Bicarbonate and fluid absorption by renal proximal straight tubules

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Bicarbonate and fluid absorption by renal proximal straight tubules. Rabbit proximal straight tubules from superficial nephrons were perfused *in vitro* in order to elucidate the mechanism of fluid and bicarbonate absorption. Both processes were greatly inhibited when sodium was replaced in the perfusate and bath by other cations, when ouabain was added to the bath, or when potassium was removed from the bath. We infer that these experimental manipulations inhibit active sodium transport, and that active sodium transport is a primary process leading to fluid and bicarbonate absorption. Fluid absorption also decreased (but only by 22 to 36%) when bicarbonate was replaced by chloride in the perfusate and bath or when acetazolamide (10^{-3}M) was added, suggesting that fluid and sodium transport depend in part on bicarbonate. We infer that the links between fluid, sodium, and bicarbonate transport are complex and involve at least two mechanisms: *1)* a sodium for hydrogen ion exchange mechanism located in the brush border membrane and *2)* the transepithelial concentration difference for bicarbonate, which results from its absorption and which acts as an additional driving force for fluid and sodium absorption. Finally, bicarbonate absorption was unaltered when chloride was replaced by nitrate in the perfusate and bath, suggesting that chloride is not necessary for acidification in this nephron segment.

Réabsorption de bicarbonate et de liquide par les tubes proximaux droits du rein. Des tubes proximaux droits de néphrons superficiels de lapins ont été perfusés in vitro afin d'étudier le mécanisme de la réabsorption de liquide et de bicarbonate. Ces deux processus sont fortement inhibés quand le sodium est remplacé, dans le perfusat et le bain, par d'autres cations, quand de l'ouabaïne est ajoutée au bain ou quand le potassium est supprimé du bain. Nous en concluons que ces manipulations expérimentales inhibent le transport actif de sodium et que le transfert actif de sodium est le processus primaire conduisant à la réabsorption de liquide et de bicarbonate. La réabsorption de liquide diminue aussi (mais seulement de 22 à 26%) quand le bicarbonate est remplacé par du chlore dans le perfusat et le bain ou quand l'acétazolamide (10^{-3}M) est ajouté, ce qui suggère que le transport d'eau et de sodium dépend en partie du bicarbonate. Nous en concluons que les liens entre les transports de liquide, de sodium et de bicarbonate sont complexes et impliquent au moins deux mécanismes: *1)* un mécanisme d'échange sodium—hydrogène dans la bordure en brosse de la membrane et *2)* la différence de concentration transépithéliale de bicarbonate qui résulte de sa réabsorption et qui agit comme une force supplémentaire pour la réabsorption de liquide et de sodium. Enfin, la réabsorption de bicarbonate n'est pas modifiée quand le chlore est remplacé par du nitrate dans le perfusat et le bain, ce qui suggère que le chlore n'est pas nécessaire à l'acidification dans ce segment du néphron.

The mechanism of fluid absorption by proximal straight tubules was previously studied directly by dissecting them and perfusing them *in vitro* [1–3]. Although the results differed in detail, depending on the composition of the fluids used and the anatomical origin of the tubules, i.e., whether they were derived from the superficial or juxtamedullary nephrons [2], the general conclusion was that active sodium transport results in fluid absorption and a voltage oriented negative in the lumen.

Isolated perfused proximal straight tubules also reabsorb bicarbonate, as initially inferred from changes in chloride concentration [1] and later directly confirmed by measurements of total carbon dioxide content [4].

Investigators have identified a number of possible interactions between the transport of fluid, sodium, and bicarbonate in renal tubules. Smith [5] and Pitts and Lotspeich [6] proposed that in distal tubules sodium is reabsorbed in exchange for hydrogen ions. Berliner [7] suggested that this mechanism was operative in the proximal nephron as well. Since hydrogen ions secreted into the tubule lumen react with bicarbonate to cause its reabsorption [8], the existence of this mechanism implies a link between sodium and bicarbonate transport. One purpose of the present studies was to test for such a link in proximal straight tubules from superficial nephrons. Another interaction was identified by Schafer, Patlak, and Andreoli [9] who noted that even when active sodium transport was completely inhibited in proximal straight tubules from superficial nephrons, reabsorption of fluid (and sodium) still occurred, provided that the concentration of bicarbonate was made higher in the bath than in the perfusate and the concentration of chloride was made reciprocally lower in the bath than in the perfusate. Following an earlier suggestion by Rector et al [10], they ascribed the fluid absorption under these conditions to a difference in reflection coefficients between bicarbonate

and chloride, the former being higher. Similar concentration differences, when they occur *in vivo*, might contribute to the fluid and sodium absorption that normally occurs in this segment.

