<sup>-1</sup> of Na between the bath and lumen. The mean  $L_p = 3.7 \times 10^{-5}$  cm sec<sup>-1</sup> atm<sup>-1</sup>.

In the third set of the studies (Tables II and III) water was added to the serum in the bathing solution to lower the osmolality 55 mOsm below that of the ultrafiltrate in the lumen. Net flow into the bath diminished and in some studies reversed.

$$L_p = \frac{\Delta J_v}{RT\sigma_{\text{NaCl}}\Delta C}$$

where  $\Delta C = -55$  mOsm. The mean value of  $L_p = 6.3 \times 10^{-5}$  cm sec<sup>-1</sup> atm<sup>-1</sup>.

The hydraulic conductivity of the rat proximal convoluted tubule in vivo was found to be  $17 \times 10^{-5}$  cm sec<sup>-1</sup> atm<sup>-1</sup> (14).

It is noteworthy that  $L_p$  was highest in the current studies when the osmolality of the bathing solution was

lowest, and lowest when the osmolality was highest. This result is analogous to that observed in the rat proximal tubule (14) and in other tissues (19).

## DISCUSSION

The results of the present studies are in many respects similar to those previously obtained in *in vivo* micro-puncture studies in other species. This of itself is gratifying and indicative of the utility of the *in vitro* preparation. The isolated perfused proximal convoluted tubule of the rabbit is highly permeable to NaCl and water and actively transports an isosmotic solution out of the lumen. As in the nephron *in vivo* no change in the concentration of Na occurs during reabsorption unless a relatively nonabsorbable solute such as raffinose is present in the perfusate. In the latter case the concentration of Na declines and reaches a minimum steady-state value 30–35 mEq liter<sup>-1</sup> less than in the bath.

This steady-state concentration difference, virtually identical with that reported in rat (12, 13), is achieved when the rate of active efflux of Na from the lumen is equal to the rate of net passive backflux from the bath to the lumen. In the present studies net backflux of Na at the steady-state minimum luminal concentration was determined from the relationship  $M_{Na_{b \rightarrow l}} = P_{Na_{b \rightarrow l}} \times \Delta C_{Na}$ , where  $M_{Na_{b \rightarrow l}}$  is net backflux of Na,  $P_{Na_{b \rightarrow l}}$  the isotopic Na permeability from bath to lumen, and  $\Delta C_{Na}$  the steady-state concentration difference between bath and lumen (33 mEq liter<sup>-1</sup>). Then,  $M_{Na_{b \rightarrow l}} = 3.13 \times 10^{-9}$  moles cm<sup>-2</sup> sec<sup>-1</sup>.

This value, assumed to be equal to the rate of active transport of Na in the steady state with raffinose in the perfusate may be compared with the net flux of sodium out of tubules bathed in rabbit serum and perfused with an ultrafiltrate of the same serum. The net flux, equal to the rate of active Na transport out of the tubule under these circumstances, was  $4.46 \times 10^{-9}$  moles cm<sup>-2</sup> sec<sup>-1</sup> or approximately 40% higher than with raffinose added to the perfusate. Ullrich (20), on the basis of similar calculations, reported that active transport of Na with raffinose added to the perfusate ( $\Delta C_{Na} = 35$  mEq liter<sup>-1</sup>) was  $9.8 \times 10^{-9}$  moles cm<sup>-2</sup> sec<sup>-1</sup> and was  $9.0 \times 10^{-9}$  moles cm<sup>-2</sup> sec<sup>-1</sup> in the absence of a concentration difference for Na. He concluded that the Na transport mechanism was saturated under both circumstances (13). Although our results are at variance with his and if accepted at face value would lead to the opposite conclusion, we are not convinced of the validity of the calculations. The rate of active transport with poorly absorbable solute in the lumen is probably an underestimate for two reasons. In the first place the calculation is dependent upon the assumption that there is no electrical driving force

(transtubular PD) in the steady state which may not be correct. Were there a negative PD as there is in the rabbit proximal tubule during isosmotic net transport (mean of -3.8, mv lumen negative, with rabbit serum in the bath and ultrafiltrate in the perfusion fluid [21]), net passive flux into the tubule would be greater than that calculated above. Secondly, net NaCl absorption, albeit at a markedly reduced rate, occurs in the rat in the steady state with raffinose (22) and were this the case in the rabbit as it appears to be (Table I, middle), the calculated rate of active Na transport would again be an underestimate.

In any event, it is of interest that the rat proximal tubule actively transports Na two to three times as rapidly as does that of the rabbit and also is two to three times as permeable to Na. Since the steady-state concentration difference for Na depends on the ratio of active transport to passive permeability, the equality of this ratio in the two species readily accounts for the virtual identity of the Na concentration differences in the presence of raffinose in rat and rabbit. On the basis of these observations, it is suggested that the transport mechanisms may be basically the same in rat and rabbit but that the tubule of the former species contains more transport units and passive permeability paths per unit length than does that of the rabbit. Consistent with this view is the observation that the reflection coefficient for Na, which like the steady-state concentration difference is independent of area, is the same in rat and rabbit, whereas the  $L_p$  and Na permeability which are functions of surface area are greater in the rat.

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