Effects of Acetazolamide on Proximal Tubule Cl, Na, and HCO_3 Transport in Normal and Acidotic Dogs during Distal Blockade

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ABSTRACT It has been suggested that the establishment of a tubular fluid to plasma chloride gradient in the late proximal tubule by the reabsorption of bicarbonate (and other anions) in the early proximal tubule is responsible for a significant part of sodium chloride and water reabsorption in the proximal tubule. In the present study the effects of acetazolamide on proximal tubule water and electrolyte excretion were examined in 6 normal dogs and 10 chronic ammonium chloride-loaded dogs during distal blockade produced by ethacrynic acid and chlorothiazide administration. During distal blockade control urine/plasma osmolality and urine/plasma sodium were close to unity in all experiments. Urine/plasma chloride and urine/plasma bicarbonate were 1.21±0.02 and 0.75±0.07 in normal and 1.24±0.01 and 0.04±0.01 in acidotic dogs, respectively. After the administration of acetazolamide (20 mg/kg i.v.), there was a significant increase in urine flow, absolute and fractional excretion of sodium, bicarbonate, and chloride in all animals. Associated with these effects, urine/plasma osmolality and urine/plasma sodium remained unchanged but urine/plasma chloride decreased significantly to 1.15±0.01 in normal and to 1.19±0.01 in acidotic dogs. In acidotic dogs there was a significant correlation between the increase in bicarbonate, sodium, or chloride excretion after acetazolamide and the plasma bicarbonate level (range 6.8–12.5 meq/liter). These data demonstrate a significant effect of acetazolamide on bicarbonate, sodium, and chloride reabsorption in the proximal tubule even in the face of severe acidosis. Moreover, the data suggest that the decrease in chloride reabsorption (and accompanying sodium) after acetazolamide is related to the decrease in bicarbonate reabsorption and the associated decrease in the transtubular chloride gradient.

INTRODUCTION

Over the past few years there have been several reports suggesting that the establishment of a tubular fluid to plasma chloride gradient in the late proximal tubule by the reabsorption of bicarbonate (and possibly other anions) in the early proximal tubule is responsible for a significant part of the sodium and water reabsorbed in the proximal tubule (1–9). That is, passive

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diffusion of chloride downhill along the established gradient will lead to a positive potential difference (lumen to blood) and, secondarily, sodium (and water) reabsorption. All of the above studies have used micropuncture and microperfusion techniques in intact kidneys or isolated tubules of superficial nephrons. In the only report using clearance methods, the investigators employed the rat isolated perfused kidney model to examine sodium and bicarbonate transport and found that the effect of altering bicarbonate reabsorption on sodium excretion was stoichiometric, suggesting that the chloride gradient mechanism was not important for the reabsorption of a significant fraction of the glomerular filtrate (10).

The present studies were undertaken to test the significance of the chloride gradient in the proximal tubular handling of sodium in intact dogs using clearance methods. To examine proximal tubule transport the technique of distal blockade was adopted. To evaluate the relationship of bicarbonate reabsorption to chloride and sodium transport, acetazolamide, a carbonic anhydrase inhibitor, was administered to normal dogs and dogs with metabolic acidosis secondary to chronic ammonium chloride loading. The data demonstrate that acetazolamide decreases sodium chloride as well as sodium bicarbonate reabsorption in normal and severely acidotic dogs. Furthermore, because of the relationship between sodium chloride excretion and the plasma bicarbonate level (and bicarbonate excretion) the data suggest that chloride reabsorption is linked to bicarbonate reabsorption and is best explained by the mechanism of the chloride gradient.

METHODS

All experiments were performed on female mongrel dogs weighing approximately 15–20 kg. 16 normal dogs were maintained on a regular kennel diet containing 60 meq of sodium and 40 meq of potassium per day. Among these, 10 dogs were kept in metabolic balance cages, weighed daily, and given 20 meq/kg/day of ammonium chloride in two equally divided doses via orogastric tube for 3–7 days to produce metabolic acidosis with a wide range of plasma HCO_3. Attempts were made to replace NH_4Cl-induced urinary loss of Na, Cl, and K to avoid extracellular fluid volume contraction. Before NH_4Cl loading, base-line anaerobic arterial blood and urine were obtained for measurement of pH, pCO_2, Na, Cl, K, and osmolality.

