

Kinetics of Na⁺ Transport in *Necturus* Proximal Tubule

KENNETH R. SPRING and GERHARD GIEBISCH

From the Department of Physiology, Yale University School of Medicine, New Haven, Connecticut 06510. Dr. Spring's present address is the National Heart, Lung, and Blood Institute, Laboratory of Kidney and Electrolyte Metabolism, National Institutes of Health, Bethesda, Maryland 20014.

ABSTRACT The dependence of proximal tubular sodium and fluid reabsorption on the Na⁺ concentration of the luminal and peritubular fluid was studied in the perfused *Necturus* kidney. Fluid droplets, separated by oil from the tubular contents and identical in composition to the vascular perfusate, were introduced into proximal tubules, reaspirated, and analyzed for Na⁺ and [¹⁴C]mannitol. In addition, fluid transport was measured in short-circuited fluid samples by observing the rate of change in length of the split droplets in the tubular lumen. Both reabsorptive fluid and calculated Na fluxes were simple, saturable functions of the perfusate Na⁺ concentration ($K_m = 35\text{--}39$ mM/liter, $V_{max} = 1.37$ control value). Intracellular Na⁺, determined by tissue analysis, and open-circuit transepithelial electrical potential differences were also saturable functions of extracellular Na⁺. In contrast, net reabsorptive fluid and Na⁺ fluxes were linearly dependent on intracellular Na⁺ and showed no saturation, even at sharply elevated cellular sodium concentrations. These concentrations were achieved by addition of amphotericin B to the luminal perfusate, a maneuver which increased the rate of Na⁺ entry into the tubule cells and caused a proportionate rise in net Na⁺ flux. It is concluded that active peritubular sodium transport in proximal tubule cells of *Necturus* is normally unsaturated and remains so even after amphotericin-induced enhancement of luminal Na⁺ entry. Transepithelial movement of NaCl may be described by a model with a saturable luminal entry step of Na⁺ or NaCl into the cell and a second, unsaturated active transport step of Na⁺ across the peritubular cell boundary.

INTRODUCTION

A model for the proximal renal tubule depicts passive sodium movement down an electrochemical potential gradient across the luminal membrane and into the cell and active transport out of the cell by a pump mechanism on the peritubular membrane (Giebisch, 1961; Whittembury et al., 1961). It has also been suggested that a rate-limiting step for transepithelial transport may be located at the level of sodium movement across the luminal cell membrane (Stroup et al., 1974).

Despite considerable effort, several kinetic aspects of proximal renal tubular sodium transport have remained unresolved and, in particular, the relationship between extracellular sodium concentration and net sodium transport rate is not clear. Evidence has been presented to suggest that proximal salt and water reabsorption may be a saturable function of extracellular sodium (Györy and

Lingard, 1976), be independent of extracellular sodium (Baldamus et al., 1969), or be linearly dependent on luminal sodium (Bentzel et al., 1974).

The purpose of our experiments was to delineate in more detail some critical aspects of the kinetic behavior of sodium transport in proximal tubules and, in particular, to gain further insight into the nature of sodium movement across the luminal and peritubular cell barriers. To this end, we studied the influence of symmetrical alterations in luminal and peritubular sodium concentrations on the rate of net fluid reabsorption by the *Necturus* proximal tubule, while minimizing chemical or electrical potential gradients. The kidney was perfused with test solutions of varying Na concentrations and a split droplet, identical in composition, was introduced into the tubule lumen. The spontaneous transepithelial potential difference (PD) was subsequently eliminated by a voltage clamp method utilizing an axial electrode inserted into the tubule lumen (Spring and Paganelli, 1972), and fluid reabsorption was assessed by observation of the rate of droplet shrinkage. Our results show that fluid reabsorption by the *Necturus* proximal tubule is a saturable function of the extracellular sodium concentration but that it is linearly related to cellular sodium concentration. This finding is consistent with the thesis that the luminal cell membrane is the site of the rate-limiting step for transepithelial Na and fluid transport.

MATERIALS AND METHODS

Since the experiments were designed to examine the effects of reducing extracellular sodium upon net fluid reabsorption across the proximal tubular epithelium it was important to find a substitute for Na⁺ which did not seriously alter intracellular K⁺ and Cl⁻ concentration or change the electrical cell polarization, i.e. the peritubular membrane PD. Two groups of experiments were performed: (a) cation substitution for Na⁺ by the chloride salts of equimolar quantities of K⁺, Li⁺, choline, and tetramethylammonium (TMA); and (b) partial removal of NaCl and the addition of appropriate amounts of mannitol to maintain normal levels of osmolality. The control perfusion solution had the following composition: NaCl 90 mM/liter; KCl 2.5 mM/liter; NaHCO₃ 10 mM/liter; NaH₂PO₄ 0.5 mM/liter; CaCl₂ 1.8 mM/liter; MgCl₂ 1.0 mM/liter; glucose 2.2 mM/liter; heparin 2,000 U/liter; polyvinylpyrrolidone 2.0 g%, pH 7.59, gassed with 1% CO₂, 99% O₂, temperature ~22°C, osmolality 214 mosmol/kg. In one group of experiments, involving the reduction of extracellular sodium to 2 mM/liter and its replacement by TMA, the NaHCO₃ buffer was replaced by HEPES (Sigma Chemical Co., St. Louis, Mo.) at a concentration of 4 mM/liter since the HCO₃⁻ salt of TMA is not available.

Adult *Necturus maculosus* were obtained, stored, fed, and anesthetized as previously described (Spring, 1973). All experiments were performed on isolated perfused kidneys in the manner described by Giebisch (1961). The aortic perfusion flow was 1.5 ml/min and the renal portal veins were perfused at 1 ml/min. Adequacy of renal perfusion was checked in every experiment by the infusion of a bolus of 0.05% FDC green no. 3 (Keystone Anilene and Chemical Co., Chicago, Ill.) into both the aortic and venous catheters. A period of 1 h of equilibration with test solution was allowed before measurements began and all experiments were terminated after 3 h of perfusion. Subsequent analysis of the kidneys for Na⁺, K⁺, and Cl⁻ revealed that the intracellular composition remained stable over the entire perfusion period after reaching a steady state during the first 60 min. All perfusion solutions were analyzed for pH, osmolality, Na⁺, K⁺, and Cl⁻.

Tissue Analysis

At the conclusion of each experiment both kidneys were removed, blotted, weighed,

dried for 24 h at 110°C, and then shaken in 1 N HNO₃ for 24 h. Additional animals were perfused with test solutions for 100 min, then their kidneys were removed and treated as above for additional use in determination of the ion content of the tubule cells. For the calculation of intracellular electrolyte concentration an extracellular space value of 0.225 g/g wet tissue was assumed, a value similar to that obtained by Whittembury et al. (1961). As a check on the constancy of extracellular space during low Na⁺ or NaCl perfusion, [¹⁴C]inulin space was determined in whole kidneys of nine animals—three control, three at 32.5 NaCl plus mannitol, three at 32.5 NaCl plus TMA. The ratio of experimental:control extracellular space was 1.12 ± 0.19 (mannitol) and 0.91 ± 0.05 (TMA); neither value is significantly different from a ratio of 1. Tissue Na⁺ and K⁺ were determined by flame emission spectroscopy (IL model 143, Boston, Mass.), Cl⁻ was determined by potentiometric titration (Buchler-Cotlove, Buchler Instruments Div., Searle Analytic Inc., Fort Lee, N. J.), and Li⁺ by atomic absorption spectroscopy (model 303, Perkin-Elmer Corp., Norwalk, Conn.).

