THE ROLE OF PLASMA CO_2 TENSION AND CARBONIC ANHYDRASE ACTIVITY IN THE RENAL REABSORPTION OF BICARBONATE *

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The reabsorption of bicarbonate by the kidneys under normal circumstances appears to be a linear function of the plasma CO_2 tension (1–3). Previous studies from this laboratory (4) demonstrated that when carbonic anhydrase was inhibited by acetazolamide the linear relationship between plasma CO_2 tension and HCO_3^- reabsorption still obtained. It was therefore proposed that the uncatalyzed, as well as the catalyzed, hydration of CO_2 was an important source of the H^+ involved in the reabsorption of HCO_3^-.

The present investigations were undertaken in an attempt to characterize more precisely the catalyzed and uncatalyzed reactions. By varying plasma CO_2 tension, carbonic anhydrase activity, and filtered HCO_3^-, three aspects of HCO_3^- reabsorption were examined: *1*) the maximal reabsorptive capacity, or the HCO_3^- Tm, with and without carbonic anhydrase activity; *2*) the relation of HCO_3^- excretion to HCO_3^- Tm with and without carbonic anhydrase activity; *3*) the capacity of high plasma CO_2 tensions to effect complete HCO_3^- reabsorption in the absence of carbonic anhydrase.

On the basis of these studies it was concluded that HCO_3^- reabsorption is mediated by two distinct processes. One process has a HCO_3^- Tm which is dependent on plasma pCO_2 and independent of carbonic anhydrase activity. The second process is dependent on carbonic anhydrase, independent of plasma pCO_2, and necessary for the establishment of sharp pH gradients between blood and urine.

METHODS

Experiments were performed on female dogs anesthetized with either Nembutal, sodium pentothal, or Fluothane. An endotracheal tube, fitted with an inflatable cuff, was inserted into the trachea and connected to a Bird assisted-respiratory anesthesia unit. Respiratory movements were inhibted by either d-tubocurare, succinylcholine, or gallium triethiodide (Flaxedil) to facilitate control of rate and depth of ventilation with the respirator. The concentration of CO_2 in inspired air varied by controlling the flow rate of 100 per cent CO_2 and 100 per cent O_2 into the respirator. In some experiments alveolar pCO_2 was monitored with a Liston-Becker infrared CO_2 analyzer.

To determine the time required for HCO_3^- reabsorption to stabilize after an abrupt change in plasma pCO_2, 2 dogs were studied for 7 periods each. Plasma pCO_2 was abruptly elevated to approximately 150 mm Hg and then maintained at this level. Bicarbonate reabsorption reached a stable value within 10 minutes and remained constant for as long as 2 hours. A 15 minute equilibration period was chosen in order not to prolong unnecessarily the length of the experiment.

In the first group of experiments on 15 normal dogs, the effect of pCO_2 on the maximal HCO_3^- reabsorptive capacity (HCO_3^- Tm) was studied. Plasma HCO_3^- concentration was elevated by the injection of 12 g $NaHCO_3$ and maintained at a high level by the constant infusion of isotonic $NaHCO_3$ at the rate of approximately 10 ml per minute throughout the experiment. Plasma pCO_2 was varied in 10 dogs from extreme respiratory alkalosis (pCO_2, 6 mm Hg) to extreme respiratory acidosis (pCO_2, 400 mm Hg) by either hyperventilation or by changing the concentration of CO_2 in the inspired air. In 6 of these dogs the study was repeated after the administration of acetazolamide, 50 mg per kg body weight. In 5 additional dogs the studies were performed only after the administration of acetazolamide. The kidneys from 4 of the dogs given acetazolamide were removed at the termination of the experiment, weighed, and assayed for carbonic anhydrase activity.

In the second group of experiments the relationship of HCO_3^- excretion to HCO_3^- reabsorption was studied as plasma HCO_3^- concentration was progressively

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elevated from low values to levels at which a HCO_3^- Tm was demonstrated. Plasma pCO_2 was maintained constant at normal or elevated levels with and without acetazolamide administration. In 3 dogs pCO_2 was maintained at normal levels (33 to 40 mm Hg) by breathing room air. In 4 dogs pCO_2 was maintained at 85 to 100 mm Hg by breathing 9 per cent CO_2 and 91 per cent O_2. Eight additional dogs were studied at the normal (4 dogs) and elevated (4 dogs) tensions of CO_2 after the administration of acetazolamide (prime dose 50 mg per kg body weight; sustaining infusion, 1 mg per minute). After plasma pCO_2 was stabilized for about 1 hour, plasma HCO_3^- concentration was slowly elevated in stepwise fashion from depressed values by injections of 6 per cent $NaHCO_3$. After each injection an equilibration period of 15 to 20 minutes was permitted before beginning a collection period.

In the third group of experiments on 4 dogs a mild metabolic acidosis was induced by administration of 10 to 15 g NH_4Cl on the day preceding the experiment. Acetazolamide (25 to 50 mg per kg body weight) was administered to inhibit carbonic anhydrase activity. The plasma pCO_2 was then progressively elevated in an attempt to obliterate HCO_3^- excretion.

Urines were collected in oiled syringes through an indwelling catheter at the mid-point of the collection period for measurement of pH and CO_2 content. At the end of the collection period the bladder was emptied by manual compression and washed with 20 ml distilled water. Heparinized blood samples were drawn anaerobically from the femoral artery. Methods used were those previously described (4).

To determine the extent to which renal carbonic anhydrase was inhibited by the administration of 50 mg per kg of acetazolamide, the kidneys were removed at the end of the experiment and perfused with 300 to 500 ml of ice-cold isotonic saline to remove all red cells. In addition, the saline perfusion served to wash acetazolamide out of the intravascular and interstitial spaces, and also out of the tubular lumen, thus minimizing enzyme inhibition by acetazolamide not located within renal tubular cells. The kidneys were then homogenized in ice-cold distilled water, diluted 1 : 50, 1 : 100 and 1 : 1,000 and assayed by the method of Davis (5). With this method the addition of 1 ml of 1 : 1,000 solution of normal kidney to 100 ml of reaction solution produced a fivefold increase in the rate constant for the hydration of CO_2, whereas the addition of 200 times this quantity of kidney (10 ml of 1 : 50 solution of kidney) from a dog given 50 mg acetazolamide per kg body weight had no measurable effect on the rate constant.

RESULTS

I. Effect of plasma CO_2 tension on the HCO_3^- Tm

To study the effects of plasma CO_2 tension on the HCO_3^- Tm, plasma HCO_3^- concentration was raised to a level such that filtered HCO_3^- always greatly exceeded the HCO_3^- Tm. Schwartz, Falbriard and Lemieux (6) have presented data suggesting that the HCO_3^- Tm is approached gradually during acute respiratory acidosis. Data presented below (Figure 4), however, show that at CO_2 tensions ranging between 85 and 100 mm Hg a Tm of 3.7 mEq per L was obtained when plasma HCO_3^- concentration (corrected for Donnan factor) reached 41 mEq per L [i.e., filtered HCO_3^- per unit glomerular filtrate (GF) exceeded the Tm by approximately 15 per cent]. Similarly, at still higher CO_2 tensions (150 to 200 mm Hg) a Tm was obtained when filtered HCO_3^- per unit GF exceeded the Tm by only 10 to 12 per cent (7). With the exception of the experiment on Dog 4 (which was designed primarily to study HCO_3^- reabsorption during respiratory alkalosis) the concentration of HCO_3^- in GF always exceeded the HCO_3^- Tm by at least 25 per cent. In animals given acetazolamide, however, the HCO_3^- Tm was reached only when the filtered HCO_3^- was approximately twice the Tm at normal CO_2 tensions and about 1.5 to 1.8 times the Tm at elevated CO_2 tensions (Figure 5). Therefore, to insure valid Tm measurements in the presence of carbonic anhydrase inhibition, the concentration of HCO_3^- in GF was always maintained at a level 2.5 to 5.0 times greater than the HCO_3^- Tm.

A. Intact carbonic anhydrase enzyme system. In 10 dogs the plasma pCO_2 was varied from 6 to 400 mm Hg. The first two protocols of Table I summarize representative experiments. Figure 1 depicts the data from all the studies. The lowest plasma pCO_2 was 6 mm Hg, a level at which HCO_3^- reabsorption was still significant (1.4 mEq per 100 ml GF). Owing to the invariable appearance of pulmonary edema and hemolysis it was impossible to study HCO_3^- reabsorption below 6 mm Hg. For this reason the intercept value at zero CO_2 tension could not be identified. Although the over-all shape of the curve suggests that it might project through the origin, it could equally well intercept the vertical axis at some point above the origin.

