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Research Article

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Proximal Tubular Bicarbonate Reabsorption and P_{CO_2} in Chronic Metabolic Alkalosis in the Rat

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ABSTRACT Studies were undertaken to define the pattern of proximal tubular bicarbonate reabsorption and its relation to tubular and capillary P_{CO_2} in rats with chronic metabolic alkalosis (CMA). CMA was induced by administering furosemide to rats ingesting a low electrolyte diet supplemented with $NaHCO_3$ and $KHCO_3$. Proximal tubular bicarbonate reabsorption and P_{CO_2} were measured in CMA rats either 4–7 or 11–14 d after furosemide injection, in order to study a wide range of filtered bicarbonate loads. A group of nine age-matched control animals, fed the same diet but not given furosemide, was studied for comparison. In a third group of controls, the filtered load of bicarbonate was varied over the same range as in the CMA rats by plasma infusion and aortic constriction. The CMA rats had significant alkalemia and hypokalemia (4–7 d: pH 7.58, HCO_3 38.3 meq/liter, K^+ 2.1 meq/liter; 11–14 d: pH 7.54, HCO_3 38.1 meq/liter, K^+ 2.5 meq/liter). Nonetheless, proximal bicarbonate reabsorption was not significantly different from that seen in control rats at any given load of filtered bicarbonate (from 250 to 1,300 pmol/min). In both control and CMA rats, 83–85% of the filtered bicarbonate was reabsorbed by the end of the accessible proximal tubule. These observations indicate that proximal bicarbonate reabsorption is determined primarily by the filtered load in chronic metabolic alkalosis. When single nephron glomerular filtration rate (SNGFR) is reduced by volume depletion in the early postfurosemide period, the filtered load and the rate of proximal bicarbonate reabsorption remain at or below control levels, maintaining metabolic alkalosis. In the late postfurosemide period, however, SNGFR returned to control levels in some instances. In these animals, both the filtered load and rate of proximal reabsorption were increased above the highest levels seen in control animals.

The P_{CO_2} gradient between the peritubular capillaries and arterial blood (Pc-Art) was significantly higher in CMA than in control, even though the rate of proximal bicarbonate reabsorption did not differ. Thus, proximal bicarbonate reabsorption did not appear to be the primary determinant of Pc-Art P_{CO_2}. P_{CO_2} in the early proximal (EP) tubule was significantly higher than in either the late proximal (LP) tubule or peritubular capillaries in both control and CMA rats. The EP-LP P_{CO_2} gradient correlated directly with proximal bicarbonate reabsorption ($P < 0.05$). The small elevation in P_{CO_2} in EP may be related to CO_2 generated at this site in the process of bicarbonate reabsorption.

INTRODUCTION

Chronic metabolic alkalosis (CMA)[1] is characterized by renal maintenance of a high plasma bicarbonate concentration, but the role of proximal tubular bicarbonate reabsorption in this process is unclear. Early micropuncture studies of chronic metabolic alkalosis in the rat suggested that proximal bicarbonate reabsorption is increased, but in these studies no measurements of single nephron glomerular filtration rate (SNGFR) or the absolute rate of bicarbonate reab-

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[1] *Abbreviations used in this paper:* APR, absolute proximal reabsorptive rate; B_0, initial Hct; CMA, chronic metabolic alkalosis; EP, early proximal; FF, filtration fraction; FL, filtered load; FR, fractional proximal reabsorptive rate; GFR, whole kidney glomerular filtration rate; Hct, hematocrit; LP, late proximal; Pc-Art, peritubular capillary-arterial; RBF, renal blood flow; SNGFR, single nephron GFR.

sorption in the proximal tubule were carried out (1–3). By contrast, a recent study in rats with chronic metabolic alkalosis induced by deoxycorticosterone acetate and sodium sulfate administration has provided evidence that the renal contribution to the maintenance of metabolic alkalosis is primarily due to a reduction in SNGFR; absolute proximal bicarbonate reabsorption was unchanged from control (4). In the present study, metabolic alkalosis was induced in the rat by furosemide and sodium bicarbonate administration. To examine further whether a reduction in SNGFR is critical for maintenance of the alkalosis, rats were studied over a variable period of time after recovery from furosemide treatment to assess proximal bicarbonate reabsorption across a wide range of SNGFR values. In addition, this study provides, for the first time, measurements of both proximal bicarbonate reabsorption and tubular and capillary P_{CO_2} in the same animal. We used these data to examine whether cortical P_{CO_2} is influenced notably by the rate of proximal bicarbonate reabsorption (5).

METHODS

All experiments were carried out with male Sprague-Dawley rats (Charles River Breeding Laboratories, Inc., Wilmington, MA). The animals were maintained for 1–2 wk on a chloride-free diet (18% casein, 70.7% sucrose, and 5% fat, plus all essential vitamins and minerals from ICN Nutritional Biochemicals, Cleveland, OH) supplemented with 2.0 mmol $NaHCO_3$ and 0.5 mmol $KHCO_3$/12 g. One group of nine rats served as controls, receiving the diet plus water to drink ad lib. an average of 10 d until the morning of study. The control rats gained an average of 4.9±0.4 g/d (mean±SE) on the diet and had a mean weight at the time of micropuncture of 267±5 g. CMA was induced in a second group of 15 rats. These animals received 75 mM $NaHCO_3$ as a drinking solution in place of water. 2 d after starting the diet and drinking solution, the rats received three intraperitoneal injections of furosemide (10 mg/kg body wt) at 12-h intervals. Eight rats were studied 4–7 d after receiving furosemide, while seven were studied 11–14 d after furosemide administration. In an effort to have all rats at the same age and weight at the time of micropuncture, we used younger rats for the 11–14-d CMA studies than those for the 4–7-d CMA studies. At the time of initiation of the furosemide injections, the 11–14-d CMA rats weighed 214±7 g, while the 4–7-d CMA rats weighed 243±8 g ($P < 0.025$). The rats lost an average of 4.2±0.7% body wt during the first 24 h after the start of these injections. At the time of micropuncture, the 4–7-d rats had not yet recovered from the weight loss and were at or below their preinjection weight, averaging 238±4 g. The 11–14-d rats, however, were back to a normal growth pattern and weighed an average of 254±5 g at the time of study. In all rats with metabolic alkalosis, the sodium bicarbonate drinking solution was removed and replaced with water 24 h before study.