Several cations were evaluated as possible Na⁺ substitutes by the following criteria: intracellular K⁺ and Cl⁻ concentrations should be minimally affected by the partial removal of Na⁺ and its replacement by the trial cation, and tissue swelling should not occur. Also, peritubular membrane potential should remain relatively constant. Since most of the kidney wet weight is made up of proximal tubules, the tissue results primarily reflect the composition of proximal tubule cells (Whittembury et al., 1961; Giebisch, 1961; Khuri et al., 1972).

Table I lists the effects of replacement of 45% of the extracellular Na⁺ by choline, K⁺, Li⁺, or of tetramethyl ammonium (TMA) on tissue H₂O, cell composition, and peritubular membrane PD. Choline, K⁺, and Li⁺ did not meet the above criteria as Na⁺ substitutes; their presence depolarized cells, caused some tissue swelling, and drastically altered intracellular K⁺ concentration. The choline and K⁺ substitution results agree with previous reports of high choline permeability (Whittembury et al., 1961) and significant K⁺ permeability of the *Necturus* proximal tubule cell (Giebisch, 1961, Whittembury et al., 1961). Cell Li⁺ concentrations rose to equal extracellular Li⁺ during these experiments (intracellular Li⁺ = 39.5 ± 3.6 mM/liter, $n = 4$), while K⁺ and Na⁺ fell. In contrast, TMA substitution for Na⁺ caused only moderate changes in cell Na⁺, K⁺, tissue H₂O, or peritubular membrane PD. In additional experiments, performed in the winter months and listed in Table IV, we observed that TMA was still not the ideal sodium substitute since its presence led to alterations in cell K⁺ and Cl⁻, indicating that some TMA entered the tubule cells.

Abolition of Spontaneous PD

In order to reduce the effects of transepithelial electrical potential differences upon net sodium transfer, short-circuit conditions were established. The apparatus and methods for short-circuiting the tubules have been described in detail previously (Spring and Paganelli, 1972; Spring, 1973). In brief, an isolated split droplet of composition identical to that of the renal perfusate was introduced into the lumen of a long, straight segment of *Necturus* proximal tubule. Castor oil stained with sudan black was used to provide mobile upstream and fixed downstream oil blocks. A platinized axial electrode was inserted across the droplet through the barrel of a pipette placed in the tubule lumen. Transepithelial potential differences were measured with conventional glass microelectrodes filled with 3 M KCl, and short-circuit conditions were achieved by the application of current through the axial electrode.

Determination of ϕ_w and ϕ_{Na}

Fluid reabsorption (ϕ_w) and net sodium reabsorption (ϕ_{Na}) across the proximal tubular epithelium were evaluated by the rate of change in length of the split-drop after

appropriate meniscus correction (Spring and Paganelli, 1972). In previous experiments in which tubules and kidneys had been perfused with normal amphibian Ringer's solution, net sodium flux had been calculated from the rate of fluid transport and the luminal sodium concentration since the reabsorbate is isosmotic and has a sodium concentration (i.e. 100 meq/liter) identical to that of the tubular fluid. The situation is more complicated in the present series of experiments in which both the luminal and peritubular sodium concentrations were lowered by substitution with poorly permeant solutes (mannitol or tetramethylammonium chloride). If active sodium transport continues and sustains the formation of an isosmotic reabsorbate in which the sodium substitutes are essentially absent, a significant fall in luminal Na^+ and a commensurate rise in luminal mannitol would be expected. Indeed, such a mode of sodium extrusion has been observed in split-drop experiments using luminal sodium chloride-mannitol mixtures (Windhager et al., 1959).

TABLE I
EFFECTS OF CATION SUBSTITUTION ON CELL COMPOSITION AND PERITUBULAR MEMBRANE PD

Solution	n	Tissue H ₂ O	Cell ions			Peritubular PD
			[Na ⁺]	[K ⁺]	[Cl ⁻]	
		%	meq/kg cell H ₂ O			mV
Control (100 NaCl)	8	86.4	42.95	81.85	50.34	-51.18
		±0.6	±4.83	±5.27	±2.70	±1.82 (39)
55 NaCl, 45 Choline Cl	4	87.5	25.84	63.16*	42.84	-33.9*
		±0.9	±8.14	±3.32	±13.56	±2.01 (20)
55 NaCl, 45 KCl	4	88.5*	25.61	97.44	39.46	-17.72*
		±0.5	±5.85	±13.11	±10.22	±1.44 (18)
55 NaCl, 45 LiCl	4	87.2	25.79	62.77*	44.31	-24.48*
		±0.3	±5.65	±5.08	±9.16	±2.71 (23)
55 NaCl, 45 TMACl	5	88.0	40.2	92.4	-	-48.3
		±0.8	±0.5	±5.7		±2.8 (9)

Experiments done during the summer.

* $P < 0.05$.

n = the number of kidneys analyzed.

The numbers in parenthesis at the far right are the number of observations of peritubular PD.

To test whether a similar relationship exists between fluid, sodium, and mannitol (or TMA) movement in the present experiments, sodium concentration changes, luminal osmolarity, and transepithelial mannitol permeability were measured in two sets of split-drops experiments. In the first series of experiments (open-circuit conditions), split-drops of normal and of reduced sodium content were deposited in proximal tubules with trace amounts of [¹⁴C]mannitol (ICN, Irvine, Calif., sp act 22mCi/mM). Samples were reaspirated after varying time periods and analyzed for sodium (helium glow spectrophotometer, Aminco, Silver Spring, Md.) and [¹⁴C]mannitol (scintillation spectrophotometer, Packard Instrument Co., Downers Grove, Ill.). In a second group of experiments split-drops of normal and reduced sodium content were short circuited for an average time of 6.94 min, and their osmolality was measured after reaspiration with a micro-osmometer (Clifton Technical Physics, Hartford, N. Y.). The length of time the tubules were short circuited was sufficient for 10-15% of the luminal fluid to be reabsorbed. The small sample size in the short-circuited droplets precluded accurate tracer and sodium concentration measurements.

Data are presented as mean \pm standard errors, and statistical analysis was performed on IBM 370/155, Olivetti P602, and Hewlett-Packard digital computers and calculators. Statistical significance was determined by Student's *t*-test, and in some instances confirmed by the Wilcoxon-Rank sum test.

RESULTS

Relationship among Transepithelial Fluid, Sodium, and Mannitol Movement

In order to calculate tubular net sodium reabsorption from the rate of fluid reabsorption it is necessary to ascertain the sodium concentration of the fluid reabsorbed. Table II summarizes relevant data. When droplets of control

TABLE II
EFFECTS OF CHANGES IN Na CONTENT ON TUBULAR MANNITOL PERMEABILITY, SODIUM CONCENTRATION, AND OSMOLALITY

Solution	Open-circuit droplets		
	[¹⁴ C]Mannitol sample/std	Mannitol permeability* $\times 10^7$ cm/s	Sodium sample/std
100 NaCl (control)	0.80 \pm 0.03‡ (7)	2.10	—
55 NaCl (mannitol)	0.82 \pm 0.02‡ (14)	1.99	0.91 \pm .04‡ (13)
55 NaCl (45 TMA)	0.83 \pm 0.05‡ (8)	1.54	0.89 \pm .04‡ (8)
Solution	Short-circuited droplets§		
	Osmolality sample/std		
100 NaCl (winter)	1.08 \pm 0.05 (3)		
37.5 NaCl (mannitol)	1.02 \pm 0.03 (5)		
37.5 Na (67.5 TMA)	1.07 \pm 0.06 (6)		

* Mannitol permeability (P) was calculated from the rate constant for mannitol disappearance (*k*) and the tubular radius (*r*) as follows $P = kr/2$.

