JCI The Journal of Clinical Investigation

Loop of Henle bicarbonate accumulation in vivo in the rat.

D Z Levine, … , J A Luisello, S Raman

J Clin Invest. 1979;63(1):59-66. https://doi.org/10.1172/JCI109278.

Research Article

We have carried out perfusion studies on hydropenic and bicarbonate-loaded rats to provide direct in vivo observations on bicarbonate accumulation in the short loops of Henle. Analysis of early distal tubular fluid was made during bicarbonate-free saline perfusion from the end proximal to the early distal site, documenting accumulation of "new" bicarbonate. During perfusion in hydropenic rats, steady-state bicarbonate concentrations were suggested by early distal values of approximately equal to mM, which were independent of perfusion rate and virtually indistinguishable from bicarbonate concentration measured during free flow when filtered bicarbonate was allowed to enter the loop. Thus, loop bicarconate accumulation was apparently sufficient to allow new bicarbonate to enter at a rate comparable to that delivered to the early distal site during free flow, recognizing of course that free-flow delivery rates are the result of complex components of filtration and bidirectional fluxes. In bicarbonate-loaded rats, however, bicarbonate accumulation rates although higher than in hydropenia, were much lower than free-flow delivery rates. Furthermore, early distal bicarbonate concentrations during bicarbonate loading fell as perfusion rate increased, presumably because of a limitation to increasing ionic bicarbonate entry.

Loop of Henle Bicarbonate Accumulation In Vivo in the Rat

D. Z. LEVINE, M. K. BYERS, R. A. MCLEOD, and J. A. LUISELLO, with statistical analyses by S. RAMAN, *Departments of Medicine and Physiology and the Ottawa General Hospital, University of Ottawa, Ottawa, Ontario, Canada*

ABSTRACT We have carried out perfusion studies on hydropenic and bicarbonate-loaded rats to provide direct in vivo observations on bicarbonate accumulation in the short loops of Henle. Analysis of early distal tubular fluid was made during bicarbonate-free saline perfusion from the end proximal to the early distal site, documenting accumulation of "new" bicarbonate. During perfusion in hydropenic rats, steady-state bicarbonate concentrations were suggested by early distal values of ≅6 mM, which were independent of perfusion rate and virtually indistinguishable from bicarbonate concentration measured during free flow when filtered bicarbonate was allowed to enter the loop. Thus, loop bicarbonate accumulation was apparently sufficient to allow new bicarbonate to enter at a rate comparable to that delivered to the early distal site during free flow, recognizing of course that free-flow delivery rates are the result of complex components of filtration and bidirectional fluxes. In bicarbonate-loaded rats, however, bicarbonate accumulation rates although higher than in hydropenia, were much lower than free-flow delivery rates. Furthermore, early distal bicarbonate concentrations during bicarbonate loading fell as perfusion rate increased, presumably because of a limitation to increasing ionic bicarbonate entry.

INTRODUCTION

Micropuncture studies have been mainly responsible for our recent insights into single nephron bicarbonate transport. The proximal tubule has been most frequently studied. In our view, the more significant contributions included studies suggesting that changes in fractional bicarbonate reabsorption in the rat proximal tubule could be altered in a variety of circumstances (1), the demonstration by Bank and Aynedjian (2) and Burg and Green (3) that bicarbonate can accumulate in proximal tubular fluid during bicarbonate-free saline perfusion, the recent demonstrations by McKinney and Burg (4) and Warnock and Burg (5) of CO_2 influx during in vitro perfusion of the pars recta, theoretical and experimental approaches to the mechanism whereby ionic bicarbonate can be removed from the tubular lumen (1, 6), and measurements of absolute net bicarbonate fluxes under a variety of conditions (7–10). Some information is also available concerning bicarbonate transport in the distal convoluted tubule (11), although these results may not be as precise as some of the proximal tubular data: distal bicarbonate concentrations are usually calculated from the pH of the tubular fluid, and are thus subjected to the error introduced by changes in pK_1' secondary to alterations in ionic strength of the tubular fluid. Finally, by comparing distal and proximal tubule reabsorptive rates, in vivo estimates of loop bicarbonate reabsorption have also been reported under several circumstances (11).

The present investigations were undertaken to examine in vivo bicarbonate accumulation in the short loops of Henle[1] by end-proximal to early distal bicarbonate-free saline perfusions.

METHODS

These studies were done on male Sprague-Dawley rats weighing between 280–340 g. The animals were bred and raised in our climate-controlled animal house, fed Purina Rat Chow (Ralston Purina Co., St. Louis, Mo.), and given free access to food and drink before the experiment. Four groups of animals were studied: hydropenic rats and bicarbonate-loaded rats for perfusion studies, and two additional groups of animals were studied (*a*) to exclude leaky upstream oil blocks during perfusion, and (*b*) to exclude an important effect of possible high in vivo P_{CO_2} on bicarbonate measurements.

This paper was presented, in part, at the Canadian Society for Clinical Investigation, January 1978, Vancouver, and at the International Congress of Nephrology, June 1978, Montreal.

Received for publication 8 December 1977 and in revised form 29 August 1978.

[1] The expression "short loop of Henle" or "loop" in this paper is used with the recognition that in addition to perfusion of the pars recta, thin descending and thick ascending components of the short loops, some late proximal and early distal epithelium is also involved (see Discussion).