Unlike previous studies (1–3), it is apparent from Figure 1 that increasing plasma pCO_2 accelerates HCO_3^- reabsorption in curvilinear fashion. Although the curve tends to become flat at higher CO_2 tensions, a point was never reached

TABLE I

The effect of CO_2 tension and carbonic anhydrase activity on the maximal bicarbonate reabsorptive capacity *

Time	Treatment	Plasma			Cin	Urine		Bicarbonate		
		HCO_3^-	pH	pCO_2		Flow	pH	Filt.	Excr.	Reab.
min		*mEq/L*		*mm Hg*	*ml/min*	*ml/min*		*μEq/min*	*μEq/min*	*mEq/100 ml GF*
Dog 4; wt. 30 kg										
0	Anesthesia, sodium pentothal and succinylcholine chloride; infuse 0.15 M $NaHCO_3$ at 10 ml/min									
60–70	Hyperventilation	24.3	7.83	14	60.8	1.66	8.26	1,475	354	1.84
70–80	Hyperventilation	22.5	7.89	11	70.6	1.18	8.49	1,585	184	1.98
80–90	Hyperventilation	21.4	7.93	10	80.5	1.30	8.49	1,725	153	1.95
105–115	Breathing room air	33.7	7.56	42	100	2.70	7.90	3,370	363	3.01
130–140	Breathing 9% CO_2	41.5	7.31	81	87.5	1.93	7.75	3,630	251	3.87
155–165	Breathing 16% CO_2	45.3	7.14	131	83.7	1.56	7.63	3,790	261	4.22
180–195	Breathing 23% CO_2	50.0	7.13	148	81.5	2.20	7.39	4,065	375	4.53
Dog 11; wt. 12 kg										
0	Anesthesia, sodium pentothal and succinylcholine chloride; prime 12 g $NaHCO_3$; infuse 0.15 M $NaHCO_3$ at 10 ml/min									
30–40	Hyperventilation	40.7	7.73	30	28.9	7.34	8.03	1,178	543	2.20
55–65	Breathing room air	46.1	7.58	48	31.0	8.34	7.75	1,430	547	2.85
80–90	Breathing 5% CO_2	54.2	7.54	62	29.5	8.07	7.72	1,595	695	3.05
105–115	Breathing 9% CO_2	61.0	7.45	86	30.0	7.47	7.65	1,830	775	3.53
130–140	Breathing 17% CO_2	66.8	7.29	136	30.0	6.48	7.55	2,005	715	4.29
155–165	Breathing 23% CO_2	68.4	7.20	172	29.7	6.22	7.36	2,030	670	4.58
180–190	Breathing 29% CO_2	70.8	7.12	214	28.7	6.69	7.29	2,030	700	4.63
206	Acetazolamide 600 mg i.v.; infuse 0.15 M $NaHCO_3$ at 10 ml/min and acetazolamide at 2 mg/min									
235–245	Breathing room air	67.7	7.73	50	21.5	11.1	7.86	1,458	1,200	1.20
260–270	Breathing 5% CO_2	71.0	7.52	85	21.3	10.2	7.63	1,512	1,088	1.99
285–295	Breathing 9% CO_2	74.9	7.48	99	20.9	9.68	7.56	1,565	1,075	2.34
310–320	Breathing 17% CO_2	76.2	7.32	145	20.2	9.21	7.51	1,540	1,005	2.65
335–345	Breathing 23% CO_2	78.9	7.28	165	18.1	8.44	7.44	1,425	930	2.75
360–370	Breathing 29% CO_2	80.3	7.16	221	19.6	8.34	7.27	1,510	965	3.09
385–393	Breathing 37% CO_2	80.1	7.06	279	18.7	8.52	7.18	1,500	935	3.22

* In Tables I–IV plasma bicarbonate concentrations have been corrected for a Donnan factor of 1.05.

at which additional increments in pCO_2 did not elicit further increases in HCO_3^- reabsorption. The straight line relationship previously reported is doubtless the result of the limited range over which plasma pCO_2 was varied.

B. Inhibited carbonic anhydrase enzyme system. In 11 dogs the HCO_3^- Tm was examined while the plasma pCO_2 was varied from 30 to 350 mm Hg after injection of large doses of acetazolamide. A sample protocol is presented in Table I; the data from all experiments are plotted in Figure 2.

Inhibition of red cell carbonic anhydrase prevented the lowering of the plasma pCO_2 below 30 mm Hg. At any given concentration of CO_2 in the inspired air, arterial pCO_2 averaged 20 to 40 mm Hg higher during acetazolamide administration than under normal circumstances.

The administration of acetazolamide tended to give somewhat scattered results when different animals were compared (Figure 2). To circumvent variations arising from different responses of dogs to acetazolamide, six dogs were studied over the complete range of plasma pCO_2 while carbonic anhydrase was intact and then restudied after administration of acetazolamide. The data from single animals generated remarkably smooth curves (Figure 3). The curve obtained after carbonic anhydrase inhibition is also curvilinear, in contrast to the straight line obtained in the previous study (4) where the plasma pCO_2 was varied over a much smaller range.

At the conclusion of the experiments, analysis of the kidneys revealed no demonstrable evidence of carbonic anhydrase activity. The absence of carbonic anhydrase activity in the kidneys of dogs

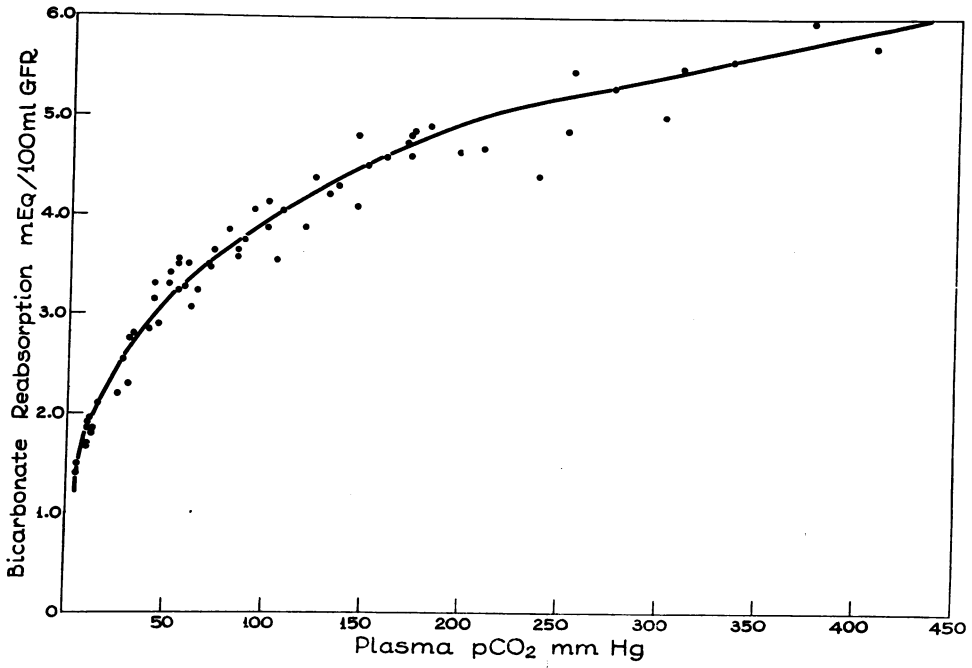

FIG. 1. RELATION BETWEEN PLASMA pCO_2 AND HCO_3^- REABSORPTION IN DOGS.

given acetazolamide cannot be cogently attributed to artifacts of the assay method. The extensive perfusion of the kidney before homogenization minimized contamination of intracellular enzyme with extracellular inhibitor. In addition, the assay method tends to err in the direction of exaggerating, not underestimating, enzyme activity, owing to dissociation of the enzyme-inhibitor complex in diluted kidney homogenates. This direct evidence of complete inhibition is consonant with the results of the recent kinetic analysis of carbonic anhydrase inhibition developed by Maren, Tarcell and Malik (8). These investigators have shown that the ratio of free enzyme [E] to inhibited enzyme [EI] is given by the expression:

$$\frac{[E]}{[EI]} = \frac{K_1}{[I]}$$

where K_1 for acetazolamide is 8×10^{-9}M and [I] is the tissue concentration of free acetazolamide. The administration of 50 mg per kg acetazolamide produces a plasma concentration of approximately 5×10^{-4} M; and Maren, Wadsworth, Yale and Alonso have shown the concentration of free inhibitor in cellular water to be the same as that in plasma (9). Therefore, $\frac{[E]}{[EI]}$ is 1/50,000; consequently the per cent inhibition is 99.998. Finally, the demonstration that doses of acetazolamide above 20 mg per kg elicit no further response strongly supports (although it does not by itself conclusively establish) the contention that the physiologic effects of carbonic anhydrase are completely blocked (9, 10).