‡ Significantly less than 1, $P < 0.05$.

§ Tubules were short circuited for an average time of 6.94 min. The mean droplet length was 431.5 μ m.

Ringer's solution containing traces of [¹⁴C]mannitol were reaspirated after periods ranging between 29.3 and 48.3 min (mean: 39.1 min), the [¹⁴C]mannitol activity declined to 80% of that in the initial perfusion solution. The fall in [¹⁴C]mannitol activity allows one to measure unidirectional fluxes as well as the permeability coefficient, which was calculated from the slope of the least-squares line (significance of slope: $P < 0.001$, $r = -0.89$) fitted to the time course of mannitol disappearance, as 2.1×10^{-7} cm/s⁻¹. Inspection of Table II indicates that there was no change in mannitol permeability when the sodium content of the luminal and peritubular fluid had been reduced to 55 mM by mannitol or TMAcI substitution.

Since the transepithelial mannitol permeability of the proximal tubule of *Necturus* is small, progression of fluid reabsorption leads to a decrease in sodium concentration of the fluid remaining in the tubule with a concomitant increase in mannitol concentration. As shown in Table II, such a decline in sodium concentration was observed in the present series of experiments as evidenced by a significant fall of the collected tubular fluid:initial tubule perfusate sodium concentration ratio. Accordingly, the sodium concentration of the reabsorbed fluid must have exceeded the initial luminal sodium concentration in those experiments in which poorly permeant solutes were present in the lumen.

Water movement due to mannitol leakage out of the tubule lumen was calculated, by the approach of Windhager et al. (1959), from the mannitol permeability and intraluminal concentration as follows: NaCl reabsorption leads to a reduction in the luminal salt concentration to 91% of that in the initial perfusate (as shown in Table II). Luminal mannitol concentration rises to maintain isosmolality since there was no change in luminal osmolality in droplets of normal and reduced sodium concentration (see Table II). After the osmotic coefficients of NaCl and mannitol have been taken into account, the increase in luminal mannitol concentration is calculated to be 12.66 mM. The product of the mannitol concentration difference and mannitol permeability yields a net mannitol flux of 2.5×10^{-12} M/cm²-s. The corresponding fluid flux calculated on the basis of isosmotic fluid reabsorption (Windhager et al., 1959) is 0.014 nl/cm²-s. This net fluid flux due to passive transepithelial mannitol leakage is less than 3% of the fluid reabsorption associated with active transport. We have assumed that the mannitol permeability at NaCl concentrations below 55 meq/liter remains unchanged. Clearly, even a severalfold increase would result in only a minimum fraction of fluid movement due to mannitol, since the driving force for transepithelial mannitol movement, the concentration difference of mannitol, becomes progressively smaller as fluid reabsorption declines at low sodium concentrations. Hence, at all extracellular sodium concentrations, most of the solute reabsorbed is NaCl. In view of the fact that by far the largest fraction of the reabsorbed solute species is sodium, one can approximate the amount of net sodium reabsorption from the measured net fluid reabsorption, assuming the reabsorbed fluid to be isosmotic and to contain 100 meq/liter of sodium.

Relationship between Extracellular Sodium Concentration and Transepithelial Fluid and Sodium Movement

Both ϕ_w determined from the shrinkage rate of split droplets and calculated ϕ_{Na} are similar functions of extracellular Na⁺ or NaCl concentration and approach saturation at high extracellular Na⁺ or NaCl levels. Fig. 1 illustrates this dependence of ϕ_w and ϕ_{Na} expressed as fractions of control values on extracellular Na⁺ or NaCl concentration, for the case of partial replacement of NaCl by mannitol and of Na⁺ by TMA at constant Cl. This mode of presenting the data was chosen in view of the marked seasonal differences between absolute transport rates in summer and winter animals. The solid and dashed curves represent regression lines fitted by the Michaelis-Menten equation directly to the data by the method

of least squares (Colquhoun, 1971). The data could not be adequately fitted by a straight line ($P < 0.001$). The Michaelis constant K_m and maximal transport rate V_{max} are indicated in each panel of Fig. 1, and it is apparent that the two curves have essentially similar kinetic constants.

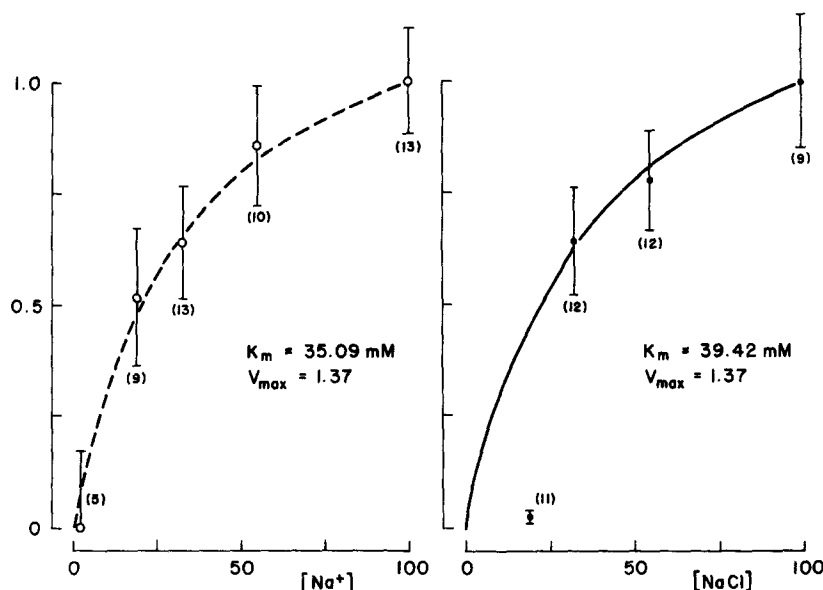


FIGURE 1. Net H₂O flux (ϕ_w) and net Na flux (ϕ_{Na}) on the ordinate, expressed as the fraction of control value, plotted against the Na⁺ concentration (left panel) or NaCl concentration (right panel) of the perfusate. Na⁺ is replaced by TMA and NaCl by mannitol; net H₂O flux is determined from the rate of shrinkage of split-droplets. Standard errors of the mean and number of observations are indicated. The solid and dashed lines are derived from a least-squares fit of the Michaelis-Menten equation to the data by using the parameters indicated in each panel. Examination of the points in the right hand panel of Fig. 1, which shows ϕ_w as a function of perfusate NaCl concentration, reveals that ϕ_w at 19 mM/liter is markedly depressed. This point was not used in the calculation of the Michaelis-Menten line. We have no satisfactory explanation for this observation other than to point out that it was difficult to short circuit split-drops for periods longer than 15 min during which time droplet volume would change by only 6%. It was not possible to observe short-circuited droplets for longer periods because of difficulties in prolonged stabilization of the pipettes and electrodes in the tubules.

Table III lists the observed values of ϕ_w and ϕ_{Na} at different extracellular sodium concentrations. The ϕ_{wmax} values predicted from the Michaelis-Menten equation differ significantly between the two groups. This difference can satisfactorily be attributed to seasonal variations in cellular Na⁺ transport which will be discussed in more detail in a subsequent section of this paper. Fluid transport during the winter months proceeds at a faster rate than during the summer, and TMA substitution experiments were performed during the fall and winter while

the mannitol replacement experiments were done during the spring-summer of the same year.