General techniques. On the morning of study, the rats were anesthetized with Inactin (100–120 mg/kg body wt) and placed on a heated table to maintain a constant body temperature of 37°C. A catheter was inserted into the left femoral artery for determination of the initial hematocrit (designated B_0), previously shown to equal that in the calm, awake rat (6, 7). This arterial catheter was subsequently used for periodic blood sampling and measurement of mean arterial pressure. Surgical preparation for micropuncture was then completed. The kidney was bathed continuously with warm mineral oil (37°C) bubbled with 7% CO_2. The P_{CO_2} of the oil at the kidney surface averaged 53±3 mmHg. During surgery, all rats received 1.3% body wt isooncotic rat plasma over 1 h to maintain arterial hematocrit (Hct) and hence plasma volume constant (6, 7). The plasma infused was obtained on the day of micropuncture from a donor rat maintained on the same dietary and pharmacologic regimen. After completion of surgery, the plasma infusion was reduced to 6–12 μl/min for the remainder of the experiment, and the rate adjusted as needed to maintain Hct at the B_0 level. The animals were maintained on a small animal ventilator during the course of the experiment, and O_2 added to the inspired air at a rate sufficient to maintain $P_{a_{O_2}}$ at ~100 mmHg (mean, 98±4 mmHg). Arterial P_{CO_2} was monitored frequently and did not vary by >5 mmHg during the course of an experimental period. 45–60 min before micropuncture measurements, the rats received a bolus injection of [^{14}C]inulin (40 μCi) in 5% dextrose in water and then were infused with [^{14}C]inulin at a rate of 60 μCi (in 0.84 ml)/h in the same diluent. After this equilibration period, two to four timed collections of urine were obtained from the left kidney for determination of flow rate; [^{14}C]inulin activity; and sodium, potassium, and total CO_2 excretion rates. Concurrently, samples of femoral arterial blood were obtained at 30- to 60-min intervals for measurement of Hct, plasma [^{14}C]inulin activity, arterial pH, $P_{a_{O_2}}$ and $P_{a_{CO_2}}$, and plasma sodium and potassium concentrations. Two to four samples of renal venous blood were obtained with a sharpened pipette with a 40-μm tip diameter for determination of renal venous [^{14}C]inulin activity and calculation of whole kidney filtration fraction (FF) and renal blood flow (RBF). Whole blood, obtained on the morning of the study from a rat previously maintained on the same diet as the experimental rat, was injected after each blood sampling to replace all blood withdrawn for analytical purposes.

Micropuncture methodology. The last loops of proximal tubules were identified by their characteristic appearance adjacent to surface efferent arteriolar star vessels. The loops were confirmed as being the last accessible proximal convolutions by the failure of a small injected oil droplet to reappear in any subsequent proximal loop on the surface of the kidney. Timed collections (5–20 min in duration) were made from two to four end-proximal tubules with sharpened glass pipettes with an outside tip diameter of 9–12 μm, after injection of an oil block. Unless the flow into the pipette was such that no suction was necessary (pipette open to atmospheric pressure) to maintain the oil block in position, the sample was discarded. The oil block was checked periodically to ascertain that it was mobile.

P_{CO_2} measurements. The P_{CO_2} microelectrodes used in these studies were constructed as described previously (8), and had outer tip diameters of 4–10 μm. The electrodes were calibrated by immersing the tip sequentially in water bubbled with CO_2-air mixtures containing ~4, 8, and 15% CO_2, respectively. The precise percentage of CO_2 in the gas mixtures was determined by Scholander analysis (9), and the P_{CO_2} in each mixture was calculated daily from the measured barometric pressure. The CO_2 standard solutions were maintained at 37°C in a glass equilibration chamber. The electrode tip was immersed in a pH 4 buffer solution before and after each calibration to assure that the tip remained impermeable to hydrogen ions, and to bring the P_{CO_2} in the

microelectrode below the lowest calibration standard. All electrodes used had a slope of 56 mV/log P_{CO_2} or greater. The average slope of the 19 P_{CO_2} electrodes used in this study was 61.0±0.7 mV/log P_{CO_2}.

The electrical potential developed in the microelectrode was measured on a Keithley electrometer (model 600B; Keithley Instruments, Inc., Cleveland, OH) and read from a chart recorder set at 50 mV full scale (1 mV = 5 mm). Readings were accepted only if they were stable (varied by <0.5 mV with no evidence of trend) for at least 2 min. The microelectrodes were calibrated before and after each set of tubule and capillary punctures. The time interval between calibrations averaged 44 min, but was no longer than 60 min in any experiment. No electrode drifted by >2.6 mV/h; the average drift (positive or negative) was 0.7±0.2 mV/h. The calibration curves were plotted as a function of time and P_{CO_2} was calculated from the millivolt reading obtained for each puncture by linear interpolation between the calibration curves bracketing the puncture in time.

The ability of our microelectrode system to detect small differences in P_{CO_2}, with the recorder set at 1 mV = 5 mm, was tested by using it to measure P_{CO_2} in three solutions equilibrated with known gas mixtures (P_{CO_2} values of 30.9, 32.4, and 35.9 mmHg as determined by Scholander analysis). The electrode was immersed alternately in the three solutions and the cycle repeated five times. Between immersions the electrode was exposed to air long enough to decrease the P_{CO_2} below the lowest standard. The results, shown in Fig. 1, demonstrate that the microelectrode can readily distinguish differences in P_{CO_2} at least as small as 1.5 mmHg at this recorder setting.

Early proximal (EP) and late proximal (LP) tubular segments were identified by the time of appearance of lissamine green dye after an intravenous bolus injection. Second- and third-order peritubular capillaries adjacent to the EP segments were also identified and mapped. The LP segment was always punctured before the EP segment. In some instances, the peritubular capillaries were punctured before the tubules and in others the tubules were punctured first. After the P_{CO_2} measurements were obtained, the EP site was injected with mineral oil stained with Sudan black to determine whether the late segment was part of the same tubule. Approximately half of the tubule segments were from the same nephron, while the other half were adjacent nephrons. No differences were noted in P_{CO_2} gradients when measurements were made in the same tubule or in adjacent tubules, and therefore the data for each animal were pooled. The order of micropuncture determinations (P_{CO_2} vs. tubular fluid collection) was varied from animal to animal.

