Metabolic Alkalosis in the Rat

EVIDENCE THAT REDUCED GLOMERULAR FILTRATION RATHER THAN ENHANCED TUBULAR BICARBONATE REABSORPTION IS RESPONSIBLE FOR MAINTAINING THE ALKALOTIC STATE

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ABSTRACT Maintenance of chronic metabolic alkalosis might occur by a reduction in glomerular filtration rate (GFR) without increased bicarbonate reabsorption or, alternatively, by augmentation of bicarbonate reabsorption with a normal GFR. To differentiate these possibilities, free-flow micropuncture was performed in alkalotic Munich-Wistar rats with a glomerular ultrafiltrate total CO_2 concentration of 46.5±0.9 mM (vs. 27.7±0.9 mM in controls). Alkalotic animals had a markedly reduced single nephron GFR compared with controls (27.4±1.5 vs. 51.6±1.6 nl/min) and consequently unchanged filtered load of bicarbonate. Absolute proximal bicarbonate reabsorption in alkalotic animals was similar to controls (981±49 vs. 1,081±57 pmol/min), despite a higher luminal bicarbonate concentration, contracted extracellular volume, and potassium depletion. When single nephron GFR during alkalosis was increased toward normal by isohydric volume expansion or in another group by isotonic bicarbonate loading, absolute proximal bicarbonate reabsorption was not substantially augmented and bicarbonaturia developed. To confirm that a fall in GFR occurs during metabolic alkalosis, additional clearance studies were performed. Awake rats were studied before and after induction of metabolic alkalosis associated with varying amounts of potassium and chloride depletion. In all cases, the rise in blood bicarbonate concentration was inversely proportional to a reduction in GFR; filtered bicarbonate load remained normal. In conclusion, a reduction in GFR is proposed as being critical for maintaining chronic metabolic alkalosis in the rat. Constancy of the filtered bicarbonate load allows normal rates of renal bicarbonate reabsorption to maintain the alkalotic state.

INTRODUCTION

The pathogenesis of chronic metabolic alkalosis may be divided into two phases (1). In the first, generation phase, there is net hydrogen ion loss from the body or bicarbonate gain so that plasma bicarbonate concentration and pH are increased. In the second, maintenance phase, all the filtered bicarbonate is reabsorbed and hence the alkalosis is sustained. This contrasts to the normal renal response to an elevated bicarbonate concentration in which excess bicarbonate is promptly excreted so that the plasma bicarbonate concentration is quickly normalized (2).

Metabolic alkalosis is usually associated with potassium and chloride deficiency, which in turn might be responsible for maintaining the alkalosis in two ways. To accommodate the increased filtered bicarbonate load, a proportional increase in bicarbonate reabsorption might occur, predominantly in the proximal nephron. It is assumed that glomerular filtration rate (GFR)[1] would remain normal in this case. Alternatively, as the plasma bicarbonate concentration rises, maintenance of metabolic alkalosis might be accomplished by a re-

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[1] *Abbreviations used in this paper:* DOCA, deoxycorticosterone acetate; EDD, electrolyte-deficient diet; FFDS, fat-free dry solid; GFR, whole kidney glomerular filtration rate; SNGFR, single nephron GFR.

ciprocal reduction in GFR. In this case, there is no increment in filtered bicarbonate load. Consequently, a normal (not enhanced) rate of proximal acidification would suffice to prevent excessive distal bicarbonate delivery and bicarbonaturia.

Differentiation of these two pathophysiological alternatives rests in measuring absolute rates of bicarbonate filtration and proximal reabsorption during chronic metabolic alkalosis. Such measurements have not been previously performed. Indeed, there is even disagreement over whether fractional proximal bicarbonate reabsorption during chronic metabolic alkalosis is increased (3), decreased (4), or normal (5).

The principal purpose of the free-flow micropuncture experiments in this study was to compare the single nephron glomerular filtration rate (SNGFR) and absolute proximal bicarbonate reabsorption in rats with chronic metabolic alkalosis accompanied by extracellular volume (chloride) and potassium depletion to values in normal euvolemic animals. Another purpose was to examine the response of proximal acidification to an increase in filtration rate. These latter experiments attempted to define the mechanism whereby isohydric expansion during metabolic alkalosis results in a bicarbonate diuresis and correction of the alkalotic state (6, 7).

The micropuncture studies revealed that SNGFR was reduced during chronic metabolic alkalosis. At comparable filtered bicarbonate loads, absolute proximal bicarbonate reabsorption during alkalosis was no higher than normal. Proximal bicarbonate reabsorption did not rise when SNGFR was caused to increase.

Since proximal bicarbonate reabsorption was not enhanced during metabolic alkalosis, the fall in filtration rate appeared to be critical for maintaining the alkalotic state. Additional clearance studies were therefore performed in awake rats to confirm that GFR falls in chronic metabolic alkalosis.

The clearance studies confirmed the micropuncture findings. As plasma bicarbonate concentration rose in conscious animals with varying amounts of potassium and chloride depletion, there was a proportional decrease in GFR. This inverse relationship continued when the alkalosis was partially or fully repaired by potassium and/or chloride repletion. Under all conditions of induction and repair of chronic metabolic alkalosis, filtered bicarbonate loads did not exceed normal values. Thus, a fall in GFR permitted normal rates of renal acidification to maintain the alkalotic state.

METHODS

Micropuncture protocols

43 Munich-Wistar rats (Timco Breeding Laboratories, Houston, TX) weighing 218±4 g were studied using free-flow micropuncture techniques.

Chronic metabolic alkalosis. To induce chronic metabolic alkalosis, animals were maintained for 11–14 d on a standard liquid electrolyte-deficient diet (EDD) of 40 ml/d, supplemented with Na_2SO_4, 2.6 meq/d (3). They also received 80 mM $NaHCO_3$ drinking water and were injected daily with 0.5 mg deoxycorticosterone acetate (DOCA) (Organon Inc., W. Orange, NJ) intramuscularly. The diet was withheld for 24 h before study to permit sulfate excretion. The first period of micropuncture followed surgical preparation of these animals. Surgically induced plasma volume losses (8, 9) were replaced using homologous plasma obtained from identically pretreated rats (10). It was found by extensive preliminary experience that failure to replace plasma losses precluded micropuncture because of the severe volume depletion sustained. The measured total CO_2 concentration of the infused plasma was 42.2±0.9 mM (n = 34). The infusion rate was 35 μl/min for the 45–60 min of surgical preparation, for a total infusion of 1% body wt. The infusion rate was then reduced to 4 μl/min for the duration of the study. Using this protocol, plasma volume was maintained constant, as reflected by stability of the hematocrit: the hematocrit immediately following arterial catherization, but before major surgery, was 49.8±0.5 vol%, while the hematocrit during the first period of micropuncture was 49.5±0.6 vol% (n = 27).

Chronic metabolic alkalosis plus isohydric expansion. Following the first micropuncture period, 10 rats underwent isohydric expansion, similar to the protocol of Cohen (6). They received a 5% body wt/h infusion of a colloid-free solution that approximated the plasma electrolyte composition during alkalosis. It contained (in milliequivalents per liter): NaCl, 88; $NaHCO_3$, 45; Na_2HPO_4, 4; KCl, 2; $MgSO_4$, 1; $CaCl_2$, 1.8. The second period of micropuncture was performed in the last half of the second hour of the infusion, thus after 7.5–10% body wt total expansion. To define the independent role of extracellular volume status on proximal acidification during expansion, seven of these rats subsequently underwent aortic constriction while volume expansion continued. The suprarenal aorta was constricted to a mean arterial pressure of 74±1 mmHg to return SNGFR to the level obtained during the first, preexpansion period. The third period of micropuncture was then performed following a 0.5-h stabilization period.

Another group of six animals were isohydrically expanded with homologous plasma to increase SNGFR following the first micropuncture period. At the same SNGFR following plasma expansion compared with colloid-free expansion, the effect of differences in peritubular Starling forces on proximal anion reabsorption could be examined. The second period commenced following 1.5 h of plasma expansion at 2% body wt/h, thus, after a total of 3–4% body wt plasma expansion.

Chronic metabolic alkalosis plus acetazolamide. In six animals, the second period of micropuncture followed 0.5 h of carbonic anhydrase inhibition with acetazolamide. Acetazolamide was given in a loading dose of 50 mg/kg, and then infused at 50 mg/kg per h in 125 mM $NaHCO_3$/25 mM $KHCO_3$ at 0.1 ml/min. When carbonic anhydrase is inhibited, normal anion concentration gradients collapse (i.e., tubular fluid/plasma anion concentrations approximate unity) (11). Under these conditions, both carbonic anhydrase-insensitive proximal bicarbonate reabsorption as well as nondiffusional proximal chloride reabsorption can be quantitated (12).

Chronic metabolic alkalosis plus severe aortic constriction. To ascertain whether the end-proximal luminal total CO_2 concentration in the animals with chronic metabolic

alkalosis had achieved a stable, steady-state value or whether a further reduction in concentration were possible, five chronically alkalotic animals were prepared for micropuncture as above. They were micropunctured during a single period only, after severe aortic constriction had been used for 20–30 min. Mean arterial pressure was reduced to 64–71 mmHg to markedly diminish SNGFR and tubular flow rate.

Acute metabolic alkalosis. This group was studied in order to examine proximal reabsorption: (*a*) at a higher SNGFR than could be obtained in chronic metabolic alkalosis even following volume expansion; and (*b*) in an alkalotic state with normal potassium stores. 16 rats received an infusion of isotonic bicarbonate (125 mM $NaHCO_3$/25 mM $KHCO_3$) at 5% body wt/h. Micropuncture began 1.5 h following commencement of infusion, for a total of 7.5–10% body wt expansion.

Clearance protocols

95 female Sprague-Dawley rats weighing 200–220 g were studied in the awake state. Each animal was studied twice by clearance techniques: when normal and following the induction of metabolic alkalosis (groups I–IV); or when alkalotic and following the partial or complete repair of alkalosis (groups V–VIII).