Dependence of Cell Composition on Extracellular Na⁺ or NaCl

Intracellular ion composition was measured on kidneys perfused with solutions of normal and reduced sodium content. As shown in Fig. 2, intracellular Na⁺ concentration (normalized by dividing by the control value) is a saturable function of extracellular Na⁺ or NaCl. The mean values of chemically determined intracellular Na⁺ concentrations are indicated by the triangle points in Fig. 3. It

TABLE III
NET H₂O FLUX AND CALCULATED NET Na FLUX IN DIFFERENT
EXPERIMENTAL CONDITIONS

Solution	n	ϕ_w	ϕ_{Na}
		<i>nl/cm²s</i>	<i>pmol/cm²s</i>
100 NaCl (control-summer)	9	-0.627 ±0.095	-62.7 ±9.5
55 NaCl (mannitol)	12	-0.487 ±0.067	-48.7 ±6.7
32.5 NaCl (mannitol)	12	-0.401 ±0.075	-40.1 ±7.5
19 NaCl (mannitol)	11	-0.016* ±0.01	-1.6* ±1.0
100 NaCl (control-winter)	13	-0.951 ±0.113	-95.1 ±11.3
55 Na (45 TMA)	10	-0.841 ±0.127	-84.1 ±12.7
32.5 Na (67.5 TMA)	13	-0.608‡ ±0.117	-60.8‡ ±11.7
19 Na (81 TMA)	9	-0.492‡ ±0.144	-49.2‡ ±14.4
2 Na (98 TMA)	5	+0.073‡ ±0.232	+7.3* ±23.2
100 NaCl (in vivo-winter)	9	-1.483 ±0.285	-148.3 ±28.5

* $P < .01$.

‡ $P < 0.05$.

n = the number of tubules studied.

can be seen that intracellular Na⁺ concentrations follow the same general pattern as ϕ_w and ϕ_{Na} , suggesting an interdependence of these variables.

Table IV summarizes the observed values of intracellular Na⁺, K⁺, and Cl⁻ as well as tissue H₂O content for both Na⁺ substitution by TMA and NaCl replacement by mannitol. Measurements of the intracellular ion content of control kidneys during summer and winter are included. The control values for intracellular composition in Table IV are in good agreement with the previous observations of Whittembury et al., 1961 (Na⁺ = 37, K⁺ = 108, Cl⁻ = 32 mM/liter); Giebisch, 1961 (Na⁺ = 40, K⁺ = 90 mM/liter); and Khuri et al., 1972 (Na⁺ = 38, K⁺ = 103 mM/liter).

It is apparent from Table IV that the cellular sodium concentration falls with

the reduction of sodium in the extracellular fluid. In addition, intracellular K⁺ and Cl⁻ concentrations underwent some alterations during the reduction of extracellular sodium. Thus, NaCl replacement by mannitol resulted in a decrease in intracellular K⁺ and Cl⁻ concentrations. These concentration changes are associated with a modest depolarization of the peritubular membrane PD (see Table V). Changes in cell K⁺ and Cl⁻ concentrations also accompanied TMA substitution for Na⁺: cell K⁺ fell slightly and Cl⁻ rose significantly. This is most probably due to some entry of TMA into the cells down its electrochemical potential gradient. TMA will thus replace some cell potassium, and the peritu-

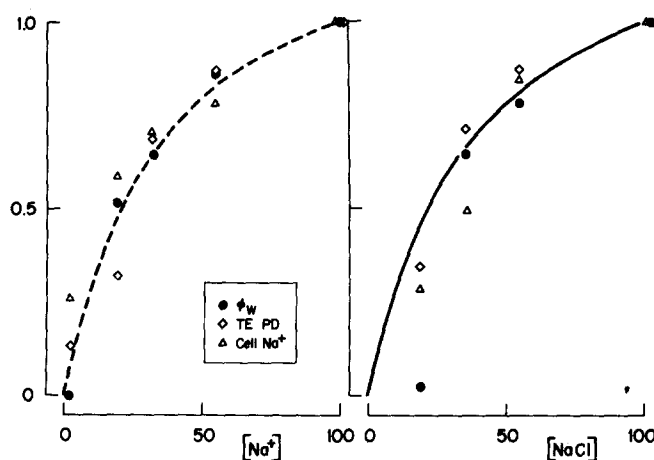


FIGURE 2. The mean values of several variables are plotted as a function of Na⁺ concentration (left panel) or NaCl concentration (right panel) of the perfusate. Each is expressed as the fraction of its corresponding control value. Net fluid flux (ϕ_w) is indicated by filled circles (●) (which also represent values of ϕ_{Na}), transepithelial potential difference (TE PD) by diamonds (◊), and chemically determined intracellular Na⁺ (cell Na⁺) by triangles (Δ). The lines are the least squares fit of the Michaelis-Menten equation to net fluid flux shown in Fig. 1 and redrawn here for purpose of comparison.

bular electrical potential difference falls. The intracellular chloride concentration will increase after this decline in cell membrane potential.

Dependence of Electrical Potential Differences upon Extracellular Sodium

Fig. 2 shows the relationship between the mean transepithelial PD (normalized by dividing by the control value) and extracellular Na⁺ or NaCl concentration. Table V lists similar values of transepithelial and peritubular PDs during the different perfusion experiments. It is apparent that transepithelial PD, similar to ϕ_w , ϕ_{Na} , and cell Na⁺ are all functions of external Na⁺ or NaCl which are consistent with saturation. Extracellular Cl⁻ is constant during TMA substitution but is sharply reduced during NaCl removal, yet it does not affect the relationship between either electrical or transport phenomena and extracellular Na

except at low NaCl concentrations. Therefore it is safe to conclude that changes in chloride concentration do not play a significant electrogenic role over the concentration range from 32.5 to 100 meq/liter.

Transepithelial PD values in control experiments at physiological sodium concentrations (see Table V) are comparable to previous observations in isolated drops used for voltage clamp experiments (-8.1 mV, Spring and Paganelli,

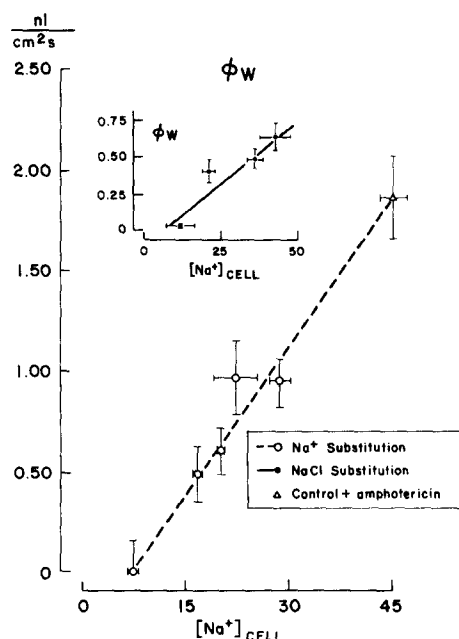


FIGURE 3. Net H₂O flux (ϕ_w) in nl/cm²·s is shown in the left panel as a function of measured intracellular sodium concentration, $[Na^+]_{CELL}$. The open circles represent the results obtained with Na⁺ substitution by TMA, and the filled circles (*inset*) show similar data for NaCl substitution by mannitol. The relationship for ϕ_w also holds for ϕ_{Na} (See Table III). The point labeled with a triangle (Δ) was obtained after treatment of the luminal cell membrane with amphotericin B (10 μ g/ml) in a solution of normal Ringer's. The lines are drawn by the method of least squares on the mean values and have the following parameters: Na⁺ substitution $-\phi_w$ vs. $[Na^+]_{CELL}$, slope = 0.05, intercept = -0.396 ; NaCl substitution $-\phi_w$ vs. $[Na^+]_{CELL}$, slope = 0.017, intercept = -0.10 .

