CHARACTERISTICS OF RENAL BICARBONATE REABSORPTION IN MAN *

By DONALD W. SELDIN, RICHARD M. PORTWOOD,† ‡ FLOYD C. RECTOR, Jr. AND ROBERT CADE §

(From The Department of Internal Medicine, The University of Texas Southwestern Medical School, Dallas, Tex.)

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The reabsorption of filtered HCO_3^- by the kidney is thought to result not from the active reabsorption of HCO_3^- ions but rather from the secretion of cellular H^+ in exchange for tubular Na^+ (1, 2). The secreted H^+ reacts with filtered HCO_3^- to form H_2CO_3 which then decomposes to CO_2 and H_2O, thereby effecting the reabsorption of $NaHCO_3$. The enzyme carbonic anhydrase, the inhibition of which blocks HCO_3^- reabsorption, is thought to maintain an adequate supply of H^+ for secretion by accelerating the hydration of CO_2 within the renal cells. In support of this theory is the fact that HCO_3^- reabsorption varies linearly with plasma pCO_2 (3–5).

Recently Schwartz, Falbriard and Relman (6) have suggested an alternative role for carbonic anhydrase. By a kinetic analysis of the effects of partial inhibition of carbonic anhydrase on HCO_3^- reabsorption during severe metabolic acidosis they found that at a given dose of Diamox® the reciprocals of HCO_3^- reabsorption and of plasma HCO_3^- concentration were linearly related. As carbonic anhydrase was progressively inhibited with increasing doses of Diamox® a family of lines typical of substrate-enzyme-inhibitor kinetics was obtained. From these relationships it was suggested that HCO_3^- was reabsorbed by some mechanism in which cellular carbonic anhydrase was the enzyme and filtered HCO_3^- (or some intermediate derived from it) was the substrate for the enzyme.

In order to examine further the role of carbonic anhydrase in the reabsorption of HCO_3^-, the effect of Diamox® on the relationship between HCO_3^- reabsorption and plasma pCO_2 was studied in normal subjects and in subjects with preexisting metabolic acidosis. During the course of these experiments, certain relations between HCO_3^- reabsorption, plasma HCO_3^- concentration and plasma pCO_2 became apparent which suggested that CO_2[1] rather than filtered HCO_3^- constituted the substrate involved in HCO_3^- reabsorption. A series of *in vitro* experiments was then performed in which the kinetics obtained by Schwartz and his associates (6) were reduplicated, although neither enzyme nor inhibitor was involved.

MATERIAL AND METHODS

A total of 24 experiments was performed on 13 normal young men. All studies were done in the morning, the subjects having fasted overnight. The subjects remained recumbent throughout except when voiding. Maximum water diuresis was maintained in all experiments by the intravenous infusion of 5 per cent fructose (in some cases glucose) in water following the oral ingestion of 1,500 to 2,000 ml. of distilled water.

In five types of experiments the effects of carbonic anhydrase inhibition alone and in combination with respiratory alkalosis, respiratory acidosis, metabolic acidosis, and mixed metabolic acidosis-respiratory alkalosis were observed. Carbonic anhydrase inhibition was produced by a single intravenous injection of 250 mg. of Diamox®. The alterations in acid-base composition were induced as follows:

1) Respiratory alkalosis—by voluntary hyperventilation, assisted by a Halliburton Intermittent Positive Pressure Breathing (IPPB) machine delivering 100 per cent oxygen.

2) Respiratory acidosis—by inhalation of 6 to 6.5 per cent CO_2 in oxygen.

3) Metabolic acidosis—by oral ingestion of a total of 20 to 25 Gm. of NH_4Cl during the 24 hours preceding the

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‡ Present address: Division of Endocrinology, School of Medicine, Duke University, Durham, N. C.

§ Present address: The Department of Physiology, Cornell University Medical College, New York, N. Y.

[1] In this paper it is assumed that plasma and cellular CO_2 tensions are identical; therefore plasma pCO_2 reflects the effective concentration of intracellular CO_2.

