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Some aspects of proximal tubular sodium chloride reabsorption in *Necturus* kidney

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Some aspects of proximal tubular sodium chloride reabsorption in *Necturus* kidney. Renal tubular reabsorption of fluid and sodium was measured by clearance methods in the doubly perfused *Necturus* kidney in which the bicarbonate concentration was varied between 0 and 60 mEq/liter. The effects of Diamox (2.2×10^{-3}M), ouabain (10^{-5}M) and ethacrynic acid (10^{-4}M) and of acidosis were also investigated. In addition to clearance experiments, stationary microperfusion experiments were carried out on proximal tubules to measure volume flow and steady-state sodium and chloride concentration differences across the tubular epithelium. In some experiments, the transepithelial electrical potential difference was also measured using an axial electrode system. The following results were obtained: *1*) Bicarbonate is not essential to the operation of renal tubular fluid and sodium transport. *2*) Total renal and proximal tubular fluid and sodium transport are partially inhibited by Diamox, ouabain and ethacrynic acid. *3*) The proximal tubule maintains a significant transepithelial sodium and chloride concentration difference and a significant electrical potential difference (lumen-negative) in the presence of a poorly permeant nonelectrolyte. The direction and magnitude of the electrical polarization fully accounts for the observed chloride concentration difference. The data support the thesis that sodium chloride transport across the proximal tubular epithelium takes place by active sodium transport and electrically coupled passive chloride reabsorption. Important species differences with respect to mammalian transport mechanisms are discussed.

Quelques aspects de la réabsorption tubulaire proximale de chlorure de sodium par le rein du *Necturus*. La réabsorption tubulaire de sodium et d'eau a été mesurée par les clearances dans le rein du *Necturus* doublement perfusé avec des concentrations de bicarbonate qui ont varié entre 0 et 60 mEq/litre. Les effets du Diamox ($2{,}2 \times 10^{-3}$M), de l'ouabaïne (10^{-5}M), de l'acide éthacrynique (10^{-4}M) et de l'acidose ont été étudiés aussi. Outre les expériences de clearance, des expériences de microperfusion stationnaire ont été réalisées sur les tubes proximaux afin de mesurer le débit de liquide et les différences de concentration de sodium et de chlore de part et d'autre de l'épithélium tubulaire à l'équilibre. Dans quelques expériences la différence de potentiel trans-épithéliale a été mesurée à l'aide d'un système d'électrode axiale. Les résultats suivants ont été obtenus: *1*) le bicarbonate n'est pas indispensable au transport tubulaire de sodium et d'eau; *2*) la réabsorption rénale globale et la réabsorption tubulaire proximale d'eau et de sodium sont partiellement inhibées par le Diamox, l'ouabaïne et l'acide éthacrynique; *3*) le tube proximal maintient une différence de concentration trans-épithéliale de sodium et de chlore et une différence de potentiel (lumière négative) significatives en présence d'un corps non ionisé peu perméant. Le sens et la grandeur de la polarisation électrique rendent complètement compte de la différence de concentration de chlore qui est observée. Ces résultats sont en faveur de la thèse selon laquelle le transport de chlorure de sodium à travers l'épithélium tubulaire proximal à lieu par un transport actif du sodium et une réabsorption passive du chlore couplée électriquement.

Several lines of evidence support the view that the mechanism of sodium chloride reabsorption along the proximal tubule of *Necturus* is characterized by active transport of sodium ions and electrically coupled passive reabsorption of chloride [1, 2]. Results in agreement with this view are the finding that the lumen of the proximal tubule in *Necturus* is electrically negative with respect to the peritubular fluid [2–12] and that in the presence of a poorly permeant solute the steady-state tubular sodium concentration drops significantly below that of plasma [13]. The latter steady-state concentration difference develops as a consequence of the operation of a pump-leak system of sodium transport characterized by active sodium transport out of the lumen and passive backflux into the lumen along both an electrical and chemical potential gradient. It is further known that intercellular pathways are the major route of passive sodium backflux [5, 6, 11, 14–17] and that the luminal entry step of sodium into the cell, although downhill an electrochemical potential gradient, is rate-limiting for overall transepithelial sodium movement [10, 18].

It has further been shown that chloride reabsorption across the proximal tubule of *Necturus* is passive in nature [19]. Since the concentration of chloride does

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not significantly increase along the proximal tubule of *Necturus* [19, 20, 28], the main driving force acting on chloride is the transepithelial electrical potential difference. The magnitude of the luminal negativity can be shown to depend on the sodium concentration of the extracellular fluid [2, 3, 10].