Additional evidence for interaction of fluid, sodium, and bicarbonate transport derives from studies of the action of acetazolamide, a carbonic anhydrase inhibitor that blocks hydrogen ion secretion. Acetazolamide caused the rate of fluid absorption to decrease in proximal straight tubules from superficial nephrons [11], which is consistent with either of the mechanisms described above. On the other hand, removal of bicarbonate from the perfusate and bath did not significantly alter the rate of fluid absorption [1], which is not consistent with an important link between bicarbonate and sodium transport. A further purpose of the present studies was to elucidate in general the interrelationships between fluid, sodium, and bicarbonate transport in proximal straight tubules from superficial nephrons.

Methods

The method of perfusion of isolated segments of rabbit nephrons used in these studies has been previously described [12–14] and is briefly summarized below. Young (1 to 2 kg), female, New Zealand white rabbits were killed by decapitation. Kidney slices were prepared and were placed in "control perfusate" (described in the following). Then, a proximal straight tubule from a superficial nephron was dissected and perfused using concentric glass pipets [15]. The mean tubule length was 3.5 mm.

The rate of fluid absorption (J_v) was measured, as previously described [13], using ^{14}C-inulin as a volume marker:

$$J_v = V_L [(C_L/C_O) - 1]/L,$$

where V_L is the rate of collection of tubule fluid, L is the length of the tubule, and C_L and C_O are the concentrations of ^{14}C in the collected and perfused fluid, respectively. The rate of perfusion, V_O, was calculated as $V_O = (C_L/C_O)V_L$.

Total carbon dioxide content of the perfusate and collected fluid was measured by the microcalorimetric method of Vurek, Warnock, and Corsey [16]. Although total carbon dioxide measured by this method includes dissolved carbon dioxide, carbonates, and carbonic acid in addition to bicarbonate, the latter predominates at the pH encountered in these studies (i.e., at pH 7.4, 95% of the total carbon dioxide is bicarbonate). Therefore, for the present purposes, bicarbonate and total carbon dioxide will be considered interchangeably, neglecting the small error introduced by this approximation.

The rate of total carbon dioxide absorption (J_{CO_2}) was calculated as:

$$J_{CO_2} = (V_O C_O - V_L C_L)/L,$$

where C_O and C_L are the concentrations of total carbon dioxide in the perfused and collected fluids, respectively.

After the tubule had been perfused for 20 to 30 min at 37°C with the control solutions, measurements were begun. Generally two or three control samples were collected over a period of approximately 15 min. Then, experimental solutions and in some cases additional control solutions were substituted; after 10 min, the collections were repeated (at the same perfusion rate). The mean total carbon dioxide content, J_{CO_2}, and/or J_v was calculated for each set of collections. In those experiments in which control measurements were made both at the beginning and end of the experiment, their mean was used for statistical evaluation. The summary data is presented as the mean of the results in the individual tubules ± SEM (N = number of tubules). Statistical significance of differences was determined by Student's paired t test.

Transepithelial voltage was measured, as previously described [13], correcting for the liquid junction potential when the concentrations of chloride and bicarbonate were different in the perfusate and bath [13]. The control perfusate contained the following in mM: sodium chloride, 114.0; sodium bicarbonate, 25; magnesium sulfate, 1.2, calcium chloride, 2.0; dibasic potassium phosphate, 2.5; glucose, 5.5; *l*-alanine, 6.0, sodium lactate, 4.0; and sodium citrate, 1.0. The control bath was identical except that: *1)* 6 g/dl of defatted bovine serum albumin was added [17], *2)* calcium chloride was 3.0 mM and *3)* sodium chloride concentration was adjusted so that the osmolality of the perfusate and bath were equal. In order to substitute other ions for sodium or chloride, solutions that were otherwise identical were prepared which contained nitrate in place of chloride, and lithium, choline, or tetramethyl ammonium (TMA) in place of sodium [18]. Solutions containing bicarbonate were gassed with 95% oxygen/5% carbon dioxide to pH 7.4. Solutions containing no bicarbonate were gassed with 100% oxygen and adjusted to a pH of 7.4 with 0.15 N hydrochloric acid.

Results

Fluid absorption and voltage

Control measurements. The average rate of fluid absorption with the control solutions was 0.36 ± 0.02 (43) nl $mm^{-1}min^{-1}$, and the average transepithelial voltage was −1.4 ± 0.2 (34) mV (lumen negative).

