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A Microperfusion Study of Bicarbonate Accumulation in the Proximal Tubule of the Rat Kidney *

NORMAN BANK † AND HAGOP S. AYNEDJIAN

(From the Department of Medicine, New York University School of Medicine, New York, N. Y.)

Summary. In order to determine whether HCO_3^- gains access to the proximal tubular lumen from a source other than the glomerular filtrate, we carried out microperfusion experiments on isolated segments of rat proximal tubules *in vivo*. The perfusion fluid was essentially free of HCO_3^- and of a composition that prevented net absorption of sodium and water.

It was found that when plasma HCO_3^- concentration and CO_2 tension (PCO_2) were normal, the HCO_3^- concentration in the collected perfusate rose to about 3 mEq per L. Inhibition of renal carbonic anhydrase did not produce an appreciable change in this value in normal rats, but when the enzyme was inhibited in acutely alkalotic rats, a mean concentration of 15 mEq per L was recovered in the perfusate. Addition of HCO_3^- to the tubular lumen might occur by either intraluminal generation of HCO_3^- from CO_2 and OH^- or by influx of ionic bicarbonate from the plasma or tubular cells. Because of the marked increase in HCO_3^- found when intraluminal carbonic anhydrase was inhibited, generation of new HCO_3^- from CO_2 and OH^- seems unlikely. We conclude, therefore, that influx of ionic bicarbonate occurred, either across the luminal membrane or through extracellular aqueous channels. These observations suggest that the proximal epithelium has a finite degree of permeability to HCO_3^- and that influx of this ion may be a component of the over-all handling of HCO_3^- by the kidney.

Introduction

Reabsorption of bicarbonate by the proximal and distal convoluted tubules of the rat kidney has been shown to be mediated by active secretion of H^+ by the lining epithelial cells (1). Bicarbonate ions in the tubular lumen react with secreted H^+ to form H_2CO_3, which then decomposes into CO_2 and H_2O. It has generally been assumed that all of the HCO_3^- taking part in the reactions in the tubular lumen is derived from the plasma by glomerular filtration. Recent studies have demonstrated, however, that the proximal epithelium of the rat kidney has a high degree of permeability to certain ions, allowing a significant influx of sodium (2, 3), chloride (2), and potassium (4) into the tubular lumen. The possibility must be considered, therefore, that HCO_3^- also enters the tubular lumen from a source other than the glomerular filtrate. If it does, the over-all processes of net reabsorption and excretion of HCO_3^- by the kidney might be subject to variables that have not previously been evaluated by clearance measurements.

In the experiments reported here, the question of addition of HCO_3^- to the tubular fluid distal to the glomerulus was studied in rats by perfusing isolated segments of cortical proximal tubules *in vivo* with a HCO_3^--free solution. Upon col-

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† Career Scientist of the Health Research Council of New York.

Address requests for reprints to Dr. Norman Bank, Dept. of Medicine, New York University School of Medicine, 550 First Ave., New York, N. Y. 10016.

lection of the perfusate, pH was measured with a quinhydrone microelectrode under conditions of known P_{CO_2}, and the HCO_3^- concentration calculated from the Henderson-Hasselbalch equation. It was found that when plasma HCO_3^- concentration and P_{CO_2} were normal, the concentration of HCO_3^- in the perfusate rose to about 3 mEq per L. Inhibition of carbonic anhydrase did not alter this value significantly, but when the enzyme was inhibited in acutely alkalotic rats, a mean HCO_3^- concentration of 15 mEq per L was found in the perfusate. On the basis of these observations we conclude that the bicarbonate found in the perfusate had entered the proximal lumen in the ionic form. Permeability of the proximal epithelium to HCO_3^- suggests that flux of this ion may be a component of the over-all handling of bicarbonate by the kidney.

Methods

Male white rats weighing 200 to 300 g were anesthetized with Inactin [sodium ethyl-(*l*-methylpropyl)-thiobarbiturate], tracheotomized, and placed on a heated animal table. The left external jugular vein was cannulated with polyethylene tubing for infusion of solutions and drug administration. The left kidney was exposed through a lateral abdominal incision, dissected free of perirenal fat tissue, and immobilized in a plastic cup packed with vaseline. The surface of the kidney was bathed in mineral oil flowing continuously from a Knisely quartz rod illuminator. Blood samples were obtained from the abdominal aorta at the beginning and end of most experiments or, in a few cases, only at the end of the experiment.