II. Bicarbonate excretion as HCO_3^- Tm is approached

A. Normal plasma pCO_2 (33 to 40 mm Hg). In dogs with a normal plasma pCO_2, a HCO_3^- Tm

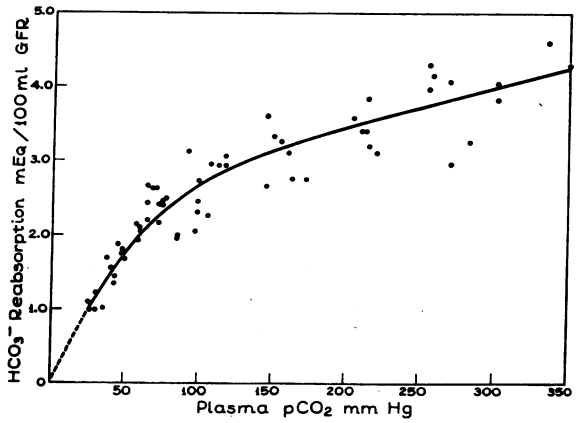

FIG. 2. EFFECT OF ACETAZOLAMIDE ON THE RELATION BETWEEN HCO_3^- REABSORPTION AND PLASMA pCO_2.

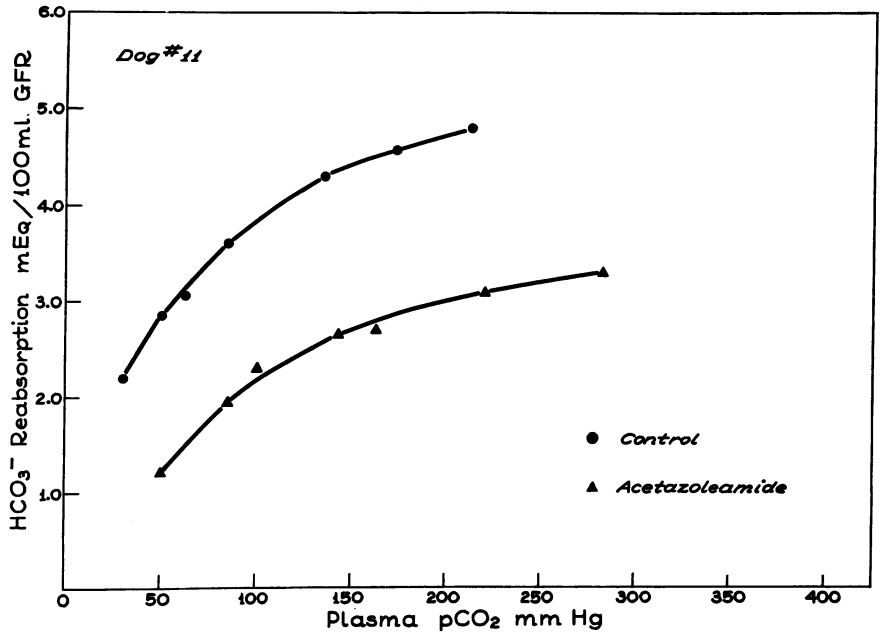

FIG. 3. COMPARISON OF THE RELATION BETWEEN HCO_3^- REABSORPTION AND PLASMA pCO_2 WITH AND WITHOUT ACETAZOLAMIDE.

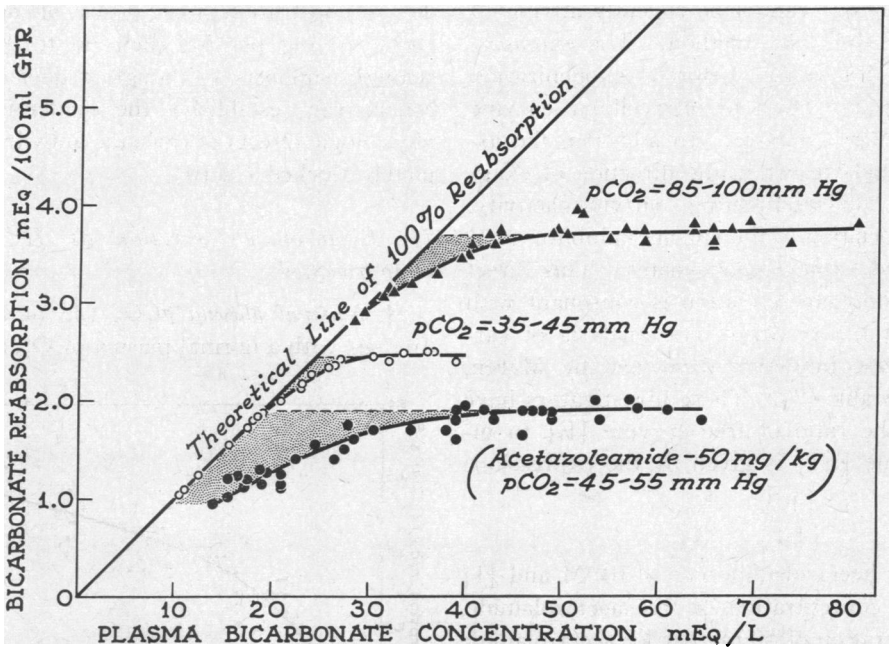

FIG. 4. EFFECT OF PLASMA pCO_2 AND ACETAZOLAMIDE ON THE RELATION BETWEEN HCO_3^- REABSORPTION AND PLASMA HCO_3^- CONCENTRATION. The stippled area indicates HCO_3^- excretion before the Tm was reached, and is termed a HCO_3^- leak (see text).

of approximately 2.6 mEq per 100 ml GF was obtained when plasma HCO_3^- concentration was progressively increased (Table II, Dog 72; Figure 4, middle curve). The stippled area on the middle curve of Figure 4 indicates that a small amount of HCO_3^- escaped reabsorption before the Tm was reached.

B. Elevated plasma pCO_2 (85 to 100 mm Hg).

TABLE II

The relationship of bicarbonate excretion to plasma bicarbonate concentration

Time	Plasma			C_{in}	Urine		Bicarbonate		
	HCO_3^-	pH	pCO_2		Flow	pH	Filt.	Excr.	Reab.
min	*mEq/L*		*mm Hg*	*ml/ min*	*ml/ min*		*μEq/ min*	*μEq/ min*	*mEq/ 100 ml GF*
Normal pCO_2: Dog 72; wt. 28 kg									
0	Anesthesia, sodium pentothal; breathing room air; infuse isotonic saline								
60–80	18.9	7.34	34	84	4.02	6.97	1,585	31	1.85
85	Inject 1.5 g $NaHCO_3$								
100–110	22.8	7.44	33	75	3.35	7.27	1,712	70	2.19
115	Inject 1.5 g $NaHCO_3$								
130–140	23.5	7.45	35	62	3.11	7.37	1,520	106	2.28
145	Inject 1.5 g $NaHCO_3$; infuse $NaHCO_3$, 250 μEq/min								
160–170	26.3	7.45	37	78	4.81	7.62	2,060	240	2.34
175	Inject 1.5 g $NaHCO_3$								
190–200	27.1	7.53	33	65	3.59	7.56	1,765	197	2.41
205	Inject 3.0 g $NaHCO_3$								
220–230	33.5	7.55	38	64	7.33	7.71	2,145	585	2.46
235	Inject 3.0 g $NaHCO_3$								
250–260	36.8	7.56	40	60	7.64	7.75	2,205	704	2.50
Elevated pCO_2: Dog 53; wt. 14.5 kg									
0	Anesthesia, continuous 1.5% Fluothane; breathing 8% CO_2 and 92% O_2								
30	Prime 12 g $NaHCO_3$; infuse 500 μEq $NaHCO_3$/min i.v.								
60–75	40.1	7.28	85	67	2.44	7.65	2,638	350	3.49
75–90	39.7	7.26	87	59	2.47	7.61	2,319	303	3.45
91	Prime 12 g $NaHCO_3$; infuse 750 μEq $NaHCO_3$/min i.v.								
120–135	49.5	7.34	90	58	4.36	7.67	2,857	680	3.77
135–150	49.2	7.35	87	56	4.25	7.68	2,757	638	3.70
151	Prime 12 g $NaHCO_3$; infuse 1,000 μEq $NaHCO_3$/min i.v.								
180–195	58.3	7.43	86	60	7.17	7.74	3,484	1,232	3.76
195–210	58.4	7.44	85	55	6.86	7.75	3,196	1,213	3.62
211	Prime 12 g $NaHCO_3$; infuse 1,250 μEq $NaHCO_3$/min i.v.								
240–255	65.3	7.46	90	54	8.75	7.74	3,529	1,530	3.70
255–270	64.9	7.46	90	56	9.62	7.76	3,635	1,643	3.56
Elevated pCO_2: Dog 70; wt. 14 kg									
0	Anesthesia, sodium pentothal and d-tubocurare; breathing 7% CO_2 and 93% O_2								
30	Infuse isotonic saline								
90–115	23.3	7.04	85	55.8	0.67	5.30	1,305		2.33
120	Inject 3 g $NaHCO_3$								
130–140	29.7	7.13	88	60.5	2.63	6.91	1,805	55	2.89
145	Infuse 250 μEq $NaHCO_3$/min								
155–165	28.5	7.15	82	63.3	3.56	6.83	1,810	56	2.78
170	Inject 1.5 g $NaHCO_3$								
180–195	32.5	7.21	80	59.0	3.46	7.12	1,905	107	3.08
200	Inject 1.5 g $NaHCO_3$								
210–220	34.5	7.23	81	58.5	3.06	7.38	2,015	155	3.18
225	Inject 1.5 g $NaHCO_3$; increase $NaHCO_3$ infusion to 500 μEq/min								
235–245	36.6	7.25	82	59.2	2.77	7.52	2,170	223	3.29
250	Inject 1.5 g $NaHCO_3$								
265–275	40.8	7.29	83	58.2	4.00	7.63	2,380	285	3.60
280	Inject 1.5 g $NaHCO_3$								
290–300	42.2	7.29	86	56.4	5.17	7.69	2,370	302	3.69
305	Inject 6.0 g $NaHCO_3$								
315–325	50.4	7.38	85	55.0	7.80	7.58	2,775	740	3.70