1972). These values are significantly lower than transepithelial PD measured in proximal tubules under free-flow conditions (-12.6 mV, Spring and Paganelli, 1972; -10.3 mV, Spring, 1973; -15.4 mV, Boulpaep, 1972; -11.5 mV, Bentzel, 1974). Small leaks in the tubule as a result of the multiple punctures required for voltage clamp experiments may be the cause of the lower stop-flow transepithelial PD.

Peritubular membrane PD is significantly lower in perfused kidneys than in vivo at the same temperature (Giebisch, 1961; Whittembury et al., 1961). The cause of the depression in peritubular PDs which accompanies perfusion of the

TABLE IV
CELL COMPOSITION IN DIFFERENT EXPERIMENTAL CONDITIONS

Solution	n	Tissue H ₂ O %	Cell ions		
			Na	K	Cl
			meq/kg cell H ₂ O		
100 NaCl (control-summer)	8	86.4 ±0.6	42.95 ±4.83	81.85 ±5.27	50.34 ±2.70
55 NaCl (mannitol)	5	85.3 ±0.7	36.10 ±2.33	85.48 ±2.92	31.98* ±2.78
32.5 NaCl (mannitol)	5	84.5* ±0.3	21.05* ±1.70	73.58 ±2.80	—
19 NaCl (mannitol)	5	85.2 ±0.5	12.11* ±4.7	68.13 ±5.2	—
100 NaCl (control-winter)	12	84.6 ±0.2	28.81 ±1.51	111.29 ±2.19	32.31 ±1.58
55 Na (45 TMA)	7	85.3 ±0.7	22.29 ±3.19	87.55* ±3.54	39.29‡ ±3.06
32.5 Na (67.5 TMA)	7	87.9* ±0.5	20.18* ±0.4	85.46* ±6.9	47.41* ±2.58
19 Na (81 TMA)	10	87.2* ±0.3	16.71* ±0.57	84.43* ±3.39	49.99* ±2.74
2 Na (98 TMA)	5	84.8 ±0.6	7.55* ±0.46	75.90* ±6.55	62.07* ±2.60

* $P < 0.02$.

‡ $P < 0.05$.

n = the number of kidneys analyzed.

TABLE V
TRANSEPITHELIAL AND PERITUBULAR PDs IN DIFFERENT
EXPERIMENTAL CONDITIONS

Solution	Transepithelial PD	Peritubular PD
	mV	mV
100 NaCl (control-summer)	-7.6 ±0.86 (24)	-51.18 ±1.82 (39)
55 NaCl (mannitol)	-7.0 ±0.62 (30)	-43.61* ±1.42 (59)
32.5 NaCl (mannitol)	-5.4 ±0.71 (23)	-43.36* ±1.47 (36)
19 NaCl (mannitol)	-2.63* ±0.38 (15)	-40.85* ±1.96 (26)
100 NaCl (control-winter)	-7.79 ±0.64 (29)	-60.23 ±2.14 (13)
55 Na (45 TMA)	-6.75 ±0.49 (32)	-48.3* ±2.27 (18)
32.5 Na (67.5 TMA)	-5.33* ±0.39 (49)	-50.42* ±1.05 (62)
19 Na (81 TMA)	-2.49* ±0.70 (31)	-45.51* ±1.30 (57)
2 Na (98 TMA)	-1.10* ±0.97 (9)	-32.45* ±1.92 (11)

* $P < 0.01$.

kidney is not known. However, peritubular PDs remain constant for 3 h or more in the perfused preparation (Giebisch, 1961; Spring and Giebisch, unpublished observations). Peritubular PDs tend to fall with the reduction of extracellular Na^+ . The present data do not allow one to distinguish whether this modest cell depolarization was due to alterations in intracellular K^+ or was a direct effect of an extracellular Na^+ removal.

Dependence of ϕ_w and ϕ_{Na} upon Intracellular Na^+

In sharp contrast to the dependence of ϕ_w and ϕ_{Na} upon extracellular sodium concentration, net H_2O and net Na flux are linearly related to intracellular Na^+ . Fig. 3 shows a plot of ϕ_w against intracellular Na^+ concentration during Na^+ substitution by TMA. The *inset* in Fig. 3 shows that a similar result is observed during reduction by mannitol of the tubular concentration in the perfusate. These results are consistent with the concept that the peritubular active sodium extrusion mechanism shows no sign of saturation and that its rate of pumping depends primarily and linearly upon the cellular sodium concentration. Since transepithelial proximal tubular sodium transport exhibits saturation kinetics and the peritubular sodium pump shows linear transport kinetics, it is most reasonable to argue that saturation of the overall transport operation occurs at the site before the pump, i.e. the entry step of Na^+ from the lumen into the tubule cell. If indeed the luminal entry step were rate limiting, it could be predicted that increased Na^+ entry would lead to stimulation of transepithelial sodium movement. To test this thesis, amphotericin B (10 $\mu\text{g}/\text{ml}$, E. R. Squibb & Co., New York) was added to the luminal split droplet and the resultant transport rate observed. Lichenstein and Leaf (1965) had previously shown that amphotericin B sharply increased net Na transport in the toad bladder by increasing luminal permeability. As shown in Fig. 3, addition of amphotericin B to the luminal drop significantly increased the intracellular Na^+ concentration.¹ Importantly, ϕ_w and ϕ_{Na} also rose to the extent expected from the previously established relationship between cellular Na concentration and transepithelial Na transport. The peritubular Na pump shows no saturation even after a nearly twofold increase in intracellular Na^+ concentration. The data summarized in Table VI show that amphotericin treatment did not seriously affect intracellular K^+ concentration, and transepithelial and peritubular PDs. Amphotericin treatment of the luminal membrane results in increased permeability to K^+ as well as to Na^+ (Stroup et al., 1974). An increased K^+ permeability of the luminal membrane tends to reduce cell K^+ and peritubular PD, as seen in Table VI. An increase in transepithelial PD may thus be prevented by exposure to amphotericin. Also included in Table VI are data that show that the amphotericin effect is primarily due to an increase in luminal membrane permeability to sodium.² This

¹ Intracellular ionic composition was determined in kidneys perfused with amphotericin containing solutions in the aortic perfusate only. Since the glomerular capillaries are supplied by the aortic perfusate, the amphotericin solution was delivered mainly to the tubule lumen. A steady-state cell composition was reached by 30 min.

² If one assumes that sodium ions enter the tubule cells from the lumen by diffusion, luminal membrane permeability may be estimated from the data in Tables IV and V by using the Goldman-Hodgkin-Katz equation. A minimum value for the unidirectional Na^+ flux into the cell was

permeability increase is responsible for the elevation of the intracellular Na⁺ concentration and the stimulation of active sodium extrusion across the peritubular cell boundary.