On the basis of perfusion experiments carried out on isolated segments of rabbit proximal tubules, a different model of proximal tubular salt and water reabsorption has been proposed [21]. Its main feature is that sodium-hydrogen ion exchange is thought to be the sole mode of active proximal tubular sodium transport. Active reabsorption of sodium, with chloride following passively to maintain electroneutrality, has been excluded as a mechanism of proximal sodium chloride transfer. The most critical consequences of this proposed model of proximal tubular sodium transport are absence of a significant sodium or chloride concentration difference in the presence of a nonreabsorbable solute and failure of proximal fluid reabsorption upon deletion of bicarbonate from the extracellular fluid.

We have examined several critical aspects of proximal tubular salt and fluid transport in *Necturus* kidneys with a view towards testing the importance of bicarbonate in this system. Utilizing renal clearance and stationary microperfusion methods in the nonperfused and the doubly perfused *Necturus* kidney—the latter a stable preparation permitting extensive ionic substitutions—we observed that bicarbonate is not essential to the operation of fluid and sodium transport. Also, passive chloride transfer out of the proximal tubule could be shown to be coupled to the active transport of sodium. This became apparent from the observation of similar transepithelial concentration differences of sodium *and* chloride in the presence of a poorly reabsorbable solute. In this situation the transepithelial electrical potential difference (lumen-negative) could fully account for the observed transepithelial chloride concentration difference. The magnitude of sodium and chloride concentration differences was unaffected by the extracellular bicarbonate concentration over a wide range. These findings, as well as the results of experiments in which the effects of some transport inhibitors were tested, are in support of the original theory of active sodium transport and electrically coupled passive chloride reabsorption across the proximal tubule of *Necturus*.

Methods

Three types of experiments were carried out to test the effects of extracellular changes in bicarbonate and of transport inhibitors. Whole kidney clearance experiments were done in which tubular sodium reabsorption was evaluated. A second group of experiments was done in which the rate of proximal tubular fluid transport was assessed in split-droplet experiments using isosmotic saline solution. Since proximal tubular fluid movement is isosmotic, volume reabsorption is also a measure of net reabsorptive sodium flux. In a third group of experiments, the ability of the proximal tubular epithelium to establish transepithelial sodium and chloride concentration differences was tested in split-droplet experiments using raffinose in the perfusion fluid.

Experiments were carried out during the summer months on nonperfused and doubly perfused *Necturus* kidneys. The methods of anesthesia, preparation of the animal and the perfusion techniques have been described in considerable detail in a previous paper from the laboratory [22]. The perfusion fluids were kept in Mariotte's bottles maintained at 40 cm of water above the level of the kidney (aortic perfusion circuit) and at 20 cm of water above the kidney (portal perfusion circuit). Perfusion rates were maintained at 1.6 ml/min in the aorta and 1.0 ml/min in the renal portal vein of the perfused kidney. Experiments were rejected when small amounts of lissamine green injected either into the portal or aortal circulation did not distribute uniformly around tubules or fill the glomeruli.

Clearance experiments. Forty animals were used to obtain the control data and four to six animals in each series to vary the bicarbonate concentration or add Diamox or to induce respiratory acidosis. Urine samples were collected into constant-bore glass capillaries (microcaps, 100 μl) to measure the urinary excretion rate of inulin and the amounts of sodium and fluid excreted. After two to three control urine collection periods (duration, 15 to 20 min), the perfusion was switched to one of the experimental perfusion fluids and several further urine samples were collected. In about half of the experiments, the sequence was reversed such that the experimental perfusion fluid was used first. The inulin clearance was used to measure glomerular filtration rate (GFR) and the amount of sodium reabsorbed was calculated as the difference between rates of filtration and excretion. Inulin was measured in urine samples and in the perfusion fluid by the fluorometric method of Vurek and Pegram [23]. Sodium was analyzed by flame photometry (Instrumentation Laboratory Inc., Boston, MA). Sodium reabsorption was expressed either as fraction of the filtered sodium load or in absolute terms and corrected for variations in wet kidney weight. To this end, after the termination of the experiment, the kidneys were quickly removed, blotted and weighed.

Split-droplet experiments to measure volume flow. In this series of experiments, the rate of proximal tubular fluid reabsorption was measured using a modification [6] of the split-droplet method originally introduced by Gertz [24]. A straight segment of a proximal tubule was selected and punctured with an oil-filled micropipette (silicone oil SF 96, 1000 centistokes, General Electric, stained with Automat Black, Patent Chemical Industries, Paterson, NJ). The tubule was filled with oil and punctured with another micropipette containing the perfusion solution. The oil droplet was split and the perfusion fluid deposited. Subsequently, the oil–fluid–oil assembly was gently displaced downstream to avoid leakage from the punctured site. The length of the fluid column between the oil menisci and the tubular diameter (d) between the brush borders were measured, the former in one-minute intervals, by means of an ocular micrometer (Leitz). A meniscus correction was applied by adding one-third of the diameter to the measured length of the fluid column. Half-times of reabsorption ($t_{\frac{1}{2}}$) of split-droplets were calculated from the linear regression of the logarithms of the relative volume V/V_0 of the droplet against time: $t_{\frac{1}{2}} = 0.301/b$, where b is the slope of the regression line ($\log V = \log V_0 - bt$), expressed in min^{-1} [6]. To estimate volume flow per unit area, we used the following expression [6]: $J_v\ (cm^3\ cm^{-2} \cdot sec^{-1}) = 0.009597 \cdot b(min^{-1}) \cdot d(cm)$.