Animals in the first four groups were used to study the induction of metabolic alkalosis. They were allowed free access to Purina rat chow (Ralston Purina Co., St. Louis, MO) and water before the first clearance study. Following the first clearance period of these normal rats, the 40 ml/d standard EDD was provided, divided into twice daily feedings. The diet was supplemented with various salts to generate metabolic alkalosis (groups II–IV) or to maintain a normal control state (group I), as shown in the top of Table I. Half of the animals in each group (except IV_C) received 80 mM $NaHCO_3$ drinking water while the other half received distilled water. During this period, rats were housed in metabolic cages under balance conditions. Urine was collected under oil with thymol as a preservative.

Group I: control. 20 rats were maintained for 9 d (group I_A) or for 21 d (group I_B) on a normal intake of NaCl (1.5 meq/d) and KCl (1.5 meq/d).

Group II: severe K and Cl deficiency. 20 rats were given Na_2SO_4 (2.6 meq/d) and were injected intramuscularly daily with 0.5 mg DOCA in sesame oil for 8 d. This diet was used to induce a high degree of K and Cl depletion and severe metabolic alkalosis, as in the rats used for micropuncture.

Group III: moderate K and Cl deficiency. K replaced Na in the diet (K_2SO_4, 2.6 meq/d) and in the drinking water (80 mM $KHCO_3$) of 10 rats. Less Cl deficiency also ensued (13). This diet was used to ameliorate the K depletion in group II.

Group IV: K deficiency. In 25 rats, dietary NaCl with DOCA (group IV_A) or without DOCA (groups IV_B and IV_C) was used to induce K depletion without Cl depletion. The subgroups with pure diet-induced K deficiency, unenhanced by DOCA, necessitated a longer treatment period. These latter diets were designed to be equivalent to group I on the basis of similar Na content (group IV_B) or Cl content (group IV_C).

The second clearance period was performed on each animal following completion of the above protocols.

The next four study groups were used to study the repair of metabolic alkalosis. In each group of five rats, K and Cl

TABLE I
Dietary Protocols for Clearance Studies

Group		Treatment	*n*	No. of days on diet	Quantity				No. receiving HCO_3^- in drinking water
					Na^+	K^+	Cl^-	SO_4	
					meq/d				
Induction									
I Control	A	EDD, NaCl, KCl	10	9	1.5	1.5	3.0	—	5
	B	EDD, NaCl, KCl	10	21	1.5	1.5	3.0	—	5
II Severe K and Cl deficiency		EDD, Na_2SO_4, DOCA	20	8	2.6	—	—	2.6	10
III Moderate K and Cl deficiency		EDD, K_2SO_4, DOCA	10	10	—	2.6	—	2.6	5
IV K deficiency	A	EDD, NaCl, DOCA	10	10	1.3	—	1.3	—	5
	B	EDD, NaCl	10	21	1.3	—	1.3	—	5
	C	EDD, high NaCl	5	21	2.6	—	2.6	—	5
Repair									
Initial preparation		EDD, Na_2SO_4, DOCA	20	7	2.6	—	—	2.6	20
V No K and Cl replacement		EDD, Na_2SO_4, DOCA	5	8	2.6	—	—	2.6	5
VI K replacement		EDD, K_2SO_4, DOCA	5	7	—	2.6	—	2.6	5
VII Cl replacement		EDD, NaCl, DOCA	5	8	2.6	—	2.6	—	5
VIII K and Cl replacement		EDD, KCl, DOCA	5	7	—	2.6	2.6	—	5

depletion with alkalosis was first induced with Na_2SO_4 and DOCA (the same protocol used in group II). The initial baseline clearance period was then performed. A second diet was then instituted for 7–8 d in order to continue the alkalotic state (group V), to partially repair the alkalosis with K or Cl (groups VI and VII), or to fully repair the alkalosis with KCl (group VIII), as shown in the bottom of Table 1. All groups received 80 mM $NaHCO_3$ drinking water.

Group V: no K or Cl replacement. The previous Na_2SO_4 and DOCA regime was continued.

Group VI: K replacement. K replaced Na in the diet (K_2SO_4) and drinking water ($KHCO_3$).

Group VII: Cl replacement. Cl replaced the SO_4 in the diet.

Group VIII: K and Cl replacement. KCl replaced Na_2SO_4.

The second clearance period was performed following completion of the above protocols.

Procedures

Micropuncture techniques. The free-flow micropuncture methodology from this laboratory has been previously published (10–12).

Clearance techniques. For the first clearance period, each rat was anesthetized with 50 mg/kg body wt Brevital i.p. (methohexital sodium, Eli Lilly & Co., Indianapolis, IN). The femoral vein and artery were catheterized (PE-50). The rat was allowed to awaken (30–90 min). It was then placed in a plexiglass cage small enough to prevent catheter dislodgement, as previously described (8, 9). Care was taken during the rest of the experiment to avoid disturbing the animal. When the animal was fully conscious, a [*methoxy*-^{3}H]inulin (Nuclear Chicago Corp., Chicago, IL) infusion was begun. Following a prime of 6 μCi, a maintenance infusion of 16 μCi/h (in bicarbonate Ringer's solution at 0.8 ml/h) was started. 1 h elapsed for equilibration. Five consecutive urine collections were then performed with intermittent arterial blood sampling for inulin, protein, Na, K, and Cl concentrations. At the end of the clearance studies, blood was obtained for blood gas determination. Finally, 0.75 μCi ^{125}I human serum albumin was injected for plasma volume measurement (8, 9).

Each rat was then reanesthetized to remove the catheters and to suture the femoral incision. The animal was placed in a metabolic cage for its assigned 1–3-wk dietary regime.

For the second clearance period, the same procedures cited above were repeated, except that the contralateral femoral artery and vein were used for cannulation.

Following the second clearance study, a small piece of muscle from the thigh of each animal was excised for subsequent determination of muscle [K]. To define muscle [K] for the first clearance period of each group, two separate sets of rats (n = 10 each) were utilized. They were maintained on identical initial diets to those used in the induction (groups I–IV) or repair (groups V–VIII) groups before excision of muscle.

Analysis

Measurements were made of [^{3}H]inulin concentration by liquid scintillation counting (Tri Carb 460C, Packard Instrument Co., Downers Grove, IL), of plasma and urine sodium and potassium concentration by flame photometry (model 343, Instrumentation Laboratory, Inc., Lexington, MA), of plasma and urine chloride concentration amperometrically (Buchler-Cotlove chloridometer, Buchler Instruments, Inc., Fort Lee, NJ), of plasma osmolality by vapor pressure osmometry (Wescor, Inc., Logan, UT), and of plasma protein concentration by refractometry (American Optical Co., Keene, NH). Arterial pH and P_{CO_2} were measured by a blood gas analyzer (model 165 Corning Medical and Scientific, Corning Glass Works, Medfield, MA). Total CO_2 concentration of an aliquot of 20–40 nl of tubule fluid, plasma, or urine in the micropuncture studies was measured by microcalorimetry (Picapnotherm) (14). Within the physiologic pH range, the total CO_2 in a sample represents bicarbonate plus dissolved CO_2 gas. To document bicarbonaturia, urine total CO_2 was measured using a Natelson microgasometer (model 600, Scientific Instruments, Inc., Springfield, MA) from two rats in each of the groups studied by clearance techniques that were receiving bicarbonate in the drinking water. Tubule fluid chloride concentration was measured by the electrometric titration method of Ramsay (15).

For the muscle potassium measurements, the muscle was minced and then dried at 80°C for at least 48 h to constant weight. Fat-free dry solid (FFDS) weight was defined after fat extraction with ether and subsequent redrying and reweighing. Finally, 0.46 N nitric acid digestion was carried out for 48 h with constant rotation of the samples. The supernatant [K] was then measured.

To ascertain whether morphological changes in the proximal tubule had occurred due to kaliopenia, kidneys from alkalotic animals (n = 2) were compared with normal controls (n = 2). Kidneys were fixed in Bouin's solution, stained with hematoxylin and eosin, and examined by light microscopy.

Calculations

SNGFR and GFR were estimated from the single nephron and whole kidney inulin clearances. Absolute and fractional rates of water, total CO_2, and chloride reabsorption and the logarithmic mean luminal total CO_2 concentration were calculated as previously described (10–12).[2] The mean length of the accessible proximal convoluted tubule in the Munich-Wistar rat is 4.5–5.0 mm.

Results are expressed as mean±SEM. Statistical significance was assessed using the paired t test for results obtained in the same animal or the unpaired t test for comparisons between groups. Multiple linear regression analysis was performed using standard techniques (16).

RESULTS

Proximal bicarbonate reabsorption during metabolic alkalosis. As shown in Table II (line 1), animals

[2] Occasional rats had insufficient surface glomeruli for direct glomerular ultrafiltrate analysis. In such cases, the glomerular ultrafiltrate total CO_2 concentration was estimated by multiplying the measured plasma water total CO_2 concentration (corrected for protein content) by an effective "Donnan factor" of 1.06. In other experiments when Bowman spaces were present, measurements during alkalosis verified that the ratio of the glomerular ultrafiltrate total CO_2 concentration to the simultaneously obtained plasma water total CO_2 concentration was 1.06±0.01 (n = 52). This coefficient is slightly higher than previously reported in normal animals, 1.05±0.01 (10), perhaps due to the increased anionic protein charge density during alkalosis. The comparable factor for chloride during alkalosis was 1.05±0.01 (n = 48).

