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The role of bicarbonate in proximal tubular sodium chloride transport

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The role of bicarbonate in proximal tubular sodium chloride transport. Microperfusion studies of single proximal tubules in rat kidney cortex slices show a progressive increase in the rate of reabsorption of isosmotic fluid and NaCl as the bathing medium bicarbonate concentration is raised from 0 to 12 mEq/liter while the pH is maintained at 7.3 to 7.4. When bathing medium bicarbonate was 25 mEq/liter, tubules maintained a significantly lower intraluminal $NaHCO_3$ concentration: 15 mEq/liter. At the same time luminal NaCl concentration was equivalently elevated above that of the bathing medium. Reabsorptive rates were decreased when acetazolamide was added either to the bathing medium or to the tubular perfusate. Intratubular carbonic anhydrase reversed the effects of acetazolamide. Active transport of $NaHCO_3$ stimulates NaCl reabsorption by generating and maintaining concentration gradients favoring passive reabsorption of this salt. The proximal tubule also possesses mechanisms for active NaCl reabsorption.

Rôle du bicarbonate dans le transport tubulaire proximal du chlorure de sodium. L'étude par microperfusion de tubes proximaux dans des tranches de cortex rénal de rat montre une augmentation progressive du débit de réabsorption de solution iso-osmotique et de Nacl alors que la concentration de bicarbonate dans le bain augmente de 0 à 12 mEq/litre cependant que le pH est maintenu à 7,3 à 7,4. Pour une concentration de bicarbonate dans le bain de 25 mEq/litre les tubules maintiennent une concentration intraluminale significativement inférieure: 15mEq/litre. Au même moment la concentration luminale de NaCl est supérieure, dans les mêmes proportions, à celle du bain. Les débits de réabsorption diminuent quand de l'acétazolamide est ajouté soit au bain soit au perfusat tubulaire. L'anhydrase carbonique intra-tubulaire annule les effets de l'acétazolamide. Le transport actif de $NaHCo_3$ stimule la réabsorption de NaCl en créant et en maintenant des gradients de concentrations qui assurent la réabsorption passive de ce sel. Le tube proximal possède aussi des mécanismes de réabsorption active pour NaCl.

Although protein oncotic, and possibly also hydrostatic, pressure gradients contribute to proximal tubular fluid reabsorption, active solute transport is ultimately responsible for the return of most of the glomerular filtrate to the bloodstream [1–5]. Sodium chloride and sodium bicarbonate are the major solutes of the proximal reabsorbate. Of these two salts there is compelling evidence that $NaHCO_3$ is reabsorbed actively. The mechanism depends largely upon the exchange of filtered Na^+ for secreted H^+ ions and the reaction of the latter with filtered HCO_3^- ions to form CO_2, which diffuses into the peritubular capillaries [6, 7]. The operation of this transport system reduces the tubular fluid bicarbonate concentration to 8 to 10 mEq/liter [8, 9]. The reabsorption of $NaHCO_3$ together with osmotically obligated water leads to a rise in intratubular NaCl concentration. A concentration gradient promoting the reabsorption of this salt, therefore, exists along most of the length of the proximal tubule. In the absence of $NaHCO_3$, this favorable concentration gradient cannot develop, and the rate of NaCl reabsorption should be slowed. Investigations of the proximal tubule using three different experimental preparations show that this is, indeed, the case. In the capillary-perfused rat kidney *in situ* [10], the isolated rabbit proximal tubule [11], and in perfused proximal tubules of rat kidney cortex slices [12, 13], substantial concentrations of extracellular bicarbonate are required for maximum rates of NaCl reabsorption. The present studies extend the observations made on rat kidney cortex slices. The results indicate that about one-half of the perfusate NaCl is reabsorbed by passive diffusion down a concentration gradient maintained by active $NaHCO_3$ transport. Theoretical calculations suggest that the situation in the *in vivo* kidney is similar: about two-thirds of the proximal tubular NaCl reabsorption can be accounted for on the basis of passive driving forces.