Before the acute clearance experiment food and water were withheld for 16 h. On the morning of the experiment, the dogs were weighed and anaerobic arterial blood and urine specimens were obtained. The animals were then anesthetized with pentobarbitol, 30 mg/kg, and light anesthesia was maintained with subsequent small doses as needed. An endotracheal tube was inserted and respiration was regulated with a Bird respirator (Bird Corporation, Palm Springs, Calif.) supplying 60% oxygen. Arterial pCO_2 was maintained at 35–40 mm Hg in normal dogs and at 25–30 mm Hg in acidotic dogs. The urinary bladder was catheterized with a no. 16 Foley catheter (C. R. Bard, Inc., Murray Hill, N. J.). All urine specimens were obtained under oil. Blood samples were obtained anaerobically through an Angiocath (Deseret Pharmaceutical Co., Sandy, Utah) secured into a femoral artery.

After initial blood and urine samples were obtained, a priming dose of inulin was administered. A sustaining solution of normal saline containing sufficient inulin to maintain adequate blood levels was infused intravenously at 0.5 ml/min with a constant infusion pump for the remainder of the experiment. After an initial 60-min equilibration period, urine and blood specimens were collected and blockade of the distal tubule was initiated. Each animal received an intravenous prime of 50 mg of ethacrynic acid and 250 mg of chlorothiazide followed immediately by a sustaining infusion of these diuretics at 40 and 200 mg/h, respectively, in normal saline infused at 1 ml/min for the entire experiment. Urine specimens were then obtained at 10-min intervals and a blood specimen was obtained after every fourth clearance period. After a diuresis commenced, intravenous replacement was begun with a solution containing 135 meq/liter Na, 123 meq/liter Cl, 22 meq/liter HCO_3, and 10 meq/liter K in normal dogs, and 120 meq/liter Na, 130 meq/liter Cl, and 10 meq/liter K in acidotic dogs. This replacement solution was infused at a rate of 2 ml/min less than the urinary flow rate after discounting the first 100 ml of urine. Maximal blockade of the distal tubule was considered achieved when the urine/plasma osmolality ratio reached unity and when urine flow stabilized with less than 1 ml/min variation over a 30-min period. After 30 min had passed in this steady state, three 5-min clearance periods were obtained with concomitant midpoint arterial bloods. Acetazolamide (ACZ),[1] 20 mg/kg, was then administered intravenously as a bolus. All additional urine output above the steady state urine flow obtained during each 5-min collection period was matched with the same replacement fluid.

All urine and blood specimens were analyzed for osmolality, Na, K, Cl, pH, and pCO_2 according to methods previously reported from this laboratory (11). Plasma HCO_3 concentration was derived from the Henderson-Hasselbalch equation with a pK′ of 6.1. Urinary HCO_3 concentration was also derived from the Henderson-Hasselbalch equation, with a pK′ estimated from $6.33-0.5\sqrt{B}$, where B represents the sum of Na + K concentrations in equivalents per liter to account for ionic strength. The solubility coefficients used to convert CO_2 tension to H_2CO_3 were 0.0301 and 0.0309 for plasma and urine, respectively.

Statistical analysis between groups was performed by the Student's *t* test and by the paired *t* test where only one experimental condition was induced. Regression lines and correlation coefficients were calculated by the method of least squares. All results are expressed as mean±SE.

RESULTS

Table I summarizes the effects of distal tubular blockade followed by ACZ administration in six normal dogs. Control values represent the mean of three consecu-

[1] *Abbreviations used in this paper*: ACZ, acetazolamide; FE, fractional excretion; FR, fractional reabsorption; U/P, urine/plasma; V, absolute urine flow; V/GFR, fractional urine flow.

TABLE I

*Effects of Distal Blockade Followed by Acetazolamide Administration in Normal Dogs**