Split-droplet experiments to measure steady-state sodium and chloride concentration differences across the tubular epithelium. These experiments were performed in nonperfused and in doubly perfused kidneys. The technique was similar to that described above (split-droplet experiments to measure volume flow) with the exception that the poorly permeant solute raffinose was added to the perfusion fluid. This reduced the rate of fluid reabsorption drastically and led to the development of stable concentration differences of sodium and chloride. This condition is useful to evaluate the ionic transport mechanisms because hydrodynamic flow and net ion movements are drastically reduced. If active transport mechanisms are present, they will manifest themselves by the establishment of significant electrochemical potential gradients which may be evaluated by means of the Nernst equation, thus, obviating the necessity of unidirectional flux measurements [13, 56, 57]. Fluid was collected after a time period varying between 20 and 30 min, adequate to achieve stable steady-state concentration differences, particularly when solutions approaching steady-state concentrations of sodium and potassium were used, and volume flow was minimized. This time interval is similar to that used in previous experiments in which steady-state concentration differences were studied [13]. After recollection the collected fluid samples were analyzed for sodium by ultramicroflame photometry [25] and for chloride by the second coulometric method of Ramsay, Brown and Croghan [26].

Perfusion fluids. In the doubly perfused kidney, the composition of the perfusion fluids was such that the sodium concentration was kept constant but that of bicarbonate and chloride was varied as shown in Table 1. In addition, these perfusion fluids contained, in mmole/liter: 0.56, Na_2HPO_4; 0.14, NaH_2PO_4; 3, KCl; 1.8, $CaCl_2$; 1.0, $MgCl_2$; 11.1, glucose; 3.3, glycine. Five solutions were prepared by the addition of different amounts of sodium chloride and sodium bicarbonate as indicated in Table 1. The control solution contained 75 mmole/liter of NaCl and 20 mmole/liter of $NaHCO_3$, a composition similar to that of *Necturus* blood [27, 28]. The perfusion solutions were gassed before and during the experiments with an appropriate concentration of CO_2 to maintain pH close to 7.4. In addition to measuring the pH of the perfusion fluid, that of the caval effluent was also checked. This latter value was found to be quite similar to that of the perfusion fluid.

1) Clearance experiments. In addition to the constituents of the perfusion fluid described above, the following were added: 15 g/liter of PVP (polyvinyl pyrrolidone, Plasdone C, GAF Corporation), 10 g/liter of inulin (Pfanstiehl) and 111 mmole/liter of mannitol. The final osmolality of the perfusion fluid was

Table 1. Concentrations of NaCl and $NaHCO_3$, and percentage of CO_2 in the $CO_2 - O_2$ mixture used to gas the fluids and to maintain pH at 7.4

Perfusion fluids			Split-droplet fluids, *mmoles/liter*		
NaCl *mmoles/liter*	$NaHCO_3$ *mmoles/liter*	CO_2 %	Raffinose	NaCl	$NaHCO_3$
95	0	—	—	—	—
91	4	1	70	60	4
75	20	5	70	45	20
55	40	10	70	25	40
35	60	15			

330 mOsm/kg. As in a previous study [22], mannitol was added to induce a moderate osmotic diuresis and permit more accurate volume flow measurements.

2) Split-droplet experiments to measure volume flow. In this series of experiments the perfusion solutions were the same as under *1*) but mannitol was deleted. The osmolality of the perfusion solution varied between 200 and 210 mOsm/kg. The same solutions were used for the kidney perfusion and for the intraluminal split-droplet microperfusion with the exception that the luminal perfusion solutions did not contain PVP.

3) Split-droplet experiments to measure steady-state sodium and chloride concentration differences across the tubular epithelium. In these experiments the kidneys were perfused with the solutions containing 4, 20 and 40 mEq/liters of sodium bicarbonate and 91, 75 and 55 mEq/liters of sodium chloride (see Table 1). In addition, these solutions contained 15 g of PVP. The matching luminal perfusion solutions used for the split-droplets all contained 70 mOsm/kg of raffinose and, respectively, 4 mmole/liter of $NaHCO_3$+60 mmole/liter of NaCl, 20 mmole/liter of $NaHCO_3$+ 45 mmole/liter of NaCl and 40 mmole/liter of $NaHCO_3$+25 mmole/liter of NaCl. The measured osmolality of these perfusion solutions was 200 mOsm/kg. Thus, in each series of experiments an initial transepithelial concentration difference of sodium chloride equivalent to 30 mEq/liter was imposed across the perfused tubular segment in the absence of a transepithelial bicarbonate gradient. In addition to experiments on the doubly perfused kidney, experiments were also done on nonperfused kidneys *in vivo*.