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Methods

Proximal tubular fluid transport. The technique for

stop-flow microperfusion of single proximal tubules in rat kidney cortex slices has been described in detail elsewhere [14, 15]. Briefly, a surface cortical slice was mounted, capsular surface uppermost, in a micropuncture chamber where is was bathed in a rapidly recirculating, oxygenated bathing medium at 38°C. The medium equilibrated with 5% CO_2 containing (in mEq/liter) the following: Na, 140; K, 5; Ca, 2; Mg, 2.4; HPO_4, 4.8; H_2PO_4, 0.6; SO_4, 2.4; alpha-ketoglutarate, 6; Cl, 110.6; and HCO_3, 25. In media equilibrated with lower partial pressures of CO_2, some of the bicarbonate was replaced with an equivalent amount of Cl, so that the pH was maintained between 7.3 and 7.4.

Surface proximal tubule segments were punctured with double-barreled glass micropipets, and the rates of reabsorption of perfusion solution, isolated between oil columns, assessed by the "shrinking droplet" method. Perfusate volumes were determined from sequential photographs. After application of a meniscus correction [14], the relative perfusate volume (V/V_0) was plotted logarithmically as a function of time. From such graphs (Fig. 1) tubular reabsorptive $t_{\frac{1}{2}}$ was obtained.

Bicarbonate and chloride concentration in collected perfusates. For these studies long proximal tubular segments lying on or close to the surface were identified by oil injection from one barrel of the perfusing pipette. An oil-filled segment remote from the site of perfusion was punctured with the collecting pipette; perfusate was then injected and sealed off from both pipettes by oil injection. After a waiting period, fluid remaining within the tubule was drawn into the collecting pipette and stored therein behind an oil seal. The time of contact of perfusate with tubular epithelium was calculated as (0.5× [time required to fill tubule + time required to aspirate sample] + waiting time), and averaged 40 seconds. After a maximum holding time of three hours, the contents of the collecting pipettes were delivered under oil onto a 30 gauge silver wire stretched across the bottom on an analysis chamber. Here the pH of each sample was determined using a microantimony electrode [9] in conjunction with a 1N $NaNO_3$-filled glass microcapillary reference electrode. The bicarbonate concentration in the sample was determined from the following formula: (HCO_3^-) mEq/liter = $0.031\ P_{CO_2} \times \log^{-1}(pH - 6.1)$, where P_{CO_2} is the partial pressure of carbon dioxide of the analysis chamber oil, which is equal to its partial pressure in the medium bathing the kidney slice. Following the pH determination, a 0.1 nl aliquot of the collected perfusate was taken up in a micropipette, delivered to an adjacent portion of the silver wire and titrated coulometrically [16]. Chloride concentrations were determined in reference to standard solutions delivered with the same pipet. Net transtubular steady state NaCl fluxes were estimated from the following formula:

$$J_{NaCl}\ (nEq\ cm^{-2}sec^{-1}) = \frac{0.0347\ R}{t_{\frac{1}{2}}} \times C^{ss}_{NaCl},$$

where R is the average internal radius of a perfused tubule, 16.9μ, [14] and C^{ss}_{NaCl}, the steady state intratubular NaCl concentration in mEq/liter.

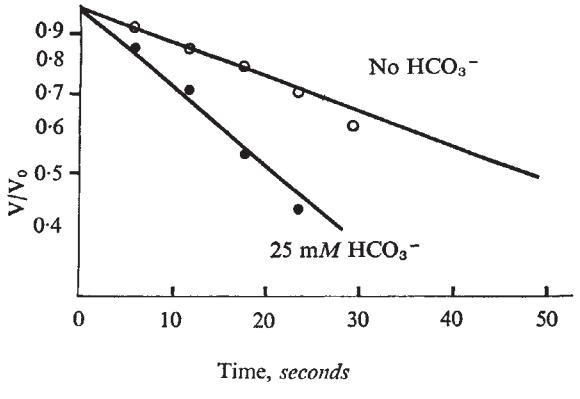

Fig. 1. *Relative perfusate volume (V/V_0) plotted as a function of time in two representative experiments, one in a bicarbonate-free and one in a 25-mEq/liter bicarbonate medium.*

Results

Fig. 1 shows the primary data from two representative experiments, one with a slice bathed in a bicarbonate-free medium, the other in a medium providing 25 mEq/liter of HCO_3^-. These experiments demonstrate the typical logarithmic decline in relative perfusate volume, and the prolongation of $t_{\frac{1}{2}}$ when $NaHCO_3$ is absent from the bathing medium. In Fig. 2, average $t_{\frac{1}{2}}$ values for the reabsorption of 140 mM NaCl perfusates are plotted as a function of medium bicarbonate concentration in the range 0 to 25 mEq/liter. Table 1 summarizes data on the effects of changes in medium bicarbonate concentration on the reabsorptive $t_{\frac{1}{2}}$ of various isosmotic perfusates.