Dog no.	V	V/GFR	U_{Cl}	U/P Cl	U_{HCO_3}	U/P HCO_3	$U_{Na}V$	FE Na	$U_{Cl}V$	FE Cl	$U_{HCO_3}V$	FE HCO_3	U_K	U_KV	GFR
	ml/min	%	*meq/liter*		*meq/liter*		*μeq/min*	%	*μeq/min*	%	*μeq/liter*	%	*meq/liter*	*μeq/min*	*ml/min*
1 C	21.1	34.6	133	1.23	21.2	0.96	2,998	34.3	2,814	42.9	354	28.2	10	211	61
E	22.4	46.1	126	1.12	20.5	0.99	3,093	43.9	2,772	53.1	493	49.5	11	246	48
2 C	17.1	29.2	121	1.19	16.5	0.77	2,297	26.7	2,057	34.5	282	22.6	14	239	58
E	22.3	44.3	115	1.12	26.9	1.16	3,131	42.1	2,565	50.0	600	51.3	14	312	50
3 C	18.2	30.3	139	1.16	9.8	0.58	2,734	30.9	2,513	35.0	176	17.3	9	164	60
E	19.2	33.8	137	1.15	12.0	0.67	2,901	34.6	2,630	38.9	231	22.6	12	214	57
4 C	19.9	31.1	132	1.21	19.1	0.90	2,968	31.0	2,641	38.0	380	28.8	10	199	64
E	21.8	40.0	128	1.15	22.9	1.04	3,244	39.5	2,790	46.3	499	41.7	11	240	54
5 C	14.8	33.6	138	1.16	15.1	0.80	2,187	32.5	2,047	39.4	203	24.8	11	163	44
E	18.5	45.1	138	1.15	18.2	0.94	2,766	43.1	2,553	51.5	337	41.6	11	204	41
6 C	18.7	23.9	147	1.30	9.5	0.50	2,839	25.1	2,742	31.2	178	11.6	10	187	78
E	20.2	32.8	139	1.23	16.0	0.84	3,097	34.6	2,808	40.3	323	27.6	11	222	62
Mean C	18.3	30.5	135	1.21	15.2	0.75	2,671	30.1	2,469	36.8	262	22.2	10.7	193	61
±SE	±0.9	±1.5	±3	±0.02	±1.9	±0.07	±142	±1.4	±138	±1.7	±37	±2.7	±0.7	±12	±4
E	21.7	41.0	129	1.15	19.8	0.94	3,039	40.4	2,686	46.7	417	40.3	11.7	240	52
	±0.1	±2.1	±3	±0.01	±1.9	±0.06	±71	±1.4	±48	±2.4	±54	±4.0	±0.6	±16	±3
P value	<0.025	<0.0005	<0.005	<0.005	<0.025	<0.01	<0.05	<0.0005	<0.05	<0.0005	<0.005	<0.0005	<0.05	<0.0005	<0.005

* C, control; E, post acetazolamide; V, urine flow rate; V/GFR, fractional urine flow rate; U_{Cl}, urine chloride; U/P Cl, urine/plasma chloride ratio; U_{HCO_3}, urine bicarbonate; U/P HCO_3, urine/plasma bicarbonate ratio; $U_{Na}V$, absolute sodium excretion; FE Na, fractional sodium excretion; $U_{Cl}V$, absolute chloride excretion; FE Cl, fractional chloride excretion; $U_{HCO_3}V$, absolute bicarbonate excretion; FE HCO_3, fractional bicarbonate excretion; U_K, urine potassium; U_KV, absolute potassium excretion; GFR, glomerular filtration rate.

tive steady-state clearance periods during maximal blockade of the distal tubule, as described in Methods. The experimental values represent the period of peak urine flow rate after ACZ administration, usually the second or third 5-min clearance period.

During maximal distal blockade in normal dogs, urine/plasma (U/P) osmolality was 0.99±0.01 and U/P Na was 0.99±0.01. Absolute urine flow (V) and fractional urine flow (V/GFR) were 18.3±0.9 ml/min and 30.5±1.5% respectively. Urine HCO_3 was 15.2±1.9 meq/liter and plasma HCO_3 was 19.9±0.8 meq/liter so that U/P HCO_3 was 0.75±0.07. Urine Cl was 135±3 meq/liter and plasma Cl was 112±3 meq/liter with a U/P Cl ratio of 1.21±0.02. Fractional Cl excretion (FE Cl) was 36.8±1.7%, which was significantly greater than the fractional Na excretion (FE Na) of 30.1±1.4% ($P < 0.001$). Fractional HCO_3 excretion (FE HCO_3) was 22.2±2.7%. Plasma K was 3.7±0.2 meq/liter and urine K concentration was 10.7±0.7 meq/liter. Urinary K excretion was 193±12 μeq/min and fractional K excretion (FE K) was 88.7±9.4%. Plasma total protein was 6.6±0.3 g/100 ml.