Plasma expansion studies. In a separate group of five rats, we studied the influence of changes in the filtered load of bicarbonate induced by changes in SNGFR on proximal bicarbonate reabsorption. The animals were maintained on the same diet as the previous group, with the exception that the sodium supplement (2 mmol/12 g) was given with chloride rather than bicarbonate, and 1 mmol KCl/12 g was

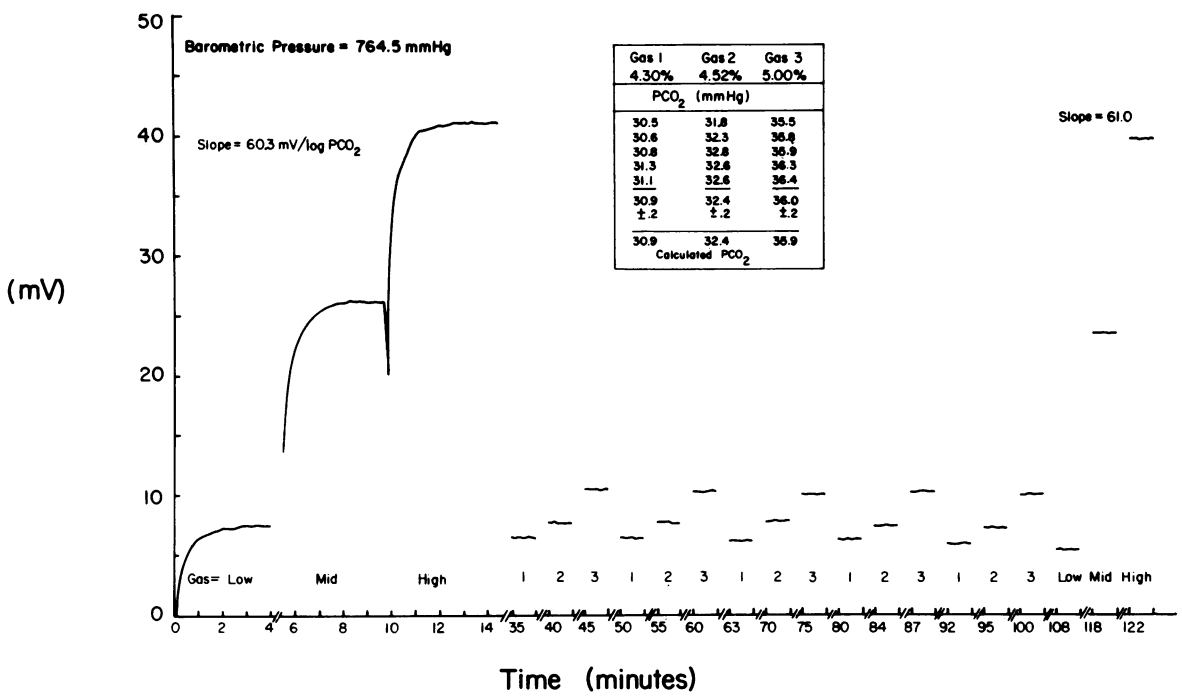

FIGURE 1 Tracing of P_{CO_2} microelectrode measurements of three gases (*1*, *2*, and *3*) of known CO_2 content. Time in minutes is shown on the abscissa, and the reading in millivolts on the ordinate. The percentage of CO_2 for *gas 1* (4.30%), *gas 2* (4.52%), and *gas 3* (5.00%) was determined by Scholander analysis and the P_{CO_2} calculated from the barometric pressure. Experimental readings are bracketed by readings in three standards (*low*, 4.30%; *mid*, 8.54%; and *high*, 15.50%). Measured P_{CO_2} values are shown in the box together with values calculated from Scholander analysis.

substituted for $KHCO_3$. These rats were prepared for micropuncture as described above, with the exception that plasma replacement was not given during surgery. In these animals, no P_{CO_2} measurements were carried out, and the [^{14}C]inulin was infused in Ringer's solution. The initial set of proximal tubular collections were carried out 45 min to 1 h after initiation of the [^{14}C]inulin infusion (termed the hydropenia period). Plasma expansion was then induced by infusion of 5% body wt of isooncotic plasma from a littermate donor at a rate of 200 μl/min, followed by a maintenance infusion at 40 μl/min. To maintain plasma potassium concentrations constant, 60 meq/liter of KCl was added to the plasma infusion (10). 30 min after beginning the maintenance infusion, samples were obtained from new late proximal tubule sites. GFR was then reduced acutely by partial aortic constriction to lower renal perfusion pressure to 75–85 mmHg, and additional tubular fluid samples were obtained. Arterial blood and urine samples were obtained as described above for the other protocols.

Analytical techniques. The volume of the collected tubule fluid was determined by injecting it, under oil, into constant bore glass capillary tubing with a known volume per length and measuring the length occupied. A 15-nl aliquot was taken for measurement of total CO_2 concentration and the remaining sample volume measured and added to scintillation fluid (11) for measurement of ^{14}C activity. Measurements of total CO_2 concentrations in tubule fluid and urine were performed by microcalorimetry. Urine and plasma sodium and potassium concentrations were measured by flame photometry. Arterial pH, Pa_{CO_2}, and Pa_{O_2} were measured with a Radiometer blood gas system (Radiometer America Inc., Westlake OH). Plasma inulin concentration was corrected for serum protein content (either determined directly by refractometry or, when not obtained, estimated to be 5 g/dl).