TABLE I

The effect of Diamox® on bicarbonate reabsorption during acute changes in plasma acid-base composition

Subject, Age, Ht., Wt., Experiment	Time	Plasma			Urine							
		pH	pCO_2	HCO_3^-	Flow	C_{in}	pH	pCO_2	HCO_3^- filt.	HCO_3^- exc.	HCO_3^- reabsorbed	
	min.		*mm. Hg*	*mEq./ L.*	*ml./ min.*	*ml./ min.*		*mm. Hg*	*μEq./ min.*	*μEq./ min.*	*μEq./ min.*	*mEq./ 100 ml. G. filtrate*
N. K.	0–20	7.38	41	23.4	26.0		5.94	68		31		
26 yr.	20–40*	7.38	40	23.0	22.5	123	6.02	73	2,829	34	2,795	2.27
1.8 M.	40–60	7.38	40	22.9	30.5	130	6.85	134	2,977	567	2,410	1.85
85 Kg.	60–80	7.37	41	22.8	21.0	108	7.00	121	2,462	496	1,966	1.82
	80–100	7.36	41	22.3	22.0	114	6.92	122	2,542	436	2,106	1.85
Diamox®	100–120	7.37	39	21.8	25.0	122	6.88	120	2,660	445	2,215	1.82
only	120–140	7.38	38	21.7	23.5	124	6.82	118	2,691	357	2,334	1.88
N. K.	0–21	7.37	35	19.7	17.9	163	5.67	51	3,211	8	3,203	1.96
26 yr.	21–41*	7.35	37	19.5	18.5	142	5.65	56	2,769	8	2,761	1.94
1.8 M.	41–62†	7.34	38	20.0	26.9		7.11	78		468		
85 Kg.	62–82	7.54	19	15.9	31.0	126	7.19	60	2,003	499	1,504	1.19
	82–102	7.55	17	14.4	26.2	110	7.21	48	1,584	354	1,230	1.12
Respiratory	102–122†	7.56	17	14.3	24.0	113	7.09	57	1,616	293	1,323	1.17
alkalosis+	122–142	7.41	30	18.1	19.8	95	6.98	65	1,720	216	1,504	1.58
Diamox®	142–162	7.36	34	18.8	21.0	123	6.91	73	2,312	218	2,094	1.70
N. K.	0–15	7.38	41	23.5	27.3		6.38	52		60		
27 yr.	15–30*	7.39	40	23.6	22.3	159	6.27	59	3,752	47	3,705	2.33
1.8 M.	30–45‡	7.39	40	23.7	28.0	151	7.16	65	3,579	512	3,067	2.03
85 Kg.	45–60	7.27	56	24.8	27.0	131	7.20	83	3,249	697	2,552	1.95
Respiratory	60–75‡	7.23	59	24.0	32.0	159	7.07	94	3,816	688	3,128	1.97
acidosis+	75–90	7.34	41	21.5	21.3	130	7.16	76	2,795	456	2,339	1.80
Diamox®	90–105	7.41	37	22.6	12.7	129	7.26	94	2,915	425	2,490	1.93
C. P.	0–20	7.24	42	17.4	37.5	209	5.83	43	3,820	22	3,798	1.81
26 yr.	20–40*	7.23	41	16.5	31.5	167	5.87	44	2,895	19	2,876	1.72
1.8 M.	40–60	7.23	42	17.1	41.0	172	6.63	86	3,090	295	2,795	1.63
80 Kg.	60–80	7.23	42	17.0	37.5	153	6.86	52	2,730	277	2,453	1.60
Metabolic	80–100	7.25	40	16.7	36.5	155	6.80	50	2,720	223	2,497	1.67
acidosis+	100–120	7.24	42	17.3	33.5	144	6.75	44	2,620	161	2,459	1.71
Diamox®	120–140	7.27	37	16.5	34.5	166	6.70	42	2,880	141	2,739	1.65
T. L.	0–20	7.29	37	17.1	19.0	147	5.63	46	2,514	8	2,506	1.70
27 yr.	20–40*	7.32	35	17.3	19.0	150	5.64	42	2,595	8	2,587	1.72
1.8 M.	40–60†	7.30	38	17.9	26.8	141	6.93	62	2,524	276	2,248	1.59
78 Kg.	60–80	7.41	23	14.3	25.8	129	7.00	54	1,845	279	1,566	1.21
NH_4Cl+	80–100	7.50	16	12.1	25.0	126	7.06	44	1,525	250	1,275	1.01
respiratory	100–115†	7.55	13	11.4	22.0	125	7.03	44	1,425	200	1,225	0.98
alkalosis+ Diamox®	115–135	7.29	34	15.9	22.0	138	6.92	59	2,194	209	1,985	1.44