TABLE II

Arterial Blood Composition and Pressure in Micropuncture Studies

	Hematocrit	[Protein]	$\overline{AP}$	Osmolality	[Na]	[K]	[Cl]	pH	pCO_a	[Total CO_2]
	vol %	*g/dl*	*mmHg*	*mosm/kg*		*meq/liter*			*mmHg*	*mM*
Chronic metabolic alkalosis (n = 22)	49.5±0.6*	5.1±0.1	102±2	303±2	155±1	2.1±0.1	88±1	7.57±0.01	43±1	41±1
+ Isohydric expansion (n = 10)	43.1±0.7	4.1±0.2	101±4	301±3	155±2	2.2±0.1	95±1	7.58±0.01	39±1	40±1
	$P < 0.001$‡	0.001	NS	NS	NS	0.025	0.001	NS	0.005	NS
+ Aortic constriction (n = 7)	41.1±0.8	3.6±0.1	74±1	306±3	157±1	1.9±0.1	96±2	—	—	36±1
	$P < 0.005$‡	0.05	0.001	NS	NS	NS	0.01			0.05
	$P < 0.001$§	0.001	0.001	NS	NS	NS	0.005			0.005
+ Plasma expansion (n = 6)	38.0±0.8	6.0±0.1	103±2	309±5	159±2	2.1±0.2	99±2	7.56±0.01	40±1	38±1
	$P < 0.001$‡	0.001	0.025	NS	NS	NS	0.01	NS	NS	NS
+ Acetazolamide (n = 6)	48.5±0.9	5.0±0.1	113±4	303±4	156±2	2.4±0.2	88±2	7.47±0.01	52±2	40±2
	$P <$ NS‡	NS	NS	NS	NS	NS	NS	0.01	0.05	NS
Acute metabolic alkalosis (n = 16)	48.0±0.6	4.1±0.1	127±3	294±2	151±2	5.0±0.1	81±1	7.59±0.01	43±1	45±1

$\overline{AP}$, mean arterial pressure.

* Mean±SEM.

‡ P values for second period compared with first period (chronic metabolic alkalosis).

§ P values for aortic constriction period compared with isohydric expansion period.

maintained on the electrolyte-deficient diet supplemented with Na_2SO_4 and given DOCA were alkalotic (arterial pH 7.57±0.01 and plasma total CO_2 concentration 41±1 mM) and potassium depleted (plasma potassium 2.1±0.1 meq/liter).

As shown in Table III (lines 1 and 8), the alkalotic animals had a markedly reduced SNGFR compared with euvolemic (similarly plasma repleted) normal animals (10) (27.4±1.5 vs. 51.6±1.6 nl/min). GFR was also greatly decreased (0.45±0.05 ml/min) compared with normal (1.42±0.03 ml/min) (Table IV). Because of the reciprocal differences in Bowman's space total CO_2 concentration (46.5±0.9 in alkalotic rats vs. 27.7±0.9 mM in normal controls), single nephron filtered total CO_2 loads in the two groups were similar. Despite significant extracellular volume and potassium depletion, absolute and fractional proximal total CO_2 reabsorption in the alkalotic animals (981±49 pmol/min and 0.77±0.01) were no higher than normal values (1,081±57 pmol/min and 0.77±0.02). As illustrated in Fig. 1, absolute proximal total CO_2 reabsorption in the alkalotic animals (open circles) overlapped the normal range (shaded area) as a function of filtered total CO_2 load. Distal total CO_2 delivery rates in the alkalotic and normal euvolemic groups were thus similar (297±27 vs. 329±36 pmol/min).

That proximal bicarbonate reabsorption was not stimulated during chronic metabolic alkalosis was surprising considering that the mean luminal bicarbonate concentration (Fig. 2) was substantially higher (32 mM, open circle) than during normal euvolemia (17 mM, closed triangle). In previous studies, proximal bicarbonate reabsorption was stimulated when the mean luminal bicarbonate concentration increased, either as a result of increased flow rate (shown by the triangles and squares in Fig. 2 [10]), or increased lu-

TABLE III
Filtration and Proximal Reabsorption of H_2O, Total CO_2, and Chloride

		Bowman's space		Filtered load			End-proximal	
	SNGFR	[tCO_2]	[Cl]	tCO_2	Cl	V	[tCO_2]	[Cl]
	nl/min	*mM*	*meq/liter*	*pmol/min*	*peq/min*	*nl/min*	*mM*	*meq/liter*
Chronic metabolic alkalosis (n = 22)	27.4±1.5°	46.5±0.9	99.0±2.0	1,276±69	2,785±184	13.2±0.9	21.5±1.1	123.3±2.1
+ Isohydric expansion (n = 10)	35.8±2.4	43.1±1.3	103.4±1.7	1,547±109	3,702±259	22.5±1.8	25.7±1.4	120.3±2.5
	P < 0.01‡	0.001	0.01	0.05	0.005	0.001	NS	NS
+ Aortic constriction (n = 7)	22.6±1.2	41.0±1.3	106.4±2.2	922±36	2,418±169	13.1±1.3	16.0±1.5	127.2±2.8
	P < 0.001‡	0.05	0.001	0.001	0.001	0.001	0.001	0.05
	P NS§	0.005	0.001	0.005	NS	0.025	0.001	0.05
+ Plasma expansion (n = 6)	37.7±2.1	41.2±1.9	106.0±3.4	1,543±86	3,988±218	20.7±1.4	21.4±2.2	128.8±4.0
	P < 0.005‡	0.05	NS	0.01	0.01	0.001	NS	NS
+ Acetazolamide (n = 6)	27.2±2.1	46.1±2.1	98.1±2.9	1,241±80	2,695±260	20.1±1.8	49.1±2.4	105.7±3.7
	P < NS‡	NS	NS	NS	NS	0.05	0.001	0.005
+ Severe aortic constriction (n = 5)	11.1±1.5	40.6±1.9	100.1±4.4	444±53	1,126±169	4.0±0.6	7.6±1.0	143.5±4.2
Acute metabolic alkalosis (n = 16)	46.4±1.9	47.6±1.0	89.4±0.9	2,250±115	4,075±251	29.7±1.6	39.1±1.0	103.2±1.2
Normal: euvolemia (n = 12)‖	51.6±1.6	27.7±0.9	—	1,412±82	—	31.8±1.1	10.3±0.9	—
Normal: hydropenia (n = 11)¶	24.1±1.4	25.0±0.9	116.1±1.4	605±40	2,736±155	11.1±0.5	4.4±0.5	140.1±2.0

tCO_2, total CO_2; $\dot{V}$, end-proximal flow rate.
° Mean±SEM.
‡ P values for second period compared with first period (chronic metabolic alkalosis).
§ P values for aortic constriction period compared with isohydric expansion period.
‖ Normal euvolemic values for comparison from reference (10).
¶ Normal hydropenic values for comparison from reference (12).

minal bicarbonate concentration when microperfusion techniques were used (17, 18). Lack of stimulation of acidification by an increase in luminal buffer concentration during alkalosis might have been due to an alteration in the luminal gradient attainable for total CO_2 concentration or pH. According to this hypothesis, the measured end-proximal total CO_2 concentration of 21.5±1.1 mM may have represented a rate-limiting, minimal luminal bicarbonate concentration, substantially above the value that normal hydropenic animals can achieve of 4.4±0.5 mM (Table III, line 9) (12). Severe aortic constriction was therefore used during chronic metabolic alkalosis and SNGFR fell to 11.1±1.5 nl/min. The gradient limitation hypothesis was rejected by finding that the end-proximal total CO_2 concentration could be reduced to 7.6±1.0 mM under these conditions (Table III, line 6).

In addition, there was a remote possibility of physical damage to the proximal nephron induced by potassium deficiency. This was excluded by light microscopy. Proximal tubules of alkalotic rats were morphologically unchanged from normal controls, in accord with others (13).

The bicarbonate reabsorptive process during metabolic alkalosis was further characterized by assessing the response to carbonic anhydrase inhibition (Table III, line 5). As had been found previously in normal animals (11), bicarbonate reabsorption during acetazolamide administration was inhibited by 80% in alkalosis. Absolute proximal total CO_2 reabsorption was also similar in magnitude (268±31 pmol/min) to that observed previously (291±16 pmol/min) when a comparable filtered total CO_2 load was achieved by plasma loading normal animals during carbonic anhydrase inhibition. In each case, due to either increased filtered bicarbonate concentration or flow rate during carbonic

TABLE III (*Continued*)

Distal delivery		Absolute proximal reabsorption			Fractional proximal reabsorption		
tCO_2	Cl	tCO_2	Cl	H_2O	tCO_2	Cl	H_2O
pmol/min	*peq/min*	*pmol/min*	*peq/min*	*nl/min*			
297±27	1,650±132	981±49	1,088±75	14.3±0.8	0.77±0.01	0.40±0.01	0.52±0.01
582±57	2,700±235	961±64	979±85	13.4±0.9	0.63±0.02	0.27±0.02	0.38±0.02
0.001	0.001	NS	NS	NS	0.001	0.001	0.001
228±33	1,665±171	694±26	752±52	9.5±0.4	0.78±0.03	0.32±0.03	0.43±0.03
0.001	0.001	0.01	0.05	0.025	0.01	0.025	NS
NS	0.005	0.001	0.025	0.001	NS	0.005	0.001
434±56	2,686±179	1,098±52	1,347±68	17.1±0.9	0.71±0.02	0.34±0.02	0.44±0.02
0.05	0.001	0.025	0.05	0.05	0.05	0.05	0.005
973±64	2,092±235	268±31	542±73	7.2±0.6	0.22±0.02	0.20±0.02	0.27±0.02
0.001	0.01	0.001	0.025	0.005	0.001	0.025	0.005
28±4	546±107	416±50	550±112	7.1±1.0	0.94±0.01	0.47±0.05	0.64±0.02
1,170±76	2,949±232	1,090±64	1,150±82	16.9±0.8	0.49±0.02	0.28±0.02	0.37±0.02
329±36	—	1,081±57	—	19.0±1.1	0.77±0.02	—	0.37±0.01
48±6	1,639±151	557±38	1,214±93	13.0±1.0	0.92±0.01	0.44±0.01	0.54±0.01

TABLE IV

GFR and Urinary Electrolyte Excretion Rates in Micropuncture Studies

	GFR	$\dot{V}$	$U_{Na}V$	U_KV	$U_{Cl}V$	$U_{tCO_2}V$
	ml/min	*μl/min*		*neq/min*		*nmol/min*
Chronic metabolic alkalosis (n = 22)	0.45±0.05*	1.4±0.2	66±11	29±5	17±3	2±1
+ Isohydric expansion (n = 10)	0.63±0.04	12.6±2.3	2,941±495	15±3	1,769±415	593±111
	$P < 0.001$‡	0.005	0.001	NS	0.025	0.005
+ Aortic constriction (n = 7)	0.39±0.02	1.8±0.3	285±96	7±1	67±18	16±9
	$P < 0.025$‡	0.05	0.025	NS	0.05	0.025
	$P <$ NS§	0.05	0.05	NS	NS	NS
+ Plasma expansion (n = 6)	0.65±0.08	11.7±6.7	2,510±1,392	19±5	1,604±972	655±437
	$P < 0.005$‡	0.05	0.05	NS	0.05	0.05
+ Acetazolamide (n = 6)	0.54±0.05	54.5±2.3	11,257±524	607±135	222±79	8,419±1,083
	P NS‡	0.001	0.001	0.05	0.05	0.001
Acute metabolic alkalosis (n = 16)	1.07±0.03	44.6±5.8	8,104±1,095	3,599±205	354±83	7,423±679

$\dot{V}$, urinary volume excretion rate; $U_{Na}V$, urinary sodium excretion rate; U_KV, urinary potassium excretion rate; $U_{tCO_2}V$, urinary total CO_2 excretion rate; $U_{Cl}V$, urinary chloride excretion rate.