After ACZ administration there was no change in U/P osmolality, U/P Na, plasma Cl, HCO_3, and plasma protein. A significant increase in V and V/GFR was seen despite a significant fall in GFR from 61±4 to 52±3 ml/min. V and V/GFR increased to 21.7±0.1 ml/min and 41.0±2.1%, respectively. Associated with these changes, urine HCO_3 and U/P HCO_3 increased significantly to 19.8±1.9 meq/liter and 0.94±0.06, respectively. In addition, there was a significant increase in Na excretion from 2,670±142 to 3,039±71 μeq/min and in FE Na to 40.4±1.4%. There was a similar rise in Cl excretion from 2,469±138 to 2,686 ±48 μeq/min and in FE Cl to 46.7±2.4%. The increase in Cl excretion was accompanied by a significant decrease in U/P Cl ratio in every experiment, to a mean of 1.15±0.01. ACZ also significantly increased urinary K concentration to 11.7±0.6 meq/liter and, therefore, K excretion increased significantly to 240 ±16 μeq/min. Plasma K remained unchanged and FE K increased significantly to 127±14.5% ($P < 0.0005$).

After NH_4Cl loading in 10 dogs, arterial pH decreased from 7.38±0.02 to 7.12±0.03 ($P < 0.005$) and arterial pCO_2 decreased from 37±2 to 29±1 mm Hg ($P < 0.005$). Plasma HCO_3 decreased from 19.7±0.6 to 9.4±0.9 meq/liter ($P < 0.001$) and plasma Cl increased from 106±3 to 124±2 meq/liter ($P < 0.005$). In these acidotic dogs there was a slight weight reduction of 3%, which was not significant. Plasma Na, K, osmolality, and hematocrit did not change significantly after NH_4Cl loading.

After NH_4Cl loading, urinary pH decreased from 6.47±0.20 to 5.52±0.07 ($P < 0.01$). No significant change was noted in urine Na or K, but urine Cl increased from 72±14 to 257±39 meq/liter ($P < 0.001$).

Table II summarizes the effects of distal blockade followed by ACZ administration in 10 acidotic dogs. During distal blockade alone, U/P osmolality was 0.98±0.01 and U/P Na was 0.97±0.08. Plasma and urine

Cl (124±2 meq/liter and 154±2 meq/liter, respectively) were significantly higher in the acidotic than in the normal dogs ($P < 0.01$). However, the U/P Cl ratio of 1.24±0.01 was not significantly different from that obtained in normal dogs (1.21±0.02). Plasma and urine HCO_3 (10.1±0.7 meq/liter and 0.5±0.2 meq/liter, respectively) were significantly lower in the acidotic than in the normal dogs ($P < 0.001$). Plasma K was 3.5±0.1 meq/liter and urine K concentration was 7.0 ±0.6 meq/liter. Urinary K excretion was 112±9 μeq/min and FE K was 69.2±4.7%. Plasma total protein was 6.8±0.6 g/100 ml, not significantly different from normal dogs.

After ACZ administration, a similar response was seen in these acidotic dogs as in the normal dogs. No changes were noted in U/P osmolality, U/P Na, plasma Cl, HCO_3, or total protein. There was a significant increase in absolute and fractional V, as well as absolute and fractional excretion of HCO_3, Cl, and Na, despite a significant fall in GFR from 46.4±3.0 to 40.5±2.7 ml/min. As in normal dogs, U/P Na and U/P osmolality ratios remained unaltered. V/GFR increased from 36.7±3.7 to 46.0±4.2% and FE HCO_3 increased from 2.1±0.9 to 15.0±3.2%. FE Na increased from 35.7±3.5 to 44.2±4.0% and FE Cl increased from 45.2±4.5 to 53.7±4.9%. The increase in Cl excretion was again accompanied by a significant fall in U/P Cl ratio in all experiments, associated with an increase in U/P HCO_3 ratio. ACZ also significantly increased urinary K concentration and K excretion to 12±1 meq/liter and 196±18 μeq/min, respectively. Plasma K remained unchanged and FE K increased significantly to 134.8±13.1% ($P < 0.0005$).

The relationship between the plasma HCO_3 level and the effect of ACZ on FE HCO_3, FE Na, and FE Cl in acidotic dogs is summarized in Fig. 1, in which plasma HCO_3 concentration is plotted against the increase in FE HCO_3 in the upper panel and against the increase in FE Na and FE Cl in the lower panel. It is apparent that the effect of ACZ on FE HCO_3 diminished as the plasma HCO_3 level was reduced, as indicated by a linear relationship, $y = 2.80x - 16.18$ ($r = 0.77$) ($P < 0.01$). A similar relationship exists for FE Na, $y = 1.06x - 2.61$ ($r = 0.73$) ($P < 0.02$), and for that of FE Cl, $y = 1.12x - 3.17$ ($r = 0.75$) ($P < 0.02$). No significant difference was noted between slopes for sodium and chloride, indicating a similar reduction in reabsorption induced by ACZ administration.