* Injection of 250 mg. Diamox® intravenously.
† Period of hyperventilation.
‡ Period of breathing 6 per cent CO_2.

experiment. In several studies chronic administration of 15 Gm. NH_4Cl daily for one to two weeks preceded the acute load.

4) Mixed metabolic acidosis-respiratory alkalosis—by hyperventilation in subjects who had previously ingested the 20 to 25 Gm. of NH_4Cl.

The analytical methods were those described in a previous paper (7).

RESULTS

The results are presented in protocol form in Table I with inclusion of a representative example of each of the several types of experiments. Since chronic NH_4Cl acidosis was associated with the same response as acute NH_4Cl loads, only one instance of the latter is charted. It is apparent from these data that, regardless of plasma acid-base composition, the administration of Diamox® was associated with a reduction in HCO_3^- reabsorption whether expressed in absolute terms or as mEq. reabsorbed per 100 ml. of glomerular filtrate.

In Figure 1 the effects of the different experimental procedures on plasma $[HCO_3^-]$ and pCO_2 are plotted. It is noteworthy that metabolic acido-

sis was accompanied by only a slight decrease in plasma pCO_2 despite a marked depression in plasma concentration of HCO_3^-. In the other experimental states the plasma $[HCO_3^-]$ was related to pCO_2 in a roughly linear fashion.

Following the administration of Diamox®, HCO_3^- reabsorption was linearly related to plasma pCO_2 (Figure 2). The regression equation describing this relationship is $Y = 0.61 + 0.028$ X. Brazeau and Gilman (3), Relman, Etsten and Schwartz (4) and Dorman, Sullivan and Pitts (5) have previously shown that HCO_3^- reabsorption was linearly related to plasma pCO_2 in the presence of normal carbonic anhydrase activity. Thus, inhibition of carbonic anhydrase does not disturb this linear relationship.

The administration of Diamox® during NH_4Cl acidosis resulted in moderate HCO_3^- excretion (200 to 300 μEq. per minute) despite the reduced concentration of HCO_3^- in plasma. As seen in Figure 2 the values for HCO_3^- reabsorption are somewhat skewed below the regression line. Although this skewed distribution is not striking, it is conceivable that the reduced concentration of HCO_3^- in glomerular filtrate during NH_4Cl acidosis partially limited the reabsorption of HCO_3^- despite the excretion of moderate amounts of HCO_3^- into the urine.

Schwartz and associates (6) have reported that at a given dose of Diamox® HCO_3^- reabsorption was approximately proportional to plasma HCO_3^- concentration. In the present experiments this same proportionality was also apparent (Figure 3). However, at approximately the same plasma $[HCO_3^-]$, the reabsorption of HCO_3^- was much less during respiratory alkalosis than during metabolic acidosis (Figure 3). In respiratory alkalosis and metabolic acidosis, plasma $[HCO_3^-]$ (and the filtered load of HCO_3^-) were comparably depressed, yet during respiratory alkalosis 30 to 40 per cent of the filtered HCO_3^- was excreted while in metabolic acidosis only 7 to 15 per cent of the filtered HCO_3^- was excreted. This difference in HCO_3^- reabsorption between respiratory alkalosis and metabolic acidosis was probably the consequence of the fact that in respiratory alkalosis plasma pCO_2 falls more than $[HCO_3^-]$ does, whereas in metabolic acidosis the depression in plasma $[HCO_3^-]$ is far greater than any decrease in plasma pCO_2 (Figure 1). These observations suggest that during HCO_3^- diuresis plasma pCO_2 is a more important determinant of HCO_3^- reabsorption than is the concentration of HCO_3^-.