Table 1. Fluid absorption by proximal straight tubules

| Experiment | Rate of fluid absorption $nl\,mm^{-1}\,min^{-1}$ | | | Number of tubules | Perfusion rate $nl\,min^{-1}$ |
	Control	Experimental	Change		
No sodium[a]	0.40	0.01	-0.39 ± 0.04^f	4	4.3
40 mM sodium[a]	0.37	0.18	-0.19 ± 0.04^f	4	5.4
No potassium in bath[b]	0.28	-0.05	-0.33 ± 0.05^f	4	10.3
No bicarbonate in perfusate or bath[c]	0.41	0.33	-0.08 ± 0.02^f	12	9.3
No bicarbonate in perfusate[c]	0.41	0.44	0.03 ± 0.02	12	9.1
Acetazolamide, 10^{-5}M[d]	0.39	0.30	-0.09 ± 0.04	7	8.6
Acetazolamide, 10^{-3}M[d]	0.39	0.25	-0.14 ± 0.04^f	7	8.6
Acetazolamide, 10^{-4}M[e]	0.36	0.25	-0.11 ± 0.02^f	6	10.6

[a] Sodium replaced by choline in perfusate and bath.
[b] Potassium replaced by sodium.
[c] Bicarbonate replaced by chloride.
[d] 25 mM bicarbonate in perfusate and bath.
[e] No bicarbonate in perfusate and bath.
[f] $P < 0.05$.

Both these results are in agreement with previous studies [1, 2, 12].

Effect of removing potassium from the bath. When potassium was replaced in the bath by sodium, the rate of fluid absorption and the voltage fell close to zero (Tables 1 and 2). Since potassium is required for operation of the sodium and potassium-activated adenosine triphosphatase (ATPase) that is involved in active sodium transport [19], we presume that active sodium transport is inhibited in the absence of potassium, accounting for the decrease in fluid absorption. Ouabain, which is an inhibitor of the sodium and potassium activated ATPase, was previously reported to have the same effect [1–3].

Effect of replacing sodium with choline. When sodium was replaced totally or in part in the perfusate and bath by choline, the rate of fluid absorption decreased to zero (Table 1). This result is also consistent with previous conclusions that fluid absorption is secondary to active sodium transport [1].

Effect of bicarbonate. When bicarbonate was replaced in both the perfusate and bath by chloride, the rate of fluid absorption decreased by approximately 22% (Table 1). Schafer, Troutman, and Andreoli [1] previously reported that removal of bicarbonate had no effect, but they might conceivably have missed an effect as small as that observed here since they reported paired studies only in four tubules. When bicarbonate was replaced by chloride in the perfusate, but not in the bath, there was no significant change in the rate of fluid absorption (Table 1).

The voltage did not change significantly when bicarbonate was removed from the perfusate and bath, but decreased (becoming positive by 0.7 mV) when bicarbonate was removed only from the perfusate (Table 2), confirming previous reports [1, 4].

Effect of acetazolamide in the presence of bicarbonate. The rate of fluid absorption decreased by 36% when 10^{-3}M acetazolamide was added to the perfusate and bath (Table 1). With 10^{-5}M acetazolamide, the change was smaller and of borderline significance ($P = 0.06$). These results are in agreement with those

Table 2. Transepithelial voltage across proximal straight tubules

| Experiment | Voltage, mV | | | No. of tubules |
	Control	Experimental	Change	
No potassium in bath[a]	-1.6	0.1	1.6 ± 0.3^d	12
No bicarbonate in perfusate and bath[b]	-1.0	-0.7	0.3 ± 0.1	12
No bicarbonate in perfusate[b]	-1.0	0.7	1.7 ± 0.3^d	12
No chloride[c]	-1.5	-1.5	0.0 ± 0.2	4
Acetazolamide, 10^{-5}M	-1.9	-1.8	0.1 ± 0.1	9
Acetazolamide, 10^{-3}M	-1.6	-1.6	0.0 ± 0.1	7

[a] Potassium replaced by sodium.
[b] Bicarbonate replaced by chloride.
[c] Chloride replaced by nitrate.
[d] $P < 0.05$.

of Schafer and Andreoli [11]. The voltage did not change with either concentration of the drug (Table 2).

Effect of acetazolamide in the absence of bicarbonate. Radtke et al [20] reported that concentrations of acetazolamide of 10^{-4}M or higher inhibited fluid absorption in rat proximal convoluted tubules in the absence of bicarbonate and concluded that the effect most likely was due to another action of the drug besides its well known ability to inhibit carbonic anhydrase. In contrast, Schafer and Andreoli [11] did not find any effect of 0.22×10^{-3}M acetazolamide on fluid absorption by rabbit proximal straight tubules in the absence of bicarbonate. In the present experiments, 10^{-4}M acetazolamide was tested with no bicarbonate or carbon dioxide in the bath or perfusate. Under these conditions, the drug caused the rate of fluid absorption to decrease by 30% (Table 1). The difference between the present results and those of Schaffer and Andreoli is unexplained.