Calculations. SNGFR and whole kidney GFR were estimated from the single nephron and whole kidney inulin clearances, respectively. Absolute proximal water reabsorption (APR_{H_2O}) was calculated as the difference between SNGFR and end-proximal flow rates. Bowman's space bicarbonate concentration was calculated from the plasma bicarbonate concentration, determined from pH and Pa_{CO_2}, multiplied by 1.05 (10). In previous studies of proximal bicarbonate reabsorption using microcalorimetry, Bowman's space and tubular fluid samples were collected under oil equilibrated with a Hepes buffer solution equilibrated with CO_2 to achieve a P_{CO_2} of 60–70 mmHg (10). Our assumption at that time was that by maintaining the tubular fluid under CO_2 we would maintain the P_{CO_2} in a physiologic range and preserve the total CO_2 content. To test the validity of this assumption in the present study, P_{CO_2} was measured in a 10 mM $NaHCO_3$ solution continuously bathed with oil equilibrated with CO_2 and the measurement repeated immediately after transfer of a small aliquot to a constant bore capillary. The transferred sample (~50 nl) was collected in the constant bore capillary tube between CO_2 equilibrated oil drops without exposure to air, and the microelectrode was inserted quickly into the sample. The P_{CO_2} measured both in the oil and in the samples in the capillary tubes fell uniformly to immeasurable levels within 1–2 min of sampling. Thus the total CO_2 content of these samples, even when collected under CO_2-equilibrated oil and measured as quickly as possible, was essentially all bicarbonate. Despite the rapid decline in P_{CO_2}, the total CO_2 concentration remaining in either these $NaHCO_3$ samples or in tubular fluid samples was very stable; repeated analyses of the total CO_2 content of samples stored under oil for >7 wk (refrigerated) revealed no significant downward trend. In the present study, we set bicarbonate concentration equal to total CO_2 concentration in tubular fluid samples.

Absolute proximal bicarbonate reabsorption (APR_{HCO_3}) was calculated as the difference between the filtered bicarbonate (SNGFR × Bowman's space [HCO_3]) and the bicarbonate delivered out of the proximal tubule (end-proximal flow rate times end-proximal [HCO_3]). Fractional proximal reabsorption of water (FR_{H_2O}) or bicarbonate (FR_{HCO_3}) was calculated as the absolute reabsorption divided by the SNGFR or filtered load of bicarbonate, respectively. Statistical significance was assessed with the paired *t* test for results obtained in the same animal or unpaired analysis of variance for comparison among groups.

RESULTS

General. Table I presents the Hct, arterial pressures, arterial acid-base composition, and arterial sodium and potassium concentrations in the control animals, the animals studied 4–7 d after induction of metabolic alkalosis (4–7-d CMA), and the animals studied 11–14 d after induction of CMA (11–14-d CMA). Hct during the micropuncture periods was maintained at B_0 level in all three groups. Both groups of rats with metabolic alkalosis had higher pH and bicarbonate concentrations and lower plasma potassium concentration than the control animals ($P < 0.001$). Plasma potassium concentration was slightly lower in the 4–7-d rats than in the 11–14-d rats (2.1 vs. 2.5 meq/liter, $P < 0.05$). Blood pressure in the 4–7-d CMA animals was lower than control ($P < 0.05$).

Table II presents the clearance data and electrolyte excretion by the micropunctured kidney in the three groups. GFR and RBF in the micropunctured kidney were significantly reduced in the 4–7-d CMA animals as compared with control, and FF was similar in all three groups. Potassium excretion was reduced in both metabolic alkalosis groups as compared with control ($P < 0.01$).[2] Total CO_2 excretion was reduced in the 4–7-d CMA group as compared with controls. In all groups, >99.8% of the filtered bicarbonate was reabsorbed.

Proximal tubular observations. Table III summarizes the micropuncture measurements in the three groups. SNGFR and APR_{H_2O} were lower in the 4–7-d

[2] The euvolemic control rats of the present study were excreting sodium and potassium at rates lower than those reported previously for euvolemic Munich-Wistar rats fed a chow diet ad lib. (10). Two factors can account for this: (*a*) the rats of the present study were infused with 5% dextrose in water rather than Ringer's solution during the experiment, and (*b*) in the current study, the diet contained comparable amounts of sodium but only 0.5 meq potassium/12 g compared with 1.8 meq/12 g found in Purina rat chow.

TABLE I
Arterial Blood Composition

	Hct		AP						
	B_0	E	B_0	E	pH	P_{CO_2}	$[HCO_3]$	$[Na^+]$	$[K^+]$
	vol %		*mmHg*			*mmHg*		*mM*	
Control (9)	47±1	47±1	124±4	119±3	7.47±0.01	42.9±0.8	30.0±0.7	143±2	3.9±0.2
CMA*									
4-7-d CMA (8)	49±1	50±1	108±7‡	104±2§	7.58±0.02‖	42.2±1.4	38.3±0.8‖	143±1	2.1±0.1‖
11-14-d CMA (7)	47±1	47±1	112±6	112±3	7.54±0.01‖¶	45.6±0.9¶	38.1±1.3‖	142±1	2.5±0.1‖¶

Values shown are means±SE. AP, arterial blood pressure; B_0, values obtained immediately after anesthesia and insertion of femoral artery catheter; E, values obtained during micropuncture. Numbers in parentheses, numbers of rats in this and all subsequent tables.
* Subgroups defined in Results.
‡ $P < 0.05$ compared with control.
§ $P < 0.01$ compared with control.
‖ $P < 0.001$ compared with control.
¶ $P < 0.05$ compared with 4-7-d CMA.
All other comparisons are not significant (NS, $P > 0.05$)

CMA rats than control, and LP bicarbonate concentration was higher in both CMA groups as compared with control. In Fig. 2, we plotted APR_{HCO_3} against the filtered load (FL_{HCO_3}) for each animal studied. As can be seen, five CMA rats had FL_{HCO_3} values well below the lowest value seen in the control animals, owing to a markedly lower SNGFR, and these CMA rats had a correspondingly lower APR_{HCO_3}. On the other hand, five CMA rats had FL_{HCO_3} values that were well above the range seen in control animals. In these animals, the SNGFR was at control levels and the higher FL_{HCO_3} was due to the increase in plasma bicarbonate concentration. APR_{HCO_3} was notably higher than in control in these CMA rats.