To examine whether the correlation between plasma $[HCO_3^-]$ and HCO_3^- reabsorption in a reciprocal plot establishes the existence of an interaction between filtered HCO_3^- and cellular carbonic anhydrase in the course of HCO_3^- reab-

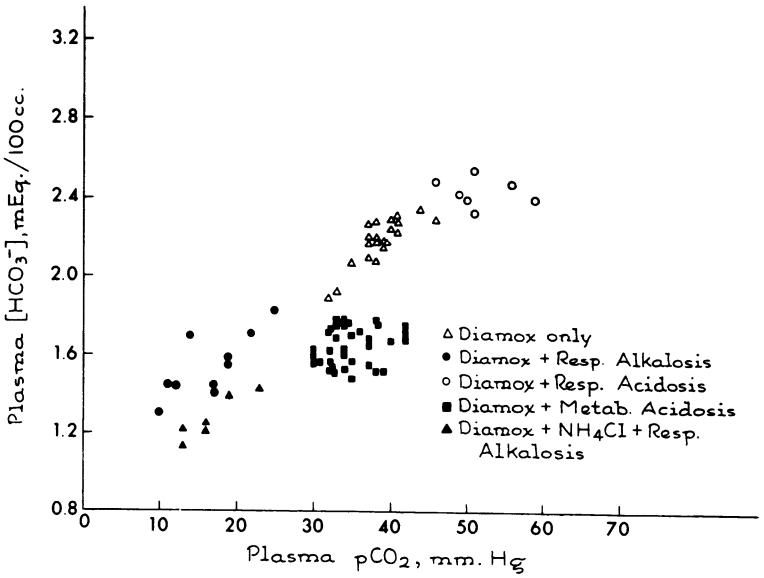

FIG. 1. EFFECT OF ACUTE CHANGES IN ACID-BASE BALANCE ON PLASMA pCO_2 AND HCO_3^- CONCENTRATION

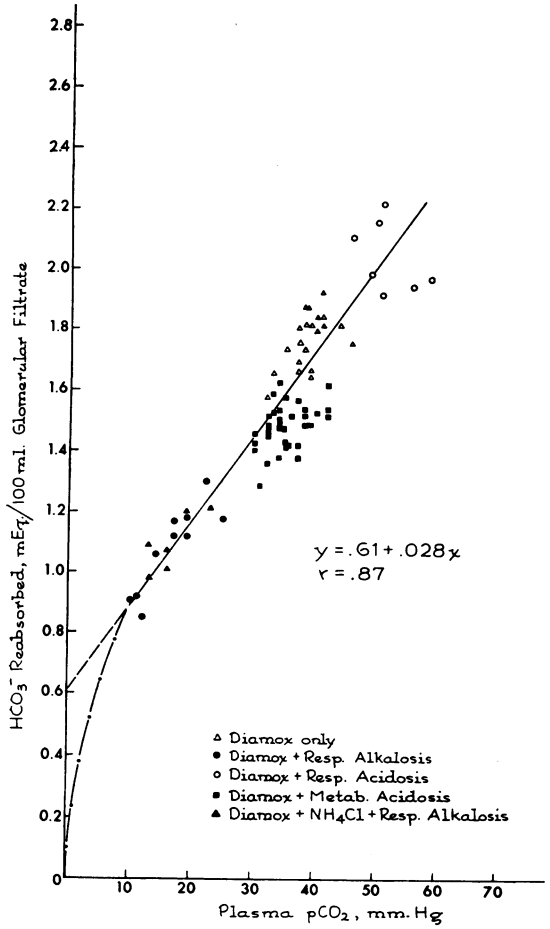

FIG. 2. RELATION OF HCO_3^- REABSORPTION TO PLASMA pCO_2 FOLLOWING DIAMOX® ADMINISTRATION DURING ACUTE CHANGES IN ACID-BASE BALANCE

The plasma pCO_2 could not be reduced below 14 mm. Hg because of the development of tetany. For this reason the configuration of the lower portion of the curve could not be examined. The dotted extensions to the vertical axis represent two alternative possibilities. This area of the curve is currently being studied in dogs.