During respiratory acidosis HCO_3^- reabsorption was increased to a maximal value of approximately 3.8 mEq per 100 ml GF (Table II, Dog 53; Figure 4, upper curve). That this value in fact represents a true HCO_3^- Tm is evidenced by the constancy of reabsorption when the filtered load of HCO_3^- was increased from 4.2 to 8.0 mEq per 100 ml GF. As previously shown by others (6), HCO_3^- excretion began before the HCO_3^- Tm was reached (Table II, Dog 70; Figure 4). The pat-

tern of the HCO_3^- leak, although greater in amount, was similar to that seen at normal CO_2 tensions.

C. Normal plasma pCO_2 plus acetazolamide. Inhibition of carbonic anhydrase by the administration of acetazolamide (50 mg per kg body weight) in amounts sufficient to produce maximal physiologic effects depressed the HCO_3^- Tm to 1.9 mEq per 100 ml GF (Table III, Dog 63; Figure 4, lower curve). Large quantities of HCO_3^- were excreted before the Tm was reached. The magnitude of the HCO_3^- leak is significantly greater than that seen at either normal or elevated CO_2 tensions. These results are similar to those obtained by Schwartz, Falbriard, and Relman (10) in that the relation between plasma HCO_3^- concentration and HCO_3^- reabsorption is curvilinear, but differ in that a distinct HCO_3^- Tm was obtained.

D. Elevated plasma pCO_2 plus acetazolamide. It has already been demonstrated (4) that HCO_3^- reabsorption increases linearly as pCO_2 is raised. If, therefore, carbonic anhydrase inhibition lowers the Tm and augments the HCO_3^- leak because of inadequate H^+ production, it should be possible to overcome the deficient production by raising plasma CO_2 tension. Increasing pCO_2 from nor-

TABLE III

The effect of acetazolamide on bicarbonate reabsorption at various concentrations of plasma bicarbonate

Time	Plasma			Urine			Bicarbonate		
	HCO_3^-	pH	pCO_2	C_{in}	Flow	pH	Filt.	Excr.	Reab.
min	*mEq/L*		*mm Hg*	*ml/min*	*ml/min*		*μEq/min*	*μEq/min*	*mEq/100 ml GF*
Normal pCO_2; Dog 63; wt. 10 kg									
0	Anesthesia, sodium pentothal; breathing room air; injection, 250 mg acetazolamide; continuous infusion 0.15 M NaCl at 5 ml/min + acetazolamide 1.0 mg/min								
30–45	22.9	7.31	44	23.9	0.91	7.94	548	214	1.40
46	Inject 3 g $NaHCO_3$; infuse 250 μEq/min $NaHCO_3$ + 1.0 mg/min acetazolamide								
55–70	28.0	7.39	45	30.1	4.4	7.60	845	323	1.70
71	Inject 3 g $NaHCO_3$								
80–95	34.1	7.48	45	29.7	7.0	7.57	1,012	496	1.74
96	Inject 3 g $NaHCO_3$								
105–120	39.4	7.53	46	31.6	8.9	7.65	1,245	639	1.91
121	Inject 3 g $NaHCO_3$; increase $NaHCO_3$ infusion to 500 μEq/min; continue acetazolamide at 1.0 mg/min								
130–145	42.9	7.58	44	26.1	7.3	7.72	112	615	1.93
146	Inject 6 g $NaHCO_3$								
155–170	54.0	7.61	52	22.5	6.6	7.78	1,215	800	1.85
171	Inject 6 g $NaHCO_3$								
180–195	64.5	7.65	56	23.5	8.1	7.78	1,515	1,069	1.88
Elevated pCO_2: Dog 77; wt. 20 kg									
0	Anesthesia, sodium pentothal and Flaxedil; breathing 9% CO_2 and 91% O_2								
30	Inject 1 g acetazolamide; infuse isotonic saline and acetazolamide at 1 mg/min								
60–80	23.6	6.93	111	52.4	1.08	7.58	1,237	208	1.96
85	Inject 3 g $NaHCO_3$								
95–105	27.7	6.98	115	53.2	2.35	7.48	1,472	373	2.07
110	Inject 3.6 g $NaHCO_3$								
120–130	31.7	7.03	118	57.7	3.37	7.47	1,832	465	2.37
135	Inject 3.6 g $NaHCO_3$; infuse $NaHCO_3$ 250 μEq/min and acetazolamide 1 mg/min								
145–155	35.0	7.05	125	54.3	3.71	7.49	1,902	500	2.58
160	Inject 5.4 g $NaHCO_3$								
170–180	41.5	7.12	125	50.2	5.37	7.51	2,083	826	2.50
185	Inject 5.4 g $NaHCO_3$								
195–205	46.2	7.15	130	54.1	6.38	7.52	2,498	1,026	2.72
210	Inject 5.4 g $NaHCO_3$								
220–230	48.3	7.18	117	58.8	7.09	7.51	2,838	1,220	2.75
235	Inject 7.2 g $NaHCO_3$								
245–255	55.3	7.21	134	57.7	8.50	7.52	3,186	1,571	2.80
260	Inject 7.2 g $NaHCO_3$								
270–280	59.0	7.27	127	53.1	8.77	7.55	3,130	1,680	2.75
285	Inject 9.0 g $NaHCO_3$								
295–305	67.5	7.32	126	43.8	8.92	7.59	2,960	1,744	2.78

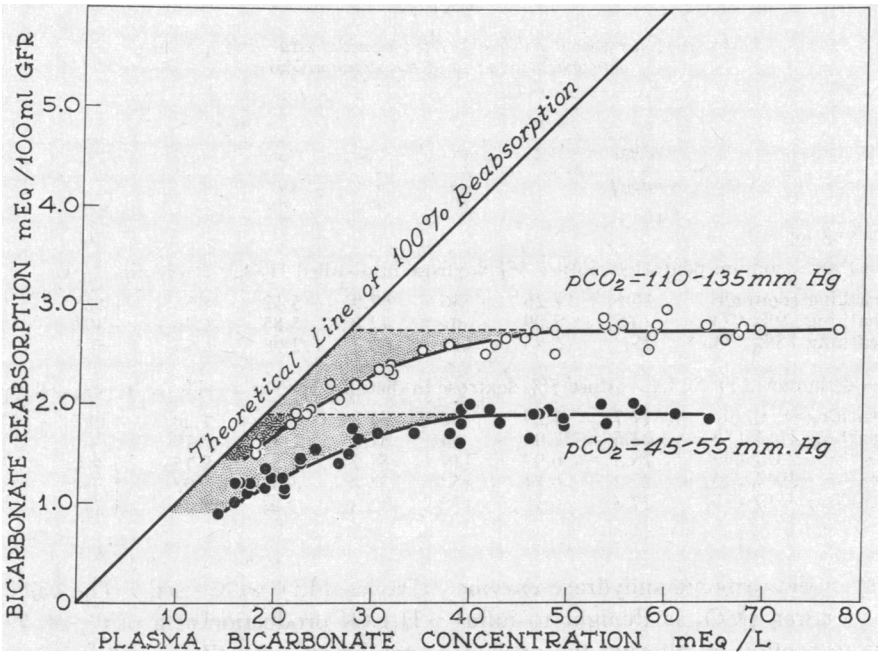

FIG. 5. FAILURE OF RESPIRATORY ACIDOSIS TO ELIMINATE THE HCO_3^- LEAK INDUCED BY ACETAZOLAMIDE.