In two groups of animals, plasma HCO_3^- concentration was maintained within normal limits by intravenous infusion of the following solution at 0.1 ml per minute: NaCl, 125 mmoles per L; $NaHCO_3$, 25 mmoles per L. In the second group of rats, 2 mg per kg body weight of the carbonic anhydrase inhibitor, 2-benzenesulfonamide-1,3,4-thiadiazole-5-sulfonamide (CL 11,366),[1] was administered intravenously 15 minutes before microperfusion of the surface tubules was started. In this group, the drug was also added to the perfusion solution in a concentration of 0.01 mg per ml. In a third and fourth group of rats, plasma HCO_3^- concentration was elevated by intravenous infusion of 0.15 M $NaHCO_3$ at 0.1 to 0.2 ml per minute for 1 hour before microperfusion of the surface nephrons and sustained by continuous infusion throughout the experiment. In the fourth group of animals, CL 11,336 was administered as described above.

The surface proximal tubules were perfused by a micropipette mounted on a continuous microperfusion pump of the type described by Sonnenberg and Deetjen (5). The perfusion solution had the following composition: NaCl, 90 mmoles per L; mannitol, 110 mmoles per L; NaH_2PO_4, 1 mmole per L; lissamine green, 1 g per L; trace amounts of ^{14}C-labeled inulin.[2] It was equilibrated with 5% CO_2. The pH was between 5.1 and 5.3, small variations being due to differences among tanks of CO_2. The rate of injection was between 12 and 20 nl per minute, which is within the normal range of tubular fluid flow (6). Two different techniques were used to achieve isolation of the perfused segments, but the results were found to be the same with the two methods. In about half of the perfusions, a small amount of heavy castor oil colored with Sudan black was injected into the lumen of a proximal tubule with a collecting pipette and the direction of flow determined by the spontaneous movement of the oil. Several segments of the nephron were then completely filled with the oil and the pipette was withdrawn, leaving a hole in the tubule wall for escape of fluid coming from the glomerulus. The same pipette was next reinserted into the most distal segment of the oil-filled tubule. The perfusion pipette was then inserted in a more proximal portion of the oil column and the perfusion started. As the entering perfusion fluid split the oil column, negative pressure was applied to the collecting pipette until the perfusion fluid reached it. A slight readjustment of the pressure on the collecting pipette was then made so that both the proximal and distal oil blocks remained in a fixed position as the perfusion fluid flowed into the collecting pipette. The second technique was that described by Marsh and Frasier (7). The direction of tubular fluid flow was determined as above by injecting a small amount of castor oil from the collecting pipette. With this pipette still in position, the perfusing pipette was inserted into the next segment of the tubule, and perfusion was started. Oil was then injected proximal to the perfusion pipette to provide the proximal block; the collecting pipette was withdrawn. After a few minutes of perfusion, the collecting pipette was reinserted into the most distal segment of the perfused proximal tubule, oil was injected and allowed to flow distally a short distance, and collection was started by adjustment of the pressure on this pipette. With both methods of perfusion, constant attention to the pressure on the collecting pipette was necessary to maintain the two oil blocks in proper position.

The collected perfusate was immediately transferred to a siliconized glass cup freshly filled with mineral oil equilibrated with 5% CO_2. Approximately 5 minutes was allowed for the sample to equilibrate with the surrounding oil, after which time about 0.01 to 0.03 μl was aspirated into a quinhydrone microelectrode filled with the same CO_2-equilibrated mineral oil. The pH was measured at 37° C as previously described (8) and the concentration of HCO_3^- calculated from the pH and the P_{CO_2} by the Henderson-Hasselbalch equation with a pK of 6.1 and a CO_2 conversion factor of 0.0309 mmoles per mm Hg. The P_{CO_2} of the mineral oil and of the original

[1] The CL 11,366 was generously supplied by Wallace Labs., Cranbury, N. J.

[2] New England Nuclear Corp., Boston, Mass.