* Mean±SEM.

‡ P values for second period compared with first period (chronic metabolic alkalosis).

§ P values for aortic constriction period compared with isohydric expansion period.

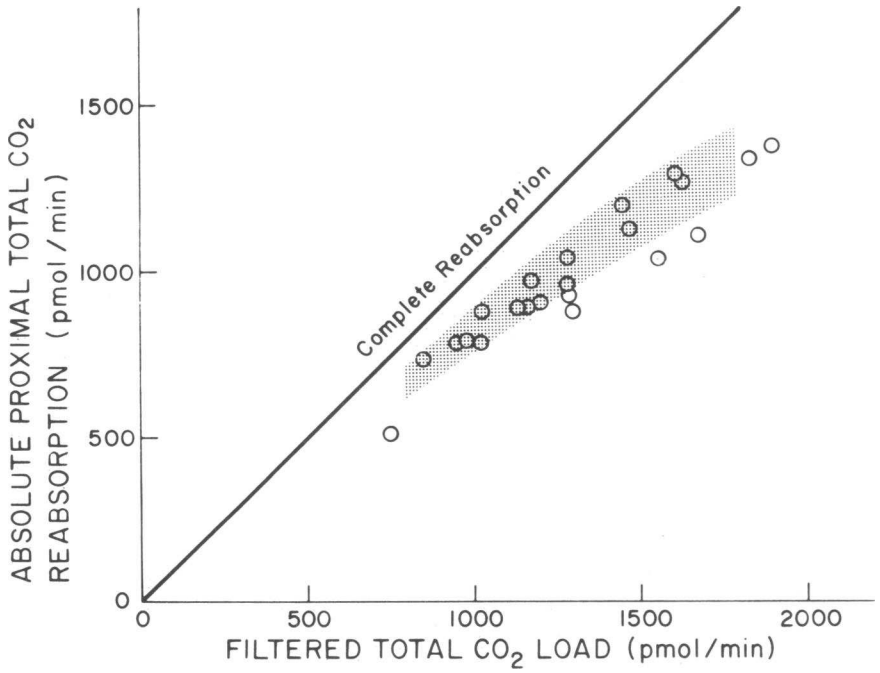

FIGURE 1 Absolute proximal total CO_2 reabsorption as a function of filtered total CO_2 load during chronic metabolic alkalosis. Shaded area encompasses the previously described proximal total CO_2 reabsorption by normal euvolemic and isohydrically expanded animals (10). Proximal bicarbonate reabsorption during metabolic alkalosis (circles) was not stimulated over normal values.

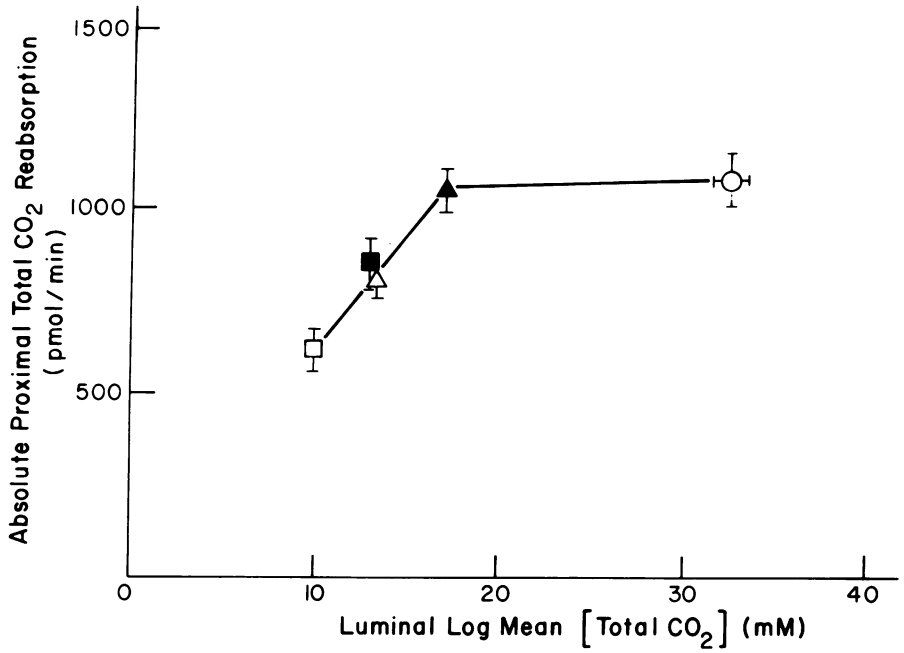

FIGURE 2 Absolute proximal total CO_2 reabsorption as a function of luminal logarithmic mean total CO_2 concentration. Values are shown for volume contracted (△) and euvolemic normal animals (▲), for contracted (□) and euvolemic acidotic animals (■) (10), and for animals with chronic metabolic alkalosis (○).

anhydrase inhibition, total CO_2 reabsorption was >70% higher than the normal hydropenic value during carbonic anhydrase inhibition of 158 pmol/min (11). These higher rates surpassed reasonable estimates of the proton secretory rate due to the uncatalyzed rate of hydration and hydroxylation of CO_2 (11).

The next series of experiments examined the response of proximal acidification to an increase in flow rate and extracellular volume expansion. Cohen (6) has shown that isohydric expansion of alkalotic dogs induced bicarbonaturia and repaired the alkalosis. It is unknown whether this reduction in fractional renal bicarbonate reabsorption was a consequence of a change in proximal acidification. 10 alkalotic animals were expanded with a colloid-free isohydric solution in a protocol similar to Cohen's. Other alkalotic animals were isohydrically expanded with homologous plasma. In both cases, SNGFR rose by ~30%, but there was little change (−2 to 12%) in absolute total CO_2 reabsorption in the two groups, as shown in Table III (lines 2 and 4). Hence, fractional total CO_2 reabsorption was diminished and distal total CO_2 delivery increased (Fig. 3). Similar to the studies of Cohen (6), bicarbonaturia developed. Whole kidney bicarbonate excretion was substantial (Table IV), representing 2±1% of the filtered load in both groups. The glomerular ultrafiltrate total CO_2 concentration fell by ~10%.

Enhanced distal total CO_2 delivery during isohydric expansion might have been the consequence of an increased filtered total CO_2 load in a setting of reabsorptive saturation. Alternatively, volume expansion may have independently inhibited total CO_2 reabsorption that otherwise would have paralleled the higher load. If the latter explanation were true, a subsequent reduction in filtered total CO_2 load during persisting expansion should be associated with continued suppression of proximal total CO_2 reabsorption. Hence, at similar flow rates, fractional proximal total CO_2 reabsorption postexpansion would be less than preexpansion. Therefore, aortic constriction was used to return SNGFR to the preexpansion level (22.6±1.2 vs. 24.0±1.4 nl/min in the seven paired studies), while isohydric expansion continued. Because of the intervening reduction in glomerular ultrafiltrate total CO_2 concentration, the filtered total CO_2 load during aortic constriction was somewhat less than the preexpansion level even though SNGFR was similar (Table III, line 3). The absolute rate of total CO_2 reabsorption was therefore less in the third period than in the first. The important observation, however, is that glomerulotubular balance was restored: fractional proximal total CO_2 reabsorption and distal total CO_2 delivery returned to preexpansion levels (Fig. 3). In addition, bicarbonaturia disappeared (Table IV), despite per-

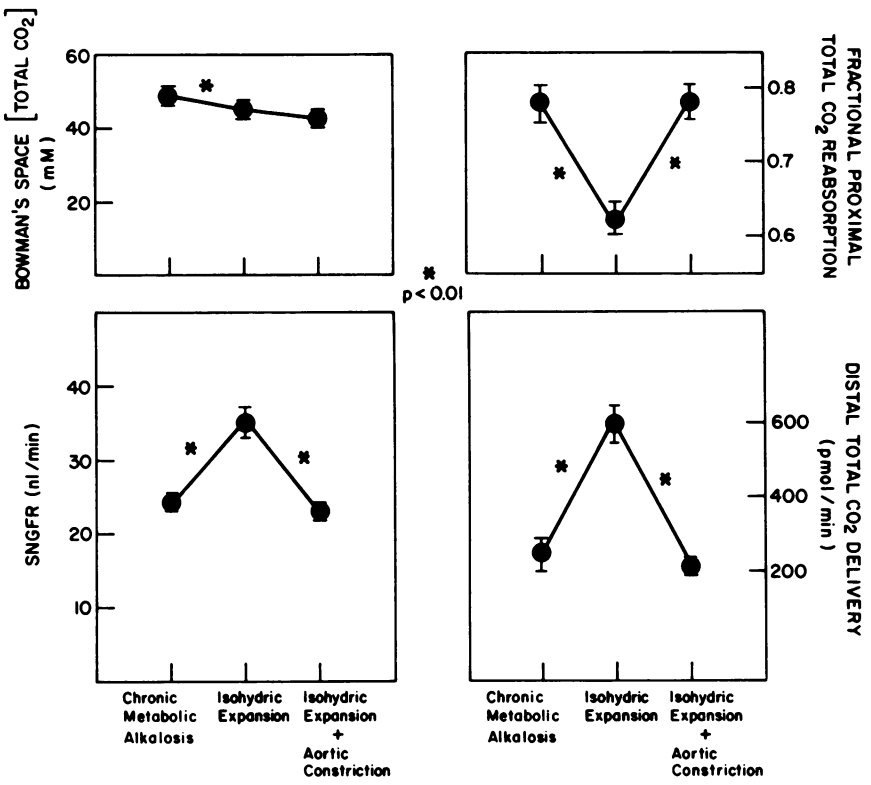

FIGURE 3 Paired changes in Bowman's space total CO_2 concentration, SNGFR, fractional proximal total CO_2 reabsorption and distal total CO_2 delivery during chronic metabolic alkalosis, following isohydric expansion, and with aortic constriction during persistent expansion.

sisting volume expansion (total 15% body wt expansion). These findings suggest that isohydric expansion had diminished fractional proximal total CO_2 reabsorption and had enhanced distal total CO_2 delivery as a consequence of increasing flow rate in the setting of static reabsorption. An inhibitory effect on net bicarbonate transport by the increased extracellular volume status per se was not observed.