Hydropenic animals were prepared for micropuncture as previously described (10) and infused with 0.15 N NaCl at a rate of 0.5% of the body weight per hour. The second group of rats was bicarbonate-loaded with an infusion of 0.15 N $NaHCO_3$ at the rate of 3% body weight per hour. In each animal, one loop was perfused, usually at from two to four perfusion rates. In all perfusion studies only one perfusion solution was used: bicarbonate-free 0.15 N NaCl that contained [^{14}C]inulin faintly stained with lissamine green. No animal was studied whose transit time was more than 12 s proximal or whose mean arterial blood pressure was <105 mm Hg.

Microperfusion technique. The technique used for microperfusion is similar to that previously described by others (12, 13). Particular attention was devoted to identifying the earliest superficial distal segments.[2] The first distal segment in a field of ≅10 mm^2 was identified by an i.v. injection of 0.02 ml of lissamine green. A 6.6-μm pipette, filled with 10% lissamine green dye was used for identification of surface segments of the late and early proximal tubule segments of the same nephron. With a larger-tipped capillary, a hole was made in an upstream proximal loop, to allow drainage of endogenous filtrate. Usually, after positioning the perfusion pipette that contained saline in the last accessible late proximal surface segment, an oil block was placed upstream between the drainage hole and the perfusion pipette. Dow Corning Silicone Oil No. 200 (Dow Corning Corp., Midland, Mich.) (60,000 C.S.) stained with blue dye to enhance visibility provided an oil block of sufficient viscosity to permit a stable position over a wide range of perfusion rates. No samples were collected until the perfusion had proceeded for several minutes. In the cases where three or four samples were obtained, the perfusion time was of the order of 15–20 min.

Collections of the perfusate were always made at the early distal site and, despite the in vitro accuracy of our perfusion pump (manufactured by W. Klotz, Munich, West Germany), collections were always quantitative. Initial flow rates were varied as to "high" or "low", so that the sequence of perfusions varied (i.e., high-low-high, low-high-low). Incremental changes were avoided. We chose a range of flows to be comparable to late proximal flow rates in hydropenia or volume expansion observed in our laboratory (10). In practice, multiple collections at different flow rates were easily obtained in the hydropenic rats, whereas in bicarbonate-loaded rats, we were not as successful in obtaining recollection samples. We presume this is because of higher intraluminal pressure in the distal nephron secondary to the diuresis caused by bicarbonate loading, impeding distal migration of the oil block. If the samples did not freely flow into the collection pipettes or if the oil block was immobile between collections, the data from that nephron were discarded.

Free-flow collections. In both hydropenic and bicarbonate-loaded rats, free-flow early distal collections were also undertaken at the early distal site identified in the same manner as for the loop perfusions (*vide supra*). Quantitative free-flow collections were made in nephrons that were not perfused.

Experiments designed to exclude bicarbonate leak around the proximal block during loop perfusion. Loops were perfused in three rats with saline that did not contain [^{14}C]inulin. While perfusion proceeded, an early distal sample was taken and did not show ^{14}C activity. A second sample was taken while a pipette that contained [^{14}C]inulin was used to enter the proximal tubule above the block and [^{14}C]inulin was injected with sufficient pressure to move the block. This sample again showed no activity. A third sample was taken while the ^{14}C-containing pipette was placed in the same segment as the perfusion pipette (below the block) and isotope was injected. This sample showed very high ^{14}C activity. This response was observed in each rat.

Analytical methods, calculations, and statistics. Most of these have been previously described (10). Our glass micro pH electrode (16) was again used in these studies but the calculations for bicarbonate were modified so that the pK_1' was adjusted for changes in ionic strength as estimated only from sodium and potassium concentrations after the method of Uhlich et al. (17): $pK_1' = pK_1 - 0.522\ \sqrt{[Na+] + [K+]}$ where sodium and potassium are expressed in molar concentrations, and $pK_1 = 6.328$. Bicarbonate concentration was then calculated as: $[HCO_3^-] = 0.0322\ P_{CO_2}$. antilog $(pH - pK_1')$.[3]

To evaluate the possible role of high in vivo P_{CO_2} (P_{CO_2} = 90 mm Hg) on in vitro bicarbonate measurements when the chamber P_{CO_2} is similar to blood (P_{CO_2} = 45 mm Hg), additional studies were carried out. Free-flow, rather than perfusion early distal samples were taken to permit maximum entry of nonbicarbonate buffers. 29 samples (14 from three hydropenic rats and 15 from three bicarbonate-loaded rats) were split and one portion was measured at the P_{CO_2} comparable to blood (P_{CO_2} = 44.8±0.57 mm Hg) and the other portion was measured at a high value, suggested by recent reports (P_{CO_2} = 87.23±0.93 mm Hg). The hydropenic samples showed a very small but significant increase in bicarbonate concentration at the high P_{CO_2} level (8.74±0.79 meq/liter increasing to 9.14±0.54 meq/liter, $P < 0.02$). No change was noted when samples from the bicarbonate-loaded rats were similarly treated: 26.20±1.18 meq/liter vs. 26.37±1 meq/liter, $P > 0.05$.