TABLE I

Simulated experiments on pH of perfusion fluid measured with glass and quinhydrone electrodes at 37° C

Added HCO_3^-	Glass electrode		Quinhydrone electrode	
	pH	Calculated HCO_3^-*	pH	Calculated HCO_3^-
mEq/L		*mEq/L*		*mEq/L*
0	5.18 ± 0.01 (9)†	0.17 ± 0.01	5.15 ± 0.05 (12)	0.17 ± 0.03
5.0	6.58 ± 0.01 (8)	4.8 ± 0.1	6.55 ± 0.06 (10)	4.4 ± 0.5
15.0	7.09 ± 0.01 (8)	15.4 ± 0.3	7.12 ± 0.06 (11)	16.5 ± 1.8

* The CO_2 tension (P_{CO_2}) of the solutions used in the calculation of HCO_3^- concentration was measured with an Instrumentation Laboratory P_{CO_2} electrode and was found to range between 44 and 51 mm Hg with different tanks of 5% CO_2.

† Data expressed as mean ± standard deviation. The numbers in parentheses are the number of measurements.

perfusion fluid was determined directly at 37° C with an Instrumentation Laboratory P_{CO_2} electrode, correction being made for the ambient barometric pressure.

The remainder of the sample was transferred to a constant bore capillary tube under microscopic visualization and the volume determined with an eyepiece micrometer, as described by Windhager and Giebisch (9). The sample was then delivered from the capillary tube into a dioxane-containing counting vial, and radioactivity was measured with a Nuclear Chicago liquid scintillation counter (Unilux). Radioactivity in the original perfusion solution was measured by the same method.

Blood pH was measured at 37° C with a capillary glass electrode and Metrohm pH meter, model E 322. Plasma CO_2 content was measured with a Natelson microgasometer, and P_{CO_2} and HCO_3^- concentration were calculated from the Henderson-Hasselbalch equation.

Results

In Table I are shown the results of simulated perfusion experiments. The perfusion fluid was delivered from the microperfusion pump into a glass cup filled with CO_2-equilibrated mineral oil and simultaneously aspirated in a collecting pipette. The fluid was then redelivered from the collecting pipette into the oil, allowed to equilibrate for 5 minutes, and finally taken up in a pipette containing a quinhydrone microelectrode. The entire time period closely approximated that required for perfusion of the tubules in the actual experiments. As can be seen, over the range of pH values and HCO_3^- concentrations studied, there was close agreement between the quinhydrone and glass electrode measurements. We conclude from these observations that the methods used to equilibrate the perfusate with CO_2 and determine its HCO_3^- concentration were accurate and reproducible.

The data obtained from 16 tubular perfusions in 7 normal rats are shown in Table II. The collected perfusate in all instances showed an increase in HCO_3^- concentration from about 0.2 mEq per L in the original solution to a mean of 2.7 mEq per L. The *in vitro* pH values shown in column 5 are probably slightly lower than was the pH *in vivo,* since the tension of CO_2 with which the samples were equilibrated was higher than most of the plasma P_{CO_2} values. This should not, however, affect the determination of HCO_3^- concentration (1). That there was no net water absorption during the perfusion period is indicated by the collected/injected inulin ratios

TABLE II

*Bicarbonate concentration in collected perfusate after passage through isolated segments of the proximal convoluted tubule**

Experiment no.	Plasma			Collected perfusate		
	pH	$[HCO_3^-]$	P_{CO_2}	pH	$[HCO_3^-]$	C/I_{In}†
		mEq/L	*mm Hg*		*mEq/L*	
1	7.36	21.5	39	6.58	4.0	0.97
				6.50	3.3	0.99
2	7.24	19.1	46	6.63	4.8	0.91
3	7.27	22.3	50	6.43	3.0	0.98
				6.77	6.7	0.98
4	7.41	20.3	33	6.01	1.2	0.98
				6.61	4.6	1.02
5	7.36	20.9	38	6.09	1.4	0.98
				5.98	1.1	1.01
	7.31	18.8	39	6.25	2.0	1.01
6	7.41	21.1	39	6.11	1.4	1.03
				6.07	1.3	0.99
				5.88	0.8	1.04
7	7.40	17.6	33	6.19	1.7	0.97
				6.35	2.4	1.04
	7.36	18.9	35	6.51	3.5	0.99
Mean ± SD				6.31 ± 0.26	2.7 ± 1.6	0.99 ± 0.03

* Bicarbonate concentrations of the perfusate were calculated from the Henderson-Hasselbalch equation, with the pH measured at a known P_{CO_2}.

† C/I_{In} = ratio of inulin concentration of collected perfusate to that of injected perfusion fluid.