DISCUSSION

The technique of distal tubular blockade, first introduced by Early et al. (12), has frequently been utilized to assess proximal tubular fluid and sodium reabsorp-

TABLE II

*Effects of Distal Blockade Followed by Acetazolamide Administration in Acidotic Dogs**

Dog no.	V	V/GFR	U_{Cl}	U/P Cl	U_{HCO_3}	U/P HCO_3	$U_{Na}V$	FE Na	$U_{Cl}V$	FE Cl	$U_{HCO_3}V$	FE HCO_3	U_K	U_KV	GFR
	ml/min	%	*meq/liter*		*meq/liter*		*μeq/min*	%	*μeq/min*	%	*μeq/liter*	%	*meq/liter*	*μeq/min*	*ml/min*
1 C	15.4	29.1	162	1.27	0.1	0.01	2,223	28.5	2,490	36.5	15	2.4	5	77	53
E	18.8	47.0	155	1.23	5.9	0.41	2,779	40.2	2,914	48.8	111	19.3	8	150	40
2 C	11.0	24.4	163	1.25	0.5	0.06	1,600	23.7	1,793	30.1	6	1.1	8	88	45
E	11.5	29.5	160	1.23	2.3	0.26	1,716	29.2	1,840	36.1	28	6.7	11	126	39
3 C	14.7	33.4	156	1.30	0.9	0.08	2,214	34.9	2,293	43.6	14	2.8	6	88	44
E	14.8	38.9	149	1.24	4.2	0.33	2,186	39.7	2,205	48.7	51	12.7	10	148	58
4 C	23.9	35.1	151	1.15	0	0	3,250	33.8	3,609	40.6	0	0	6	143	68
E	25.8	41.6	146	1.10	1.0	0.12	3,594	40.6	3,767	45.9	31	6.1	10	258	62
5 C	13.6	28.9	152	1.28	0	0	1,832	28.0	2,067	36.8	0	0	8	109	47
E	14.1	33.6	146	1.24	2.0	0.20	1,918	32.9	2,057	40.7	25	6.2	12	169	42
6 C	18.0	41.9	156	1.22	1.6	0.13	2,590	40.9	2,808	51.3	28	5.6	6	108	43
E	20.5	52.6	147	1.15	6.5	0.52	2,993	51.8	3,014	61.1	132	27.4	8	164	39
7 C	12.2	30.5	160	1.21	0.1	0.01	1,739	29.4	1,952	36.6	1	0	9	110	40
E	14.3	40.9	152	1.14	4.5	0.36	2,019	39.1	2,174	46.4	64	14.6	18	257	35
8 C	10.6	30.2	145	1.23	0.9	0.08	1,437	28.0	1,537	36.8	10	2.4	11	117	35
E	12.5	40.3	143	1.22	3.9	0.35	1,680	37.1	1,788	49.4	49	14.3	16	200	31
9 C	28.2	54.2	149	1.25	1.9	0.15	4,024	52.2	4,202	67.3	54	8.3	6	169	52
E	30.2	67.1	140	1.16	6.6	0.55	4,297	64.9	4,228	77.6	199	36.9	10	302	45
10 C	22.1	59.7	146	1.23	0.1	0.01	2,975	57.5	3,227	72.4	1	0.4	5	111	37
E	23.4	68.8	141	1.20	1.4	0.13	3,145	66.4	3,299	82.6	33	8.7	8	187	34
Mean C	17.0	36.7	154	1.24	0.5	0.04	2,388	35.7	2,598	45.2	12	2.1	7.0	112	46
±SE	±1.9	±3.7	±2	±0.01	±0.2	±0.01	±268	±3.5	±271	±4.5	±6	±0.9	±0.6	±9	±3
E	18.6	46.0	148	1.19	3.7	0.31	2,633	44.2	2,729	53.7	71	15.0	12.0	196	41
	±2.0	±4.2	±2	±0.01	±0.7	±0.04	±278	±4.0	±268	±4.9	±18	±3.2	±1.2	±18	±3
P value	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.005	<0.005	<0.0005	<0.025	<0.0005	<0.005	<0.0005	<0.0005	<0.0005	<0.0005

* For explanation, see Table I.