In the absence of bicarbonate in the bathing medium both unbuffered and phosphate-buffered NaCl perfusates are reabsorbed at approximately one-half the control rate. Based on perfusate NaCl concentrations of 140, and 134.6 mEq/liter, respectively, and the $t_{\frac{1}{2}}$ values given in Table 1, the NaCl fluxes are 1.82 and 1.55 nEq cm^{-2} sec^{-1}. The rate of fluid and NaCl transport rises progressively as the medium bicarbonate concentration is raised from 0 to 12 mEq/liter. The rate of fluid reabsorption does not increase further as the medium bicarbonate is raised from 12 to 25 mEq/liter. At this physiological bicarbonate concentration, the rate of NaCl reabsorption (based on

Table 1. Effects of changes in bathing medium bicarbonate concentration and P_{CO_2} on tubular reabsorptive $t_{\frac{1}{2}}$ and composition of collected perfusate[a]

Bathing medium		$t_{\frac{1}{2}}$, *seconds*	Collected perfusate		
HCO_3^-, *mEq/liter*	P_{CO_2} *mm Hg*		HCO_3^-, *mEq/liter*	pH	Cl, *mEq/liter*
Perfusate: 140 mEq/liter of NaCl					
0.0	0.0	45±4 (14)	—	—	—
0.25	0.7	36±3 (7)	—	—	—
6	11	34±2 (23)	—	—	—
12	22	20±1 (24) (*NS*)	—	—	—
25	45	21±2 (14)	15±2	7.20	125±4 (23)
Perfusate: isomotic phosphate buffered saline, pH 7.35 (5.4 mM PO_4)					
0.0	0.0	51±5 (12)	—	—	—
Perfusate: 115 mEq/liter of NaCl, 25 mEq/liter of $NaHCO_3^-$, pH 7.35					
25	45	22±4 (18) (*NS*)	16±1 (*NS*)	7.22 (*NS*)	— (6)
Perfusate: 100 mEq/liter of NaCl, 40 mEq/liter of $NaHCO_3$, pH 7.60					
25	45	24±1 (11) (*NS*)	—	—	—

[a] The pH of all bathing media was 7.35. Values given are means ±SEM. The number of tubules perfused is given in parentheses. *NS* indicates data not significantly different ($P > 0.01$) from control studies where the bathing medium $NaHCO_3$ concentration was 25 mEq liter; P_{CO_2}, 45 mm Hg; and the tubular perfusate, a 140 mM solution of NaCl.

the steady state chloride concentration of 125 mEq/liter) is 3.5 nEq cm^{-2} sec^{-1}. Fluid reabsorptive $t_{\frac{1}{2}}$ values are the same for NaCl solutions as for perfusates where 25 or 40 mEq $NaHCO_3$ is substituted for an equivalent concentration of NaCl.

When the bathing medium bicarbonate concentration is 25 mEq/liter, re-collected perfusates contain 15 to 16 mEq/liter $NaHCO_3$. This bicarbonate concentration reflects net bicarbonate entry into the NaCl perfusates and net bicarbonate reabsorption against a concentration gradient from perfusates originally containing 25 mEq/liter of bicarbonate. Presumably, therefore, this is a steady state concentration where passive bicarbonate entry is just balanced by active bicarbonate extrusion.