To examine whether a further increase in filtered total CO_2 load and in mean luminal total CO_2 concentration would stimulate total CO_2 reabsorption, normal animals were infused with isotonic bicarbonate. A higher SNGFR (46.4±1.9 nl/min) and hence filtered total CO_2 load could be achieved in this acute model of metabolic alkalosis than had been possible in chronic metabolic alkalosis even after isohydric expansion (Table III, line 7). However, little augmentation in absolute total CO_2 reabsorption (1,090±69 pmol/min) was found, as shown in Fig. 4A (closed square), even at the markedly higher filtered load and mean luminal bicarbonate concentration of 43.2 mM. Thus, the fraction of total CO_2 reabsorbed in the superficial proximal convoluted tubule fell to 0.49±0.02. The very high distal bicarbonate delivery was associated with substantial bicarbonaturia (Table IV). However, it should be noted that total renal bicarbonate reabsorption was 0.86±0.01 of the filtered load (Fig. 4B, closed square), indicating a large bicarbonate reabsorptive capacity by nephron segments distal to the superficial proximal convoluted tubule and/or by deeper nephrons. Nevertheless, a qualitative relationship in all groups between distal bicarbonate delivery and urinary bicarbonate excretion clearly existed, as has been previously found (10, 11).

Proximal chloride and water reabsorption during metabolic alkalosis. In previous studies, absolute proximal chloride reabsorption was divided into two components: a passive component, dependent principally on the magnitude of the lumen-to-blood chloride concentration gradient; and an active transport (or possibly convection) component. The active component can be quantitated when the chloride gradient is minimized by the use of carbonic anhydrase inhibition (12). As shown in Fig. 5A, absolute proximal chloride reabsorption during metabolic alkalosis (closed symbols) was similar to normal hydropenic controls

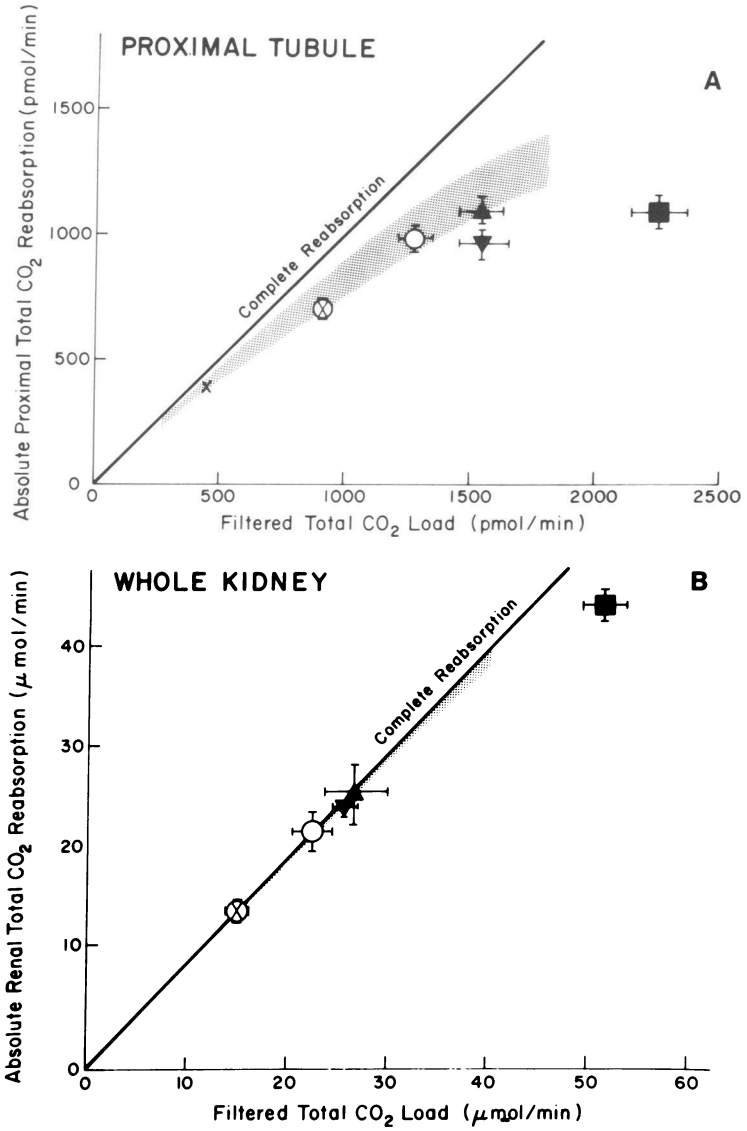

FIGURE 4 Absolute proximal (top panel) and whole kidney (bottom panel) total CO_2 reabsorption as a function of filtered total CO_2 load in metabolic alkalosis. Represented are animals during acute metabolic alkalosis (■); during chronic metabolic alkalosis (○); and during chronic metabolic alkalosis with subsequent plasma expansion (▲), with subsequent isohydric colloid-free expansion (▼), with isohydric expansion and aortic constriction (⊗), or with severe aortic constriction (×). Shaded area is the normal proximal and whole kidney total CO_2 reabsorption rates (10).

(open symbols) (12) before (circles) and after (diamonds) acetazolamide. It is important for this analysis that comparisons be made at similar flow rates because of the flow dependence of both total and acetazolamide-insensitive absolute proximal chloride reabsorption (11). Mean values for SNGFR in the four groups ranged from 24.1 to 27.4 nl/min.

The passive diffusional chloride reabsorptive component appeared normal. Both the principal electrochemical driving force, the chloride gradient (~25 meq/liter), as well as the proximal chloride permeability (estimated by the slopes of the lines in Fig. 5A) were similar in alkalotic and control rats.

The active component of proximal chloride reabsorption can be estimated from the vertical intercepts of Fig. 5A, when the diffusive driving force is mini-

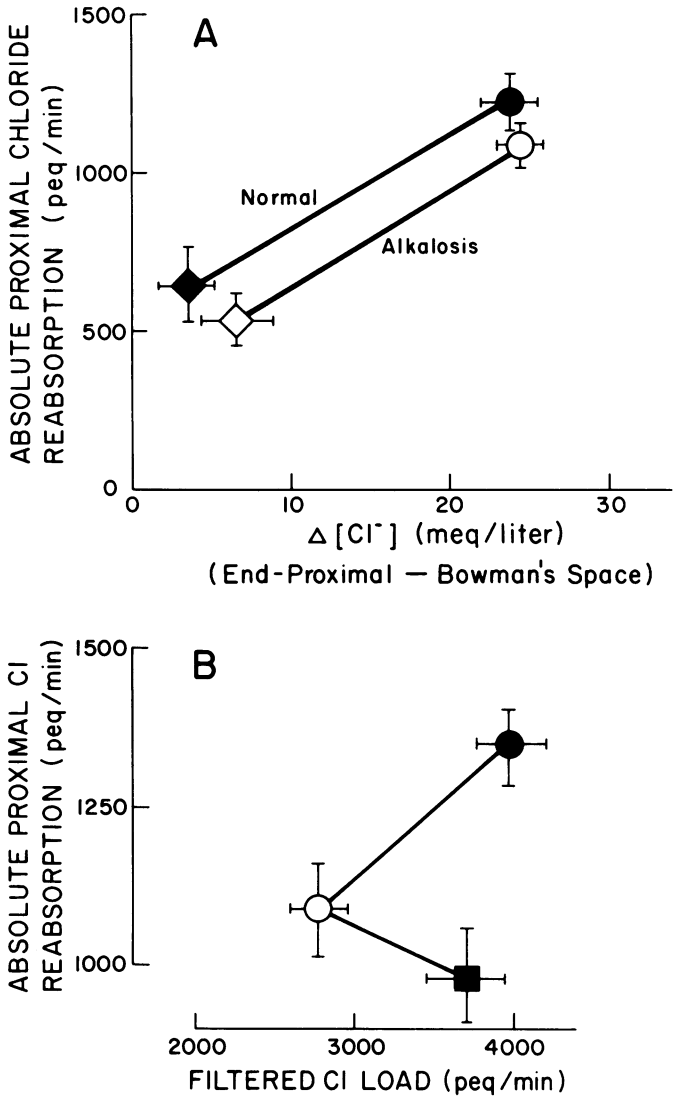

FIGURE 5 (A) Absolute proximal chloride reabsorption as a function of the chloride concentration gradient (end-proximal minus Bowman's space). Values both before (circles) and after (diamonds) acetazolamide administration are shown for normal hydropenic animals (filled symbols) (12) and animals with chronic metabolic alkalosis (open symbols). Mean SNGFR in the four groups were similar. (B) Absolute proximal chloride reabsorption as a function of SNGFR and filtered Cl load during chronic metabolic alkalosis (○) and following isohydric plasma expansion (●) or isohydric colloid-free Ringer expansion (■).

mized. Previous studies in normal and acidotic rats found this component of reabsorption to be an isochloric process (11). In agreement, the ~20% reduction from normal in the vertical intercept in Fig. 5A was proportional to the fall in the blood chloride concentration during metabolic alkalosis.