Bicarbonate absorption

Control measurements. Tubules were perfused with 25 mM bicarbonate in the bath and 0 or 25 mM alternatively in the perfusate (Fig. 1). The average collection rate was 0.5 nl mm^{-1} tubule length min^{-1}. The mean total carbon dioxide content of the collected fluid was 6.9 ± 1.5 (8) mM/liter with 0 mM in the perfusate and 9.2 ± 1.5 (8) mM/liter with 25 mM bicarbonate in the perfusate. Although these results are significantly different ($P < 0.05$), the mean paired difference was only 2.2 ± 0.7 mM/liter, indicating
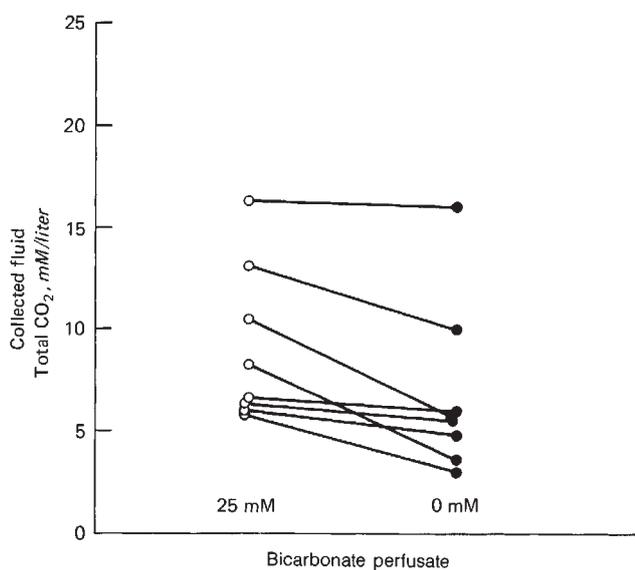

Fig. 1. *Effect of perfusate bicarbonate concentration on concentration of total carbon dioxide in fluid collected from proximal straight tubules perfused at slow rates with 25 mM bicarbonate in the bath.*

that a steady state concentration was approached at these slow rates of perfusion. In many of the studies that follow, the tubules were perfused at similar slow rates with 25 mM bicarbonate in the perfusate and bath, and the near steady state total carbon dioxide concentration in the collected fluid was measured. Under these conditions, inhibition of bicarbonate transport should result in an increase in total carbon dioxide concentration.

The rate of bicarbonate absorption was measured in other experiments at faster rates of perfusion.[a] The concentration of bicarbonate was 25 mM in the perfusate and bath; the mean collection rate was 4.3 ± 0.6 nl min^{-1}. The mean concentration of bicarbonate in the collected fluid was 16.6 ± 1.0 mM and the rate of bicarbonate absorption was 3.3 ± 0.4 (10) pEq cm^{-1} of tubule length s^{-1}, similar to that found previously [4].

Effect of chloride replacement by nitrate. It was suggested previously [21, 22] that bicarbonate might be absorbed in proximal convoluted tubules in part secondary to reaction with hydrochloric acid secreted into the tubule lumen. In order to test whether chloride is required for bicarbonate absorption in proximal straight tubules from superficial nephrons, the tubules were perfused at slow rates with the control solutions and then with solutions in which chloride was replaced by nitrate in both the perfusate and bath. There was no difference in the near steady state concentration of total carbon dioxide in the collected fluid when chloride was eliminated (Fig. 2 and Table 3). Thus, there is no evidence in these experiments that removal of chloride affected bicarbonate transport or that hydrochloric acid secretion is important for bicarbonate reabsorption in proximal straight tubules, as had been previously proposed for proximal convoluted tubules.

Effect of removing sodium from the bath and perfusate. As was shown earlier, fluid (and presumably sodium) absorption was greater in the presence of bicarbonate than in its absence. In order to test whether the converse is also true, i.e. whether bicarbonate absorption is greater in the presence of sodium than in its absence, sodium in the perfusate