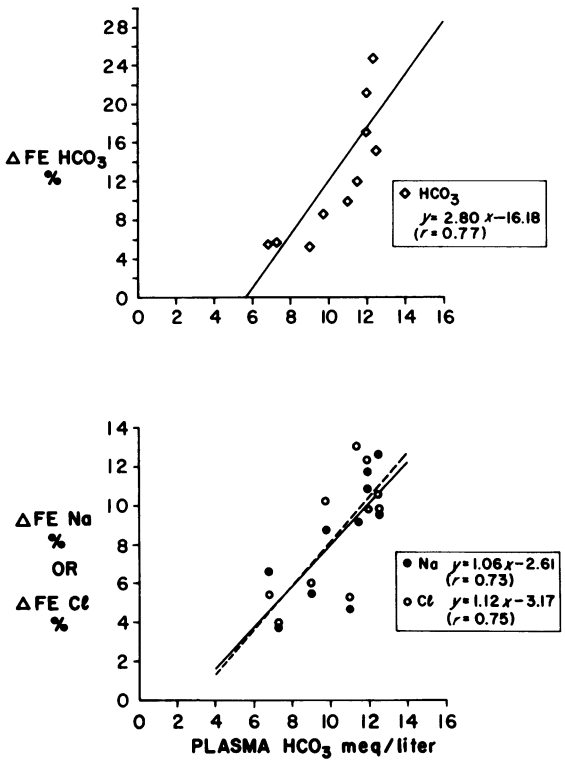

FIGURE 1 The effects of acetazolamide on the fractional excretion of bicarbonate (FE HCO_3), sodium (FE Na), and chloride (FE Cl) at various plasma bicarbonate levels during distal blockade in acidotic dogs.

tion, but has not been used as a means of evaluating proximal Cl transport (13–17). Traditionally, distal blockade was considered to be secondary to inhibition of Na transport in the diluting segment by ethacrynic acid and chlorothiazide. It is now recognized that Cl transport in the thick ascending limb of Henle is active, leading to a positive voltage in the tubular lumen and passive Na transport (18, 19). The in vitro microperfusion studies have concluded inhibition of active Cl transport by ethacrynic acid in this nephron segment (20) and micropuncture studies have demonstrated a major inhibitory effect of chlorothiazide on Na and Cl transport in the distal tubule (21), making the technique of distal blockade particularly useful for assessing proximal tubule handling of Cl. Ideally, distal blockade should be accomplished with drugs that have no effect on proximal tubular fluid and ion transport. This may not be precisely the case for ethacrynic acid and chlorothiazide (21–23), but it is unlikely that the relatively small proximal tubular effect of those two agents administered together before ACZ significantly altered the results or interpretation. During distal blockade alone the U/P Na ratio was close to unity in all experiments (Tables I and II), indicating that most, if not all, distal Na reabsorption was blocked by the two drugs. Furthermore, during the steady state approximately 70% of the filtered sodium was reabsorbed in the normal dogs (Table I), a value in close proximity to that predicted for total proximal tubular sodium reabsorption by micropuncture studies (24–26).

During control distal blockade, U/P Cl was 1.21 ±0.02 in normal dogs and 1.24±0.01 in acidotic dogs,[2] values almost identical with the end proximal tubular fluid/plasma (TF/P) Cl ratios obtained by micropuncture in normal and acidotic rats (induced by chronic NH_4Cl loading; 33, 34). Bernstein and Clapp (35), employing the quinhydrone electrode in micropuncture studies, have reported late proximal tubule TF/P HCO_3 not different from unity in the normal dog. This data has been used by other authors as evidence against the presence of a significant Cl gradient in the proximal tubule of the dog (10, 36). However, Marchand et al. (37) recently reported similar proximal TF/P Cl ratios in the rat and the dog, similar to those obtained in the present study.

The larger FE Cl than FE Na in the control state in the present study suggests a greater fractional reabsorption of Na (FR Na) than of Cl (FR Cl) in the proximal tubule. As seen in Fig. 2, in which FR Cl was plotted against FR Na, FR Cl invariably falls below the identity line, both in normal and acidotic dogs. The difference between the fractional reabsorptive rates for Na and Cl in the present clearance study of 6.7% is almost identical with the values obtained in micropuncture studies in the rat (34).