Thus, absolute proximal chloride reabsorption during metabolic alkalosis was similar to or slightly less than normal hydropenic controls. Fractional proximal chloride reabsorption in metabolic alkalosis was virtually identical to normal hydropenic controls, in accord with others (4, 5). Low distal chloride delivery rates and, hence, avid renal chloride reabsorption appears to result from both the low GFR and hypochloremia in chronic metabolic alkalosis.

Sodium chloride reabsorption had previously been found to increase when filtration rate was raised by plasma expansion, but not by colloid-free expansion

(10, 11). As shown in Fig. 5B, absolute proximal chloride reabsorption increased with plasma expansion (closed circle), but not with colloid-free expansion (closed square) during metabolic alkalosis. Looked at in a different way, at the higher flow rate (36 nl/min), the greater plasma and peritubular protein concentration during plasma expansion than during colloid-free expansion was associated with a significant accentuation in absolute proximal chloride reabsorption (1,347±68 vs. 979±85 neq/min, $P < 0.05$). Absolute proximal water reabsorption was also higher during plasma expansion (17.1±0.9 vs. 13.4±0.9 nl/min, $P < 0.05$). However, absolute proximal total CO_2 reabsorption was not significantly different between the two groups. Thus, the peritubular Starling forces have an important influence over proximal sodium chloride reabsorption, although not over proximal sodium bicarbonate reabsorption (10, 11, 19, 20).

Despite the difference in proximal sodium and chloride reabsorption during the two types of expansion, urinary sodium and chloride excretion rates were comparable (Table IV, lines 2 and 4). Therefore, nephron segments other than the superficial proximal convoluted tubule played an important role in modulating renal sodium chloride excretion during volume expansion in metabolic alkalosis. Further support for this concept comes from an analysis of chloride and bicarbonate reabsorption during expansion. Whole kidney chloride reabsorption was stimulated compared with bicarbonate reabsorption by colloid-free expansion, resulting in partial repair of the alkalosis (6, 7). Nevertheless, in the superficial proximal convoluted tubule, there was no enhancement in the ratio of chloride/bicarbonate reabsorbed during isohydric expansion compared with the base-line alkalotic state (1.02 vs. 1.11).

Clearance studies

Induction of alkalosis. The previous micropuncture studies found absolute proximal bicarbonate reabsorption to be normal. Reabsorption was not stimulated when flow rate was made to increase toward normal. The maintenance of metabolic alkalosis in this setting was dependent upon a reduction in SNGFR. To confirm that GFR falls during chronic metabolic alkalosis in unanesthetized rats, additional clearance studies were performed.

As shown in Table V, the control group (group I) maintained on the artificial diet with NaCl and KCl sustained no significant alteration in acid-base status, GFR, plasma volume, muscle [K] or plasma [K]. In this and the following groups, a change in muscle [K] was used as an index of K depletion and a change in plasma volume as a reflection of Cl depletion. There was no difference in the results of rats receiving supplemental bicarbonate in the drinking water from those not receiving extra bicarbonate. Rats in this and other groups typically ingested and excreted 1.0 meq/d of bicarbonate with a mean urinary bicarbonate concentration of 50±5 meq/liter. Also, there were no significant differences in the rats maintained for 9 d (groups I_A) compared with those maintained for 21 d (groups I_B) so that results were combined for these groups in Table V.

The Na_2SO_4 and DOCA-treated group II developed a significant metabolic alkalosis (plasma bicarbonate concentration rose from 24.6±0.4 to 39.2±1.1 meq/liter), as shown in Table V. There was also marked K depletion (muscle $[K^+]$ fell from 42.1±0.5 to 22.7±0.7 meq/100 g FFDS) and Cl depletion (plasma volume declined from 8.6±0.5 to 6.2±0.1 ml). Weight loss of 7% compared with controls occurred. Supplemental bicarbonate sufficient to induce bicarbonaturia caused no greater degree of alkalosis in group II. Thus, the plasma bicarbonate level stabilized at the same level whether the excess blood bicarbonate was generated endogenously or was provided exogenously. In both cases, the rise in blood bicarbonate concentration was accompanied by a proportional fall in GFR, from 2.9±0.1 to 1.8±0.1 ml/min ($P < 0.001$). This inverse relationship between plasma bicarbonate concentration and GFR is illustrated in Fig. 6 (the mean value for all normal animals in groups I–IV during the first study period is represented by the large black circle with error bars, while the individual group II animals with severe metabolic alkalosis in the second study period are represented by squares).

Since the changes in plasma bicarbonate concentrate and GFR were reciprocal, the filtered load of bicarbonate before and after induction of alkalosis was identical (Table V). Thus, a normal rate of renal hydrogen ion secretion was sufficient to maintain the high plasma bicarbonate concentration of group II.

To ameliorate the K deficiency, K salts replaced Na salts in the diet and drinking water in group III. In comparison to group II, group III sustained less of a reduction in muscle [K] and plasma volume (13). A more moderate degree of alkalosis resulted (plasma bicarbonate concentration 29.5±0.3 meq/liter). As had been observed with group II, supplemental bicarbonate had no additional effect on acid-base or electrolyte parameters, so the results are combined in Table V. Mirroring the smaller increment in plasma bicarbonate concentration, the decline in GFR was also less (hexagons in Fig. 6). Again, filtered bicarbonate loads before and after alkalosis were comparable.

TABLE V

Acid-Base Status, GFR, Plasma Volume, and K Stores during Clearance Studies

Group		Arterial pH		[HCO_3]		GFR		Filtered bicarbonate load		Plasma volume		Muscle [K]		Plasma [K]	
Period:		1	2	1	2	1	2	1	2	1	2	1	2	1	2
				meq/liter		*ml/min*		*μeq/min*		*ml*		*meq/100 g FFDS*		*meq/liter*	
Induction															
I Control		7.44* (±0.01)	7.44 (±0.01)	24.4 (±0.5)	26.0 (±0.5)	2.7 (±0.1)	2.6 (±0.1)	69 (±2)	68 (±2)	8.9 (±0.4)	8.7 (±0.3)	42.1 (±0.5)	42.3 (±0.5)	4.2 (±0.1)	4.4 (±0.1)
II Severe K and Cl deficiency		7.45 (±0.01)	7.62‡ (±0.01)	24.6 (±0.4)	39.2‡ (±1.1)	2.9 (±0.1)	1.8‡ (±0.1)	71 (±2)	69 (±2)	8.6 (±0.5)	6.2‡ (±0.1)	—	22.7‡ (±0.7)	4.4 (±0.1)	2.1‡ (±0.1)
III Moderate K and Cl deficiency		7.42 (±0.01)	7.53‡ (±0.01)	24.4 (±0.5)	29.5‡ (±0.3)	2.9 (±0.1)	2.3‡ (±0.1)	69 (±3)	70 (±2)	8.6 (±0.3)	7.0‡ (±0.3)	—	33.0‡ (±0.8)	4.3 (±0.1)	3.3‡ (±0.1)
IV K deficiency	A	7.44 (±0.01)	7.54‡ (±0.01)	25.4 (±0.6)	31.6‡ (±0.9)	2.9 (±0.1)	2.3‡ (±0.1)	74 (±3)	73 (±3)	9.7 (±0.6)	9.6 (±0.6)	—	28.1‡ (±0.8)	3.9 (±0.1)	2.2‡ (±0.1)
	B	7.42 (±0.01)	7.51‡ (±0.01)	24.9 (±0.6)	28.7‡ (±0.5)	2.8 (±0.1)	2.3‡ (±0.1)	70 (±3)	66 (±3)	9.5 (±0.6)	9.3 (±0.4)	—	29.5‡ (±0.8)	4.0 (±0.1)	2.3‡ (±0.1)
	C	7.43 (±0.01)	7.53‡ (±0.01)	25.4 (±0.7)	29.7‡ (±0.8)	2.6 (±0.1)	2.3‡ (±0.1)	66 (±3)	68 (±3)	9.1 (±0.2)	10.0 (±0.5)	—	32.0‡ (±0.9)	4.1 (±0.1)	2.9‡ (±0.2)
Repair															
V No K and Cl replacement		7.54 (±0.02)	7.63‡ (±0.01)	33.6 (±0.8)	40.6‡ (±0.6)	2.1 (±0.1)	1.7‡ (±0.1)	72 (±1)	70 (±1)	7.9 (±0.1)	6.6‡ (±0.1)	26.4 (±0.4)	23.1‡ (±0.3)	2.3 (±0.1)	1.8‡ (±0.1)
VI K replacement		7.54 (±0.01)	7.51§ (±0.01)	33.0 (±0.7)	29.9‡ (±0.3)	2.2 (±0.1)	2.4‡ (±0.1)	73 (±1)	72 (±1)	7.3 (±0.1)	8.0 (±0.3)	—	32.5‡ (±0.5)	2.3 (±0.1)	3.2‡ (±0.1)
VII Cl replacement		7.55 (±0.01)	7.52§ (±0.01)	35.0 (±0.6)	30.8‡ (±0.1)	2.0 (±0.1)	2.3‡ (±0.1)	70 (±1)	71 (±1)	7.8 (±0.2)	10.0‡ (±0.5)	—	29.1‡ (±0.5)	2.3 (±0.1)	2.0 (±0.1)
VIII K and Cl replacement		7.54 (±0.01)	7.46‡ (±0.01)	31.3 (±0.6)	26.2‡ (±0.4)	2.2 (±0.1)	2.7‡ (±0.1)	71 (±1)	71 (±1)	7.6 (±0.2)	9.4‡ (±0.2)	—	40.7‡ (±1.0)	2.3 (±0.1)	4.1‡ (±0.1)

* Mean±SEM.

‡ $P < 0.001$ for second period compared with the first.

§ $P < 0.05$ for second period compared with first.