TABLE III

*Effect of carbonic anhydrase inhibition on bicarbonate concentration of collected perfusate**

Experiment no.	Plasma			Collected perfusate		
	pH	$[HCO_3^-]$	P_{CO_2}	pH	$[HCO_3^-]$	C/I_{In}
		mEq/L	*mm Hg*		*mEq/L*	
8	7.26	23.9	55	6.53	3.8	1.02
9	7.24	19.1	46	6.63	4.8	0.99
10	7.30	19.8	41	6.61	4.1	1.00
				6.81	6.5	1.01
				6.87	7.5	1.00
	7.42	22.2	35	6.62	4.2	1.02
11	7.32	22.2	44	6.46	2.9	1.00
				6.30	2.0	0.96
				6.42	2.7	1.02
	7.48	25.5	35	6.51	3.3	0.99
12	7.21	20.1	52	6.64	4.4	1.01
				6.38	2.4	1.03
				6.56	3.7	0.99
				6.46	2.9	1.00
	7.46	23.0	33	6.69	4.9	1.00
13	7.35	22.5	42	6.42	2.7	0.96
				6.20	1.6	0.95
Mean ± SD				6.54 ± 0.14	3.8 ± 1.5	1.00 ± 0.07

* Carbonic anhydrase was inhibited by intravenous and intraluminal administration of 2-benzenesulfonamide-1,3,4-thiadiazole-5-sufonamide (CL 11,366).

(C/I_{In}) shown in the last column. These data also serve to rule out the possibility of contamination of the perfusate with tubular fluid coming from the glomerulus or retrograde from other nephrons. Since net absorption of water was reduced to zero, bidirectional movement of sodium was presumably in a steady state condition. Bicarbonate ions, however, may not have reached their final steady state concentration during the perfusion. It has been found in "split oil drop" studies in which solutions low in Na^+ concentration were injected into isolated segments of the rat proximal tubule (3) that Na^+ concentration rises exponentially with time. In the first 10 seconds, the Na^+ concentration rose to about two-thirds of its steady state concentration, but took about 50 to 60 seconds before it finally stabilized at 90 to 100 mEq per L. In the present experiments, a rough approximation of the contact time of the perfusion fluid with the tubular epithelium can be made from the rate of perfusion (15 nl per minute), the mean tubular diameter (24 μ), and an average length of perfused nephron segment (5 mm). This calculation yields a time of contact of about 10 seconds. If HCO_3^- accumulation occurs at a rate comparable to that of Na^+ influx, steady state concentrations of HCO_3^- would have been approached but not reached during the perfusion period, and the final concentrations would be even higher than those found. However, it seems unlikely that the true steady state HCO_3^- concentration is very much higher, since the value calculated from steady state $[Na^+] + [K^+] - [Cl^-]$ in normal rats is approximately 4.5 mEq per L (2).

In the second group of normal rats, shown in Table III, renal carbonic anhydrase was inhibited by intravenous and intraluminal injection of 2-benzenesulfonamide-1,3,4-thiadiazole-5-sulfonamide (CL 11,366). This drug has been shown to have no significant effect on red blood cell carbonic anhydrase at the dose used here but to have a maximal effect on the kidney (10). Plasma P_{CO_2} did not rise, therefore, as it does with acetazolamide administration; it actually fell in most of the experiments, probably due to a decrease in the depth of anesthesia and resultant

TABLE IV

Effect of $NaHCO_3$ infusion on bicarbonate concentration of collected perfusate

Experiment no.	Plasma			Collected perfusate		
	pH	$[HCO_3^-]$	P_{CO_2}	pH	$[HCO_3^-]$	C/I_{In}
		mEq/L	*mm Hg*		*mEq/L*	
14	7.45	33.9	50	6.14	1.7	1.03
	7.60	41.3	43	6.51	4.1	1.04
15	7.35	36.4	68	6.46	3.6	1.03
16	7.52	35.3	45	6.69	6.1	1.03
	7.74	51.9	40	6.59	4.9	1.02
17	7.42	27.2	43	5.90	1.0	1.02
				6.59	4.9	1.05
				6.79	7.7	1.01
				6.69	6.1	1.02
	7.63	30.4	30	5.81	0.8	0.98
18	7.48	31.7	44	6.51	4.1	1.01
				6.58	4.8	1.01
				6.20	2.0	0.97
	7.64	36.5	35	6.50	4.0	0.96
19	7.51	28.9	37	6.63	5.3	1.04
				6.45	3.5	1.01
				6.61	5.1	1.01
	7.60	30.9	32	6.74	6.9	0.95
20	7.33	26.7	52	6.58	3.8	1.01
				6.61	4.1	1.04
				6.84	7.0	0.98
	7.63	40.3	40	6.29	2.0	1.03
Mean ± SD				6.49 ± 0.16	4.3 ± 1.9	1.01 ± 0.06