Effects of Elevated Extracellular Calcium

As shown in Table VI an increase in the calcium concentration of the perfusion solution to 8 mM/liter by the addition of CaCl₂ causes a depression in ϕ_w and ϕ_{Na} . Associated with this reduction in net transport rate is a significant fall in transepithelial PD and the estimated luminal membrane permeability. These observations suggest significant inhibition of Na⁺ entry across the luminal membrane by Ca⁺⁺ ions in the lumen. Since the increase in luminal Na⁺ permeability induced by amphotericin leads to a rise in cell Na⁺, a decreased luminal Na

TABLE VI
EFFECTS OF AMPHOTERICIN B AND OF ELEVATED Ca⁺⁺

Solution	ϕ_{Na}	Tissue H ₂ O	Cell ions			Peritubular PD	Transepithelial PD	P _{Na} (luminal)
			Na	K	Cl			
	<i>pmol/cm²·s</i>	%		<i>mM/kg</i>		<i>mV</i>	<i>mV</i>	<i>cm/s × 10⁶</i>
Control	-95.09 ±11.33 (13)	0.846 ±0.002 (12)	28.81 ±1.51 (12)	111.29 ±2.19 (12)	32.31 ±1.58 (12)	-60.23 ±2.14 (13)	-7.79 ±0.64 (29)	1.12 ±0.10 (15)
10 μg/ml Amphotericin B	-187.02 ±21.24 (9)	0.857 ±0.004 (12)	45.27 ±2.15 (12)	101.55 ±4.28 (12)	42.15 ±1.75 (12)	-52.53 ±2.51 (17)	-7.26 ±0.31 (36)	2.75 ±0.21 (9)
<i>P</i>	<0.001	<0.05	<0.001	ns	<0.001	<0.05	ns	<0.001
8 mM Ca ⁺⁺	-67.04 ±5.38 (10)	0.841 ±0.006 (6)	30.99 ±1.01 (6)	116.58 ±2.45 (6)	40.98 ±0.73 (6)	-61.44 ±1.39 (24)	-2.79 ±0.42 (16)	0.79 ±0.05 (10)
<i>P</i>	<0.05	ns	ns	ns	<0.02	ns	<0.001	<0.05

ns = not significant.

permeability would be expected to reduce intracellular Na. No such decrease is observed in the high calcium experiments (Table VI). One possibility is that elevated calcium inhibits peritubular Na⁺ extrusion by an amount sufficient to obviate the decrease in cell Na⁺ due to impaired entry. It is at present uncertain whether additional effects of elevated calcium on the Na transport system occur. Curran et al. (1963) also observed that net sodium transport in the frog skin was inhibited by elevating external calcium and they interpreted their results by postulating competition of Ca⁺⁺ for an Na⁺ carrier at the entry step across the outer surface of the frog skin. Our results also confirm the studies of Fülgraff and Heidenreich (1967). These investigators found that fourfold elevation of the

approximated from the measured net Na⁺ flux (Table III). The permeability values obtained from the measured net Na⁺ flux must be considered only as minimal values since the unidirectional Na⁺ flux into the cell should be somewhat greater than the net flux. A recent estimate of control luminal membrane Na permeability from tracer experiments— 2.4×10^{-6} cm/s (Spring and Giebisch, 1977)—compares favorably with the values in Table VI. Clearly, any component of nondiffusional Na⁺ entry would tend to reduce the estimated apparent Na permeability.

luminal Ca^{++} concentration in split droplets deposited in rat proximal tubule caused a prolongation of the half-time of shrinking from 9 to 12.5 s. They also observed a similar decrease in tubular fluid reabsorption during elevation of plasma calcium to 9.7 mM/liter.

Seasonal Variations

Significant differences in control values of ϕ_w , ϕ_{Na} , and cell composition have been noted when results of experiments performed during the summer months are compared to those carried out in the winter. As shown in Fig. 4, ϕ_w and ϕ_{Na} are less dependent on the cell Na^+ concentration during the summer months

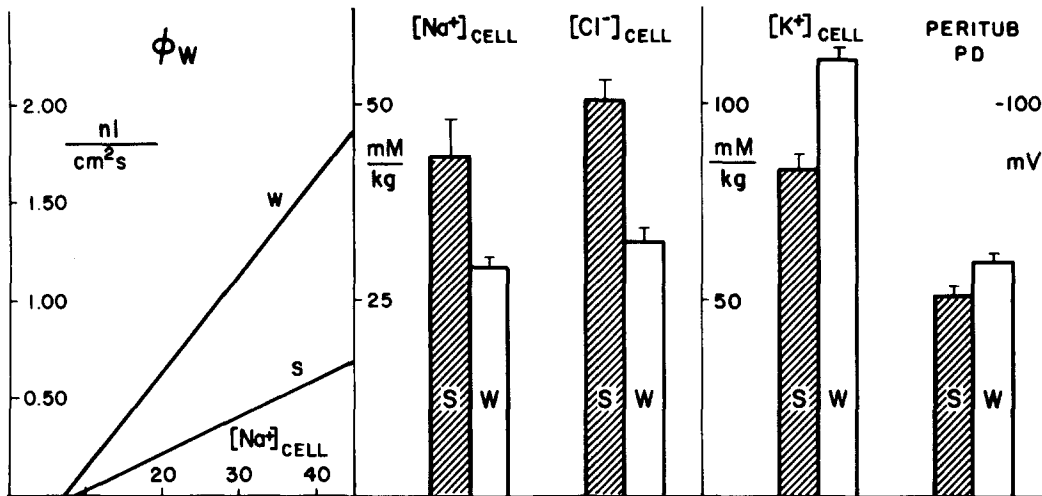


FIGURE 4. A comparison of several parameters measured in summer and winter *Necturus*. The panels show the regression line for ϕ_w vs. $[\text{Na}^+]_{\text{cell}}$ during both seasons. Intracellular ion composition during summer and winter for control animals is also summarized and standard errors are indicated. Seasonal difference in peritubular membrane potential is also shown in millivolts. All seasonal differences in cellular composition and potential difference are significant at $P < 0.01$.

than during the winter. In addition, cell Na^+ and Cl^- concentrations are significantly elevated in the summer, while cellular K^+ levels are reduced. The cell $\text{Na}^+:\text{K}^+$ ratio varies from approximately 1:2 in the summer to 1:4 during the winter. The absolute level of the peritubular membrane PD is also significantly elevated in the winter animals. As shown in Table VII, K_m values for ϕ_w and the calculated luminal membrane Na^+ permeability are unchanged from one season to the other. In contrast, $\phi_{w\text{max}}$ and $\phi_{\text{Na max}}$ are markedly increased in winter. This suggests that seasonal alteration may result from changes of the number of peritubular sodium transport sites. Strong support for this hypothesis comes from the recent work of Spector et al. (1974) who showed that Na, K-ATPase levels are reduced in *Necturus* kidneys of summer animals roughly in proportion to the observed reduction in fluid transport. The observation of constancy of K_m and P_{Na} values in the present study also supports the concept that the luminal

carrier site affinity for Na⁺ is unchanged and that luminal sodium entry is not subject to seasonal variations. These observations of seasonal changes in fluid and sodium transport, cell composition, and of ATP levels support the findings of other investigators in the *Necturus* kidney who have similarly observed seasonal variations in fluid reabsorption (Shipp et al., 1958; Boulpaep, 1972) and in organic acid transport (Tanner and Kinter, 1966; Tanner, 1967; Kinter, 1959).