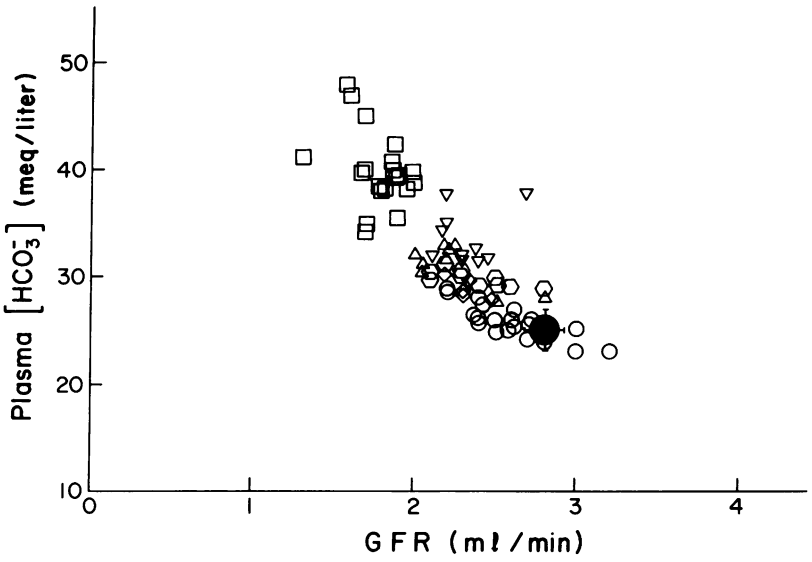

FIGURE 6 Relationship of plasma bicarbonate concentration to GFR during clearance studies in normal controls and in chronic metabolic alkalosis. The mean±SEM for the data of all 75 normal animals in the first clearance period is represented by the large black circle with error bars (●). Shown are the individual data in the second clearance period for group II (□); group III (○); group IV_A (▽); group IV_B (△); and group IV_C (◇).

The three subsets of animals comprising group IV were used to induce isolated K deficiency without Cl deficiency. Each subgroup sustained a reduction in muscle [K^+] while plasma volume did not change. Weight loss compared with controls was −4%. A moderate alkalosis occurred in the three subgroups. As illustrated in Fig. 6 (triangles and diamonds), the counterbalancing fall in GFR (2.3±0.1 ml/min in each) kept filtered bicarbonate load constant. Animals given excess bicarbonate were no more alkalotic than those receiving just water so that the data have again been combined. Neither the usage of DOCA (group IV_A) nor the higher NaCl diet with a slightly higher plasma volume in group IV_C than in IV_B disrupted the inverse relationship between changes in plasma bicarbonate concentration and GFR.

Repair of alkalosis. The next four groups of experiments were designed to assess whether the reversal of the K and/or Cl deficiencies modulated the plasma bicarbonate concentration and GFR in a qualitatively similar fashion (but in opposite directions) to that which occurred during the induction experiments. These rats were first made alkalotic with Na_2SO_4 and DOCA in a manner similar to group II. In the first clearance period, somewhat milder degrees of K and Cl depletion, alkalosis and GFR reduction were found than had occurred in Group II. The latter had received two rather than one surgical preparations and clearance studies. However, continuing Na_2SO_4 and DOCA for another week in group V exacerbated the decrease in muscle [K] and plasma volume. A greater degree of alkalosis (plasma bicarbonate concentration 40.6±0.6 meq/liter) and GFR reduction resulted (1.7±0.1 ml/min), indistinguishable from group II. As before, the inverse relationship between plasma bicarbonate concentration and GFR was retained, as shown in Fig. 7 (the mean value for the first alkalotic period of all rats in groups V–VIII is represented by the large stippled circle with error bars, while the individual results for the second period of group V are represented by squares; the lightly shaded area represents the range of values obtained during the induction experiments of groups I–IV, from Fig. 6). Filtered bicarbonate load in group V remained normal.

Dietary K replacement without Cl replacement in group VI increased muscle [K] without appreciably affecting the plasma volume. A significant but incomplete repair of the alkalosis occurred (bicarbonate concentration fell from 33.0±0.7 to 29.9±0.3 meq/liter). There was significant improvement, but without normalization of the GFR (from 2.2±0.1 to 2.4±0.1 ml/min). The relationship between plasma bicarbonate concentration and GFR in Group VI is shown by the hexagons in Fig. 7. Filtered bicarbonate load stayed at a normal level.

Selective NaCl replacement in group VII allowed an increase in plasma volume without appreciably affecting K status. As with K replacement, Cl repletion caused a significant partial recovery of the alkalosis (plasma bicarbonate concentration fell from 35.0±0.6

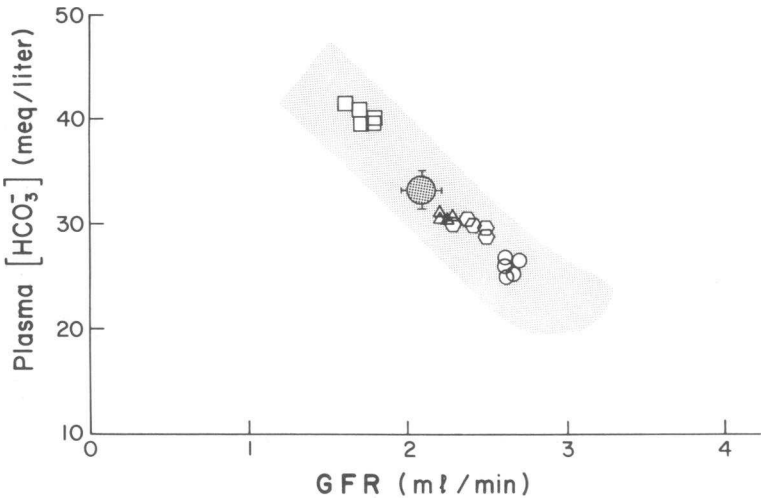

FIGURE 7 Relationship of plasma bicarbonate concentration to GFR during clearance studies in chronic metabolic alkalosis and following recovery from alkalosis. The mean±SEM for the data of all 20 alkalotic animals in the first clearance period is represented by the large stippled circle with error bars (⊕). Shown are the individual data in the second clearance period for group V (□); group VI (○); group VII (△); and group VIII (○). The shaded area encompasses the range of data points from Fig. 6.

to 30.8±0.1 meq/liter). GFR also improved (from 2.0±0.1 to 2.3±0.1) with maintenance of a normal filtered bicarbonate load. These results are illustrated by the triangles in Fig. 7.

Finally, repletion of both K and Cl in group VIII led to complete normalization of K and Cl stores with complete recovery of acid-base status (bicarbonate concentration fell to 26.2±0.4 meq/liter) and hemodynamics (GFR rose to 2.7±0.1 ml/min), as shown by the circles in Fig. 7. All values were then indistinguishable from the controls of group I.

DISCUSSION

The maintenance of metabolic alkalosis requires that tubular bicarbonate reabsorption be increased or filtration rate decreased. The micropuncture studies reported here demonstrated that absolute proximal bicarbonate reabsorption was not increased in rats with chronic metabolic alkalosis. Proximal bicarbonate reabsorption was not stimulated by a high luminal bicarbonate concentration, extracellular volume and potassium depletion, or when flow increased. The alternative pathophysiological mechanism for maintaining alkalosis was therefore suggested, that filtration rate was diminished. SNGFR and GFR were, in fact, found to be depressed in metabolic alkalosis so that filtered bicarbonate loads were normal. The 95 clearance studies in awake animals during the induction and repair of metabolic alkalosis confirmed that an elevation of the blood bicarbonate concentration was accompanied by a reciprocal fall in GFR.

The following discussion will first consider factors that control proximal bicarbonate reabsorption in metabolic alkalosis. The observation of a reduction in GFR will then be considered, especially in reference to the accompanying potassium and chloride deficits that are frequently present in metabolic alkalosis.

Determinants of proximal bicarbonate reabsorption. On the basis of previous observations, the higher mean luminal total CO_2 concentration in metabolic alkalosis compared with normal euvolemic controls (32 vs. 17 mM) was expected to stimulate proximal bicarbonate reabsorption (10, 17, 18). That an increased luminal bicarbonate concentration failed to increase acidification (Fig. 2) might be ascribed to intrinsic saturation of the hydrogen ion secretory system; i.e., a maximum transport rate (V_{max}) may have already been attained. Another possible explanation is that the magnitude of the maximal bicarbonate concentration or pH gradient in the proximal tubule had been altered during metabolic alkalosis. A new, higher steady-state luminal bicarbonate concentration may have occurred (e.g., 21.5 mM in alkalosis instead of 4–5 mM in normal controls), as might happen if bicarbonate back-leak had markedly increased. Such was found not to be the case since an end-proximal total CO_2 concentration as low as 7.6 mM was achievable when flow rate was severely reduced.

Yet another explanation is that the increase in peritubular bicarbonate concentration and pH during met-

abolic alkalosis independently suppressed the proximal acidification process. An increase in the peritubular bicarbonate concentration might inhibit acidification by slowing bicarbonate exit from the cell and alkalinizing the cell. If cellular pH increased, the rate of proton secretion would be relatively depressed at any level of luminal bicarbonate concentration or pH. Proximal acidification might even appear saturated as a function of luminal bicarbonate concentration at a subnormal level (20). Thus, antagonism by peritubular alkalinity of the stimulatory effect of luminal alkalinity would be observed.

This thesis, that peritubular alkalinity suppressed proximal hydrogen ion secretion, is consonant with observations of split droplet studies in the proximal tubule (4, 21), of in vivo microperfusion studies (20) and in vitro microperfusion studies (22) using proximal tubules, as well as of studies using the turtle bladder (23). Thus, suppression of acidification by peritubular alkalinity may explain why reabsorptive saturation was observed in the proximal tubule as filtered bicarbonate concentration was simultaneously increased, and similarly why a reabsorptive plateau (a "Tm") has been observed in the whole kidney during bicarbonate titration studies (2).