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[a] The *rate* of bicarbonate absorption cannot be determined from the near steady state measurements. Ideally, the *rate* should be measured at perfusion rates great enough to prevent a significant decrease in concentration of total carbon dioxide in the lumen, but then accurate measurements would be impossible. As a compromise, we chose a rate of perfusion slow enough to measure accurately the decrease in total carbon dioxide concentration that occurred, but fast enough to prevent reaching a steady state. We recognize that the rate of bicarbonate absorption could be greater (theoretically) at faster perfusion rates which lead to less change in concentration.
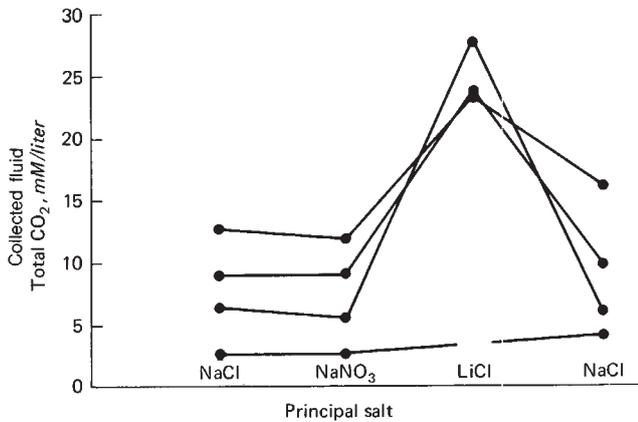
Fig. 2. *Effect of anion and cation replacement in the perfusate and bath on total carbon dioxide content in fluid collected from proximal straight tubules perfused at slow rates with 25 mM bicarbonate in the perfusate and bath.*
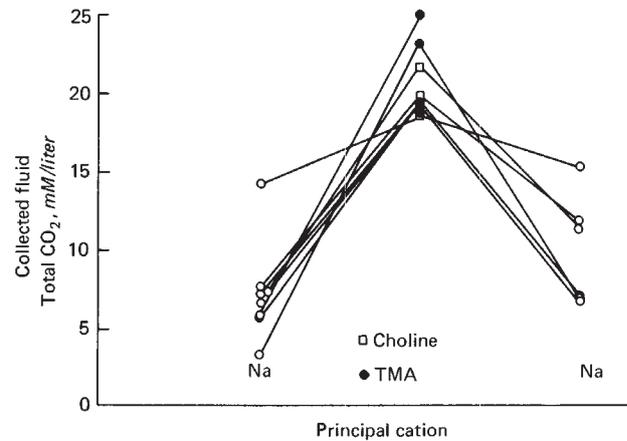

Fig. 3. *Effect of cation replacement in the perfusate and bath on total carbon dioxide content of fluid collected from proximal straight tubules perfused at slow rates with 25 mM bicarbonate in the perfusate and bath.*

and bath was replaced by lithium, TMA, or choline. When at slow flow rates, sodium was replaced by these cations, the near steady state total carbon dioxide content of the collected fluid was higher. The individual experiments are shown in Figures 2 and 3 and the mean results in Table 3. As shown in the figures, the effect was reversible. Although higher in the absence of sodium, the mean total carbon dioxide content in the collected fluid (21.8 ± 0.9 (11) mM, Table 3) was significantly less ($P < 0.05$) than in the perfusate and bath (26.2 mM), indicating that some bicarbonate absorption continued under these conditions. Evidently, the absorption of bicarbonate is strongly dependent on sodium, but not completely so.

The purpose of the following experiments was further to elucidate the relationship between transport of bicarbonate and sodium. The rate of fluid absorption was measured as an estimate of sodium transport since they are directly related in proximal straight

tubules [1]. The rates of fluid and bicarbonate absorption were measured first under control conditions and then when the sodium in the perfusate and bath was replaced entirely or in part (40 mM sodium remaining) by choline. When the sodium concentration was changed, the rates of fluid and bicarbonate absorption changed in a correlated fashion (Fig. 4), consistent with a link between sodium and bicarbonate transport.

Effect of ouabain. When at slow flow rates 10^{-5}M ouabain was added to the bath, the near steady state total carbon dioxide content of collected fluid was markedly higher (Fig. 5 and Table 3), but still lower (18.7 ± 0.8 (5) mM) than that in the perfusate and bath (26.2 mM, $P < 0.01$). Therefore, bicarbonate transport was inhibited substantially but not completely. Evidently the inhibition of fluid and sodium absorption caused by ouabain is accompanied by inhibition of bicarbonate absorption.