mal values to 110 to 130 mm Hg increased the HCO_3^- Tm from 1.9 to approximately 2.8 mEq per 100 ml GF but did not diminish the magnitude of the HCO_3^- leak (Table III, Dog 77; Figure 5, upper curve). It would appear, therefore, that in the absence of carbonic anhydrase, raising the plasma pCO_2 can restore HCO_3^- Tm to a normal value, but cannot diminish the HCO_3^- leak.

III. Effect of raising plasma pCO_2 on an acetazolamide-induced HCO_3^- diuresis during metabolic acidosis

The demonstration in the first group of experiments that the reabsorption of HCO_3^-, despite carbonic anhydrase inhibition, could be increased by raising pCO_2 formed the basis for the third group of experiments. In these studies a mild metabolic acidosis was induced, carbonic anhydrase was inhibited with acetazolamide and a HCO_3^- diuresis ensued. Attempts were then made to obliterate the HCO_3^- diuresis by increasing plasma pCO_2. A representative protocol is presented in Table IV.

During the control period when plasma pCO_2 was normal, HCO_3^- reabsorption was virtually complete. Raising plasma pCO_2 from 36 to 113 mm Hg increased both filtered and reabsorbed HCO_3^- without altering excretion. Simultaneous blood and urine pH's both fell, with a proportionately greater drop in urine pH. Respiratory acidosis, however, failed to elicit the maximal urinary acidity that has been observed in the dog during severe metabolic acidosis (11).

Following the administration of acetazolamide, HCO_3^- excretion rose to 249 μEq per minute. Raising plasma CO_2 tension from 36 to 184 mm Hg in stepwise fashion increased HCO_3^- reabsorption, but had only a modest effect on HCO_3^- excretion. The fall in HCO_3^- excretion to 76 μEq per minute in the last period was in part the result of a fall in glomerular filtration rate (GFR) from 61 to 42 ml per minute. Blood and urine pH were both reduced as pCO_2 was increased, but in the face of carbonic anhydrase inhibition, urine pH never fell below that of blood.

DISCUSSION

The reabsorption of $NaHCO_3$ is regarded as the consequence of the secretion of cellular H^+ in exchange for tubular Na^+ (11, 12). Two of the principal determinants of H^+ secretion thus far identified are the CO_2 tension of plasma (1–3)

TABLE IV

The effect of elevated pCO_2 on acetazolamide-induced bicarbonate diuresis during mild metabolic acidosis

Time	Treatment	Plasma			Cin	Urine		Bicarbonate		
		HCO_3^-	pH	pCO_2		Flow	pH	Filt.	Excr.	Reab.
min		*mEq/L*		*mm Hg*	*ml/ min*	*ml/ min*		*μEq/ min*	*μEq/ min*	*mEq/ 100 ml GF*
Dog 28; wt. 13.7 kg										
0	Anesthesia, sodium pentothal; infuse 5% dextrose in distilled H_2O at 5 ml/min									
60–70	Breathing room air	16.6	7.26	36	67.9	5.42	6.56	1,125	10	1.65
85–95	Breathing 9% CO_2	20.5	7.09	67	72.8	5.85	6.48	1,490	16	2.02
110–120	Breathing 23% CO_2	25.3	6.95	113	60.7	2.98	6.14	1,535	5	2.53
121	Acetazolamide 350 mg i.v.; infuse 5% dextrose in distilled H_2O at 5 ml/min + acetazolamide 1 mg/min									
140–150	Breathing room air	17.7	7.29	36	61.2	7.23	7.38	1,085	249	1.37
165–175	Breathing 16% CO_2	23.5	7.00	93	57.8	4.22	7.10	1,360	143	2.11
190–200	Breathing 23% CO_2	25.3	6.93	119	57.7	3.54	7.15	1,460	177	2.23
215–225	Breathing 29% CO_2	27.3	6.84	153	56.8	3.65	6.93	1,550	146	2.48
240–250	Breathing 38% CO_2	27.8	6.78	185	42.3	2.43	6.79	1,175	74	2.60

and the activity of the carbonic anhydrase enzyme system (11). Plasma pCO_2 is thought to influence HCO_3^- reabsorption by altering the production of H^+ ions, predominantly via the catalyzed hydration of CO_2. In the present studies, however, inhibition of carbonic anhydrase did not prevent the augmentation of HCO_3^- reabsorption when plasma pCO_2 was increased. In fact, the regulatory effect of pCO_2 on the capacity to reabsorb HCO_3^- appeared to be entirely independent of carbonic anhydrase activity.

A comparison of the relationship between maximal HCO_3^- reabsorptive capacity and plasma CO_2 tension in the presence and absence of carbonic anhydrase activity (Figure 6) clearly discloses that the difference between the upper (carbonic anhydrase intact) and lower (carbonic anhydrase inhibited) curves is constant at all CO_2 tensions studied. This means that the contribution of the carbonic anhydrase enzyme system to HCO_3^- reabsorption is independent of plasma pCO_2 and amounts to 1.4 mEq per 100 ml GF, as is shown in the inset of Figure 6. It also follows from an analysis of this figure that since an increase in pCO_2 accelerates HCO_3^- reabsorption to the same extent in the presence or absence of carbonic anhydrase activity, its action is mediated entirely by the uncatalyzed hydration of CO_2.

To determine whether the uncatalyzed hydration of CO_2 could, in fact, account for all HCO_3^- reabsorption observed in the absence of carbonic anhydrase activity, calculations similar to those of Davies (13) were used. The calculated rates of H_2CO_3 production at a pCO_2 of 40 mm Hg [assuming an intracellular pH of 7.0 to 7.2, an intracellular HCO_3^- concentration of 2.6 to 5.0 mEq per L, and a rate constant, K_{CO_2}, for the reaction $CO_2 + H_2O \rightleftarrows H_2CO_3$, of 0.11 $second^{-1}$ at 38° C (14)] varied from 0.06 to 0.13 μmoles per second per ml intracellular water. The observed rates of HCO_3^- reabsorption at CO_2 tensions of 40 mm Hg and plasma HCO_3^- concentrations of approximately 50 mEq per L were 0.33 μEq per second per ml of estimated intracellular water.[1] Thus the observed rates of HCO_3^- reabsorption were three to five times greater than the calculated rates of H_2CO_3 production.

The discrepancy between the calculated rate of H_2CO_3 production and the observed rate of HCO_3^- reabsorption suggests that some source other than the hydration of CO_2 was contributing H^+ to the transport process. In Figure 2, extrapolation of the curve to zero CO_2 tension intercepts the ordinate at approximately the origin. While such an extrapolation is admittedly a crude estimate of HCO_3^- reabsorption at zero CO_2 tension (i.e., the intercept could be either slightly above or slightly below the origin), it does indicate that

[1] Intracellular water was estimated by removing the kidneys from four dogs previously given acetazolamide. The kidneys were then weighed and dried to determine total kidney water. Intracellular water was assumed to be 50 per cent of the total kidney water because of the relatively higher extracellular fluid volume of the kidney.