TABLE V

*Effect of carbonic anhydrase inhibition and $NaHCO_3$ infusion on bicarbonate concentration of collected perfusate**

Experiment no.	Plasma pH	Plasma $[HCO_3^-]$	Plasma P_{CO_2}	Collected perfusate pH	Collected perfusate $[HCO_3^-]$	Collected perfusate C/I_{in}
		mEq/L	*mm Hg*		*mEq/L*	
21	7.48	34.9	48	6.94	10.9	1.12
				7.03	13.4	0.82
	7.60	37.5	39	7.10	15.7	1.13
22	7.37	33.0	59	7.20	19.8	1.02
				7.07	14.7	0.97
				7.18	18.9	1.02
	7.72	47.2	38	7.31	25.5	1.03
23	7.45	30.5	45	7.05	14.0	1.01
				7.08	15.0	1.03
				7.11	16.1	0.97
	7.70	42.7	36	6.90	9.9	1.04
24	7.65	43.3	41	6.94	10.9	1.01
25	7.41	27.4	45	6.84	8.6	1.00
				7.03	13.4	0.98
				6.92	10.4	1.00
				6.95	11.1	0.98
	7.75	44.0	33	6.81	8.1	1.00
26	7.47	33.7	48	7.16	18.1	1.00
				7.20	19.8	1.01
				7.07	14.7	1.16
				7.31	25.5	0.98
27	7.54	37.2	45	7.16	18.1	0.94
	7.73	45.5	36	7.07	14.7	0.95
28	7.52	29.4	37	7.18	18.9	0.97
				7.07	14.7	0.98
				6.87	9.3	1.01
	7.74	44.4	34	7.02	13.1	0.90
Mean				7.06	14.9	1.00
± SD				0.14	4.6	0.08

* Carbonic anhydrase was inhibited by intravenous and intraluminal administration of CL 11,366.

hyperventilation.[3] The average HCO_3^- concentration in the collected perfusate was slightly higher than in the animals with intact enzyme activity, but the difference was not statistically significant ($p > 0.8$). Urinary pH, on the other hand, rose from a control value of 6.19 to 7.58 after enzyme inhibition, indicating that the drug had a potent effect on the free flowing tubules. The absence of any significant effect on the isolated perfused tubules probably means that H^+ secretion was at minimal levels under these experimental conditions, and further that the total accumulation of HCO_3^- was also small.

[3] It is also possible that hyperventilation was the result of a cerebrospinal fluid acidosis. This might occur by delayed dehydration of H_2CO_3, similar to the mechanism in the proximal tubule (1), if carbonic anhydrase is present at the interface between blood and spinal fluid.

Induction of acute metabolic alkalosis in the presence of an intact carbonic anhydrase system resulted in only a slight rise in the pH and HCO_3^- concentration of the perfusate (Table IV). With a comparable degree of metabolic alkalosis, inhibition of carbonic anhydrase resulted in a significantly greater recovery of HCO_3^- than in any of the other three groups of animals ($p < 0.001$).

The composition of the perfusion fluid used in the experiments in Tables II to V was such that no net absorption of water (or sodium) occurred. Under these conditions, the effects of solute flux asymmetry and solvent drag are minimized or eliminated (11). It is possible to conclude, therefore, that the rise in HCO_3^- concentration during perfusion was not due simply to absorption of water. The question arises, however, whether net reabsorption of solute and water as occurs during free flow might prevent HCO_3^- influx. For example, bulk movement of water might create a solvent drag effect in which flux of ions in the direction opposite to the flow of water is impeded (12). In order to examine this possibility, we used an isotonic NaCl perfusion fluid in the experiments shown in Table VI. As can be seen, in most of the collections, much more HCO_3^- was recovered than can be attributed to absorption of water from the original perfusion fluid, indicating that HCO_3^- accumulation was occurring in spite of bulk flow of water. The variable rise in inulin and HCO_3^- concentration may have been due in part to differences in the length of the perfused segments (13).