After the administration of ACZ in normal dogs, there was a significant rise in absolute and fractional V and excretion of Cl, Na, and HCO_3. Although micropuncture studies by Malnic et al. (33) and Kunau (34) in rats have also shown a significant effect of carbonic anhydrase inhibition on proximal Cl reabsorption, early clearance studies failed to demonstrate increased Cl excretion after ACZ (38, 39) despite the findings of a decrease in sodium and fluid reabsorption in the proximal nephron in later studies (26, 40, 41). In the clearance study of Rosin et al. (42), increased Cl excretion became apparent only after significant volume expansion, which presumably depressed Cl reabsorption in the distal nephron to some extent (43), suggesting that the absence of a chloruresis in previous

[2] The U/P Cl ratio of 1.24±0.01 in the presence of acidosis and a plasma HCO_3 of 10.1±0.7 meq/liter might be explained by the increased reabsorption in the proximal tubule of other anionic species. There are indirect data to suggest that lactate, phosphate, and citrate reabsorption may be increased in the proximal tubule in acidosis (27–32).

studies must then be largely accounted for by enhanced Cl reabsorption in the loop of Henle, where active Cl transport takes place (18, 19). It is, therefore, not surprising to observe ACZ increasing delivery of Cl out of the proximal nephron comparable to that of Na when distal blockade was utilized in the present study.

The findings of a significant fall in U/P Cl and a significant rise in U/P HCO_3 ratios upon the administration of ACZ are in agreement with those of micropuncture studies in which carbonic anhydrase was inhibited by either ACZ or benzolamide (33, 34). It should be noted that the increase in V produced by ACZ in the present experiments is accompanied by an essentially unchanged U/P osmolality and U/P Na, suggesting that the primary effect of ACZ is in the proximal tubule.

It is well known that the response to ACZ is greatly reduced or eliminated in the presence of metabolic acidosis and that the acidosis resulting from the administration of ACZ is an important factor in the development of resistance to continued treatment with this drug (38, 39). The apparent reversion of urine electrolytes to pretreatment values after continuous administration of ACZ (38, 39) does not necessarily mean that the proximal tubule no longer responds to this drug. Rector et al. (44) and Schwartz et al. (45) have demonstrated that ACZ significantly reduced HCO_3 reabsorption in acidotic dogs even at a low plasma HCO_3 level. Moreover, the persistence of acidosis during chronic administration of ACZ and the subsequent correction of the acidosis only after cessation of treatment indicate that the mechanism for urinary acidification has been continuously inhibited. Thus, it would appear that other factors come into play to alter the urinary excretion pattern after ACZ in acidotic dogs. It seems reasonable to argue that the initial diuresis induced by ACZ produces extracellular fluid volume contraction which can mask the effect of ACZ in the proximal tubule due to enhanced NaCl reabsorption in the distal nephron, a phenomenon shown in clearance and micropuncture studies in salt-depleted dogs and rats (46, 47). The advantage of distal blockade in the present study is to unmask the effects of ACZ on proximal tubular reabsorption of Cl as well as Na, HCO_3, and water in severely acidotic dogs.

Recently, a number of micropuncture and microperfusion studies have provided evidence that HCO_3 plays an important modulating role in enhancing fluid and Na transport in the mammalian superficial proximal convoluted tubule (1–9, 36, 48). It has been proposed that preferential tubular reabsorption of HCO_3 and other anions in the very first portion of the proximal tubule mediates an increase in luminal Cl concentration significantly above that of plasma in the late portion of proximal tubule. The diffusion of Cl down its concentration gradient in this Cl-permselective nephron segment generates a lumen-positive transepithelial voltage, which serves as an electrical driving force for Na reabsorption. Furthermore, the reabsorbed HCO_3, having a higher reflection coefficient than NaCl, effects some Na reabsorption by solvent drag. Thus, it would appear that in addition to active Na transport, proximal tubular fluid movement is dependent upon HCO_3 reabsorption which supports additional Na transfer both by bulk flow and by enhancing passive Cl reabsorption.