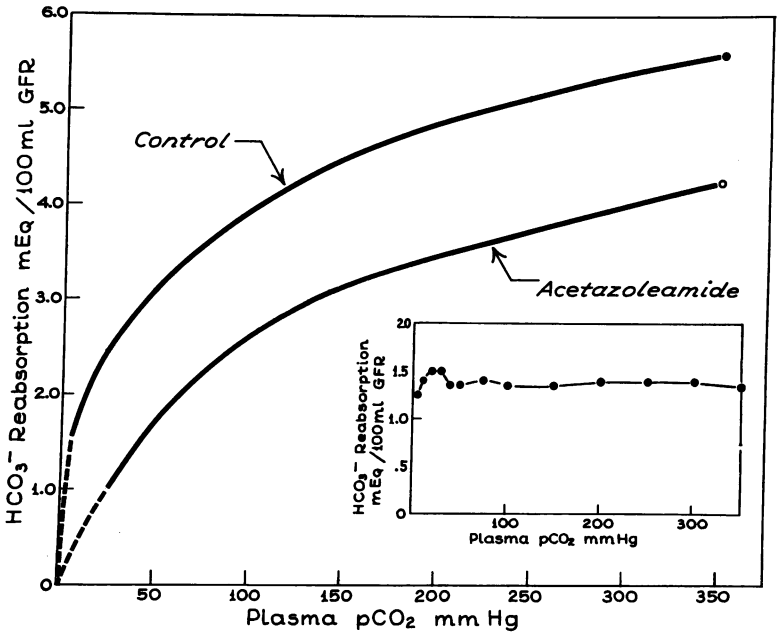

FIG. 6. COMPARISON OF THE RELATION BETWEEN HCO_3^- REABSORPTION AND PLASMA pCO_2 WITH AND WITHOUT ACETAZOLAMIDE. The upper curve (carbonic anhydrase intact) was taken from Figure 1, while the lower curve (carbonic anhydrase inhibited) was taken from Figure 2. Complete inhibition of carbonic anhydrase reduces the HCO_3^- Tm by a constant amount at all CO_2 tensions so that the lower curve parallels the upper curve. The intercept point, while not precisely defined for the upper curve, approximates the origin for the lower curve. The contribution of the carbonic anhydrase enzyme system at all levels of plasma pCO_2 is plotted in the inset as the difference between the upper and lower curves.

most, if not all, HCO_3^- reabsorption is in some way dependent upon CO_2 when carbonic anhydrase is maximally inhibited. Therefore, if other metabolic processes contribute H^+ to HCO_3^- reabsorption, their contribution is relatively minor and certainly not nearly of sufficient magnitude to account for the three- to fivefold calculated deficit in H^+ production.

Actually it is not necessary to postulate a special source of H^+ to compensate for the deficit in H_2CO_3 production from CO_2. The reabsorption of HCO_3^- secondary to H^+ secretion involves the formation of H_2CO_3 in the tubular lumen, some of which will return to the cell, contributing directly to the supply of intracellular H_2CO_3. Figure 7 depicts the two mechanisms whereby H^+ secretion, in the process of mediating HCO_3^- reabsorption, leads to the return of non-ionized H_2CO_3 to the tubular cell. First, as in the case of other undissociated acids, H_2CO_3 will back-diffuse into the cell by a process of non-ionic diffusion through the lipoid luminal membrane. Second, the reabsorption of large amounts of filtrate will sweep H_2CO_3 back into the cell through aqueous-filled pores as a result of solvent drag. This recycling of H_2CO_3 back into the cell completes the process of HCO_3^- reabsorption initiated by the secretion of H^+ and at the same time markedly reduces the need for high rates of H_2CO_3 formation from the uncatalyzed hydration of CO_2.[2]

[2] In a cyclic process such as this the rate at which H_2CO_3 must be produced from CO_2, in order to accomplish the observed rates of HCO_3^- reabsorption, needs only be as great as the rate at which H_2CO_3 is lost from the cycle by decomposition in the tubular lumen. If, therefore, only 75 per cent of the luminal H_2CO_3 returned to the cell by diffusion and solvent drag and the remaining 25 per cent decomposed to CO_2 and H_2O, then only 25 per cent of the H^+ mediating HCO_3^- reabsorption need be produced from CO_2. Recycling of this magnitude could readily account for the calculated three- to fivefold deficit in H^+ production from CO_2.

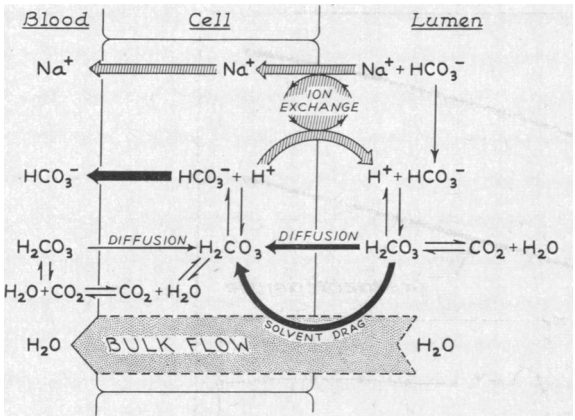

FIG. 7. ROLE OF BACK-DIFFUSION OF H_2CO_3 IN THE SUPPLY OF INTRACELLULAR H^+. By recycling into the cell, H_2CO_3 can furnish H^+ to the exchange process, and to that extent reduce the requirements for CO_2 hydration. The pCO_2 will nevertheless determine the steady state concentration of H_2CO_3 at which the system operates.

The capacity to reabsorb HCO_3^- in the absence of carbonic anhydrase activity increases as pCO_2 is elevated, but not in linear fashion (Figure 6, lower curve). The curvilinear character of this relationship could be due to a nonlinear relationship between: *1*) pCO_2 and H^+ production; *2*) H^+ production and intracellular H^+ concentration (resulting from more effective intracellular buffering at higher CO_2 tensions); or *3*) intracellular H^+ concentration and H^+ transport (progressive saturation of H^+ transport). The first two possibilities seem unlikely, since the production of H^+ via the uncatalyzed hydration of CO_2 is linearly related to pCO_2 *in vitro* (5), and since the titration of a thick homogenate of kidney with CO_2 showed that H^+ concentration of the homogenate was linearly related to pCO_2 (7). The curvilinear shape of the lower curve, therefore, probably reflects the kinetic characteristics of the H^+ transport mechanism as it becomes saturated with H^+. Plotting the reciprocal of HCO_3^- reabsorption against the reciprocal of plasma pCO_2 (Lineweaver-Burke plot) generates a straight line when carbonic anhydrase is inhibited (Figure 8). While the straight-line relationship does not permit identification of the specific process involved, it does suggest that the characteristics of this reabsorptive process are determined by a single rate-limiting step. A similar plot of data obtained from animals with normal carbonic anhydrase activity did not give a linear relationship (Figure 8). It would appear that HCO_3^- reabsorption in the presence of an intact carbonic anhydrase enzyme system is qualitatively as well as quantitatively different from HCO_3^- reabsorption in the absence of carbonic anhydrase.

In contrast to the uncatalyzed reaction, the reabsorption of that fraction of HCO_3^- mediated by carbonic anhydrase is strikingly independent of variations in pCO_2 (Figure 6). One possible explanation for this apparent insensitivity to changes in pCO_2 is that carbonic anhydrase becomes completely saturated with CO_2 at very low tensions (< 10 mm Hg) and thereafter contributes a constant quantity of H^+ to the reabsorptive process. This, however, would imply a Km for renal carbonic anhydrase of approximately 1×10^{-4} M, a value at great variance with the Km at 38° C of 760×10^{-4} M for purified red cell enzyme (15). From this latter Km it can be estimated that at plasma CO_2 tensions as high as 2,500 mm Hg carbonic anhydrase would be only half-saturated. To attribute the constant contribution of the carbonic anhydrase enzyme system to HCO_3^- reabsorption to early saturation of the enzyme would therefore appear to be untenable.

Actually, there is reason to believe that carbonic anhydrase is not simply contributing a constant quantity of H^+ in excess of that supplied by the uncatalyzed hydration of CO_2. Because the curve relating HCO_3^- reabsorption to pCO_2 in the absence of carbonic anhydrase flattens at high plasma CO_2 tensions, it was concluded that the H^+ transport mechanism was becoming saturated by an excess of H^+ ions. Increasing or decreasing the supply of H^+ in the range of H^+ excess should have little or no effect on HCO_3^- reabsorption. If carbonic anhydrase were simply contributing a fixed quantity of H^+ to the transport mechanism the two curves (Figure 6) should converge at higher CO_2 tensions, rather than remain parallel. The contribution of carbonic anhydrase, therefore, does not appear to be a simple addition of a constant quantity of H^+ to the same transport mechanism supplied by the uncatalyzed hydration of CO_2.