To determine further whether the relationship between FL_{HCO_3} and APR_{HCO_3} was altered in CMA rats, FL_{HCO_3} was varied over a comparable range in an additional group of five control rats by varying SNGFR over a wide range. Plasma composition and single nephron data in each of the three experimental periods (hydropenia, plasma expansion, and plasma expansion plus aortic constriction) in these rats are summarized in Table IV. SNGFR increased significantly from 19.7 to 31.7 nl/min after plasma expansion, and fell to 17.6

TABLE II
Whole Kidney Clearances and Excretion Rates

Group	GFR	RBF	FF	Sodium excretion	Potassium excretion	Total CO_2 excretion	Transit time
	ml/min				*nmol/min*		*s*
Control (9)	1.16±0.05	9.38±0.93*	0.24±0.02*	173±35	403±58	77±27	8.8±0.6
CMA							
4-7-d CMA (8)	0.73±0.13‡	6.38±0.95§	0.23±0.02	101±30	133±31‖	4±2¶	18.0±3.3‡
11-14-d CMA (7)	1.02±0.12	8.77±0.91	0.24±0.03	195±128	159±31‡	20±13	12.1±1.8

Values shown are means±SE.
* RBF and FF measured in eight animals.
‡ $P < 0.01$ compared with control.
§ $P < 0.05$ compared with control.
‖ $P < 0.001$ compared with control.
¶ $P < 0.025$ compared with control.
All other comparisons are not significant.

TABLE III
Single Nephron Function and Proximal Bicarbonate Reabsorption

Group	SNGFR	APR_{H_2O}	FR_{H_2O}	FL_{HCO_3}	APR_{HCO_3}	FR_{HCO_3}	$TF[HCO_3^-]$
	nl/min			*peq/min*			*mM*
Control (9)	27.1±1.0	15.7±0.6	0.587±0.033	853±33	727±30	0.854±0.023	11.0±1.2
CMA							
4–7-d CMA (7)	17.2±2.5*	11.3±1.2‡	0.691±0.045	704±106	579±73	0.850±0.034	18.9±2.1*
11–14-d CMA (7)	23.9±3.3	14.1±1.5	0.626±0.048	948±157	761±105	0.828±0.028	18.2±2.3‡

Values shown are means±SE. $TF[HCO_3^-]$, end-proximal tubule fluid bicarbonate concentration.
* $P < 0.01$ compared with control.
‡ $P < 0.025$ compared with control.
All other comparisons are not significant.

nl/min with aortic constriction.[3] These animals had normal plasma acid-base and potassium values throughout all periods of study. APR_{HCO_3} is also plotted against FL_{HCO_3} for each of these rats in Fig. 2. As can be seen, no difference was noted in the relationship of APR_{HCO_3} to FL_{HCO_3} between these animals and the CMA animals. Over a comparable range of FL_{HCO_3} values, APR_{HCO_3} increased in a similar fashion in response to an increase in FL_{HCO_3}. Mean fractional HCO_3 reabsorption was 0.87, 0.86, and 0.86, respectively, in the three experimental periods in these five additional rats. These values were not significantly different from those found in the control animals (0.85, Table III), the 4–7-d CMA rats (0.85), or the 11–14-d CMA rats (0.83).

Tubular and capillary P_{CO_2} measurements. Tubular and capillary P_{CO_2} measurements were carried out in eight of the control animals, five of the 4–7-d CMA animals, and six of the 11–14-d CMA animals (Table V). The P_{CO_2} gradients between the surface tubules or peritubular capillaries and systemic arterial blood were higher in both metabolic alkalosis groups than in the control animals. In the 4–7-d CMA rats, the tubule and capillary-arterial P_{CO_2} gradients were twice as large as in the control animals.

In both the control and 11–14-d CMA animals, a significant P_{CO_2} gradient was found between the EP and LP tubule (1.2 mmHg, $P < 0.02$, and 2.4 mmHg, $P < 0.001$), and between the EP tubule and the adjacent peritubular capillary (2.1 mmHg, $P < 0.01$ and 2.8 mmHg, $P < 0.01$). In Fig. 3, we plotted the EP-LP P_{CO_2} gradient against APR_{HCO_3} for each animal studied. As can be seen, a significant correlation was found between the magnitude of the EP-LP gradient and the rate of proximal bicarbonate reabsorption.

DISCUSSION

The present studies have defined the pattern of proximal bicarbonate reabsorption and tubular and capillary P_{CO_2} in a model of CMA in the rat induced by furosemide administration. Our discussion will focus first on the pattern of bicarbonate reabsorption, and then examine the relationship between bicarbonate reabsorption and renal cortical P_{CO_2}.

The results of the present study indicate that the relation between bicarbonate delivery and reabsorption in the proximal tubule is not altered by chronic metabolic alkalosis. As shown in Fig. 2, for any given delivery rate, APR_{HCO_3} was not different from control rats in which bicarbonate delivery was systematically varied over a comparable range. Bicarbonate delivery was altered by changes in SNGFR in the control animals, and by spontaneous variations in both SNGFR and bicarbonate concentration in the rats with metabolic alkalosis. Over the range of deliveries studied, APR_{HCO_3} appears to be indifferent to whether delivery is altered by the changes in concentration or by flow. When SNGFR is varied acutely in normal rats, the parallel changes in bicarbonate reabsorption have been shown to correlate directly with the mean bicarbonate concentration along the tubule (log mean $[HCO_3]$) (10). From these observations, it was proposed that the delivery-related increase in reabsorption was due primarily to exposure of a greater fraction of the tubule to high intraluminal bicarbonate concentrations, thus

[3] SNGFR values in these Charles River Sprague-Dawley rats are lower than has been reported for the Munich-Wistar rat (7, 10, 12, 13). This difference is not related to differences between laboratories. We have measured SNGFR in euvolemic Munich-Wistar rats and found the mean value (40.9±2.5 nl/min, $n = 9$) to be similar to values reported previously. Because superficial glomeruli are not accessible in the Charles River Sprague-Dawley rat, we cannot determine the cause for the lower SNGFR in this strain.