TABLE VI

*Accumulation of bicarbonate in an isotonic NaCl perfusate**

Perfusion no.	pH	$[HCO_3^-]$	C/I_{in}
		mEq/L	
1	7.02	10.5	1.59
2	6.56	3.7	1.28
3	6.01	1.0	1.15
4	6.98	9.6	1.25
5	5.94	0.9	1.17
6	6.72	5.3	1.43
7	6.20	1.6	1.10
8	6.63	4.3	1.33
9	6.75	5.7	1.78
10	6.82	6.7	1.90

* Data were obtained from four normal rats. Carbonic anhydrase was inhibited by intravenous and intraluminal administration of CL 11,366.

Discussion

The results of the present study indicate that HCO_3^- can gain access to the proximal tubular lumen from a source distal to the glomerulus. When isolated segments of cortical proximal tubules of normal rats were perfused with a solution containing less than 0.2 mEq per L of HCO_3^- and from which there was no net absorption of sodium or water, the HCO_3^- concentration rose to about 3 mEq per L. Inhibition of renal carbonic anhydrase did not significantly alter the recovery of HCO_3^- in the perfusate in normal animals, but when the enzyme was inhibited in acutely alkalotic animals, HCO_3^- recovery increased to a mean value of 15 mEq per L. Considering that the time of contact of the perfusion fluid with the tubular epithelium was only about 10 seconds, the concentrations of HCO_3^- found in each of the experimental groups may be slightly lower than the true steady state concentrations, and the magnitude of the postglomerular addition of HCO_3^- might be underestimated to some degree by the present observations.

The precise mechanism of the addition of HCO_3^- to the luminal fluid cannot be established with certainty from these data, but several possibilities may be considered. In the discussion that follows, reference will be made to the following series of reactions, which are thought to occur within the lumen of the proximal tubule [4]:

$$\underset{\text{(secreted)}}{H^+} + HCO_3^- \rightleftharpoons H_2CO_3 \overset{CA}{\rightleftharpoons} CO_2 + H_2O.$$

$$\underset{\text{(plasma)}}{CO_2}$$

First, HCO_3^- may have been generated in the tubular lumen due to either passive or active transfer of H^+ out of the lumen or to addition of OH^- from the epithelial cells. Either of these processes would have the effect of shifting the above series of reactions toward the left, and, because of the ready availability of CO_2, generating new bicarbonate ions. However, this explanation seems unlikely for two reasons. It has been shown that carbonic anhydrase is accessible to the fluid in the proximal tubule in rats and that its inhibition results in an acid disequilibrium pH in the lumen, due to continued net secretion of H^+ and delayed dehydration of intraluminal H_2CO_3 (1). Movement of H^+ out of the lumen or of OH^- into the lumen as a mechanism for HCO_3^- generation would lead to an alkaline disequilibrium pH in the enzyme-inhibited animals, contrary to the observed effect of carbonic anhydrase inhibition (1). More important, however, is the fact that inhibition of carbonic anhydrase markedly increased the recovery of HCO_3^- in the alkalotic animals (Table V vs. Table IV). It is clear from the above reactions that generation of new HCO_3^- from CO_2, whether due to removal of H^+ or addition of OH^- from the cells, should be reduced whenever intraluminal carbonic anhydrase is inhibited. Our observations in alkalotic rats are thus incompatible with this hypothesis. Although the combination of alkalosis and enzyme inhibition may have increased the alkalinity of the tubular cells to a greater degree than did alkalosis alone, and therefore enhanced the movement of OH^- from cell to tubular lumen, the absence of intraluminal carbonic anhydrase activity would be expected to slow the reaction

$$CO_2 + OH^- \longrightarrow HCO_3^-$$

drastically and thereby limit the formation of HCO_3^-. Thus, transfer of H^+ out of the lumen or transfer of OH^- into the lumen seems inadequate to explain the data.