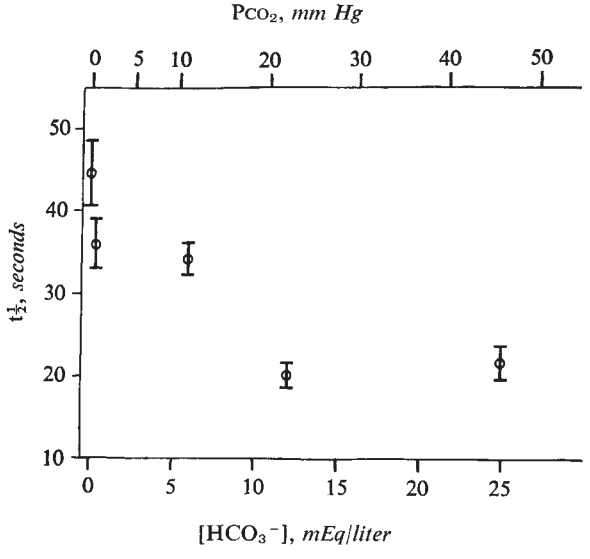

Fig. 2 *Average fluid reabsorptive $t_{\frac{1}{2}}$ value for isosmotic saline perfusates plotted as a function of the bathing medium bicarbonate concentration. Vertical bars* extend ±1 standard error from the mean.

Table 2 shows the effects of pH changes and ionic substitutions on proximal fluid reabsorption. The substitution of SO_4^{--} for Cl^- in both luminal and peritubular fluid prolongs the reabsorptive $t_{\frac{1}{2}}$, but is without effect on the steady state bicarbonate concentration.

A reduction of medium bicarbonate concentration at constant P_{CO_2} to yield a pH of 7.00 slows fluid reabsorption, while a comparable HCO_3^- reduction at constant pH is without effect. Raising the HCO_3^- concentration at constant P_{CO_2} to yield a medium pH of 7.8 resulted in no change in transport rate.

Table 3 summarizes the effects of acetazolamide on

Table 2. Effect of pH and ionic substitution on tubular fluid reabsorptive $t_{\frac{1}{2}}$

Bathing medium			$t_{\frac{1}{2}}$, *seconds*	Collected perfusate		
HCO_3^-, *mEq/liter*	pH	anion		HCO_3^-, *mEq/liter*	pH	Cl, *mEq/liter*
Perfusate: 140 mEq/liter of NaCl						
11	7.00	Cl^-	33±2 (14)	—	—	—
62	7.80	Cl^-	19±2 (14) (*NS*)	—	—	—
Perfusate: SO_4 bathing medium						
25	7.35	SO_4^{--}	39±2 (20)[a]	15±2 (*NS*)	7.20 (*NS*)	6±2 (10)

[a] Data from reference 15.

Table 3. Effects of acetazolamide on tubular reabsorptive $t_{\frac{1}{2}}$ and composition of the collected perfusate[a]

Bathing medium (HCO_3^-), *mEq/liter*	Acetazolamide concentration, *mg/liter*		$t_{\frac{1}{2}}$, *seconds*	Collected perfusate		
	Bath	Perfusate		HCO_3^-, *mEq/liter*	pH	Cl, *mEq/liter*
Perfusate: 140 mEq/liter of NaCl						
12	2	0	37±3 (13)	—	—	—
25	2	0	33±2 (21)	19±2 (*NS*)	7.29 (*NS*)	122±6 (*NS*) (13)
12	5	0	44±3 (14)	—	—	—
0	5	0	51±3 (26)	—	—	—
25	0	2	18±2 (17) (*NS*)	—	—	—
25	0	5	25±2 (15) (*NS*)	—	—	—
25	0	10	32±2 (16)	—	—	—
25	0	20	39±4 (15)	—	—	—
Perfusate: 100 mEq/liter of NaCl, 40 mEq/liter of $NaHCO_3$, pH 7.60						
25	5	0	58±5 (13)	—	—	—
Perfusate: 140 mEq/liter of NaCl with carbonic anhydrase, 400 e.u./ml						
25	5	0	23±1 (31) (*NS*)	—	—	—

[a] The pH of all bathing media was 7.35.

proximal tubular fluid transport. Whether it is added to the tubular perfusate or the bathing medium, this carbonic anhydrase inhibitor slows fluid reabsorption.

Acetazolamide appears to be effective at lower concentration when added to the bathing medium rather than to the perfusate.

Addition of carbonic anhydrase to the tubular perfusate abolished the effect of acetazolamide in the bathing medium.

Although the tubular fluid HCO_3^- concentration is higher in the presence of actazolamide, 19 mEq/liter, a significant difference from the control could not be demonstrated. Failure to achieve significance may have been due to the limited number of experiments and to inherent problems with the microanalytical methods available.