Sodium and potassium concentrations in tubular fluid samples were determined by an Aminco helium glow photometer (American Instrument Co., Silver Spring, Md.) specially modified for our use. In the 17 experiments reported here the mean coefficient of error of replicate determinations on 60 micropuncture samples was 2.4%. Water movements were determined by measuring inulin concentrations of the perfusate and collected fluid. The concentration of isotope was always greater than eight times background. Acid-base measurements of whole blood were different from that previously reported. Total CO_2 was measured in the plasma of these rats by an Ericsen CO_2 Analyzer (Ericsen Instruments, Ossining, N. Y.) in 50 or 100 μl of plasma. Blood pH was measured by the Radiometer PHM-72 system (Radiometer Co., Copenhagen, Denmark). Plasma bicarbonate and P_{CO_2} were calculated with the Henderson-Hasselbalch equation and a solubility coefficient for CO_2 gas in plasma of 0.0301. We assessed the accuracy and precision of this machine in separate studies on rat plasma and urine at varying concentrations of total CO_2. Replicate samples showed a coefficient of error of 1% and the accuracy was determined by comparing aliquots of the same plasma or urine wherein the total CO_2 was determined by calculations from pH and P_{CO_2} and this value correlated with the total CO_2 as reported by the Ericsen

[2] 18 early distal samples obtained in this fashion had a mean potassium concentration of 1.68 meq/liter and a tubular fluid to plasma potassium concentration (TF/p K) = 0.33. Use of transit time and tubular fluid potassium concentration has been shown by Kunau et al. (14) and Wright (15) to identify early distal segments as determined by dissection.

[3] In the experiments reported here, the mean mV response of the electrodes was 98.0 ± 0.42% of the theoretical value, the mean electrical drift during measurement was 0.88 ± 0.16 mV, and the mean P_{CO_2} drift during measurement was 0.79 ± 0.23 mm Hg.

CO_2 Analyzer. The agreement was invariably within 1 mmol/liter and the correlation coefficient was 0.995.

The early distal collected flow rate was determined by measuring the volume of a timed sample. With this value and the ratio of concentrations of inulin in the perfusate and the collected fluid, the initial perfusion rates were determined. Because bicarbonate was not present in any perfusion, all bicarbonate appearing in the samples collected at the early distal site during loop perfusion was caused by accumulation. Bicarbonate influx was calculated as the product of early distal collected flow rate and bicarbonate concentration of the sample. Under free-flow conditions, early distal delivery rates for bicarbonate were calculated in a similar fashion after early distal tubules were quantitatively sampled.

We have been acutely aware of problems concerning analysis of variance in micropuncture experiments (18). In view of the homogeneity among the responses of individual tubules in our experiments, we have disregarded variation among the animals. We have analyzed our results with standard regression techniques as well as paired and unpaired t tests. Results are expressed as mean±1 SEM.

RESULTS

Acid-base and electrolyte values for hydropenic and bicarbonate-loaded rats are shown in Table I. Micropuncture data are presented in Table II.

In the results and discussion we use the term "influx" to represent not only diffusion by ionic bicarbonate across the tubular epithelium, but also to include "accumulation" of new bicarbonate by the interaction of diffused CO_2 with base.

Hydropenic rats. It must be emphasized (see Methods) that the initial perfusion rates were varied as to high (≅35 nl/min) and low (≅15 nl/min) so as to result in collected early distal flow rates of ≅23 and 9 nl/min. Further, it is worth noting that these flow rates were selected so as to be similar to the rate of flow of fluid leaving the end proximal tubular site as determined in our previous studies during hydropenia and volume expansion (8). Figs. 1 and 2 and Table II show the replicate bicarbonate concentrations for the individual tubules at different perfusion rates. The concentration is reasonably constant over a large flow range (slope = −0.07, $r = 0.21$, $P > 0.05$). The mean bicarbonate concentration is 6.45±0.66 meq/liter ($n = 19$). Fig. 1 shows a regular horizontal orientation of the flow rates in all but one animal. This constancy is further strengthened by the small SEM (0.66 meq/liter). Fig. 2 shows early distal bicarbonate concentrations obtained under free-flow conditions in hydropenic rats. In addition, the perfusion values (Fig. 1) are also included. The free-flow concentrations are again reasonably constant over a large flow range (slope = 0.15, $r = 0.46$, $P > 0.05$). The mean free-flow bicarbonate concentration is 5.26±0.40 meq/liter. Fig. 3 shows the same tubules as in Fig. 1, plotted as influx against increasing flow rates. The stability of the increasing influx rates is evident, corroborating the homogeneity referred to in Methods. Influx rates of new bicarbonate during perfusion were highly correlated with early distal collected flow rates ($y = 6.76 + 7.76x$, $r = 0.69$, $P < 0.001$). During free-flow, the rate of early distal bicarbonate delivery was also highly correlated with flow ($y = -25.76 + 7.80x$, $r = 0.90$, $P < 0.001$), and did not have a slope which was significantly different than that for the perfusion line (*vide supra*).

TABLE I
Various Experimental Parameters in Hydropenic and Bicarbonate-Loaded Rats

	Hydropenic rats ($n = 5$)	Bicarbonate-loaded rats ($n = 6$)
Blood pH	7.41±0.01	7.57±0.01
Blood P_{CO_2}, *mm Hg*	48.8±1.1	50.6±1.2
Plasma electrolytes		
Na, *meq/liter*	142±0.3	143±1.0
K, *meq/liter*	4.9±0.05	3.2±0.08
Cl, *meq/liter*	98±2.1	82±0.8
HCO_3^-, *meq/liter*	29.7±0.5	44.9±0.7
Urine flow rate, *μl/min*	1.85±0.30	75.25±15.0

Bicarbonate-loaded rats. The identical perfusion procedure with bicarbonate-free 0.15 N NaCl was carried out in these bicarbonate-loaded animals. In contrast to what was observed in hydropenic rats, bicarbonate concentration fell as perfusion rate increased (Fig. 4, Table II). The regression line for $[HCO_3^-]$ during loop perfusion was: $y = 18.45 - 0.48x$ ($r = 0.85$, $P < 0.001$).