From this analysis of the effect of pCO_2 on the maximal HCO_3^- reabsorptive capacity, the HCO_3^- Tm can be characterized as the sum of two inde-

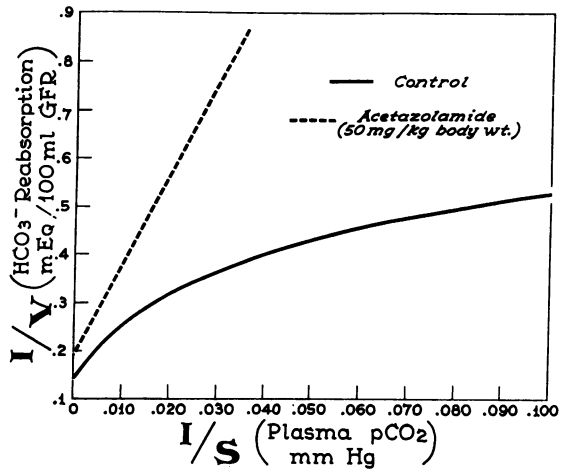

FIG. 8. DOUBLE RECIPROCAL PLOT OF THE RELATION BETWEEN HCO_3^- REABSORPTION AND PLASMA pCO_2 WITH AND WITHOUT ACETAZOLAMIDE. The solid line is the mean curve obtained by a double reciprocal of data from control animals (taken from Figure 1) and the broken line is the mean curve obtained by a similar plot of data from animals given acetazolamide (taken from Figure 2).

pendent reactions. One reaction is catalyzed by carbonic anhydrase, the other is uncatalyzed. As pCO_2 is increased the Tm increases, but solely as the result of an increase in the capacity of the uncatalyzed reaction, the capacity of the catalyzed reaction remaining constant. The regulation of HCO_3^- reabsorption in respiratory acidosis and alkalosis, therefore, is entirely independent of the carbonic anhydrase enzyme system.

Two mechanisms with such diverse reabsorptive properties suggest the existence of two different transport systems, one pCO_2-insensitive, the other pCO_2-sensitive. The fact that acetazolamide depresses the HCO_3^- Tm by a constant amount at all CO_2 tensions indicates that one transport system (pCO_2-insensitive) has a fixed capacity which is critically dependent upon the activity of carbonic anhydrase. Since changes in plasma pCO_2 have no effect on the capacity of this system, the H^+ transport mechanism must be saturated with H^+ and consequently insensitive to the changes in intracellular pH produced by variations in CO_2 tension. The dependence on carbonic anhydrase activity could result from the requirement of the transport system for a very rapid rate of H^+ supply (such as might occur if the ratio of transport capacity to cell volume were very high). A second transport system located elsewhere in the nephron is sensitive to changes in plasma pCO_2, suggesting that, in contrast to the pCO_2-insensitive mechanisms, it is unsaturated with respect to H^+ and therefore responsive to changes in intracellular pH. This system, which functions well in the absence of carbonic anhydrase, can be adequately supplied with H^+ by the uncatalyzed hydration of CO^+ supplemented by the recycling of H_2CO_3. Inhibition of carbonic anhydrase, therefore, has little effect on the reabsorptive capacity of this pCO_2-sensitive system.[3]

To characterize the two distinct mechanisms involved in HCO_3^- reabsorption from the vantage point of parameters other than maximal reabsorptive capacity, the relationship of HCO_3^- excretion to HCO_3^- reabsorption was studied when plasma HCO_3^- was maintained at high levels and carbonic anhydrase uninhibited (Figure 4, upper curve); HCO_3^- excretion began before the Tm was reached (magnitude of HCO_3^- leak indicated by stippled area). Schwartz, Falbriard and Lemieux (6), in similar experiments, found that in respiratory acidosis as plasma HCO_3^- concentration was increased from 26 to 55 mEq per L there was a curvilinear rise in HCO_3^- reabsorption without a definite Tm being attained. A double reciprocal plot (Lineweaver-Burke) of their data generated a straight line, which was interpreted as evidence that the high CO_2 tension had in some fashion altered the H^+ transport mechanism so that carbonic anhydrase had become the rate-limiting step in HCO_3^- reabsorption. In contrast, when the plasma HCO_3^- concentrations were increased over a far greater range in the present investigations, a Tm was clearly obtained in respiratory acidosis, with HCO_3^- reabsorption remaining constant as plasma HCO_3^- was increased from 42 to 80 mEq per L. The fact that a Tm was reached means that a double reciprocal plot would not generate a straight line. Consequently, this type of curvilinear relationship cannot be used as evidence that renal carbonic anhydrase is rate-limiting and, therefore, responsible for the shape of the curve.

[3] This does not imply that the cells involved in this transport process contain no carbonic anhydrase. Evidence will be presented later indicating that carbonic anhydrase may be present on the luminal border of the renal tubular cells. Its action, however, would not influence the HCO_3^- Tm.

TABLE V

Ability of HCO_3^- reabsorptive mechanism to effect complete HCO_3^- reabsorption (100% of filtered HCO_3^-) as the maximal reabsorptive capacity (Tm) is approached

HCO_3^- reab. / HCO_3^- Tm ×100	Filtered HCO_3^- reabsorbed		
	Normal pCO_2	Elevated pCO_2	Acetazolamide
%	%	%	%
50	100	100	65
70	99	100	60
75	99	99	59
80	98	98	58
85	95	96	56
90	92	92	55
95	90	90	50
100	83	83	42

When carbonic anhydrase was uninhibited and the pCO_2 maintained at normal values (Figure 4, middle curve), a distinct HCO_3^- leak was also demonstrated, HCO_3^- excretion commencing when plasma HCO_3^- concentration approached 18 mEq per L. This HCO_3^- leak does not appear to differ in any way from that observed during respiratory acidosis. It would appear that a HCO_3^- leak in each instance supervenes because the reabsorptive mechanism, when functioning at near-capacity levels, cannot effect complete HCO_3^- reabsorption, but can reabsorb some fixed percentage of the filtered HCO_3^- (possibly because of sensitivity to luminal pH when transporting H^+ at near-capacity rates). Thus, bicarbonate excretion begins in both the normal state and respiratory acidosis when the transport system is functioning at approximately 75 per cent of capacity (Table V). As reabsorption approaches 100 per cent of capacity the percentage of filtered HCO_3^- that is reabsorbed falls to approximately 83. The relationship between the percentage of capacity utilized and percentage of filtered HCO_3^- reabsorbed is identical in the normal state and respiratory acidosis. Although the magnitude of the HCO_3^- leak, in absolute quantities, is higher in respiratory acidosis, this is the consequence of the greater filtered HCO_3^- loads rather than a direct effect of pCO_2 on the characteristics of the transport mechanism.

The nature of the HCO_3^- leak following the administration of acetazolamide, however, appears to be quite different, qualitatively as well as quantitatively, from that observed in the normal state and in respiratory acidosis. It cannot be attributed to a simple exaggeration of the normal characteristics of H^+ secretion. It is evident from Figure 4 and Table V that inhibition of carbonic anhydrase impairs the ability of the reabsorptive process to produce HCO_3^--free urines at all levels of HCO_3^- reabsorption. This large HCO_3^- leak does not appear to be due to decreased H^+ production alone, since raising plasma CO_2 tension to 110 to 130 mm Hg restored the HCO_3^- Tm to a normal value of 2.7 mEq per 100 ml GF but did not reduce the magnitude of the leak (Figure 5, upper curve). Recently Schwartz, Lemieux and Falbriard (16) have shown that decreasing H^+ production by means of respiratory alkalosis without inhibiting carbonic anhydrase lowers the HCO_3^- Tm but does not produce a comparable HCO_3^- leak. It seems unlikely, therefore, that diminished H^+ production could account for the HCO_3^- leak.

To test more rigorously whether acetazolamide might augment the HCO_3^- leak by eliminating H^+ production via carbonic anhydrase, the capacity of the uncatalyzed reaction was increased by elevating plasma pCO_2 under circumstances where filtered HCO_3^- was comparatively low. The data from Table IV are schematically presented in Figure 9. The value of the maximal capacity of the the uncatalyzed reaction (black bar) at each pCO_2 was obtained from the lower curve in Figure 6 and serves as a basis for comparison with the observed HCO_3^- reabsorption (clear bar). Despite the fact that, as pCO_2 was raised, the capacity of the uncatalyzed reaction always exceeded the filtered HCO_3^- (cross-hatched bar) by a considerable amount, HCO_3^- reabsorption remained incomplete and HCO_3^- excretion continued. The enhanced HCO_3^- leak following the administration of acetazolamide, therefore, cannot be the consequence of deficient H^+ production.