In additional renal clearance and split-droplet experiments, the effect of respiratory acidosis, ouabain, acetazolamide (Diamox) and ethacrynic acid upon total renal tubular sodium reabsorption and proximal tubular volume flow were investigated. Ouabain (10^{-5}M, Sigma Chemical Company), Diamox (2.2×10^{-3}M, Lederle) and ethacrynic acid (10^{-4}M, Merck, Sharp and Dohme) were added to control perfusion fluids containing 20 mEq/liter of sodium bicarbonate (pH, 7.4).

Electrical potential measurements. Transepithelial electrical potential differences were measured with Ling-Gerard microelectrooles whose luminal location was confirmed by the axial electrode system of Spring and Paganelli [7, 9, 10] in two experimental conditions in which steady-state sodium chloride concentrations differences were present. These were luminal perfusion experiments *in vivo* in nonperfused kidneys in which an initial NaCl gradient of some 30 mEq/liter was imposed (luminal perfusion fluid, 20 mEq/liter of $NaHCO_3$, 45 mEq/liter of NaCl and 70 mmole/liter of mannitol). This assumes a plasma sodium concentration of 95 mEq/liter and a plasma chloride concentration of 75 mEq/liter [27, 28]. Electrical potential differences were also measured across perfused segments of proximal tubules in the doubly perfused *Necturus* kidney in which, similar to the *in vivo* situation, an initial concentration difference of 30 mEq/liter with respect to sodium chloride was established at a peritubular bicarbonate concentration of 20 mEq/liter.

Mean values are expressed ±SEM and statistical differences evaluated by Student's *t* test.

Results

Glomerular filtration rates (GFR) and renal excretion data are summarized in Table 2. Figs. 1 and 2 provide graphic summaries of fractional and absolute

Table 2. Summary of GFR and urinary sodium data in the doubly perfused *Necturus* kidney

Experimental condition	HCO_3^- *mEq/liter*	pH	Experimental periods (*N*)	GFR *μl/g. min*	Na reabsorption		Urine Na *mEq/liter*
					Fractional %	Absolute *μEq/g· min*	
Control	20	7.4	(127)	11.7±0.5	89.3±0.7	953±42	14.4±1.0
	0	7.4	(14)	12.7±0.5	83.2±2.7	1021±52	22.5±2.9
	4	7.4	(22)	14.6±0.6	79.6±2.4	1100±56	24.9±2.8
	40	7.4	(51)	12.8±0.6	79.4±1.3	912±54	26.0±1.4
	60	7.4	(14)	11.3±0.7	76.1±4.2	801±24[a]	31.2±4.4
Diamox (2.2×10^{-3}M)	20	7.4	(24)	9.6±0.9	65.4±4.4	691±88[b]	31.2±2.4
Acidosis	20	6.8	(31)	7.9±0.7	70.6±3.0	555±55[b]	39.2±3.5
Ouabain[c] (10^{-5}M)	20	7.4		12.4±2.3	2.2±3.1	32±33[b]	88.0±1.0
Ethacrynic acid[c] (10^{-4}M)	20	7.4		11.7±1.0	10.0±1.0	72±7[b]	77.0±1.0

[a] $P<0.05$.
[b] $P<0.01$.
[c] Data from [22].

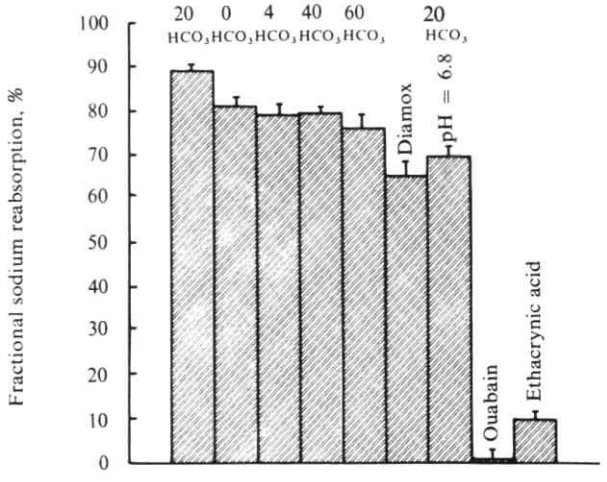

Fig. 1. *Summary of fractional urinary sodium reabsorption rates of the doubly perfused, isolated* Necturus *kidney using different solutions.* Height of the *bars* corresponds to mean reabsorption rates, height of the *lines* above bars represents SEM. *Numbers* on top of bars indicate concentration of bicarbonate in mEq/liter. For comparison, data of ouabain and ethacrynic acid experiments, taken from [22], are included.