Discussion

The role of $NaHCO_3$ in proximal tubular NaCl transport in vitro. These experiments on single perfused proximal tubules of rat kidney cortex slices clearly show that the rate of fluid and NaCl transport is significantly reduced when bicarbonate is absent from the bathing medium. The findings are in essential agreement with those of Ullrich, Radtke and Rumrich on the capillary perfused rat kidney *in situ*. In that preparation the rate of NaCl reabsorption in the absence of bicarbonate was about one-third of that at 25 mEq/liter of bicarbonate [10]. Similarly, Kokko, Rector and Seldin, using the isolated rabbit proximal tubule, found that bicarbonate was necessary for maximum rates of fluid reabsorption. In their studies, however, NaCl transport was reduced essentially to zero in the absence of bicarbonate [11].

There are several possible explanations for the stimulatory effect of bicarbonate on tubular NaCl transport: *1*) The synthesis of some metabolic intermediate essential for transport systems may depend on CO_2 fixation. The biochemical reactions would utilize either a biotin-activated enzyme (e.g., pyruvate carboxylase) or carbamyl phosphate synthetase (E.C. 2.7.2a). The biotin enzymes achieve maximum reaction rates at relatively low concentrations of bicarbonate (K_m values on the order of 1 to 2 mM are typical [17]) while the rate of carbamyl phosphate synthesis is limited by the normally low tissue concentration of NH_3. Maximum rates of fluid transport, on the other hand, require bicarbonate concentrations in excess of 6 mM, a value well above the probable rate-limiting levels for these reactions. A mechanism of this sort, therefore, seems unlikely. *2*) Bicarbonate and CO_2 may play a direct role in the transport of Na^+ and Cl^- ions. There could be, for example, a dual ion exchange mechanism by which H^+ and HCO_3^- ions generated within the tubular cells by the hydration of CO_2 are secreted in exchange for luminal Na^+ and Cl^-. The secreted ions could be reabsorbed as CO_2 following their recombination within the tubule lumen. A dual ion exchange system in some ways similar to this probably accounts for NaCl transport in the gills of fresh water teleosts [18]. We can adduce no direct evidence for such a mechanism in proximal renal tubule. *3*) Enhanced NaCl transport may be entirely due to the concentration gradient favoring outward diffusion of this salt which can develop in the presence

of bicarbonate. When $NaHCO_3$ makes up a large fraction of the bathing medium's compliment of solute, the intratubular NaCl concentration is significantly higher than that in the peritubular fluid. The concentration gradient results from the tubule's ability to maintain an intratubular $NaHCO_3$ concentration below that in the surrounding medium. Since the tubule is freely permeable to water, the reduced $NaHCO_3$ concentration must be associated with an increased NaCl concentration.

In these *in vitro* experiments the proximal tubular fluid bicarbonate is about 10 mEq/liter lower than the bathing medium. The intratubular bicarbonate concentration of 15 to 16 mEq/liter is achieved either by the active extrusion of bicarbonate ions from perfusates originally containing 25 mEq/liter of bicarbonate, or by the passive diffusion of HCO_3^- ions into the originally bicarbonate-free perfusates. This concentration, therefore, represents a steady state where active bicarbonate efflux is just balanced by passive influx. The steady state luminal NaCl concentration is correspondingly higher than in the bathing medium: 125 *vs.* 110.6 mEq/liter. The contribution of this concentration difference to NaCl reabsorption can be calculated from the following equation:

$$J_{NaCl}^{passive} = \bar{C} P_{NaCl} \ln \frac{C_L}{C_P}, \qquad (1)$$

where C_L and C_P are the concentrations of NaCl in luminal and peritubular fluids, respectively, and $\bar{C}$ is their mean (117.8 mEq/liter). The sodium chloride permeability coefficient, P_{NaCl}, is calculated from data given by Frömter, Müller and Wick for Na^+ and Cl^- permeability of rat proximal tubule *in vivo* to be 10.8×10^{-5} cm sec^{-1} [19]. Assuming the NaCl permeability coefficient in the *in situ* kidney can be applied to proximal tubules of kidney cortex slices, the rate of passive NaCl diffusion is 1.6 nEq cm^{-2} sec^{-1}. These calculations suggest that in the absence of bicarbonate, NaCl transport should be slowed to $(3.5-1.6)=1.9$ nEq cm^{-2} sec^{-1}. The observed fluxes under bicarbonate-free conditions, $1.6-1.8$ nEq cm^{-2} sec^{-1} are not far different from this predicted value. Thus, the concentration gradient theory of bicarbonate stimulation of NaCl reabsorption is at least consistent with the present experimental results.