Thus, during loop perfusion bicarbonate influx was not flow-correlated ($y - 137.0 + 1.05x$, $r = 0.20$, $P > 0.05$), with the mean bicarbonate influx being 155.33 ±11.4 peq/min, indicating constancy of the influx rate as a function of collected flow rate (i.e., the SEM is only 7% of the mean).

In bicarbonate-loaded rats, free-flow early distal bicarbonate delivery rates were high and closely linked with flow ($y = -222.7 + 43.91x$, $r = 0.98$, $P < 0.001$). Thus, in contrast to the hydropenic rats, the rate of bicarbonate delivery to the early distal site during free-flow greatly exceeded the rate of bicarbonate accumulation during loop perfusion.

DISCUSSION

In 1967, when referring to their study on proximal tubular bicarbonate accumulation Bank and Aynedjian (2) commented that ionic bicarbonate influx "may be a component of the overall handling of bicarbonate by the kidney". In the present studies we made quantitative collections of tubular fluid at the early distal site during free-flow or bicarbonate-free loop perfusion. We were able to identify the rate of bicarbonate delivery to the early distal site caused by new bicarbonate ac-

TABLE II

Summary of Micropuncture Data

Loop perfusion data*							Free-flow data						
Rat no.	Tubule no.	$\dot{V}$	[Na+]	pK_1'	$[HCO_3^-]$	HCO_3^- accumulation rate	Rat no.	Tubule no.	$\dot{V}$	[Na+]	pK_1'	$[HCO_3^-]$	HCO_3^- delivery rate
		nl/min	*mM*		*mM*	*peq/min*			*nl/min*	*mM*		*mM*	*peq/min*
Hydropenic rats													
1	1	8.3	59.1	6.200	7.0	57.8	6	2	7.7	40.0	6.222	4.9	35.4
		24.9	116.1	6.148	6.2	155.1		3	7.9	68.1	6.190	5.9	46.5
		8.2	59.0	6.200	9.8	80.6							
							7	1	12.2	41.1	6.220	4.6	55.4
2	1	25.5	114.3	6.146	4.9	124.6		2	7.1	67.1	6.192	3.4	23.9
		8.1	67.3	6.189	6.0	48.6							
		29.4	114.5	6.147	7.4	217.1	8	1	23.0	87.6	6.171	8.2	187.5
								2	12.1	48.7	6.211	6.4	77.2
3	1	33.2	117.9	6.148	2.4	77.9							
		11.8	118.9	6.147	1.5	17.5	9	1	8.5	53.8	6.205	5.8	48.8
		16.4	114.7	6.151	1.3	21.5		2	3.0	39.7	6.196	5.1	15.7
								3	6.8	34.1	6.230	5.0	33.7
4	1	20.0	112.6	6.150	6.5	128.9		4	11.1	45.3	6.215	5.3	58.4
		10.2	93.4	6.166	7.7	77.9							
		27.9	112.8	6.150	11.0	305.5	10	1	15.4	50.3	6.209	5.5	84.7
								2	7.7	48.6	6.208	3.0	22.8
5	1	22.2	118.4	6.145	9.1	202.5		4	17.0	43.8	6.217	5.9	100.5
		6.3	63.2	6.194	7.7	48.7							
		28.3	108.8	6.153	6.3	177.1	11	1	15.8	71.3	6.187	6.4	100.5
		11.3	97.6	6.162	7.4	83.4		2	14.3	49.7	6.211	1.7	24.1
								3	14.3	51.5	6.207	7.1	101.9
6	1	13.6	121.4	6.144	4.3	58.6							
		4.8	88.4	6.170	11.6	55.1							
	2	19.6	112.6	6.151	4.6	89.7							
Bicarbonate-loaded rats													
12	1	9.0	54.2	6.197	16.1	145.3	12	1	12.1	58.0	6.201	8.6	103.2
		26.0	91.6	6.169	7.8	203.8		2	11.3	42.9	6.219	14.3	161.4
		13.2	72.2	6.187	14.7	193.5		3	16.6	56.5	6.204	25.1	416.1
13	1	15.6	93.1	6.167	12.7	197.9	13	2	14.9	64.5	6.195	33.1	492.1
14	1	10.6	51.5	6.206	11.3	119.2	14	2	16.6	62.7	6.194	35.9	594.7
								3	15.3	60.7	6.199	36.4	555.6
15	1	21.3	56.9	6.197	8.1	173.0		4	18.1	58.7	6.200	36.2	655.2
	2	25.3	88.0	6.171	4.0	102.0							
		16.6	86.8	6.173	8.4	139.3	15	3	11.9	56.9	6.202	19.7	234.9
16	1	25.8	97.9	6.162	6.2	158.9	16	3	17.5	57.2	6.202	31.0	541.3
	2	11.1	79.1	6.179	10.8	120.3		4	17.0	62.8	6.196	30.7	523.0
								5	13.6	70.3	6.188	29.2	395.5
							17	1	22.6	63.4	6.195	37.2	839.8
								2	23.1	64.3	6.193	33.2	766.9
								3	55.5	108.3	6.154	39.0	2166.3
								4	16.6	64.2	6.194	37.2	617.0

Initial perfusion rates were measured to be 35.08±1.29 nl for a pump setting at 35 nl/min and 17.74±0.89 nl for a pump setting at 15 nl/min. See also Methods and Results.