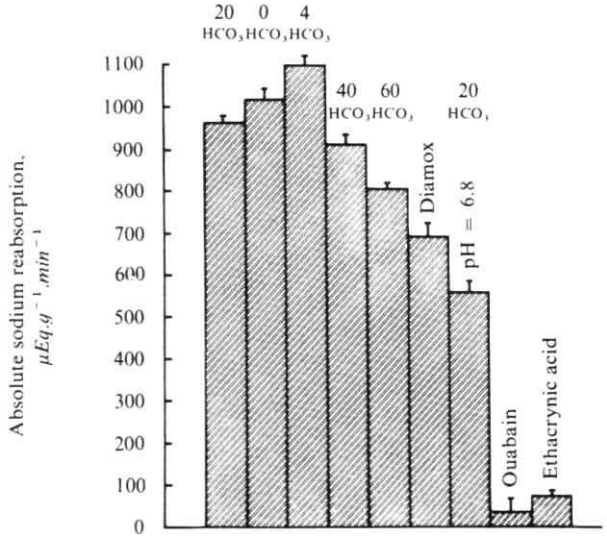

Fig. 2. *Summary of absolute rates of urinary sodium reabsorption of the doubly perfused, isolated* Necturus *kidney using different solutions.* The presentation of data is similar to that of Fig. 1.

reabsorption rates of sodium ions. As in a previous study [22], we observed in the present series of perfusion experiments similar fluid and sodium reabsorption rates as in blood-perfused animals [28]. This fact and the finding of relatively low urinary sodium concentrations indicate that renal perfusion and the addition of mannitol do not seriously impair the ability of the tubules to reabsorb most of the filtered sodium and to establish fairly steep transepithelial sodium concentration differences. Data obtained during control periods varied little from experiment to experiment and were pooled for comparison (see Table 2).

Inspection of Table 2 indicates that the rate of glomerular filtrate formation in the perfused kidney was not affected by variations of the extracellular bicarbonate concentration. With the exception of Diamox, which caused a moderate fall of GFR, and of respiratory acidosis, in which the fall of GFR was the largest, none of the other experimental conditions depressed GFR.

Sodium reabsorption, expressed in fractional terms, declined moderately as the bicarbonate concentration in the perfusion fluid rose. It is noteworthy that the dramatic reduction of the extracellular bicarbonate concentration to very low levels did not significantly reduce overall renal tubular sodium reabsorption. Thus, compared to a control reabsorption rate of 953 μEq·min^{-1}·g^{-1}, reduction of the bicarbonate concentration to 4 and 0 mEq/liter did not result in a decrease of the absolute amount of sodium reabsorbed by the tubular epithelium. The moderate reduction of fractional sodium reabsorption was probably related to the small increase in GFR since the absolute rate of tubular sodium reabsorption remains unchanged. At the two higher bicarbonate concentrations in the perfusion fluid (40 and 60 mEq/liter), there occurred a moderate fall in both fractional and absolute tubular sodium reabsorption. Most likely this was due to the osmotic effect of bicarbonate at concentrations at which its reabsorption is likely to be incomplete. The fact that the urinary sodium concentrations are increased at the higher bicarbonate concentrations is consistent with the interpretation that larger than normal loads of sodium are delivered to the distal nephron. At least from studies on mammalian distal tubules, it is known that the delivery of increased sodium loads out of the proximal tubule uniformly increases the luminal sodium concentration along the distal tubule [29, 30].

Table 2 also summarizes data from experiments in which the effects of the carbonic anhydrase inhibitor Diamox and of respiratory acidosis were investigated.

With respect to Diamox and respiratory acidosis, a significant fall in tubular sodium reabsorption, both in fractional and absolute terms, was observed. Despite a fall in GFR, fractional reabsorption of sodium fell from a control value of 89.3 to 65.4 and 70.6%, respectively. The absolute rate of sodium reabsorption declined and urinary sodium concentrations increased.