Effect of removing potassium from the bath. When

Table 3. Total carbon dioxide content of fluid collected from proximal straight tubules perfused at slow rates with 25 mM bicarbonate (26.2 mM total carbon dioxide) in the perfusate and bath

Experiment	Total CO_2 in collected fluid, mM			No. of tubules	Collection rate $nl\,mm^{-1}min^{-1}$
	Control	Experimental	Change		
No chloride[a]	8.6	7.3 ± 2.0	− 1.2 ± 0.5	4	0.53
No sodium[b]	8.7	21.8 ± 0.9	13.1 ± 1.5[d]	11	0.52
Ouabain	6.9	18.7 ± 0.8	11.8 ± 1.0[d]	5	0.37
No potassium in bath[c]	6.6	18.9 ± 1.0	12.3 ± 1.0[d]	5	0.43
Acetazolamide, 10^{-5}M	8.2	23.2 ± 1.4	15.0 ± 1.0[d]	4	0.55
Acetazolamide, 10^{-4}M	7.8	32.3 ± 0.6	24.4 ± 0.5[d]	5	0.55

[a] Chloride replaced by nitrate.
[b] Sodium replaced by choline (3 experiments), tetramethylammonium (4 experiments), or lithium (3 experiments).
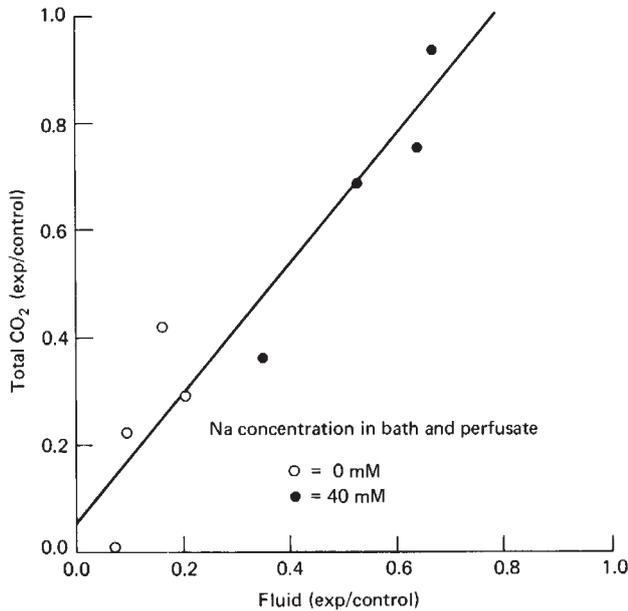[c] Potassium replaced by sodium.
[d] $P < 0.05$.

Fig. 4. *Effect of sodium concentration in the perfusate and bath (sodium replaced by choline) on rates of total carbon dioxide and fluid absorption by proximal straight tubules.* The data are presented as the ratio of the experimental rates (reduced sodium concentration) divided by the control rates (normal sodium concentration). The line is the best least squares fit, $Y = 0.057 + 1.19X$, $r = 0.94$.

potassium was removed from the bath, the total carbon dioxide content of the collected fluid was also higher (Fig. 5 and Table 3), indicating inhibition of bicarbonate transport. The inhibition was not complete, however, since the total carbon dioxide content of the collected fluid remained less (18.9 ± 1.0 (5) mM) than in the perfusate and bath (26.2 mM, $P < 0.01$).

Effect of acetazolamide. When 10^{-5}M acetazolamide was added to the perfusate and bath, the total carbon dioxide content of the collected fluid was not significantly different ($P > 0.25$) from that in the
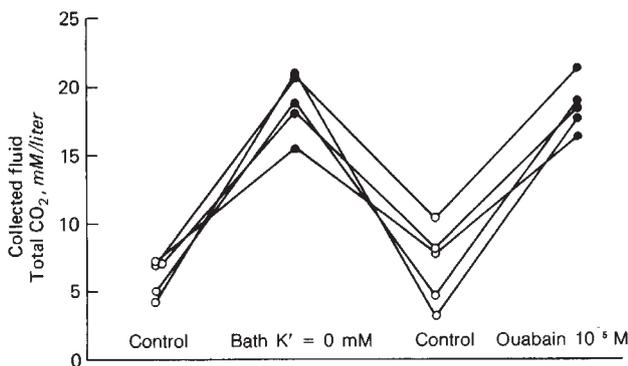
perfusate and bath (Fig. 6 and Table 3), consistent with inhibition of bicarbonate transport. When 10^{-4}M acetazolamide was added (Fig. 6), the mean total carbon dioxide content of the collected fluid was 32.3 ± 0.6 (5) mM, which is significantly greater than that in the bath or perfusate ($P < 0.001$). The inulin concentration in the collected fluid increased to approximately the same degree as did the bicarbonate concentration. The ratio of collected/perfused concentration was 1.36 ± 0.05 for inulin compared to 1.30 ± 0.03 for total carbon dioxide. These values do not differ significantly from each other (mean difference, 0.06 ± 0.02 (4) $P > 0.05$), but are both significantly greater than 1, $P < 0.001$. Therefore, 10^{-4}M acetazolamide virtually completely inhibited bicarbonate absorption but did not completely inhibit the fluid absorption.