The finding of Ullrich et al [10] that sulfamerazine and glycodiazine can substitute for bicarbonate in promoting NaCl reabsorption also favors the concentration gradient theory. Like bicarbonate, the protonated forms of these buffers are lipid soluble, and presumably cross cell membranes readily. Their sodium salts can therefore be reabsorbed by the same H^+ ion secretory mechanism which reabsorbs $NaHCO_3$, and comparable NaCl concentration gradients should develop. It is difficult to reconcile the stimulatory effects of these compounds with either the CO_2^- fixation or dual ion exchange mechanisms which we have outlined herein.

Passively diffusing NaCl probably moves between, rather than across, cells. Several lines of evidence suggest that the high ionic permeability of proximal tubular epithelium, which makes it possible for small concentration gradients to induce large salt fluxes, is a reflection of the properties of the intercellular regions rather than of the cell membranes: *1*) The electrical resistance between tubule lumen and peritubular fluid is actually lower than that between intracellular and peritubular fluid, a finding which suggests the existence of an intercellular shunt pathway [20, 21]. *2*) Electron microscopic studies in which lanthanum is used as a permeability marker indicate that this ion, although it is excluded from proximal tubular cells, is able to move from the tubule lumen into the intercellular peritubular spaces—presumably by traversing the tight junctions [22, 23].

Although passive diffusion makes a substantial contribution to proximal tubular NaCl reabsorption, over one-half of the total flux must be accounted for by other means. In the *in vitro* preparation used here, there was no protein or other solute present in the peritubular fluid which could have provided an effective osmotic pressure gradient favoring the bulk flow of salt solution out of the tubule; nor are other driving forces in evidence. It must be concluded then that the proximal tubule possesses a mechanism for active transport of NaCl.

Mechanisms of proximal tubular reabsorption of NaCl and water in vivo: A perspective on the role of $NaHCO_3$. These *in vitro* experiments suggest that $NaHCO_3$ transport plays an important role in the overall fluid reabsorptive processes of the *in situ* proximal tubule. Theoretical calculations based on available permeability data seem consistent with this view and are presented in detail in the appendix. Fig. 3 depicts the mechanisms of transcellular transport of NaCl, $NaHCO_3$ and water which may be operative in the proximal tubule of the living rat kidney [5]. Three modes of NaCl reabsorption are considered: *1*) passive diffusion down a concentration gradient created by active $NaHCO_3$ reabsorption; *2*) coupling to water flow induced by the transcellular protein oncotic pressure gradient (solvent drag); and *3*) active transport. In addition to its flow along a protein oncotic pressure gradient, water is assumed to be reabsorbed by co-diffusion with NaCl and by local osmotic coupling to actively transported sodium salts.

Under physiologic conditions NaCl can diffuse

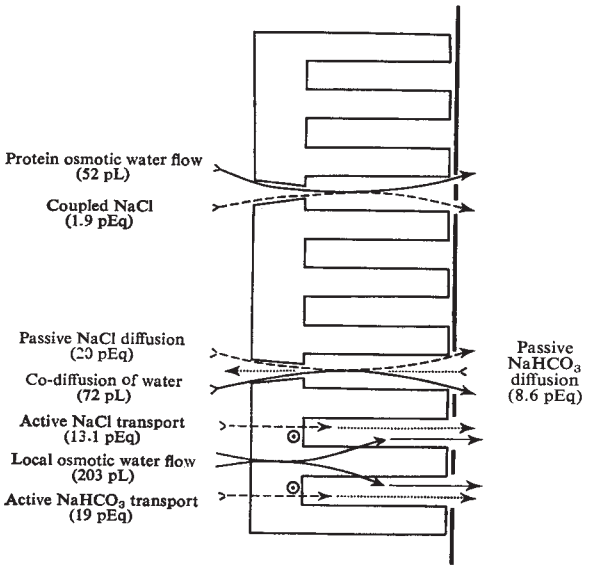

Fig. 3. *Modes of transport of $NaHCO_3$, NaCl and water across rat proximal tubular epithelium.* The numbers indicate fluxes per cm of tubule length per second. See text and appendix for details.