* $\dot{V}$ = collected flow rate.

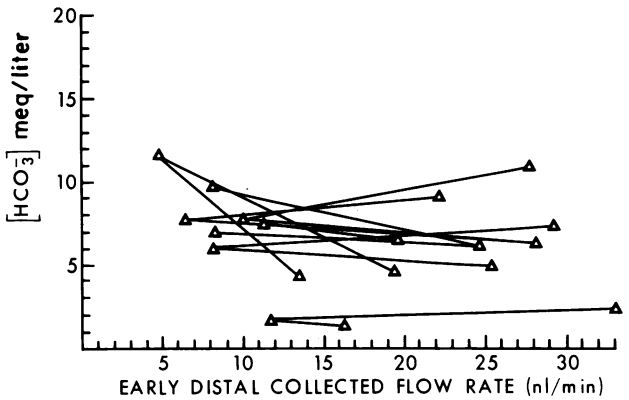

FIGURE 1 Bicarbonate-free loop perfusions in hydropenic rats: early distal bicarbonate concentrations in individual tubules. The bicarbonate concentrations of the collected perfusate of individual tubules are shown as a function of flow rate. See Results.

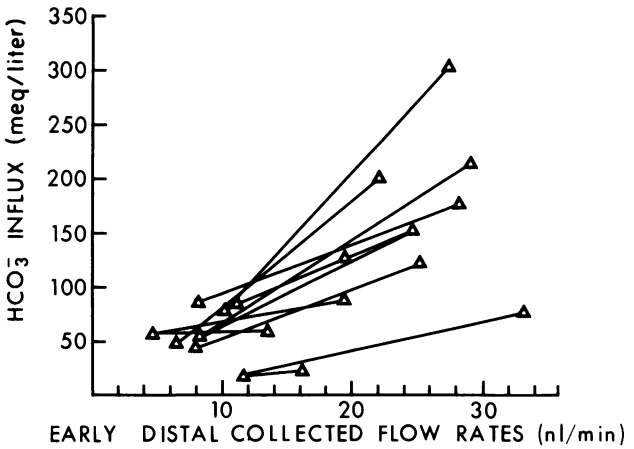

FIGURE 3 Bicarbonate-free loop perfusions in hydropenic rats: bicarbonate influx in single tubules. Bicarbonate accumulation (or influx) plotted against increasing flow rates in same tubules shown in Fig. 1.

cumulation in the loop in hydropenic and bicarbonate-loaded rats and evaluate the response of early distal bicarbonate concentrations to different flow rates. The studies presented here demonstrate (*a*) that in hydropenic rats, loop perfusion with saline is associated with early distal bicarbonate concentrations of 6 mM, which are independent of early distal flow rates over the range of 5–30 nl/min and similar to free-flow concentrations observed in a lower range of flow rates, (*b*) accumulation of new bicarbonate in hydropenic rats is brisk during loop perfusion and comparable to the rate of filtered bicarbonate escaping reabsorption and reaching the early distal site during free-flow, and (*c*) in bicarbonate-loaded rats, loop perfusion with saline is associated with a fall in bicarbonate concentration as early distal flow rate increases from 9 to 26 nl/min.

Methodological considerations. We have been meticulous in identifying the earliest possible surface distal segments in these studies (see Methods), and in no way does our loop perfusion technique seem less rigorous than that used by others (12, 13). Yet, there are certain important limitations to this technique which we feel compelled to identify. In fact, bicarbonate-free saline perfusions, as were undertaken in all of our experiments from the end proximal tubule to the early distal tubule, may involve the perfusate traversing epithelial segments which are not characterized with respect to either length or histology. Specifically, we have no way of knowing if, or how much convoluted proximal or convoluted distal subsurface tubular epithelium was

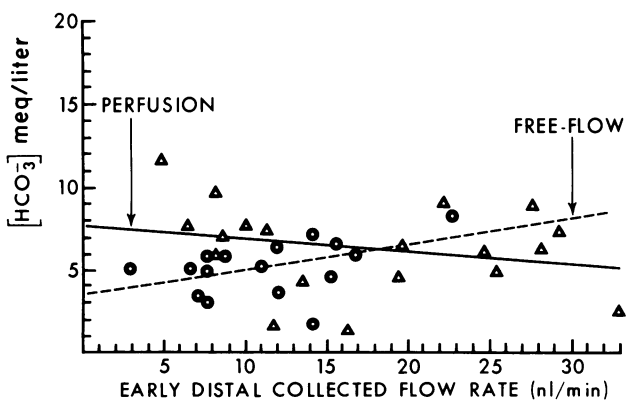

FIGURE 2 Bicarbonate-free loop perfusions and free-flow collections in hydropenic rats: early distal bicarbonate concentrations in individual tubules. Perfusion data is represented by open triangles; free-flow data by open circles.