Similar but more dramatic inhibitory effects upon renal tubular sodium reabsorption had been observed after administration of ouabain and ethacrynic acid

Table 3. Summary of data from proximal tubular microperfusion experiments under the different experimental conditions[a]

Experimental condition		Experimental periods	$t_{\frac{1}{2}}$ *min*	Volume flux $10^{-6} cm^3/cm^2 . sec$
Control	20 $NaHCO_3$	(13)	49.7±3.4	0.82±0.03
	4 $NaHCO_3$	(11)	50.7±6.4	0.87±0.10
	40 $NaHCO_3$	(10)	48.6±5.2	0.89±0.09
Acidosis	20 $NaHCO_3$ pH=6.8	(3)	217±63	0.18±0.06[b]
Ouabain (10^{-5}M)		(9)	639±186	0.13±0.04[b]
Ethacrynic acid (10^{-4}M)		(13)	195±40	0.26±0.05[b]

[a] Rates of transepithelial fluid movement are expressed as $t_{\frac{1}{2}}$ and in terms of absolute transport rates.
[b] $P<0.01$.

[22, 31]. These data are included in Table 2 for comparison. In the presence of an unchanged GFR, both fractional and absolute rates of sodium reabsorption were drastically reduced. At the same time, urinary sodium concentrations were increased and, in the case of ouabain, approached the level of the sodium concentration in the perfusion fluid.

Table 3 summarizes micropuncture data on reabsorptive proximal tubular volume flow. Table 4 contains transepithelial steady-state sodium and chloride concentration ratios and differences obtained in the presence of the poorly reabsorbable solute raffinose. These data were collected in the same group of animals in which the clearance experiments, summarized in Table 2, were carried out.

With respect to the perfusion experiments in which the effects of changes in bicarbonate concentrations were explored, it is apparent that variations over a tenfold change from 4 to 40 mEq/liter had no significant effect upon $t_{\frac{1}{2}}$ and net volume flux. The $t_{\frac{1}{2}}$ values in these experiments are similar to control values observed by us [10] and others [6] in summer animals.

Inspection of the $(TF/P)_{Na}$ and $(TF/P)_{Cl}$ ratios indicates that the proximal tubular epithelium generates significant transepithelial concentration gradients of both ion species ($P<0.001$). With respect to sodium, such limiting steady-state concentration differences have been observed previously when the poorly reabsorbable nonelectrolyte mannitol has been added to the perfusion fluid [13]. The magnitude of the concentration differences is less in the perfused than in the nonperfused kidney. The observation that similar sodium and chloride concentration differences are established indicates the absence of significant changes in luminal bicarbonate concentration during split-droplet experiments. Variations of the extracellular bicarbonate concentration over a tenfold range from 4 to 40 mEq/liter do not affect the magnitude of the transepithelial sodium concentration difference.

It is also apparent that the proximal tubular epithelium of *Necturus* establishes significant ($P<0.001$) steady-state transepithelial chloride concentration differences. They are of similar magnitude as those of sodium. Inspection of Table 4 also indicates that these steady-state chloride concentration gradients are independent of the bicarbonate concentration in the extracellular fluid over the range used.

Electrical potential measurements were carried out *in vivo* and *in vitro* during conditions in which steady-state transepithelial concentration gradients of sodium and chloride developed at normal bicarbonate concentrations. *In vivo* a mean proximal transepithelial potential difference of -12.6 ± 0.95 mv (14 measurements), lumen-negative, was observed. In the perfused kidney, the corresponding value was -8.4 ± 0.54 mv (24 measurements). These values are significantly

Table 4. Steady-state transepithelial tubular fluid to plasma (TF/P) concentration ratios and plasma minus tubular fluid concentration differences of sodium and chloride obtained in split-droplet experiments

Experimental condition	Experimental periods (*N*)	$(TF/P)_{Na}$	$(TF/P)_{Cl}$	$(P-TF)_{Na}$ *mEq/liter*	$(P-TF)_{Cl}$ *mEq/liter*
Nonperfused	(27)	0.701±025	0.729±0.017	32.3±2.9	24.5±1.8
Perfused 20 $NaHCO_3$	(19)	0.824±0.012	0.817±0.017	15.0±1.4	15.4±1.5
4 $NaHCO_3$	(17)	0.858±0.009	0.820±0.016	13.5±1.0	16.9±1.6
40 $NaHCO_3$	(18)	0.847±0.016	0.825±0.013	17.3±1.2	15.4±1.0

different from each other ($P < 0.05$) and similar to free-flow values using similar methods of measurement [6, 7, 9].

The presence of such an electrical potential difference indicates that there is a significant driving force available to transfer chloride out of the lumen and to lower its intratubular concentration below that of the peritubular fluid (see Table 4). On the other hand, the observation that the luminal sodium concentration falls below that of the peritubular fluid demonstrates the presence of an active pumping mechanism acting on this ion.

Discussion

The most important findings of our experiments are *1*) the observation that, over a wide range of the extracellular bicarbonate concentration, the operation of proximal tubular sodium reabsorption in *Necturus* is independent of the extracellular bicarbonate concentration and *2*) that passive chloride distribution may be accounted for by the electrical driving force. Our results also indicate that in the proximal tubule of *Necturus* sodium bicarbonate transport is not the sole mode of active sodium transfer.