sorption, as suggested by Schwartz and colleagues (6), or whether this same relationship might also be the result of H^+ secretion, *in vitro* studies (Table II) were performed to simulate the effects of secretion of H^+ ions into bicarbonate-containing tubular fluid. Fifty ml. aliquots of seven different HCO_3^- solutions, each containing 15 mMoles per L. sodium phosphate (pH 7.4), were placed in 100 ml. fritted glass funnels and aerated with 8.23 per cent CO_2 for 15 minutes. At the end of this period 3 ml. samples were aspirated into oiled syringes for measurement of the initial $[HCO_3^-]$ which ranged from 93.96 to 8.97 mEq. per L. Next, 0.1 ml. 2.5 N HCl was added to each funnel. Again the samples were aerated for 15 minutes with 8.23 per cent CO_2 and 3 ml. aliquots were once more removed for HCO_3^- determinations. An additional 0.1 ml. 2.5 N HCl (giving a cumulative value of 0.50 mEq. HCl) was then added to each funnel and the procedure repeated. Altogether a total of 0.4 ml. of 2.5 N HCl was added to each funnel in increments of 0.1 ml. The difference between the initial HCO_3^- concentration and the HCO_3^- concentration after each increment of acid represents the quantity of HCO_3^- dissipated by the addition of HCl.

It is clear from Table II that the quantity of HCO_3^- decomposed is not equivalent to the quantity of acid added, but more nearly approximates it at the higher initial HCO_3^- concentrations. The data in Table II are plotted in Figure 4, where the initial $[HCO_3^-]$ concentrations are plotted along the horizontal axis and the quantities of HCO_3^- decomposed (mEq. per L.) by each addition of acid are plotted along the vertical axis. It is apparent from this figure that the quantity of HCO_3^- decomposed for each quantity of acid added is related to the initial $[HCO_3^-]$ in a curvilinear fashion. If the reciprocal of the decomposed HCO_3^- (1/V) is plotted against the reciprocal of the initial HCO_3^- concentration (1/S), a family of four straight lines is obtained, one for each total quantity of acid added (Figure 5). These lines intercept at the same point on the 1/S axis in a fashion typical of lines obtained by the kinetic analysis of noncompetitive enzyme inhibition, despite the fact that neither enzyme nor inhibitor participated in the *in vitro* reactions. The results of a similar experiment in which the concentration of sodium phosphate was 5 mMoles per L. rather than 15 mMoles per L. are plotted reciprocally in Figure 6. The lines are similar to those in Figure 5. A comparison of Figures 5 and 6 reveals that decreasing the buffer concentration decreases the slope of the lines. The results of these *in vitro* studies are in accord with what have been predicted from the kinetics of buffer equilibria; *i.e.*, the addition of HCl to buffered bicarbonate solutions can yield kinetics characteristic of noncompetitive enzyme inhibition.

These *in vitro* reactions are analogous to the reabsorption of HCO_3^- by the secretion of H^+ into