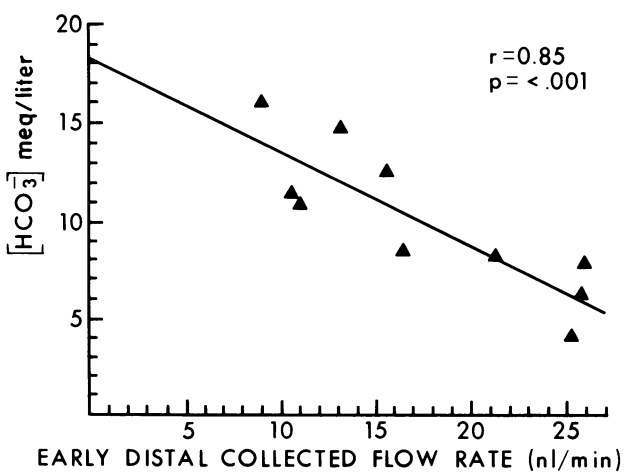

FIGURE 4 Bicarbonate-free loop perfusion in bicarbonate loaded rats: early distal bicarbonate concentrations. The equation for the line is: $y = 18.45 - 0.48x$.

present in each of the tubules we studied.[4] Further, we do not know whether the perfusate traversed any distal epithelium which was more representative of ascending limb or mid-distal tubule histology, a matter of current controversy (19). In fact, the only way to establish the histological identity and length of participating "nonloop" segments would be to determine their length by latex fixation and also carry out electron microscopy on each loop perfused. Because this arduous task was not undertaken by us (or to our knowledge by any other investigators), it is important to realize that in addition to the components of the short loops we studied (i.e., pars recta, thin descending limb, and thick ascending limb) some subsurface proximal or distal epithelium may have been perfused.

One potential source of error in bicarbonate measurements in our experiments relates to the P_{CO_2} and buffer concentration of early distal tubular samples. To the extent that the P_{CO_2} in the early distal tubule may be higher than the value used in our in vitro equilibration chamber (16) and to the extent that there is phosphate and other nonbicarbonate buffer in our samples, another kind of bicarbonate error can be present: downward titration of buffer by the in vivo-in vitro P_{CO_2} difference would tend to falsely depress the calculated bicarbonate concentration. Indeed, Karlmark (20) has presented data suggesting that the P_{CO_2} of proximal tubular fluid may be higher than that of blood, whereas Dubose et al. (21) have very recently presented results strongly suggesting distal tubular P_{CO_2} may also be significantly higher than that of blood. Samples taken at the early distal site under free-flow conditions in hydropenia showed only a trivial rise in bicarbonate concentration when the in vitro P_{CO_2} was increased by >40 mm Hg. No change was detected in samples from bicarbonate-loaded rats (see Methods).

Finally, bicarbonate concentration may also be in error unless correction is made for changes in ionic strength of early distal tubular fluid. In these experiments the pK_1' has been corrected for changes in sodium and potassium concentrations, thus avoiding an error that can be as high as 16% in the bicarbonate concentration as calculated from our micro pH measurements and the P_{CO_2} of the equilibration chamber.

What is the source of the observed bicarbonate accumulation? For the purposes of the following analysis, we will ignore proximal convolutions or early distal convolutions which may have been traversed by the perfusion fluid although it should be recalled that the proximal convoluted tubule has already been shown to be permeable to bicarbonate during bicarbonate-free perfusion in vivo and in vitro (2, 3). The superficial short loops of Henle consist of three segments: the pars recta of the proximal tubule, the thin descending limb, and the thick ascending limb. Only for the pars recta are there data that bear directly on bicarbonate accumulation. The pars recta has been recently studied by Warnock and Burg (5) in the rabbit kidney. These investigators used the isolated tubule preparation and determined total CO_2 influx or efflux with bath and perfusion solutions of varying bicarbonate composition. They showed that there was indeed very significant CO_2 influx when the perfusate contained no bicarbonate and when the bath contained 25 meq/liter. This represents very closely the transtubular gradient that existed in our saline perfusions with normal rats. Further, Warnock and Burg (5) showed that at higher flow rates the concentration of total CO_2 in the perfusate was relatively constant and seemed to be in a range consistent with our observations (*vide infra*). Thus, it appears appropriate to conclude that the pars recta could be one source of the bicarbonate accumulation we have documented, although its quantitative contribution during in vivo perfusion in the rat is unclear.

How much bicarbonate accumulates during loop perfusion? It is, of course, not surprising to measure some "new" bicarbonate during saline perfusion. We are, however, impressed with how brisk the accumulation rate is. One way of assessing the magnitude of loop bicarbonate accumulation (and hence of loop permeability) is to compare it with the rate of bicarbonate delivered which normally reaches the early distal site after escaping reabsorption under free-flow conditions. This comparison, in essence, compares net influx (during perfusion) with the difference between net reabsorption and bicarbonate load (during free-flow). Our results show that during hydropenia, accumulation rates are almost identical to free-flow delivery rates but in bicarbonate-loaded rats accumulation rates are much lower.

Early distal bicarbonate concentrations in hydropenic rats during loop perfusion. In the present studies we have measured bicarbonate concentrations at the early distal site after bicarbonate-free perfusion of the short loops of Henle. It is obvious that the measurement of bicarbonate concentrations and the calculation of accumulation rates can provide only limited insight into transport processes in different loop segments. Certainly, a given concentration may be compatible with a variety of flow-induced alterations in the unidirectional influx and efflux components.

Notwithstanding the foregoing, in view of recent in vitro perfusion results on rabbit proximal straight tubules by Warnock and Burg (5) and McKinney and

[4] Indeed, evidence already exists (2, 3) that bicarbonate can accumulate in the proximal convoluted tubule during bicarbonate-free perfusion.