The results of the present work reveal some noteworthy differences between the amphibian and mammalian proximal tubule. These concern the bicarbonate sensitivity of proximal sodium transport. Other species differences such as the relative rates of bicarbonate and chloride reabsorption and the electrical potential profiles along the proximal tubule are also relevant and will be discussed.

In contrast to the results of the present experiments, sodium reabsorption along the proximal convoluted tubule has been found to be sensitive to the peritubular reduction of bicarbonate ions in the rat [32–34] and the isolated perfused proximal convoluted tubule of the rabbit [21]. In contrast, such bicarbonate sensitivity has not been observed in isolated perfused straight proximal tubules [35]. It is of interest that in the perfused rat tubule *in vivo*, a significant fraction of proximal tubular sodium transport persists in the absence of bicarbonate [32–34]. Similarly, a large fraction of overall renal tubular sodium reabsorption persists during perfusion of the isolated rat kidney with bicarbonate-free solutions [36]. Thus, only a fraction of tubular sodium transport, in some species, depends on the extracellular bicarbonate concentration.

The reason for the variable behavior of different proximal tubular preparations concerning the bicarbonate requirement of volume reabsorption is not clear but has recently attracted considerable attention. It is possible that some or all of the following factors are involved. It has been observed that there are enzymatic differences between the mammalian and amphibian proximal tubule. The most important one concerns the absence of a cytochrome oxidase system in the proximal tubule of *Necturus* [37]. Conceivably, the higher rate of sodium transport and its more critical dependence upon aerobic metabolism in the mammalian proximal convoluted tubule would make this preparation bicarbonate-sensitive, although at present the mechanism of such an action is not understood. The proximal convoluted tubule of the rat is also characterized by a relatively higher sodium than chloride permeability [17], whereas both the proximal tubular epithelium of *Necturus* [38, 39] and that of the straight part of the proximal tubule have a relatively higher chloride permeability [35]. This relatively high chloride permeability as well as the lower absolute reabsorption rates of these preparations may explain that chloride transport alone may be adequate to sustain a normal rate of active proximal tubular sodium transport [35]. Clearly, additional studies are necessary to elucidate the precise mechanism of the bicarbonate effect in those preparations in which this ion stimulates active sodium and fluid translocation.

We have also observed that respiratory acidosis, achieved by perfusion with a solution of normal bicarbonate but high CO_2 content, depresses sodium and fluid reabsorption. The microperfusion experiments reported here indicate that one site of action of the high P_{CO_2} tension is the proximal tubule since the $t_{\frac{1}{2}}$ values of isotonic droplet disappearance were markedly prolonged. It has also been observed that in the rat proximal tubule sodium reabsorption is diminished in metabolic acidosis [40]. This transport inhibition was associated with a low peritubular bicarbonate level in contrast to our observations in which the bicarbonate level was kept constant. Our experiments do not elucidate the mechanism by which acidosis blocks a significant fraction of proximal tubular sodium transport.

Administration of the carbonic anhydrase inhibitor Diamox also led to a moderate reduction of overall tubular sodium reabsorption (see Table 2). Experiments on mammalian nephrons have shown that this transport inhibition takes place at the proximal tubular level (41–44). If this were also the site of action of Diamox in *Necturus*, it is most likely that luminal accumulation of bicarbonate acts as an osmotic diuretic. This interpretation is indirectly supported, but not proven, by our finding that at higher bicarbonate concentrations in the perfusion medium tubular sodium reabsorption declined. In the mammalian proximal tubule, the reflection coefficient of bicarbonate is

larger than that of chloride and approaches unity [45], making this anion a strong osmotic diuretic.

Another important difference between the amphibian and mammalian nephron concerns the relationship between proximal chloride and bicarbonate concentrations. Whereas the concentration of chloride along the proximal tubule of *Necturus* remains constant and quite similar to that in a plasma ultrafiltrate [20, 28], it rises early along the proximal tubule of various mammalian species [46–50]. It has been shown that this behavior of chloride ions is related to preferential bicarbonate reabsorption along mammalian proximal tubules [41, 44, 51–53]. Closely related to this elevation of the proximal tubular chloride concentration in mammalian tubules is the change in electrical polarization along the nephron. It has been reported that whereas the early part of the mammalian proximal tubule maintains a significant transepithelial negativity, this potential difference becomes smaller and even reverses (lumen-positive) towards the end of the proximal convoluted tubule [54]. Calculations indicate that the electrochemical potential difference of chloride across the mammalian proximal tubule is sufficiently large to account for chloride reabsorption by passive transport [17].