DISCUSSION

The main conclusion to be drawn from these experiments is that the entry step of sodium ions across the luminal cell membrane of proximal tubule cells is a saturable process and rate limiting for overall transepithelial sodium and fluid movement. It is this saturable luminal transport process which imposes nonlinearity upon the relationship between extracellular sodium concentration and net sodium and water reabsorption. Our thesis is based on the observation that the

TABLE VII
COMPARISON OF TRANSPORT AND ELECTRICAL PARAMETERS BETWEEN
SUMMER AND WINTER ANIMALS

Variable	Control perfusion		P
	Summer	Winter	
ϕ_{Na} pmol/cm ² -s	62.69±9.48	95.09±11.33	<0.05
[Na] cell mM/kg cell H ₂ O	42.95±4.83	28.81±1.51	<0.02
[Cl] cell mM/kg cell H ₂ O	50.34±2.70	32.31±1.58	<0.001
[K] cell mM/kg cell H ₂ O	81.85±5.27	111.29±2.19	<0.001
Transepithelial PD mV	-7.6 ±0.86	-7.79±0.64	ns
Peritubular PD mV	-51.18±1.82	-60.23±2.14	<0.01
ϕ_{wmax} nl/cm ² -s	0.86	1.3	-
K_m mM	39	35	-
P_{Na} cm/s (luminal) × 10 ⁶	0.88±0.10	1.12±0.10	ns

rate of peritubular sodium extrusion is linearly dependent upon the cellular concentration, and remains far from saturation even at dramatically elevated cellular sodium levels achieved by the amphotericin-induced increase in sodium uptake across the luminal cell membrane. We conclude that the regulation of intracellular sodium content depends on a two-step transport system in which a rate-limiting luminal entry step and a peritubular nonsaturated active pump mechanism are arranged in series. Our findings confirm the thesis of Stroup et al. (1974) who suggested, on the basis of an increase in net fluid absorption after amphotericin treatment, that the rate-limiting step for transepithelial sodium chloride transport was the luminal cell membrane. Hence, the earlier model of passive and unrestricted sodium entry across the luminal cell boundary must be revised accordingly (Giebisch, 1961; Whittembury et al., 1961).

Curran et al. (1963) also observed that frog skin Na⁺ transport was a saturable function of external Na⁺ but a linear function of the estimated cellular Na⁺ pool and concluded that the outer facing membrane was rate limiting. Their conclusion was substantiated by subsequent observations on the kinetics of the unidirectional influx of Na⁺ into the frog skin (Biber and Curran, 1970; Biber and

Cruz, 1973). The Na^+ entry step was shown to be the sum of a facilitated transport process and a small amount of simple diffusional entry.

Several investigators have assessed the relationship between extracellular sodium concentration and proximal tubular sodium transport. Györy and Lingard (1976) described, in rat kidneys, a saturable dependence of the steady-state limiting sodium concentration difference (measured under conditions of zero net volume and sodium flux) upon the luminal sodium concentration. Saturation was observed at luminal sodium concentrations greater than 80 meq/liter and the K_m was 57 mM. In view of the different species used these values are very close to those obtained in *Necturus* proximal tubule in the present study. Baldamus et al. (1969) had previously elevated the sodium concentration of luminal and peritubular perfusion fluids in the rat kidney above normal plasma levels and noted no increase in net fluid and sodium reabsorption in split-drop experiments. These findings are in accord both with the observations of Györy and Lingard (1976) cited above and with our findings of saturation of sodium transport at lower tubular sodium concentrations. Another relevant work is that of Bentzel et al. (1974) who studied in *Necturus* the relationship between steady-state limiting Na concentration difference and the rate of fluid entry into initially sodium chloride free split-droplets. By assuming a linear dependence of active sodium reabsorption on luminal sodium concentration over a range from 0 to 60 meq/liter, they calculated a linear rate coefficient for active sodium transport of $1.56 \times 10^{-6} \text{ cm, s}^{-1}$. The corresponding value determined from a linear approximation to our results over the same concentration range is $1.51 \times 10^{-6} \text{ cm, s}^{-1}$.

It has been emphasized that the luminal membrane permeability for sodium ions imposes a significant restraint upon transepithelial sodium and fluid movement. The important observation of the present study was that the luminal sodium permeability is not constant over the range of experimental sodium concentrations but increases with a fall in the extracellular sodium concentration. Fig. 5 summarizes results of calculations in which minimal permeability values were estimated from the net sodium fluxes and the electrochemical potential difference across the luminal cell membrane. It is apparent that the luminal sodium permeability increases when the extracellular sodium concentration is reduced to 55 meq/liter or less. The mechanism of this permeability change is at present unknown, but it can be explained by a simple saturating site in the luminal membrane controlling Na^+ entry. A similar relationship between luminal sodium permeability and external sodium concentration has also been observed in frog skin (Curran et al., 1963; Lindemann and Gebhardt, 1973; Lindemann, 1977), the toad skin (Larsen, 1973) and the rabbit urinary bladder (Lewis et al., 1976). This mechanism is of considerable importance as it minimizes the fall in net sodium transport which would result from a reduction in external sodium concentration. Clearly, the overall efficiency of transepithelial sodium reabsorption is augmented as the luminal sodium permeability increases with the decline in external sodium concentration. Little is known about the nature of the luminal entry process of sodium, although evidence from other studies supports the notion of several processes other than simple electrodiffusion. Thus, cotransport of sodium ions with sugars and amino acids across the luminal mem-

brane has been observed in both amphibian and mammalian proximal tubules (Ullrich, 1976). The possibility of a luminal cation exchange process, involving sodium and hydrogen ions, must also be considered. It is presently unresolved to what extent such coupled transport systems, neutral salt movement, or facilitated diffusion of either Na⁺ or neutral salt (NaCl) may be involved in the process of luminal sodium translocation.

The present experiments allow some characterization of the peritubular sodium extrusion mechanism. Under the conditions of our experiments the net Na⁺ flux may be assumed to be a valid (minimum) measure of the active Na⁺ transport rate. The minimum amount of work done by the proximal tubule cells

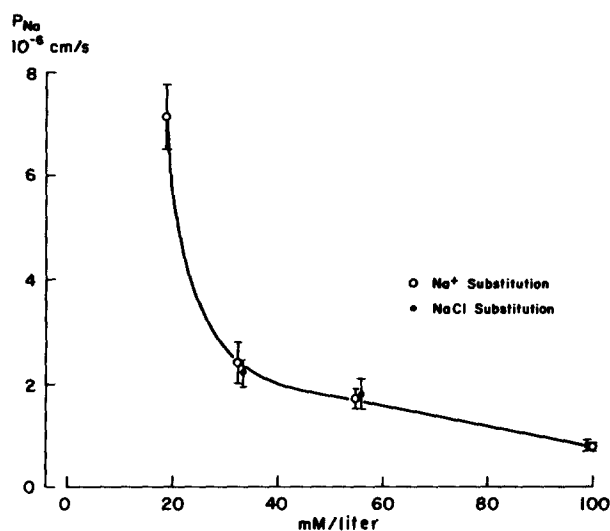


FIGURE 5. Minimum estimated luminal membrane sodium permeability is plotted against extracellular Na⁺ or NaCl concentration on the abscissa. P_{Na} was calculated from net flux data given in Tables III-V by using the Goldman-Hodgkin-Katz equation. The line is drawn by eye.

is then given by the product of the net Na⁺ flux and the electrochemical gradient for Na⁺ across the peritubular membrane, since the active Na⁺ extrusion mechanism has been previously shown to be located at this cell boundary (Giebisch, 1961; Whittembury et al., 1961). We neglect any work done to overcome the "internal resistance" of the Na⁺ pump since the flux ratio of unidirectional Na fluxes across the peritubular cell membrane was not measured. At least two possible relationships may exist between active Na transport-related work and the chemical or electrical gradients across the peritubular membrane. First, Na⁺ transport could increase as the electrochemical potential gradient across the peritubular membrane is diminished by experimental maneuvers. The amount of work would increase proportionately as the electrochemical potential gradient decreased. An alternative is that active Na⁺ extrusion is relatively independent of the electrochemical gradient against which it operates. Sodium transport rate

would be determined mainly by the availability of cellular Na^+ at the peritubular pump site.