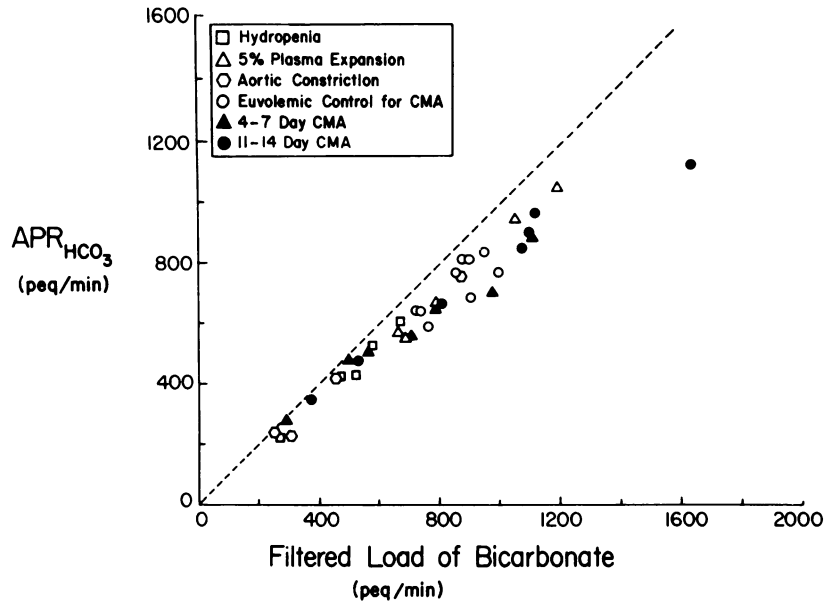

FIGURE 2 Relationship between APR_{HCO_3} and the filtered load of bicarbonate. Each point represents the mean of several measurements obtained from each animal. Open symbols represent rats without metabolic alkalosis, and filled symbols represent values from rats with CMA.

facilitating transport. More recent in situ microperfusion studies in the rat, however, have provided evidence that flow and concentration are two separate factors that influence bicarbonate reabsorption (14). The present results also appear to demonstrate these two influences on APR_{HCO_3}. When APR_{HCO_3} is plotted against SNGFR (Fig. 4), separate lines emerge for the control and CMA rats, indicating that for any given SNGFR, APR_{HCO_3} is higher in metabolic alkalosis than in control ($P < 0.01$ by covariance analysis). The increase in reabsorption per unit flow in metabolic alkalosis most likely reflects the contribution of luminal bicarbonate concentration to bicarbonate reabsorption. Log mean [(HCO_3)] was significantly higher in metabolic alkalosis than in control (27.8±1.2 vs. 19.3±1.0 mM, respectively; $P < 0.001$). Moreover, regression analysis of APR_{HCO_3} against both SNGFR and log mean [HCO_3] indicates a significant partial correlation with each of these factors, $r = 0.951$ and 0.728, respectively ($P < 0.01$ for each). Fig. 5 depicts the separate influences of SNGFR and log mean [HCO_3] on APR_{HCO_3}. In this figure, we subdivided the rats into groups according to SNGFR. As can be seen, APR_{HCO_3} rises within each group when log mean [HCO_3] is increased (from control values to those seen in metabolic alkalosis). These observations indicate

TABLE IV
Plasma Composition and Renal Function in Normokalemic Sprague-Dawley Rats

	Hydropenia	5% plasma expansion	5% Plasma expansion plus aortic constriction
n	5	5	4
Hct*, *vol %*	54.7±0.4	40.3±0.6	42.0±1.2
pH	7.38±0.02	7.42±0.01	7.41±0.01
P_{CO_2}, *mmHg*	41.9±1.2	42.1±0.9	41.2±0.5
Plasma [HCO_2], *mM*	24.3±0.5	26.2±0.5	25.3±0.8
Plasma [K], *mM*	4.8±0.1	5.0±0.2	5.3±0.3
SNGFR, *nl/min*	19.7±2.8	31.7±3.7	17.6±5.0
APR_{H_2O}, *nl/min*	10.8±2.0	17.7±2.0	10.3±1.6
APR_{HCO_3}, *pmol/min*	437±64	760±102	408±124

* B_0 Hct = 46.9±0.5. Body weight averaged 256±6 g.

TABLE V
Tubular and Capillary P_{CO_2}

	P_{CO_2}			ΔP_{CO_2}				
	EP	LP	Pc	EP-Art	LP-Art	Pc-Art	EP-LP	EP-Pc
				mmHg				
Control (8)	52.6±2.0	51.2±2.0	50.0±2.6	9.5±1.6	8.1±1.2	7.0±1.5	1.2±0.4	2.1±0.4
CMA								
4-7-d CMA (5)	59.1±2.7	58.6±2.6*	57.4±2.3	18.2±2.2‡	17.3±2.1§	16.6±2.2‡	0.5±0.5	1.8±0.8
11-14-d CMA (6)	59.7±1.6*	57.3±2.1	56.9±2.0	14.5±0.6*	12.1±0.8‖	11.7±0.8*	2.4±0.5‖	2.8±0.9

Values shown are means±SE. Pc, second- or third-order peritubular capillary adjacent to EP; Art, systemic arterial blood; ΔP_{CO_2}, gradient in P_{CO_2} between indicated structures.
* $P < 0.05$ compared with control.
‡ $P < 0.005$ compared with control.
§ $P < 0.001$ compared with control.
‖ $P < 0.05$ compared with 4-7-d-CMA.
All other comparisons are not significant.

that APR_{HCO_3} varies in direct relation to variations in either SNGFR or log mean $[HCO_3]$.

Cogan and Liu (4) have recently presented observations that the relationship between log mean $[HCO_3]$ and APR_{HCO_3} is disrupted in rats with chronic metabolic alkalosis; in their study, log mean $[HCO_3]$ was increased without any increase in APR_{HCO_3}. It is of interest that without accounting for variations in SNGFR, our results are identical to theirs. Log mean $[HCO_3]$ is higher than control in the CMA rats and mean APR_{HCO_3} in the CMA rats is not significantly different from control (670±66 and 727±30 peq/min). However, when variations in SNGFR are accounted for, the correlation of APR_{HCO_3} with log mean $[HCO_3]$ is clearly apparent (Fig. 5). Whether a similar relationship was obscured by variations in SNGFR in the work of Cogan and Liu (4) cannot be ascertained from the data provided.