In the amphibian proximal tubule, chloride reabsorption can also be accounted for by a passive mechanism. However, in contrast to the mammalian proximal tubule, the luminal chloride concentration does not increase and electrical driving forces are of primary importance. The following calculation shows that the measured transtubular potential differences are of adequate sign and sufficient magnitude to explain the steady-state transtubular chloride concentration differences when an impermeant nonelectrolyte is added to the tubule lumen. From the observed values of the transepithelial electrical potential difference of −8.4 mv, lumen-negative (perfused kidney), and −12.6 mv (*in vivo*), using the Nernst equation, steady-state tubular fluid/plasma (or perfusion fluid) chloride ratios of 0.71 and 0.60 obtain. These values are lower than the ratios actually observed under identical experimental conditions (see Table 4). Hence, the electrical potential difference accounts quantitatively for passive chloride distribution across the proximal tubular epithelium in *Necturus*.[1]

The development of a luminal chloride concentration below plasma levels—assuming passive chloride distribution—in the presence of a poorly reabsorbable solute such as mannitol or raffinose depends at least upon two factors. First, the presence of an active sodium transport system is necessary to maintain both a significant transepithelial sodium concentration gradient and the luminal electronegativity. Second, bicarbonate ions in the lumen must not be reabsorbed at a rate in excess of chloride. Otherwise, the concentration of chloride will rise to maintain isotonicity. In *Necturus*, the present demonstration of chloride concentrations below those of plasma when a poorly reabsorbable nonelectrolyte is present in the lumen is facilitated by the fact that the concentration of bicarbonate does not decrease in the proximal tubule [20]. In contrast, it is difficult to observe transepithelial chloride tubular fluid/plasma concentration ratios of less than unity in the mammalian proximal tubule. This is due to the fact that the compensatory increase of the chloride concentration [46–50], due to the disappearance of bicarbonate ions, is fortuitously of similar magnitude as the fall that would be expected from the magnitude of the limiting sodium concentration difference in the presence of a poorly reabsorbable solute [47, 57, 58]. As a consequence, these opposing concentration changes result in luminal chloride concentrations similar to that in plasma [57, 59, 60]. Thus, only when hydrogen ion secretion has been compromised will it be possible to observe a reduction of the luminal chloride concentration below plasma values. This has actually been noted by Weinstein in Diamox-treated rats in which proximal chloride concentration ratios below unity were observed [50]. In these experiments, bicarbonate reabsorption was extensively blocked yet sodium reabsorption proceeded at a rate adequate to lower the luminal chloride concentrations. In principle, however, this experimental approach is limited by the fact that Diamox potentially inhibits a significant fraction of proximal sodium transport, thus making it difficult to demonstrate coupling of chloride to sodium transport by this technique.

Several lines of evidence support the active nature of sodium transport across the proximal tubular epithelium and the close relationship between this transport and generation of the transepithelial electrical potential difference (lumen-negative). The present observation that the transepithelial electrical potential difference is negative and the finding that in the presence of a poorly permeant nonelectrolyte the concentration of sodium decreases to values significantly less than that in peritubular fluid indicates the ability of the proximal tubular epithelium of *Necturus* to establish a transepithelial distribution of sodium which cannot be

[1] The finding that the observed transepithelial chloride concentration difference is less than that calculated on the basis of the electrical potential difference could be due to the fact that electrical and concentration measurements were done on different proximal tubules, or to the presence of an element of exchange diffusion or active secretion of chloride ions into the tubule [19, 56].

accounted for by passive transport [13]. The sodium concentration also drops below plasma levels in the proximal tubule of mammals when water movement is retarded [47, 57, 58, 61]. In agreement with previous observations, the present work also shows that agents which inhibit active sodium extrusion across the peritubular cell membrane of proximal tubule cells, such as ouabain and ethacrynic acid [62, 63], block net sodium reabsorption [22, 31, 64] including that occurring at the proximal tubular level (see Table 3 and [11, 64]). Finally, both metabolic inhibition of proximal tubular sodium transport by potassium iodoacetate and ouabain [11] and symmetrical reduction of the sodium concentration in both the lumen and the peritubular fluid [10] have been shown to reduce net tubular sodium transport and luminal negativity. These observations support the view that the electrical potential difference across the proximal tubule of *Necturus* is generated by active sodium transport.

We conclude that some key aspects of our original cell model of proximal sodium chloride transport [1, 2] (i.e., active sodium movement and electrically coupled passive chloride reabsorption) are supported by findings of the present series of experiments. We believe that this basic transport mechanism is present both at the level of the amphibian and mammalian tubule. In the latter, however, it is modified by an inherently higher rate of hydrogen ion secretion and its consequences—preferential proximal tubular bicarbonate reabsorption, elevation of tubular chloride concentration and generation of a more positive transepithelial potential difference—all of which add an additional mechanism by which higher rates of sodium and fluid movement are sustained.

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