Our data show that a weak positive correlation exists between Na^+ transport work and the electrochemical potential gradient for Na^+ . Both the Na^+ flux and work increase as the opposing gradient for Na^+ becomes larger ($W = 5.9 \pm 4.4 + 1.66 \pm 0.66 \times 10^{-3} \Delta\mu_{\text{Na}}$, where W = work done in erg/cm²-s, and $\Delta\mu_{\text{Na}}$ is the electrochemical potential gradient in joules; $r = 0.66$, $P = 0.04$). Hence these data do not support the concept of an Na^+ pump rate determined by the magnitude of the electrochemical potential gradient against which the pump operates, in agreement with the findings of Labarca et al (1977).

The alternative considered is that peritubular Na^+ transport rate is only dependent on the supply of Na^+ at the intracellular pump rate. Thus a plot of Na^+ transport work against cellular Na^+ concentrations should be linear. Such a plot is shown in Fig. 6 which depicts this relationship for a variety of experimental conditions. In winter animals, $W = 3.22 \pm 0.17 + 0.356 \pm 0.058 [\text{Na}]_{\text{cell}}$, $r = 0.95$, $P < 0.003$; in summer animals, $W = -1.04 \pm 0.93 + 0.118 \pm 0.031 [\text{Na}]_{\text{cell}}$, $r = 0.94$, $P = 0.06$. Further support for the relationship between cellular Na^+ concentration and active transport work comes from a comparison of the results of two experimental groups in which the extracellular Na^+ concentration was constant at 100 mM. The peritubular electrochemical gradient was virtually constant in the control experiments and the amphotericin-treated tubules, yet Na^+ transport work differed significantly between these groups. As may be seen from the two uppermost points in Fig. 6, these differences in work are directly proportional to differences in cellular Na^+ concentration.

Fig. 6 shows that the work done by the tubule cells increases linearly with the intracellular Na^+ concentration at concentrations above 9 mM/kg cell H_2O . Over the range of electrochemical gradients in our experimental conditions the Na^+ pump operates as a constant current source. The independence of the Na^+ transport rate of the opposing electrochemical gradient is consistent with a low Na^+ -permeability of the peritubular cell membrane. Otherwise backflux of Na^+ in the direction opposite to net transport, i.e. from peritubular fluid into the cell, would increase with electrochemical potential and reduce the net sodium extrusion. Recent Na^+ tracer experiments are relevant which showed the peritubular Na^+ permeability to be exceedingly low (Spring and Giebisch, 1977) with most of the backflux proceeding via the intercellular shunt route.

Some uncertainties in the present study concern the relationship between chemically determined sodium concentration and cellular sodium activity or the cellular sodium transport pool. Relevant studies are presently not available but measurements of potassium and chloride activities in *Necturus* proximal tubule cells indicate some reduction of ion activities relative to the respective chemical concentrations (Khuri et al., 1972; Khuri et al., 1975). There are still uncertainties about the relationship between intracellular Na^+ activity and the transport pool for Na^+ (Armstrong, 1976). Measurements of cell Na^+ activity in a leaky epithelium (bullfrog small intestine) yielded an Na^+ activity about $2/3$ of that observed in free solution (Armstrong, 1976). We have assumed that *Necturus* proximal tubule cell Na^+ activity varies proportionally with the measured cell Na^+ concen-

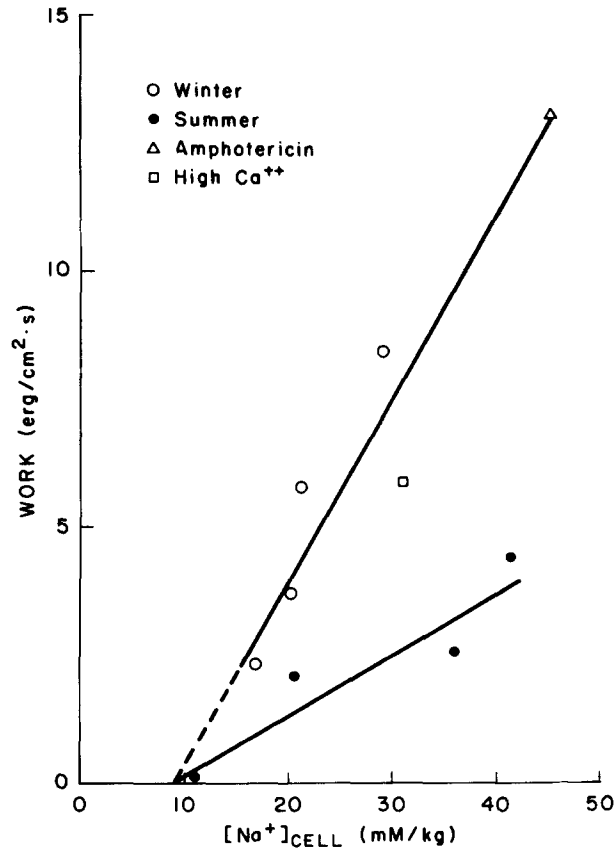


FIGURE 6. Work performed by the sodium pump in the peritubular membrane is plotted against intracellular Na^+ concentration (from Table IV). The work is defined as the product of the flux across the peritubular membrane multiplied by the electrochemical gradient across that membrane $-W = \phi_{\text{Na}} (RT \ln [\text{Na}]/[\text{Na}]_{\text{cell}} + E_{\text{pt}}F)$, where W is the work done, $[\text{Na}]$ is the extracellular Na^+ concentration, $[\text{Na}]_{\text{cell}}$ is the intracellular Na^+ concentration, E_{pt} is the peritubular membrane potential difference; R , T , F have their usual meaning. Work done to overcome the "internal resistance" of the pump is neglected in this calculation. Each point on the graph represents the mean of 9-13 experiments. The lines are drawn by the method of least squares and have correlation coefficients of 0.95 and 0.94 for winter and summer animals, respectively. Both lines intersect the x-axis at 9 mM/kg cell H_2O . Amphotericin and high Ca^{++} points were calculated from Table VI and were included in the determination of the least-squares line for the winter animals.

tration. The observed Na^+ concentration changes may then be taken as an indication of the alterations in Na^+ activity. Resolution of this relationship awaits measurements of intracellular Na^+ activity in *Necturus* proximal tubule under conditions utilized in this investigation, as well as establishment of the relevance of cell activities to the sodium transport pool.

Another point concerns the problem of whether the ionic composition of a

short-circuited tubule differs from its open-circuited neighbors. In view of the fact that the peritubular membrane potential is virtually unaffected by the transepithelial passage of electric current (Spring, 1973), we consider it virtually certain that the cellular composition is not seriously altered during short circuiting. In toad bladder cells, the Na^+ concentration did not differ between the open and short-circuited states (Herrera, 1968; Robinson and MacKnight, 1976). Measurements of the intracellular ions in frog skin in both open and short-circuited conditions showed an increase in the apparent intracellular Na^+ pool during short circuiting (Biber et al., 1966). It should be noted, however, that the transition from the open-circuited to the short-circuited state in this latter preparation was associated with large changes of the potential differences across the outer cell border.

In summary, it is proposed that alterations in the rate of entry of Na^+ into the tubule cell lead to proportionate changes in intracellular Na^+ concentration, peritubular transport rate, and overall transepithelial Na^+ and fluid transport. In the absence of transepithelial electrochemical gradients the proximal tubule cells are the major determinants of the transport characteristics of the epithelium. The transport process can be represented as a rate-limiting and saturable luminal entry step and a peritubular sodium pump which is far from saturation.

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