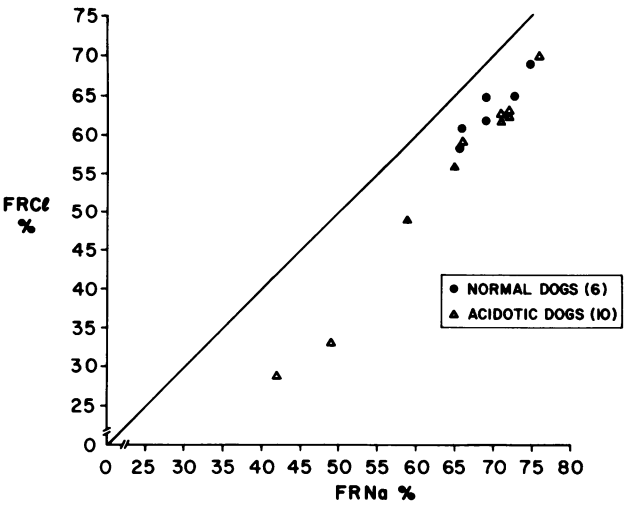

FIGURE 2 Fractional reabsorption of chloride (FR Cl) vs. fractional reabsorption of sodium (FR Na) during distal blockade in normal and acidotic dogs.

In the present study the ACZ-induced increase in V, HCO_3, Na, and Cl excretion during distal blockade was accompanied by a significant decrease in U/P Cl ratio. It is conceivable that as ACZ inhibits proximal HCO_3 reabsorption, there results a less effective osmotic drag for fluid and Na translocation along with a decrease in luminal Cl concentration. Passive outward diffusion of Cl, normally driven by a large chemical concentration gradient, would be reduced leading to a further decrease in Na and water reabsorption. In support of this proposal is the finding that administration of ACZ prevents a rise in tubular fluid Cl concentration and potential difference along the late portion of proximal tubule in micropuncture and microperfusion studies (5, 49). Inasmuch as the final urine in the present study is the composite of all nephron populations, the contribution of a decreased Cl diffusion gradient produced by ACZ to the reduction of proximal tubule fluid and NaCl reabsorption can only be determined qualitatively and not quantita-

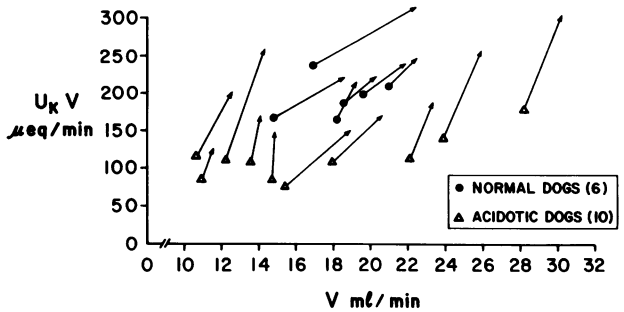

FIGURE 3 The effects of acetazolamide on K excretion in normal and acidotic dogs during distal blockade.

tively in view of intrinsic functional differences between the proximal tubule of the superficial and juxtamedullary nephrons (50). Alternatively, ACZ may also directly inhibit proximal NaCl reabsorption independent of the effect on HCO_3 reabsorption, particularly if some of the effect on HCO_3 transport takes place in the distal tubule. However, as plasma HCO_3 concentration diminished in acidotic dogs, there was a proportional reduction in the effect of ACZ on fractional NaCl excretion as well as on fractional HCO_3 excretion (Fig. 1), strongly suggesting that ACZ inhibits NaCl reabsorption by a mechanism related to its effects on proximal HCO_3 transport. Moreover, it is unlikely that the effect of ACZ on HCO_3 reabsorption in the distal nephron would result in a significant fall in U/P Cl ratio.

In the present study, ACZ administration during distal blockade also augmented urinary K excretion in normal and acidotic dogs. Beck et al. (51) have reported that ACZ did not change fractional reabsorption of K in the proximal tubule. In addition it is generally accepted that the bulk of urinary K is derived from a distal secretory process (40, 52, 53) and that K secretion is dependent upon urinary flow rate, per se (54–56). Fig. 3 demonstrates that K excretion increases as V increases in the present experiments. It might be argued that increased Na excretion associated with the increase in V after ACZ accounts for the increased K excretion. However, before ACZ administration, during distal blockade, there was already sufficient sodium available for exchange so that this could not be the limiting factor. Moreover, Na concentration did not change after ACZ. Another factor responsible for the increased K excretion might be the increased amounts of HCO_3 in the distal tubule which would behave as a nonreabsorbable anion further increasing luminal negativity leading to increased K secretion (53, 57). Furthermore, K secretion in the cortical collecting tubule has been shown to increase when the luminal pH is raised (58). Finally, increased phosphate excretion after ACZ would lead to the same effect (26, 59).

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