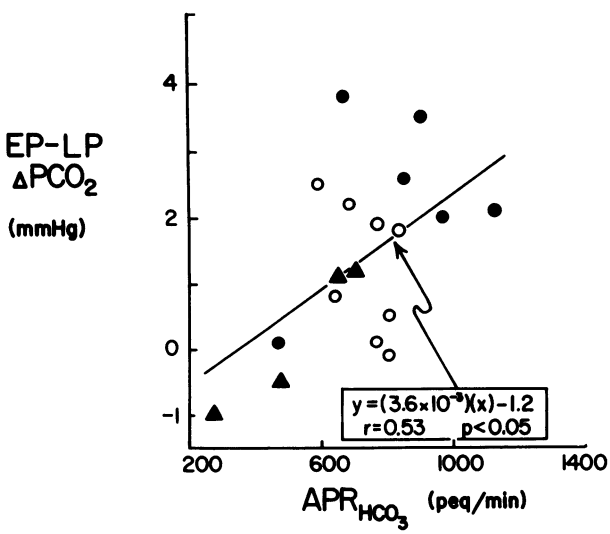

FIGURE 3 EP-LP P_{CO_2} gradient plotted against APR_{HCO_3} for each animal studied. Open circles denote values obtained in control animals; filled circles denote values in 11-14-d CMA rats, and filled triangles denote values in 4-7-d CMA rats.

The present observations do not provide any insight into the mechanisms by which changes in flow influence APR_{HCO_3}. It is still conceivable that this effect operates through some change in the bicarbonate concentration along the tubule. Log mean $[HCO_3]$ may not be a reflection of the true average bicarbonate concentration along the nephron, particularly at low flow rates when the luminal concentration may drop to its minimal value very early in the tubule. The in situ microperfusion studies of Chan and co-workers (14), however, make this possibility unlikely, because a very short segment of tubule was perfused and the collected $[HCO_3]$ was higher than the limiting gradient at all perfusion rates studied. In their study, therefore, it seems probable that the calculated log mean $[HCO_3]$ was a very close approximation of the true bicarbonate concentration, and under these conditions they showed a clear effect of perfusion rate on APR_{HCO_3} without a change in log mean $[HCO_3]$.

Given the relationship between delivery and reabsorption in CMA shown in Fig. 2, the high plasma bicarbonate concentration in these animals could be maintained by one of two mechanisms. Either SNGFR is reduced, maintaining delivery and therefore reabsorption of bicarbonate at or below control levels, or in the absence of a reduction in SNGFR, both delivery

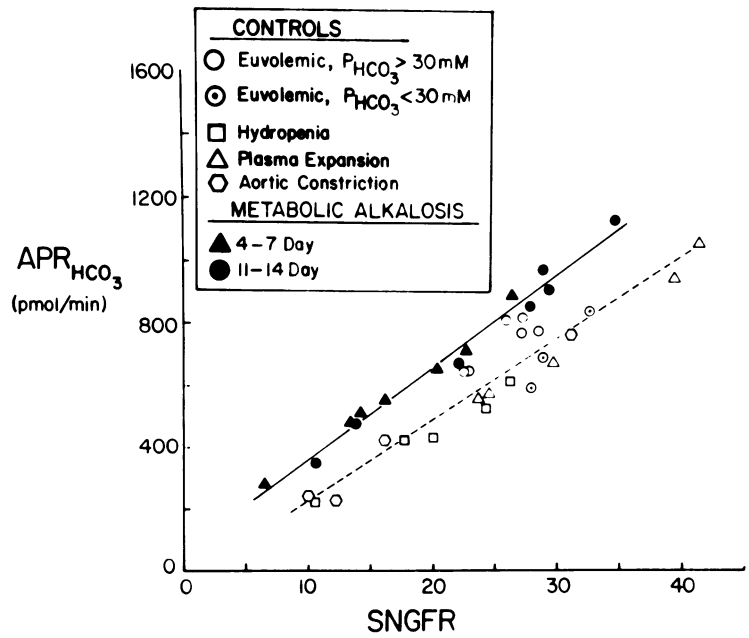

FIGURE 4 Relationship between SNGFR and APR_{HCO_3} in control rats (open symbols and dotted line) and in rats with CMA (filled symbols and solid line). The lines drawn through the data points were obtained by least-squares analysis. The two lines have similar slopes, but significantly different intercepts ($P < 0.01$) by covariance analysis.

and reabsorption are increased above control. The recent studies of Cogan and Liu (4) cited earlier have provided evidence suggesting that a reduction in SNGFR is critical. In their study, metabolic alkalosis was induced by desoxycorticosterone and sodium sulfate administration and SNGFR was reduced to ~50% of control values. As a result, bicarbonate delivery and reabsorption in the proximal tubule were unchanged from control. It should be noted, however, that the control animals received a different experimental pretreatment than did the rats with metabolic alkalosis.[4]

Whether this difference in treatment magnified the difference in SNGFR is uncertain. As noted earlier, the present results indicate that APR_{HCO_3} is higher for any given SNGFR in metabolic alkalosis than in controls (Fig. 4). This relationship appears to hold even for rats with SNGFR values in the control range. Although these observations suggest that metabolic alkalosis may, in some instances, be sustained by a delivery-dependent increase in APR_{HCO_3}, this question remains unresolved. On the other hand, it is clear from the present study and the previous work discussed above that when SNGFR is reduced, metabolic alkalosis is maintained by continued reabsorption of bicarbonate by the proximal tubule in a fashion not different from control.

It should be emphasized that this pattern of proximal bicarbonate reabsorption differs notably from that seen during acute metabolic alkalosis induced by alkali infusion. In the latter instance, micropuncture and microperfusion studies have demonstrated that proximal bicarbonate reabsorption is depressed (4, 15). In addition, the fraction of bicarbonate reabsorbed in the proximal tubule is depressed after acute isometric extracellular fluid or plasma volume expansion in rats with CMA, an effect attributed to the increase in SNGFR induced by this maneuver (4). The relevance of these acute observations to CMA is unclear. Our

[4] The metabolic alkalosis rats in the study of Cogan and Liu (4) were compared with a group of control rats studied at an earlier time by Cogan et al. (10). Unlike the rats with metabolic alkalosis, the control rats were not fasted for 24 h before micropuncture, a factor that may have influenced SNGFR. In this regard, it is of interest that they report values for GFR per kidney in awake fed rats with metabolic alkalosis that are twice as great as the starved rats they studied by micropuncture, whereas their values for fed awake control rats are nearly identical to those reported for fed micropuncture control rats (10). Finally, the values for SNGFR, APR_{HCO_3}, etc. reported for the control rats (10) were all corrected for kidney weight because of marked variation in body weight (from 191 to 321 g), whereas no correction was made in the rats with CMA. Given these differences, it is difficult to assess specific comparisons between their control and CMA rats.