down a concentration gradient and out of the proximal tubule at a rate of about 20 pEq sec^{-1} per cm of tubule length. In addition, a small amount of NaCl leaves the tubule by solvent drag in association with the water being reabsorbed along the transtubular protein oncotic pressure gradient. The estimated contribution of this transport mode is 1.9 pEq cm^{-1} sec^{-1}. Passive driving forces can then account for an NaCl reabsorptive rate of 21.9 pEq cm^{-1} sec^{-1}. This is about two-thirds of the total NaCl transport of 35 pEq cm^{-1} sec^{-1}. It is to be assumed, therefore, that in the rat proximal tubule *in vivo* one-third of the NaCl is reabsorbed actively. These theoretical considerations are then roughly consistent with the present *in vitro* results indicating that the rate of NaCl reabsorption is approximately halved in the absence of $NaHCO_3$.

Passive diffusion of NaCl out of the tubule makes an important contribution to water reabsorption through the process of co-diffusion. Although the outward diffusion of NaCl is to some extent counterbalanced by the inward diffusion of $NaHCO_3$, there is a net diffusion of sodium salts from the tubule lumen to the peritubular space at a rate of about 21 pOsm cm^{-1} sec^{-1}. Assuming a tubular fluid osmolarity of 290 mOsm/liter, the corresponding water flow will be 72 pliter cm^{-1} sec^{-1}.

Co-diffusion together with protein oncotic water flow accounts for about one-third of the total water reabsorption in rat proximal tubule. Since there is no basis for postulating active water transport in proximal renal tubule [24, 25], the remaining water reabsorption must in some way be coupled to active solute transport. A likely means for accomplishing this is local osmosis, a special case of the Curran and MacIntosh double membrane system for the coupling of solvent to solute flow [26]. Diamond and Tormey have presented considerable evidence favoring local osmosis as a mechanism of isosmotic fluid transport in the gall bladder [27, 28]. An analogous system for the proximal renal tubule would center around the active transport of NaCl and $NaHCO_3$ from the tubule lumen into the basilar pericellular space. This space (the local osmotic compartment) is bounded by the relatively solute-impermeable basilar cell membrane and by the freely permeable tubular basement membrane. Because of the deep infoldings of the basilar cell membrane, the local osmotic space is quite extensive, reaching in some areas nearly to the apex of the cell. Once transported into the local osmotic compartment, the sodium salts exert a higher effective osmotic pressure across the cell membrane than across the basement membrane. Therefore, there will be a net flow of water into the local osmotic compartment from the tubule lumen. The resultant local increase in hydrostatic pressure forces water and dissolved solute preferentially across the freely permeable basement membrane and, ultimately, into the peritubular capillaries. In rat proximal tubule local osmosis will have the task of transporting 203 pliter cm^{-1} sec^{-1} of water in conjunction with the active transport of 19 pEq of $NaHCO_3$ and 13.1 pEq of NaCl.

In summary: In the rat proximal tubule *in situ*, active $NaHCO_3$ transport accounts both directly, and indirectly through the generation of NaCl concentration gradients, for about two-thirds of the total solute reabsorption. Active NaCl transport is responsible for the remainder. Together the active transport of these salts brings about water reabsorption by local osmosis. About one-third of the water reabsorption occurs independently of this mechanism: by flow along a protein oncotic pressure gradient, and by co-diffusion with NaCl.

The role of carbonic anhydrase in proximal tubular reabsorption of NaCl and water. Recent micropuncture studies have shown that carbonic anhydrase inhibitors slow NaCl reabsorption in rat proximal tubule [29, 30]. An increase in tubular fluid bicarbonate concentration and a fall in intratubular chloride concentration accompany this reduction in transport rate. The present *in vitro* studies also demonstrate a fall in the rate of salt transport during carbonic anhydrase inhibition, though an associated decline in intratubular chloride could not be demonstrated. The reduced reabsorptive rate in the *in vivo* studies may be

due, at least in part, to the decrease in the concentration gradient favoring passive outward diffusion of NaCl. This facile explanation cannot apply to the present *in vitro* studies. If confirmed, the failure of intratubular chloride concentration to fall will suggest that acetazolamide has a direct effect on NaCl transport. The possibility of a direct action of carbonic anhydrase inhibitors on chloride transport has previously been suggested on the basis of observations on turtle bladder [31] and rabbit cornea [32].