A second hypothesis is that molecular NH_3 and possibly the cations of weak organic bases or anions of weak organic acids entered the perfusion fluid by a process of nonionic diffusion (14–16). In doing so, they would tend to combine with free H^+ in solution, as for example in the reaction

$$NH_3 + H^+ \longrightarrow NH_4^+,$$

and thereby liberate OH^-. This would result in the generation of an equivalent amount of HCO_3^- by the reaction

$$OH^- + CO_2 \overset{CA}{\longrightarrow} HCO_3^-.$$

Since pH gradients determine to a large extent the distribution of weak acids and bases across semipermeable membranes (17, 18), it might be expected that the largest intraluminal accumulation of NH_3 occurred in those experiments in which the tubular cells were most alkaline relative

[4] Carbonic anhydrase (CA) may actually catalyze the reaction $HCO_3^- \overset{CA}{\rightleftharpoons} CO_2 + OH^-$, but the conclusions drawn in the Discussion would be the same.

to the *in situ* pH of the perfusion fluid, i.e., the alkalotic rats in which carbonic anhydrase had been inhibited. Although NH_3 diffusion may have been favored under these experimental conditions, generation of HCO_3^- from OH^- and CO_2 should have been markedly impaired, due to the inhibition of intraluminal carbonic anhydrase. Thus, as discussed in the preceding section, the observation that HCO_3^- recovery increases rather than decreases in the presence of carbonic anhydrase inhibition argues against generation of HCO_3^- intraluminally from OH^-.

The experimental observations seem to be best explained by influx of ionic bicarbonate, either from the tubular cells or from the peritubular capillary blood. It would be expected if this were the mechanism that the amount of HCO_3^- recovered in the perfusate would vary inversely with the rate of H^+ secretion by the tubular cells. This was clearly the case in the alkalotic rats, in which inhibition of carbonic anhydrase and subsequent reduction in H^+ secretion markedly increased the recovery of HCO_3^- in the perfusate (Table V), presumably because less of the entering ionic bicarbonate was converted into CO_2 and H_2O during the perfusion period. The absence of any clear-cut response to carbonic anhydrase inhibition in normal rats (Table III) could also be explained by this mechanism, if it is assumed that HCO_3^- influx was much smaller when plasma HCO_3^- concentration was normal and that, consequently, the rate of H^+ secretion was at minimal levels. According to this interpretation, the magnitude of HCO_3^- influx would be influenced by either the transcellular or transtubular concentration gradient of bicarbonate ions. Since in the alkalotic animals with an intact carbonic anhydrase system the residual concentration of HCO_3^- in the collected perfusate was almost as low as in normal rats (Table IV vs. Table II), it could be concluded that HCO_3^- influx is dependent upon the intracellular HCO_3^- concentration and that acute alkalosis did not produce a significant intracellular rise within the time of the experiment. The combination of alkalosis and carbonic anhydrase inhibition might have produced a greater rise in intracellular HCO_3^-. Alternatively, HCO_3^- influx could have increased in the alkalotic animals with an intact carbonic anhydrase system, but H^+ secretion also increased in direct proportion to the larger amount of HCO_3^- entering the lumen. This view is compatible with the suggestion that, because of the availability of carbonic anhydrase to the proximal tubular fluid, the rate of H^+ secretion in this segment of the nephron varies directly with the buffer content of the luminal fluid and is not limited by steep pH gradients (1).

If our conclusion is correct that ionic bicarbonate leaked into the tubular lumen, this ion would be behaving in a manner similar to sodium, potassium, and chloride, which have all been shown to diffuse back into the lumen of the rat proximal tubule (2–4). The precise mechanism (or mechanisms) accounting for the influx of these ions is presently uncertain, and it is not clear whether the leak occurs at the luminal membrane or through aqueous channels passing either through or between the cells (19). Evidence for the presence of extracellular aqueous channels has been found by Windhager and Klose (20) in *Necturus* kidney tubules, but whether similar shunt pathways exist in rat kidney tubules or in other species is unknown. Thus, the relative importance of intracellular vs. extracellular ion concentrations and of electrical potentials in influencing ionic influx cannot be stated at this time.

The conclusion that the proximal tubular epithelium is permeable to HCO_3^- raises the possibility that flux of this ion may be a component of the over-all handling of HCO_3^- by the kidney. Although net accumulation of HCO^-_3 in the tubular lumen might not occur under ordinary free flow conditions, there could nevertheless be a significant influx of the ion opposite to the direction of net reabsorption. Since in the present experiments the magnitude of influx appeared to vary directly with the concentration gradient (either transtubular or transcellular), the importance of an influx component would be expected to depend upon the particular experimental conditions. In any event, inward movement of HCO_3^- would have to be considered in estimates of the actual kinetics of H^+ secretion at the tubular level. Further studies seem indicated to determine the precise role of flux of ionic bicarbonate in various physiological and pathological conditions.