Discussion

Fluid Absorption. It was proposed previously that active sodium transport is the primary process leading to fluid absorption and development of the voltage in proximal straight tubules from superficial nephrons. The present studies in which removal of sodium from the perfusate and bath or removal of potassium from the bath inhibited fluid absorption provide additional support for this concept. Similar results were found previously in proximal convoluted tubules [18].

Although sodium transport may be the primary process needed for fluid absorption, bicarbonate transport also has an important role, linked to sodium and fluid transport. Thus, removal of bicarbo-
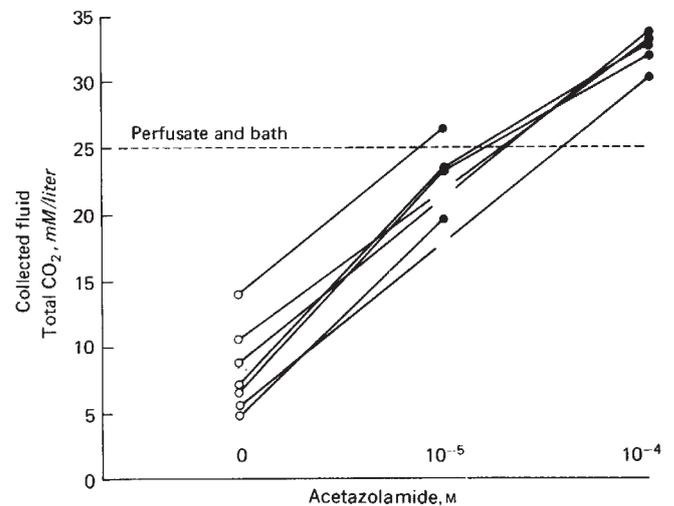

Fig. 5. *Effect on the concentration of total carbon dioxide in the collected fluid of removing potassium from or adding ouabain, 10^{-5}M, to the bath surrounding proximal straight tubules perfused at slow rates with 25 mM bicarbonate in the perfusate and bath.*

Fig. 6. *Effect of acetazolamide in the perfusate and bath on the concentration of total carbon dioxide in the fluid collected from superficial proximal straight tubules perfused at slow rates with 25 mM bicarbonate in the perfusate and bath.*

nate from the perfusate and bath or addition of acetazolamide, which inhibits bicarbonate transport, led to partial inhibition of fluid (and presumably sodium) absorption. There are at least two plausible explanations for this inhibition. First, there may be a mechanism coupling secretion of hydrogen ion to reabsorption of sodium, as reviewed in the introduction. Second, the transepithelial concentration differences for bicarbonate and chloride that result from sodium bicarbonate and fluid absorption may cause additional absorption of salt and water, as proposed by Schafer et al [9]. Both mechanisms are inhibited when there is no bicarbonate or when its transport is prevented by acetazolamide, providing an explanation for the resultant decrease in fluid and salt absorption. In contrast, omitting bicarbonate from the perfusate, but retaining it in the bath did not inhibit fluid absorption. We presume that omitting bicarbonate only from the perfusate inhibited the sodium for hydrogen ion exchange but enhanced the effect of bicarbonate and chloride concentration differences. Therefore, the rate of fluid absorption did not change (Table 1), because the inhibition of one process counterbalanced the enhancement of the other.

Although, as just discussed, fluid absorption may be enhanced by concentration differences for bicarbonate and chloride across the epithelium, the fluid absorption continued even when these concentration differences were reversed. Thus, when $10^{-4}M$ acetazolamide virtually completely inhibited bicarbonate absorption, the bicarbonate concentration difference was opposite from the normal, yet substantial fluid absorption continued.

We conclude that active sodium transport is the primary process causing fluid absorption and that the transport of bicarbonate is both linked to sodium absorption and contributes to it in a complex fashion.

Effect of acetazolamide on bicarbonate absorption. There is considerable evidence that bicarbonate reabsorption is driven by hydrogen ion secretion [8]. The hydrogen ions are presumed to originate in the tubule cells as a result of reactions dependent on carbonic anhydrase. Theoretically, in the absence of carbonic anhydrase, the supply of hydrogen ions is severely limited, and bicarbonate reabsorption should virtually cease [8]. Acetazolamide is a potent inhibitor of carbonic anhydrase, yet high concentrations of the drug (or similar agents), which appear to inhibit completely the enzyme in renal tissue [23], reduce bicarbonate reabsorption *in vivo* by only 25 to 50% [8, 23]. A number of theories were proposed to explain the discrepancy [8], and the subject remains controversial [23, 24]. In the present studies, acetazola-

mide virtually completely inhibited bicarbonate reabsorption, which differs from the previous results *in vivo* in which there was only partial inhibition. We are unable to explain the divergent results, but wish to emphasize that in proximal straight tubules of superficial nephrons from rabbits, at least, transport of bicarbonate is completely eliminated by this inhibitor of carbonic anhydrase.[b]