The present studies unfortunately do not add to our understanding of how carbonic anhydrase may be incorporated into proximal tubular transport systems for $NaHCO_3$ or NaCl. The inhibitor is active whether added to the bathing medium or to the tubular perfusate. The higher concentrations required in the latter situation do not rule out an enzyme site along the luminal membrane, for in this preparation acetazolamide is likely to diffuse rapidly out of the tubule into the inhibitor-free bathing medium. The ability of intraluminal carbonic anhydrase to reverse the inhibition might be taken as evidence, in agreement with histochemical findings [33], that the luminal membrane is the site of normal enzyme activity in the proximal tubule. However, the enzyme, when added to the perfusate, might simply be operating to remove inhibitor from an intracellular binding site.

Appendix[1]

1. *Rate of NaCl diffusion from tubule lumen to peritubular space.* The *in vivo* tubular fluid: plasma ultrafiltrate NaCl concentration ratio, C_L/C_P, is 1.23 and $\bar{C}$ is 121 mEq/liter [34]. P_{NaCl} obtained from ionic conductance data is 7.9×10^{-7} cm^2 sec^{-1} [19]. Insertion of these numbers into equation 1 yields a value for J_{NaCl} of 20 pEq cm^{-1} sec^{-1}.

2. *Protein oncotic water flow and NaCl reabsorption by solvent drag.* Calculation based upon an average peritubular capillary protein oncotic pressure of 30 mm Hg [2] and a proximal tubular hydraulic conductivity of 1.72 pliter cm^{-1} sec^{-1} mm Hg^{-1} [35] yields a value of 52 liter cm^{-1} sec^{-1} for protein oncotic water flow, $J_V^{oncotic}$. Net NaCl transfer produced by this water flow is given by the following:

$$J_{NaCl}^{solvent\ drag} = (1-\sigma)\,\bar{C}\,J_V^{oncotic}.$$

Taking the NaCl reflection coefficient, σ, as 0.7 [36] and $\bar{C}$ as 121 mEq/liter, NaCl transport by solvent drag is seen to equal 1.9 pEq cm^{-1} sec^{-1}.

3. *The net rate of solute diffusion and water transport by co-diffusion.* The net rate of transcellular solute diffusion is the algebraic sum of outward NaCl diffusion and inward $NaHCO_3$ diffusion. The latter process, as estimated using equation 1 with $P_{NaHCO_3} = 4.6 \times 10^{-7}$ cm^2 sec^{-1} [19] and $C_L/C_P = 8/25$ [8, 9], occurs at a rate of 8.6 pEq cm^{-1} sec^{-1}. The net rate of outward diffusion of sodium salts is then $(20-8.6) =$ 11.4 pEq cm^{-1} sec^{-1} or about 21 pOsm cm^{-1} sec^{-1}. The corresponding rate of water flow, assuming a tubular fluid osmolarity of 290 mOsm/liter is 72 pliter cm^{-1} sec^{-1}.

4. *NaCl and water reabsorption by rat proximal tubule in vivo.* On the basis of a single nephron GFR of 410 pliter sec^{-1} [37], 80% proximal reabsorption of the glomerular filtrate [38] and a tubule length of 1 cm, proximal tubular water reabsorption occurs at a rate of 328 pliter cm^{-1} sec^{-1}. Taking the glomerular filtrate (Cl^-) as 112 mEq/liter and end-proximal tubule (Cl^-) as 136 mEq/liter [34, 39], the overall rate of NaCl reabsorption is 35 pEq cm^{-1} sec^{-1}.

[1] In Fig. 3 and in the calculations which follow, flux and permeability data are normalized to tubule length rather than to the luminal surface area. This convention has been adopted in light of the observations of Ullrich et al on the *in situ* rat proximal tubule that the reabsorptive rates of fluid and Cl^- ions remain approximately constant per unit of tubule length in spite of wide variations in tubule diameter, and, hence, of the apparent luminal surface area [40]. Where necessary, translation from surface area to length units has been made on the basis of tubular diameter of 23.2 μ [41].

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