The most likely explanation for the singular importance of carbonic anhydrase in maintaining the ability of the H^+ transport system to render the urine HCO_3^--free hinges about its possible luminal action (17). If, in addition to its distribution in the cytoplasm of renal tubular cells, the enzyme were also present on the luminal border of the cells, H_2CO_3 could not accumulate in luminal fluid. As a consequence, tubular pH would not fall to limiting values until $NaHCO_3$ reabsorption was virtually complete. However, when carbonic anhydrase was inhibited, H_2CO_3 would accumulate in the tu-

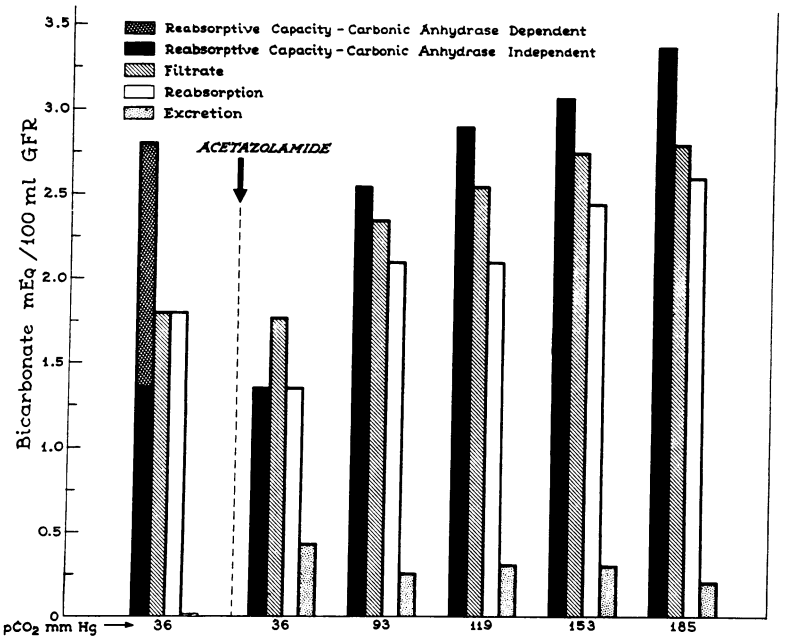

FIG. 9. FAILURE OF INCREASING PLASMA pCO_2 TO OBLITERATE BICARBONATE EXCRETION IN THE ABSENCE OF CARBONIC ANHYDRASE. See text for explanation.

bular lumen, lowering luminal pH to a limiting value despite the presence of significant amounts of $NaHCO_3$.[4] As a result of the low tubular pH, net H^+ secretion would stop, thereby preventing the complete removal of HCO_3^- from the urine.

The repression of net H^+ secretion resulting from inhibition of the luminal action of carbonic anhydrase could be eventually overcome, however, by raising the concentration of HCO_3^- in glomerular filtrate. As HCO_3^- concentration is increased, the ionization of the accumulated H_2CO_3 is repressed, permitting further H^+ secretion, until finally the maximal reabsorptive capacity is realized.[5] Inhibition of the luminal action of carbonic anhydrase, therefore, would always augment a HCO_3^- leak but would not alter the HCO_3^- Tm.

The inability to produce HCO_3^--free urines in the absence of carbonic anhydrase suggests that the pCO_2-sensitive system, whose capacity is independent of an intracellular action of carbonic anhydrase, is sensitive to luminal pH and, therefore, dependent upon the luminal action of carbonic anhydrase. Since high urinary CO_2 tensions have been observed duing HCO_3^- diuresis produced by the administration of $NaHCO_3$ in the presence of an intact carbonic anhydrase enzyme system (11), it is unlikely that carbonic anhydrase exerts a luminal action in the distal tubule. It seems probable, therefore, that the pCO_2-sensitive system, which is dependent upon the luminal action of carbonic anhydrase, is located in the more proximal portions of the nephron.

To explain the results of the present studies it is proposed that HCO_3^- reabsorption is mediated by two distinct H^+ secretory systems, one located in the proximal tubule, the other in the distal tubule. The proximal tubular mechanism appears to be sensitive to changes in intracellular as well as luminal pH. Alterations in plasma CO_2 tension, by changing the concentration of H_2CO_3 in the cell, elicit prompt changes in H^+ secretion.

[4] Under such circumstances, therefore, although bladder urine would be alkaline, the tubular fluid would be acid because of the accumulation of H_2CO_3. The high pCO_2 of such bladder urine indicates that in the tubular lumen, H_2CO_3 must have been present in significant amounts.

[5] In this manner filtered HCO_3^- would, in a sense, be competing with cellular processes for available H^+ and, as previously suggested (4), result in a curvilinear relationship between HCO_3^- reabsorption and plasma HCO_3^- concentration.

The sensitivity to luminal pH prevents transport of H^+ against sharp pH gradients. Carbonic anhydrase, by catalyzing the dehydration of H_2CO_3 as it is formed at the luminal surface of the cell, minimizes acidification of the urine, thereby facilitating continued H^+ transport; the enzyme may also be located within the cell, thereby augmenting H_2CO_3 production. Inhibition of carbonic anhydrase would diminish the rate of formation of H_2CO_3 from the hydration of CO_2 as a result of its intracellular action, and at the same time cause the accumulation of H_2CO_3 in the tubular fluid because of its luminal action. The latter effect, by enhancing the return of H_2CO_3 to the cell by a process of back-diffusion and solvent drag, could maintain H^+ transport despite reduced H^+ production. The HCO_3^- Tm of the proximal tubular transport system, therefore, would be unaffected by inhibition of carbonic anhydrase.

The distal tubular transport system, by contrast, is relatively insensitive to both intracellular and luminal pH. Consequently, the secretion of H^+ is neither influenced by alterations in plasma CO_2 tension nor dependent upon a luminal action of carbonic anhydrase. The capacity of this transport system is geared to a very rapid supply of H^+ and is, therefore, critically dependent upon the intracellular action of carbonic anhydrase.

Inhibition of carbonic anhydrase produces two distinct effects: *1*) a reduction in HCO_3^- Tm resulting primarily from diminution of distal tubular H^+ secretion, and *2*) an exaggerated HCO_3^- leak resulting from a combination of decreased distal tubular H^+ secretion and H_2CO_3 accumulation in the proximal tubular fluid (preventing thereby complete HCO_3^- reabsorption). Alterations in pCO_2, on the other hand, affect HCO_3^- reabsorption solely by changing the rate of H^+ secretion by the proximal tubular system.

SUMMARY

Renal HCO_3^- reabsorption was examined by three types of experiments. In the first group the effect of plasma pCO_2 on maximal HCO_3^- reabsorptive capacity (HCO_3^- Tm) was assessed in 15 dogs before and after inhibition of carbonic anhydrase. Bicarbonate reabsorption increased curvilinearly as plasma pCO_2 was elevated. Inhibition of carbonic anhydrase depressed the HCO_3^- Tm by a constant amount at all CO_2 tensions. From these studies it appeared that the contribution of carbonic anhydrase to the HCO_3^- Tm was completely independent of pCO_2, and that the regulatory effects of pCO_2 were mediated entirely through the uncatalyzed hydration of CO_2. In the absence of carbonic anhydrase activity all HCO_3^- reabsorption was dependent upon CO_2.

In the second group of experiments the effects of variations in plasma pCO_2 and inhibition of carbonic anhydrase on the excretion of HCO_3^- as the HCO_3^- Tm was approached were studied in 15 dogs. When plasma pCO_2 was maintained constant at a normal level, HCO_3^- excretion began before the Tm was reached. A similar leak was noted in respiratory acidosis. Carbonic anhydrase inhibition, however, caused a HCO_3^- leak which was of greater magnitude and which occurred even at very low concentrations of plasma HCO_3^-. Increasing H^+ production by elevating plasma pCO_2 to 110 to 130 mm Hg failed to obliterate the HCO_3^- leak.

In the third group (four dogs) elevating plasma pCO_2 as high as 200 mm Hg when carbonic anhydrase was inhibited did not significantly diminish HCO_3^- excretion despite marked reductions in the filtered HCO_3^- load as a result of metabolic acidosis.

It was concluded that HCO_3^- reabsorption was accomplished by two distinct processes. One process, presumably located in the proximal tubule, has a HCO_3^- Tm which is dependent upon plasma CO_2 tension and independent of carbonic anhydrase, and a transport system sensitive to the pH of tubular fluid. Carbonic anhydrase, by catalyzing the dehydration of carbonic acid at the luminal surface, prevents drastic lowering of the pH, thereby facilitating HCO_3^- reabsorption. A second process, apparently located in the distal tubule, has a fixed HCO_3^- Tm which is dependent upon carbonic anhydrase, independent of changes in plasma pCO_2 and can operate efficiently despite sharp pH gradients.

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