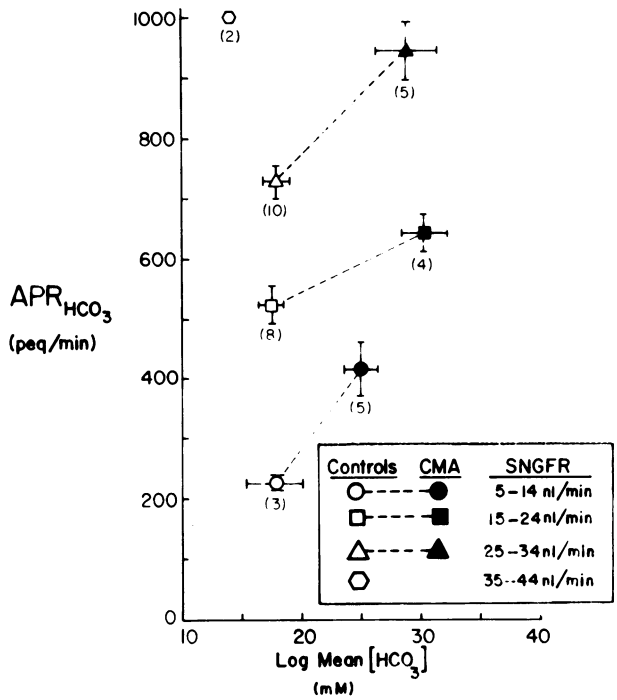

FIGURE 5 Separate effects of SNGFR and log mean luminal bicarbonate concentration (log mean [HCO_3]) on APR_{HCO_3}. Control rats (open symbols) and rats with CMA (filled symbols) were subdivided into groups according to SNGFR values. For each subdivision, APR_{HCO_3} is plotted against log mean [HCO_3]. The numbers in parentheses in the graph indicate the number of rats in each subdivision. As can be seen, at any given level of SNGFR, APR_{HCO_3} is higher in the CMA rats than control, and these rats have a higher log mean [HCO_3]. At the same time for any given log mean [HCO_3], APR_{HCO_3} is higher in the rats with higher SNGFR values, both for control and CMA rats.

results and those discussed earlier (4) show no evidence that proximal bicarbonate reabsorption is depressed in CMA.

The hypokalemia associated with metabolic alkalosis has been implicated as a factor stimulating proximal bicarbonate reabsorption (1–3, 14). The present results, however, provide little evidence to support this contention. Plasma potassium concentrations ranged from 1.7 to 3.1 meq/liter in the rats with metabolic alkalosis, and no correlation was found between potassium concentration and either the absolute or fractional rate of proximal bicarbonate reabsorption. In addition, no difference was noted in the load-reabsorption relationship between the normokalemic rats (potassium values near 5.0, Table IV), and the CMA rats with potassium values near 2.0. These results are consistent with the more recent findings of Cogan and Liu (4).

In the present study, tubular and capillary P_{CO_2} were measured in rats having a wide range of proximal bicarbonate reabsorption rates. As in our previous studies (8), two P_{CO_2} gradients were identified: a large gradient between the tubules and capillaries and systemic arterial blood, and a small gradient between the early proximal tubule and the remaining cortical structures. In the control animals, the values observed for both the large and small gradients were similar to the values we have reported previously (8). In discussing the large gradient, we will focus primarily on the peritubular capillary-arterial (Pc-Art) P_{CO_2} gradient, although the tubular-arterial gradients demonstrate the same pattern.

As shown in Table V, the Pc-Art P_{CO_2} gradient was significantly higher in both CMA groups than in control animals. Given the wide range of proximal bicarbonate reabsorption in the three groups of rats studied, we plotted the Pc-Art P_{CO_2} gradient against APR_{HCO_3} for each animal in which both variables were measured to determine whether Pc-Art P_{CO_2} varied directly with the rate of proximal reabsorption. No correlation was noted; if anything, Pc-Art P_{CO_2} was higher in the rats with low APR_{HCO_3} values. These data indicate that the peritubular capillary P_{CO_2} is not a simple function of the rate of proximal bicarbonate reabsorption. This conclusion is not surprising, because P_{CO_2} in the peritubular capillary at any given moment must be determined not only by the rate of CO_2 addition, but also by its rate of removal. CO_2 may be added to the capillary not only as a result of bicarbonate reabsorption but also from cellular metabolic CO_2 production. CO_2 is removed from the capillary by titration of hemoglobin and plasma proteins, and its removal rate is influenced as well by the rate of RBF. A reduction in RBF for any given rate of CO_2 production would be expected to increase Pc-Art P_{CO_2}. In the present studies, RBF was significantly reduced in the 4–7-d CMA rats, and these animals had the highest Pc-Art P_{CO_2} gradients. Evaluation of the specific influence of changes in blood flow on P_{CO_2}, however, requires simultaneous evaluation of the effects of all the other factors which can influence P_{CO_2}. Such an evaluation involves an estimate of metabolic CO_2 production in the renal cortex in addition to the variables measured in this study, and also necessitates the development of a complete model describing the interaction of these factors. Moreover, a reduction in RBF is only one possible explanation for the higher P_{CO_2} values. Recent work has implicated chemical disequilibrium between CO_2 and HCO_3 in the renal vasculature and vascular-vascular exchange of CO_2 as factors contributing to the high P_{CO_2} in the renal cortex (16, 17). Whether these factors contribute to the differences in cortical

P_{CO_2} between control and CMA rats remains a subject for future investigation.

In contrast to the capillary-arterial P_{CO_2} gradient, the small P_{CO_2} gradient found between the EP tubule and the other cortical structures seems likely to be the result of CO_2 generated in the lumen in the process of bicarbonate reabsorption. A transepithelial CO_2 pressure gradient in the range observed in the present study is consistent with measurements of the permeability of the proximal tubular epithelium to CO_2 and the predicted rate of luminal CO_2 production from bicarbonate reabsorption (18–20). Consistent with the view that this gradient may be related to the rate of bicarbonate reabsorption, we found a direct correlation between proximal bicarbonate reabsorption and the P_{CO_2} gradient between the early and late proximal tubule (Fig. 3). Further studies in which bicarbonate reabsorption is systematically altered are required to evaluate this hypothesis more completely.

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