Burg (4) it is of interest to further consider the results obtained in hydropenic rats. These investigators measured total CO_2 concentrations (*a*) as a function of flow rate without bicarbonate being present in the tubular lumen, (*b*) as a function of flow rate when both the lumen and bath concentrations contained 25 mM bicarbonate, and (*c*) when flow was fixed at a low rate but with perfusate bicarbonate concentration of 25 or 0 mM. In our hydropenic rats, perfusion of the loop with bicarbonate-free saline resulted in a constant bicarbonate concentration of about 6 mM in the collected fluid over a wide range of flow, similar to the observations of Warnock and Burg (5). Furthermore, in our free-flow experiments, the concentrations were similar to those observed during loop perfusion despite the presence of large quantities of bicarbonate in the fluid entering the loop.[5] These observations, which are suggestive of steady-state bicarbonate concentrations, are again similar to data obtained by McKinney and Burg (4) who found comparable concentrations of total CO_2 during perfusion of proximal straight tubules both with bicarbonate-containing and bicarbonate-free perfusates. Thus, assuming that under free-flow conditions there is net removal of bicarbonate by the loop (11), and accepting that there is net addition of bicarbonate during perfusion as shown in these studies, the constant early distal bicarbonate concentrations are particularly noteworthy. Specifically, it is of interest that a net acidification process (free-flow) and a net alkalinization process (loop perfusion) both result in an early distal bicarbonate concentration of about 6 mM, which is independent of flow.

In these hydropenic rats, the fact that the bicarbonate concentration did not fall as flow rate increased over a wide range is worthy of further comment. In an attempt to explain this finding, we propose that an increase in the influx component and a decrease in the efflux component occur to maintain bicarbonate concentrations constant. Thus, by increasing the rate of perfusion, dilution of bicarbonate that has accumulated in the lumen at low flow rates will occur. This tendency for bicarbonate concentration to fall at higher flows, will be mitigated by further bicarbonate influx down a favorable concentration gradient. In addition, the concentration may also be maintained as flow rate increases because any tendency for bicarbonate concentration to fall will be associated with a steeper hydrogen ion gradient across the luminal membrane with less bicarbonate reabsorbed. That tubular flow rate can alter reabsorption of bicarbonate, sodium, and fluid has already been suggested in another context by Malnic and de Mello-Aires (22) and Imai et al. (23) for the proximal convoluted tubule.

What accounts for the fall in early distal bicarbonate concentration and the fixed accumulation rate in bicarbonate-loaded rats? We presume that the unidirectional influx rate for bicarbonate is at a near maximal level even at low flows because of the greater transtubular bicarbonate gradient and possibly because of enhanced passive ion flows caused by volume expansion (24). Indeed, the highest accumulation rates (about 140 peq/min) achieved by the hydropenic animals at maximal flow rates are already present at the lowest flows in these bicarbonate-loaded rats. Thus, we expect that the dilution of accumulated bicarbonate with increasing flows cannot elicit even greater influx rates, as is the case in the hydropenic rats, unless CO_2 gas diffusion can become a progressively more important source of new bicarbonate accumulation (*vide infra*).

In the foregoing considerations we have not made reference to the important report of Warnock and Rector (25) which draws attention to the different permeability characteristics of the pars recta in vitro to ionic bicarbonate or CO_2 gas diffusion. Accepting that CO_2 gas is extremely permeable, it is very likely that in the bicarbonate-loaded rats, perfusion at a high enough rate would result in bicarbonate accumulation whose magnitude is dependent primarily on CO_2 diffusion and intraluminal generation of bicarbonate rather than bicarbonate ion influx, per se. That is, at extremely high flow rates bicarbonate concentration may be independent of flow, with accumulation rates increasing progressively as ionic bicarbonate entry (caused by limited permeability) makes a minimal contribution to the total accumulation. In this regard, it is also worth recognizing that a portion of the accumulated bicarbonate which we measured in the hydropenic rats is also likely attributable to CO_2 diffusion. Accordingly, at perfusion rates much lower than we used, it is also possible that bicarbonate concentrations could increase as a result of a relatively greater contribution of bicarbonate ion influx. Indeed, Warnock and Burg (5) have already reported higher concentrations of bicarbonate at low flow rates during in vitro perfusion of the pars recta with bicarbonate-free perfusate. Thus, the flow-independence of bicarbonate concentrations that we demonstrated in hydropenic rats, as well as the fall in bicarbonate concentrations in bicarbonate-loaded rats, may not occur when flow rates are explored over the range of, say, from 4 to 150 nl/min.

In addition to the foregoing, it is worth emphasizing that volume expansion per se in the bicarbonate-loaded rats may modulate the response to flow rate of

[5] In our previous study (10) end proximal fluid of similarly treated hydropenic rats contained a mean $[HCO_3^-] = 12$ mM at a flow rate of 16 nl/min. Thus, it is most likely the entry rate of bicarbonate during free-flow greatly exceeded the early distal delivery rates (Table II) and was associated with a fall in concentration by about 50%.

early distal bicarbonate concentration. As already noted above, the enhanced passive ion flows induced by volume expansion, reported for *Necturus* proximal tubule (24), could also be present in our loop segments. Accordingly, it is conceivable that volume expansion without elevation of plasma bicarbonate concentration could be associated with characteristics of loop bicarbonate accumulation we have attributed to bicarbonate loading.

Thus, it is obvious that an assessment of the validity of the foregoing discussion awaits more investigation, despite the new insight already offered by the present data. Indeed, we envision two complementary groups of future experiments: those which are designed to explore the effects of different perfusion rates and different perfusate composition, and those which alter the physiologic state of the loop epithelium. Thus, it would be of interest to use perfusates that contain bicarbonate and other buffers in varying concentrations to alter $[H^+]$ and $[HCO_3^-]$ gradients over a wider flow range. Furthermore, it is of interest to explore the specific effects of volume expansion without an increase in bicarbonate concentration, the effects of an increased transtubular bicarbonate gradient without volume expansion, and the possible roles of elevated blood pH, and hypokalemia. Experimental approaches involving saline expansion, hypertonic bicarbonate loading, desoxycorticosterone acetate-induced metabolic alkalosis, and potassium depletion all seem pertinent and worthy of pursuit.