Is there sodium-hydrogen ion exchange? This process had been proposed to occur in renal tubules, as noted in the introduction, but until recently there was little direct evidence bearing on the point. Then, Murer, Hopfer, and Kinne [25] identified a sodium-hydrogen ion exchange mechanism in vesicles prepared from brush borders of proximal tubules. Since their starting material was rat renal cortex which includes both convoluted and straight proximal tubules, it is unclear which of the tubule segments contained the exchange activity or whether both did. The results of the present studies support the concept that there is a sodium for hydrogen ion exchange mechanism in rabbit proximal straight tubules from superficial nephrons. The evidence necessarily is indirect. We

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[b] In a paper published subsequent to the submission of the present studies, Schafer and Andreoli [11] concluded that acetazolamide did not completely inhibit bicarbonate absorption in rabbit proximal straight tubules from superficial nephrons. They did not measure bicarbonate, but argued from measurements of chloride concentration and fluid absorption. The bath solution that they used contained (in mM) sodium chloride, 105; sodium bicarbonate, 25; sodium acetate, 10; dibasic sodium phosphate/monobasic sodium phosphate, 4; potassium chloride, 5; calcium chloride, 1.8; magnesium sulfate, 1.0; glucose, 8.3; alanine, 5.0; acetazolamide, 0.22; and albumin, 6 g/dl. The perfusate was identical except that sodium isethionate replaced sodium acetate, urea replaced glucose and alanine, and there was no albumin. Under these conditions the mean results were $V_O = 10.6$ nl/min, $V_L = 9.97$, $[Cl]_O = 117.8$ mM, $[Cl]_L = 119.7$. Assuming that only sodium chloride and sodium bicarbonate were absorbed and that the sodium concentration in the lumen did not change, they calculated that one-third of the net sodium flux was accompanied by bicarbonate. By contrast, in the present studies (using somewhat different solutions and measuring bicarbonate directly) little if any bicarbonate was absorbed in the presence of acetazolamide. We question whether Schafer and Andreoli actually estimated bicarbonate flux with sufficient accuracy to justify convincingly their conclusion, in view of the indirect nature of their measurements and the number of assumptions involved. For example, they observed that $[Cl]_L$ was 119.7 mM, leading to the conclusion that there was substantial bicarbonate absorption. It is easily calculated from their experimental conditons and assumptions, however, that for *zero* bicarbonate absorption $[Cl]_L$ is 116.3, which differs by less than 3% from their observed result. An additional problem with their approach is that solute with an aggregate concentration of approximately 205 mM was not measured in their experiment. For their analysis, they assumed that the concentration of the unmeasured solutes remained absolutely constant. If, in fact, the concentration of unmeasured solutes decreased by only 3.38 mM (which is less than 2%), the bicarbonate concentration could have increased by 1.5 mM and bicarbonate absorption could have been zero, even accepting the measured concentrations of chloride as absolutely accurate.

assume that sodium absorption is directly proportional to fluid absorption, as demonstrated by Schafer et al [1] and that hydrogen ion secretion is directly proportional to bicarbonate absorption according to current concepts of urinary acidification [8]. Accepting these assumptions, the proposed exchange mechanism provides a plausible explanation for the following observations: *1)* Omission of bicarbonate from the perfusate and bath caused fluid absorption to decrease. *2)* Acetazolamide caused both bicarbonate and fluid absorption to decrease. *3)* Omission of sodium from the perfusate and bath caused both bicarbonate and fluid absorption to decrease. *4)* Omission of potassium from or addition of ouabain to the bath caused both bicarbonate and fluid [1, 3] absorption to decrease.

The sodium for hydrogen ion exchange mechanism is presumed to exist at the luminal brush border membrane. At this location it could serve a dual function. First, it could facilitate the entry of sodium into the tubule cells from the lumen down the sodium activity gradient. The sodium that enters the cells presumably is then pumped out at the basolateral border. (The sodium pump in this location is sensitive to potassium and ouabain, explaining their action on sodium and hydrogen ion transport.) Second, the exchange mechanism could be responsible for the transport of hydrogen ions against their concentration gradient from the tubule cells into the lumen. Coupling between sodium and hydrogen ions via this mechanism could drive the uphill transport of hydrogen ions using energy derived from the downhill transport of sodium.

Acknowledgments

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