Whether proximal acidification would respond to an increase in filtered bicarbonate load and in mean luminal bicarbonate concentration was examined in the three expansion protocols. Bicarbonate reabsorption failed to keep pace with the increase in filtered bicarbonate load. This lack of proximal glomerulotubular balance might have been due to an effective saturation of the hydrogen secretion process as noted above, either intrinsic or induced by alkalemia. Alternatively, it has been suggested that extracellular volume expansion, by promoting bicarbonate back-leak, can independently suppress net proximal bicarbonate reabsorption (1). In the latter case, the enhanced bicarbonate back-leak and relative inhibition of acidification should persist if flow rate were reduced during expansion. However, when aortic constriction was used and flow rate decreased, the end-proximal bicarbonate concentration fell and absolute proximal bicarbonate reabsorption assumed a value appropriate to the filtered load (Fig. 4A). Fractional proximal reabsorption and distal bicarbonate delivery normalized. Thus, volume expansion did not prevent the return of proximal glomerulotubular balance (Fig. 3). These results are in accord with previous in vivo studies in the rat and in vitro studies in the rabbit: if tubular flow were maintained constant, extracellular volume status and peritubular protein concentration had little impact on proximal bicarbonate absorption (10, 19, 20). Aortic constriction also caused disappearance of bicarbonaturia, despite persistent extracellular volume expansion. The failure to abolish bicarbonaturia when Cohen (7) combined aortic constriction with isohydric expansion during metabolic alkalosis in the dog may be due to the difference in a species studied but is otherwise unexplained.

The physiologic importance of the effect of flow rate during metabolic alkalosis may be summarized as follows. When SNGFR was incrementally increased above the value recorded during chronic metabolic alkalosis, the proximal acidification process behaved as if saturated (20). Progressively larger distal bicarbonate delivery rates resulted as filtered bicarbonate loads exceeded normal levels. Although other nephron segments were capable of reabsorbing a proportion of this excess bicarbonate delivery, bicarbonaturia nevertheless developed (Fig. 4). Since proximal bicarbonate reabsorption during metabolic alkalosis was not flow dependent, these studies underscore the critical importance of a reduction in SNGFR for mediating the persistence of chronic metabolic alkalosis. The reduction of SNGFR acted to prevent the filtered load and, thus, distal bicarbonate delivery from exceeding normal values. A bicarbonate diuresis with correction of the alkalosis was thereby prevented.

Finally, a comment should be made regarding the role of potassium deficiency in proximal bicarbonate reabsorption. Clearance studies had suggested that potassium depletion stimulates renal bicarbonate reabsorption (24–26). In the present studies, there was no significant difference in absolute proximal bicarbonate reabsorption when the isohydrically expanded, chronically alkalotic, hypokalemic group was compared with the expanded (10% body wt), acutely alkalotic, normokalemic group. However, Kunau et al. (3) found increased fractional bicarbonate reabsorption during isotonic bicarbonate expansion of potassium-depleted rats compared with similarly expanded normokalemic controls. The apparent discrepancy in results can be easily explained by the different filtration rates measured in the two groups in the present studies. When the potassium-deficient animals with isohydric volume expansion are compared with the acutely alkalotic animals, (Table III, lines 2 and 7), the difference in fractional bicarbonate reabsorption in the two cases (0.63 vs. 0.49) was attributable to markedly different filtered loads (SNGFR). Absolute rates of bicarbonate reabsorption were not significantly different. Thus, the present studies failed to observe a stimulation in absolute proximal bicarbonate reabsorption when potassium-depleted rats were compared with potassium replete rats with comparable extracellular volume expansion and alkalemia. The difference in chronicity of the alkalemia in the two groups may have masked an effect of potassium deficiency. However, other free-flow micropuncture studies by Levine et al. (27) have

not demonstrated any change in absolute proximal bicarbonate reabsorption when nonalkalotic, potassium-deficient animals were compared with nonalkalemic controls or were acutely infused with potassium. Ullrich et al. (21) similarly found no abnormality in proximal acidification when potassium-depleted rats were assessed with split-droplets. However, Chan et al., (28) using in vivo luminal and pericapillary microperfusion have reported a 23% stimulation of proximal bicarbonate reabsorption in potassium-deficient alkalotic rats. Thus, the available evidence suggests that chronic potassium deficiency may stimulate absolute proximal bicarbonate reabsorption, but the effect appears to be relatively small.

Importance of a reduction in GFR for maintaining the alkalotic state. The micropuncture studies found a fall in SNGFR and GFR and no stimulation of absolute proximal bicarbonate reabsorption during metabolic alkalosis. Possible limitations, however, were that only a single model of metabolic alkalosis was utilized and animals were anesthesized. Therefore, clearance studies in awake rats with varying degrees of potassium and chloride depletion and metabolic alkalosis were performed.

These clearance experiments were not designed to explore how the excess blood bicarbonate was generated. Indeed, half of the animals received supplemental bicarbonate to ensure that net acid excretion by the distal nephron would not be rate limiting for increasing the blood bicarbonate concentration. Thus, the excess bicarbonate permitted the maximum degree of alkalosis to be achieved and the maximal rate of renal bicarbonate reabsorption (principally by the proximal nephron) to be expressed.

The inverse relationship between blood bicarbonate concentration and GFR (Fig. 6) held in the conscious animals when the metabolic alkalosis was associated with K depletion alone (group IV) or with both K and Cl depletion of moderate (group III) or severe degree (group II).

That isolated dietary K depletion, as in groups IV_B and IV_C, can sustain metabolic alkalosis has long been known for the rat (5, 29, 30) and for man (31, 32), though not for the dog. Mineralocorticoid treatment (e.g., DOCA), as in group III_A, is also a well established cause of persisting metabolic alkalosis associated with K deficiency (13, 29, 33), simulating primary hyperaldosteronism. Yet, the mechanism by which K depletion maintains metabolic alkalosis has not been previously established. The present studies suggest that K depletion may maintain metabolic alkalosis in the rat by diminishing GFR. Reduction in renal blood flow and GFR by selective K depletion is a well described phenomenon in rats (34, 35) and dogs (25, 26, 36). K depletion apparently decreases GFR by selective renal vasoconstriction. For instance, K depletion causes hyperreninemia, which may induce intrarenal vasoconstriction by activating angiotension II (37). K depletion also stimulates the renal synthesis of the potent vasoconstrictor thromboxane B_2 (38). Recently, Linas and Dickmann (39) have presented strong evidence that both of these systems are operative in the K-deprived rat to increase renal vascular resistance and depress renal blood flow (39). While some degree of K wastage is ubiquitous in clinical forms of metabolic alkalosis (1), the possible contribution of K depletion to decrease GFR and maintain alkalosis in humans is undefined at present. This issue is especially important in the pathophysiology of certain "chloride-resistant" metabolic alkaloses such as primary hyperaldosteronism. The results obtained in group III_A suggest that metabolic alkalosis in states of primary mineralocorticoid excess may be mediated by a reduction in GFR rather than enhanced tubular bicarbonate reabsorption. Further studies of GFR in K-depleted alkalotic humans is warranted.

When Cl depletion is added to K depletion, as in groups II and III, metabolic alkalosis can be well maintained in all species studied (3, 4, 6, 7, 13, 33, 40, 41). Cl deficiency reduced extracellular volume and plasma volume, the latter in turn causing a fall in renal blood flow and GFR (8, 9).[3]

Controversy exists regarding whether human forms of chronic metabolic alkalosis associated with both K and Cl depletion are also maintained hemodynamically, by a depression in GFR. There is conflicting information in chronic metabolic alkalosis on whether GFR is normal (24, 41) or reduced (31, 43). Again, further systematic studies are needed on this important clinical pathophysiologic question.

In the clearance experiments examining the repair of alkalosis, the opposite changes in plasma bicarbonate concentration and GFR occurred to mirror the effects in the induction series (Fig. 7). Selective repletion with K or Cl only partially repaired the alkalosis and GFR, while KCl repaired both, as has been previously described (13, 30, 33, 42). The rat is more "chloride-resistant" than dog or man with respect to repair of alkalosis with NaCl (13, 29, 30).

The normality of filtered bicarbonate loads during

[3] Cl depletion has also been suggested to stimulate distal hydrogen ion and K secretion by limiting anion delivery to the distal exchange sites (33, 40, 41). However, the Cl-deficient groups (groups II, III, V, VI) receiving supplemental bicarbonate did not achieve a blood bicarbonate concentration disproportionately higher than that expected for a normal filtered bicarbonate load and normal rate of renal acidification. Thus, no enhanced net renal acidification was revealed by bicarbonate loading, even with DOCA administration.

all stages of alkalosis and repair, even in animals given supplemented bicarbonate, supported the thesis that the rate of absolute renal acidification was unchanged by K and/or Cl depletion. Rather, that state of K and Cl stores modulated GFR so the metabolic alkalosis was present only when GFR was diminished.

If all the clearance studies are taken as a whole, it appeared that K depletion and Cl depletion had apparently independent and additive roles in affecting GFR. In the following analysis, an attempt is made to illustrate the empiric relationship of changes in GFR that were evoked by alterations in K and/or Cl stores. For this purpose, alteration in K stores is reflected by changes in muscle [K] (30), and alteration in Cl stores is reflected according to its impact on renal hemodynamics by changes in plasma volume (8, 9). The effect of changes in K and Cl stores on changes in GFR can be expressed quantitatively using multiple linear regression analysis. The equation was: $\Delta GFR = 0.68\ \Delta$ muscle [K] $+ 0.28\ \Delta$ plasma volume $+ 2.2$, $r^2 = 0.80$, $P < 0.001$. The change in GFR was significantly and independently related to both changes in muscle [K] and plasma volume. The best correlation, however, was with both variables combined, as indicated in the equation above. The conclusion that K and Cl depletion were additive factors in maintaining metabolic alkalosis in the rat agrees with that of Luke and Levitan (13). The present studies suggest that K and Cl depletion exerted their impact on the severity of metabolic alkalosis by modulation of GFR.

In conclusion, in all conditions of chronic metabolic alkalosis in the rat, the elevation in the plasma bicarbonate concentration was found to be inversely proportional to the fall in GFR. Filtered bicarbonate loads remained constant, so that a normal rate of renal acidification was sufficient to maintain the alkalosis.

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