References

1. Rector, F. C., Jr., N. W. Carter, and D. W. Seldin. The mechanism of bicarbonate reabsorption in the

proximal and distal tubules of the kidney. J. clin. Invest. 1965, **44**, 278.

2. Kashgarian, M., H. Stöckle, C. W. Gottschalk, and K. J. Ullrich. Transtubular electrochemical potentials of sodium and chloride in proximal and distal renal tubules of rats during antidiuresis and water diuresis (diabetes insipidus). Pflügers Arch. ges. Physiol. 1963, **277**, 89.
3. Giebisch, G., R. M. Klose, G. Malnic, W. J. Sullivan, and E. E. Windhager. Sodium movement across single perfused proximal tubules of rat kidneys. J. gen. Physiol. 1964, **47**, 1175.
4. Marsh, D. J., K. J. Ullrich, and G. Rumrich. Micropuncture analysis of the behavior of potassium ions in rat renal cortical tubules. Pflügers Arch. ges. Physiol. 1963, **277**, 107.
5. Sonnenberg, H., and P. Deetjen. Methode zur Durchströmung einzelner Nephronabschnitte. Pflügers Arch. ges. Physiol. 1964, **278**, 669.
6. Thurau, K., and P. Deetjen. Intratubular urine flow rates and transtubular water flux in parts of the individual mammalian nephron. Free communication no. 255. 22nd International Physiological Congress, Leiden, 1962.
7. Marsh, D., and C. Frasier. Reliability of inulin for determining volume flow in rat renal cortical tubules. Amer. J. Physiol. 1965, **209**, 283.
8. Bank, N. Relationship between electrical and hydrogen ion gradients across the rat proximal tubule. Amer. J. Physiol. 1962, **203**, 577.
9. Windhager, E. E., and G. Giebisch. Micropuncture study of renal tubular transfer of sodium chloride in the rat. Amer. J. Physiol. 1961, **200**, 581.
10. Travis, D. M., C. Wiley, B. R. Nechay, and T. H. Maren. Selective renal carbonic anhydrase inhibition without respiratory effect: pharmacology of 2-benzenesulfonamido-1,3,4-thiadiazole-5-sulfonamide (CL 11,366). J. Pharmacol. exp. Ther. 1964, **143**, 383.
11. Giebisch, G., and E. E. Windhager. Renal tubular transfer of sodium, chloride and potassium. Amer. J. Med. 1964, **36**, 643.
12. Koefoed-Johnsen, V., and H. H. Ussing. Ion transport *in* Mineral Metabolism, C. L. Comar and F. Bronner, Eds. New York, Academic Press, 1960, p. 169.
13. Windhager, E. E., K. Hierholzer, and M. Wiederholt. Microperfusion study of reabsorption of Na and fluid in single proximal tubules of rat kidneys. Fed. Proc. 1966, **25**, 459.
14. Glabman, S., R. M. Klose, and G. Giebisch. Micropuncture study of ammonia excretion in the rat. Amer. J. Physiol. 1963, **205**, 127.
15. Hayes, C. P., Jr., J. S. Mayson, E. E. Owen, and R. R. Robinson. A micropuncture evaluation of renal ammonia excretion in the rat. Amer. J. Physiol. 1964, **207**, 77.
16. Hayes, C. P., Jr., E. E. Owen, and R. R. Robinson. Renal ammonia excretion during acetazolamide or sodium bicarbonate administration. Amer. J. Physiol. 1966, **210**, 744.
17. Orloff, J., and R. W. Berliner. The mechanism of the excretion of ammonia in the dog. J. clin. Invest. 1956, **35**, 223.
18. Milne, M. D., B. H. Scribner, and M. A. Crawford. Non-ionic diffusion and the excretion of weak acids and bases. Amer. J. Med. 1958, **24**, 709.
19. Windhager, E. E., and G. Giebisch. Electrophysiology of the nephron. Physiol. Rev. 1965, **45**, 214.
20. Windhager, E. E., and R. M. Klose. Measurements of electrical conductances in single proximal tubules of *Necturus* kidney. Fed. Proc. 1965, **24**, 520.