ACKNOWLEDGMENTS

It is a pleasure to acknowledge the expert secretarial assistance of Mrs. Phyllis Morrissey and the valuable suggestions of Dr. John Seely.

This work was supported by the Medical Research Council and the Ontario Heart Foundation.

REFERENCES

1. Rector, F. C., Jr. 1973. Acidification of the urine. *Handb. Physiol.* (Sect. 8, Renal Physiology). 431–454.
2. Bank, N., and H. S. Aynedjian. 1967. A microperfusion study of bicarbonate accumulation in the proximal tubule of the rat kidney. *J. Clin. Invest.* **46:** 95–102.
3. Burg, M., and N. Green. 1977. Bicarbonate transport by isolated perfused rabbit proximal convoluted tubules. *Am. J. Physiol.* **233**(4): F307–F314.
4. McKinney, T. D., and M. B. Burg. 1977. Bicarbonate and fluid absorption by renal proximal straight tubules. *Kidney Int.* **12:** 1–8.
5. Warnock, D. G., and M. B. Burg. 1977. Urinary acidification: CO_2 transport by the rabbit proximal straight tubule. *Am. J. Physiol.* **232**(1): F20–F25.
6. Malnic, G., and P. R. Steinmetz. 1976. Transport processes in urinary acidification. *Kidney Int.* **9:** 172–188.
7. Levine, D. Z. 1971. Effect of acute hypercapnia on proximal tubular water and bicarbonate reabsorption. *Am. J. Physiol.* **221:** 1164–1170.
8. Levine, D. Z., and L. A. Nash. 1973. Effect of chronic NH_4Cl acidosis on proximal tubular H_2O and HCO_3 reabsorption. *Am. J. Physiol.* **225:** 380–384.
9. Levine, D. Z., T. Walker, and L. A. Nash. 1973. Effects of KCl infusions on proximal tubular function in normal and potassium-depleted rats. *Kidney Int.* **4:** 318–325.
10. Levine, D. Z., L. A. Nash, T. Chan, and A. H. E. Dubrovskis. 1976. Proximal bicarbonate reabsorption during Ringer and albumin infusions in the rat. *J. Clin. Invest.* **57:** 1490–1497.
11. Malnic, G., M. de Mello Aires, and G. Giebisch. 1972. Micropuncture study of renal tubular hydrogen ion transport in the rat. *Am. J. Physiol.* **222:** 147–158.
12. Morgan, T., and R. W. Berliner. 1969. A study by continuous microperfusion of water and electrolyte movements in the loop of Henle and distal tubule of the rat. *Nephron.* **6:** 388–405.
13. Schnermann, J. 1968. Microperfusion study of single short loops of Henle in rat kidney. *Pfluegers Arch. Eur. J. Physiol.* **300:** 255–282.
14. Kunau, R. T., Jr., H. L. Webb, and S. C. Borman. 1974. Characteristics of the relationship between the flow rate of tubular fluid and potassium transport in the distal tubule of the rat. *J. Clin. Invest.* **54:** 1488–1495.
15. Wright, F. S. 1971. Increasing magnitude of electrical potential along the renal distal tubule. *Am. J. Physiol.* **220:** 624–638.
16. Levine, D. Z. 1972. Measurement of tubular fluid bicarbonate concentration by the cuvette-type glass micro pH electrode. *Yale J. Biol. Med.* **45:** 368–372.
17. Uhlich, E., C. A. Baldamus, and K. J. Ullrich. 1968. Verhalten von CO_2-druck und bicarbonat im gegenstromsystem des nierenmarks. *Pfluegers Arch. Eur. J. Physiol.* **303:** 31–48.
18. Raman, S., G. Mousseau, and D. Z. Levine. 1977. Statistical models for renal micropuncture studies. *Am. J. Physiol.* **233**(4): F349–353.
19. Wright, F. S. 1977. Sites and mechanisms of potassium transport along the renal tubule. *Kidney Int.* **11:** 415–432.
20. Karlmark, B. 1977. Renal tubular pCO_2. *In* Proceedings of the International Union of Physiological Sciences. **XII:** 206.
21. Dubose, T. D., Jr., L. R. Pucacco, D. W. Seldin, N. W. Carter, and J. P. Kokko. 1978. Direct determination of pCO_2 in the rat renal cortex. *In* Proceedings of the American Society of Nephrology. 103A. (Abstr.)
22. Malnic, G., and M. de Mello-Aires. 1971. Kinetic study of bicarbonate reabsorption in proximal tubule of the rat. *Am. J. Physiol.* **220:** 1759–1767.
23. Imai, M., D. W. Seldin, and J. P. Kokko. 1977. Effect of perfusion rate on the fluxes of water, sodium, chloride and urea across the proximal convoluted tubule. *Kidney Int.* **11:** 18–27.
24. Boulpaep, E. L. 1972. Permeability changes of the proximal tubule of *Necturus* during saline loading. *Am. J. Physiol.* **222**(3): 517–531.
25. Warnock, D. G., and F. C. Rector, Jr. 1977. CO_2 permeability of the rabbit proximal straight tubule. *Kidney Int.* **12:** 576A. (Abstr.)