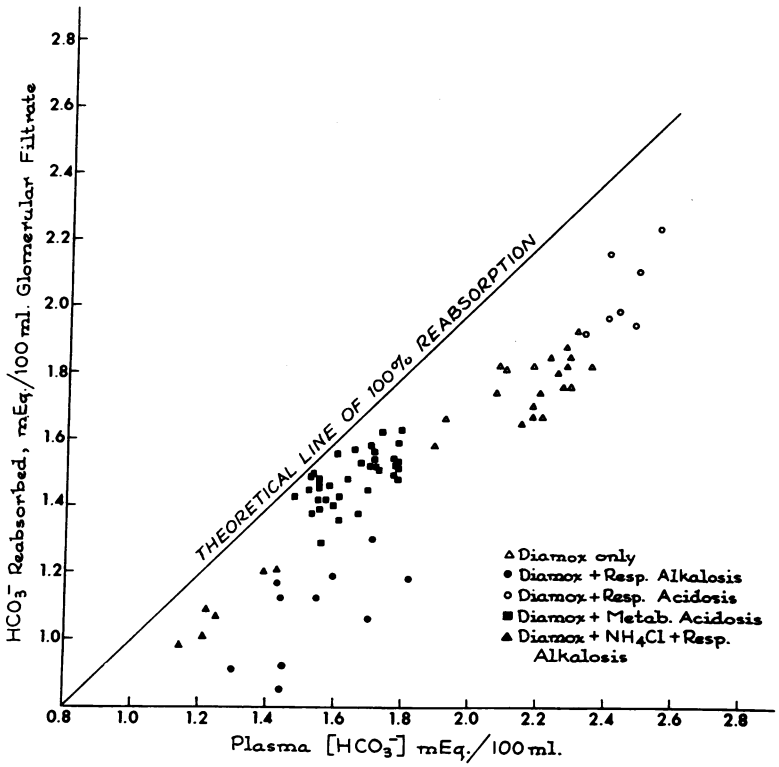

FIG. 3. RELATION OF BICARBONATE REABSORPTION TO PLASMA HCO_3^- FOLLOWING DIAMOX® ADMINISTRATION DURING ACUTE ALTERATIONS IN ACID-BASE BALANCE

the tubular lumen. It should not be inferred, however, that the *in vitro* model exactly duplicated the conditions existing in the nephron; for instance, the concentration of phosphate buffer used in these studies greatly exceeded the concentration of phosphate usually present in glomerular filtrate. The studies simply demonstrated that the reciprocal relationship between plasma HCO_3^- concentration and HCO_3^- reabsorption during the administration of Diamox® could arise from competition between bicarbonate and nonbicarbonate buffer systems for secreted H^+. The competing buffering action, however, did not necessarily originate in the glomerular filtrate, but could, instead, have arisen as

TABLE II

The addition of HCl to phosphate-buffered bicarbonate solutions

Initial $[HCO_3^-]$*†	47 ml. HCO_3^- sol. + 0.25 mEq. HCl (5.32 mEq./L.)		44 ml. HCO_3^- sol. + 0.50 mEq. HCl (11.38 mEq./L.)		41 ml. HCO_3^- sol. + 0.75 mEq. HCl (18.30 mEq./L.)		38 ml. HCO_3^- sol. + 1.00 mEq. HCl (26.30 mEq./L.)	
	$[HCO_3^-]$*	HCO_3^- decomposed	$[HCO_3^-]$*	HCO_3^- decomposed	$[HCO_3^-]$*	HCO_3^- decomposed	$[HCO_3^-]$*	HCO_3^- decomposed
mEq./L.	*mEq./L.*	*mEq./L.*	*mEq./L.*	*mEq./L.*	*mEq./L.*	*mEq./L.*	*mEq./L.*	*mEq./L.*
93.96	89.75	4.21	82.74	11.22	77.07	16.89	70.71	23.25
71.63	67.58	4.05	60.75	10.88	55.60	16.03	49.28	22.35
47.99	44.12	3.87	37.67	10.32	32.84	15.15	26.94	21.05
29.09	25.68	3.41	20.27	8.82	15.50	13.59	10.34	18.75
20.12	17.12	3.00	12.05	8.07	7.87	12.25	3.60	16.52
12.07	9.60	2.47	5.65	6.42	2.34	9.73		
8.97	6.88	2.08	3.43	5.53				

* After equilibration with 8.23 per cent CO_2.
† All solutions contained 15 mMoles per L. sodium phosphate pH 7.4.

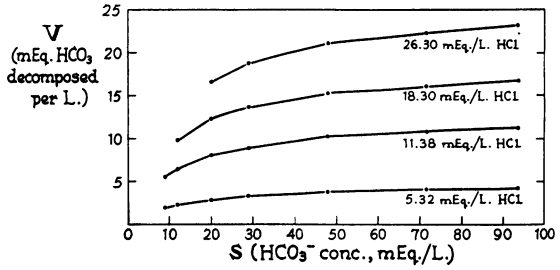

FIG. 4. TITRATION OF PHOSPHATE-BUFFERED BICARBONATE SOLUTIONS WITH HYDROCHLORIC ACID

a result of cellular processes. If increasing inhibition of carbonic anhydrase by Diamox® progressively diminished the available supply of H^+ ions and at the same time rendered the mechanism for net H^+ secretion sensitive to intraluminal pH,[2] then filtered HCO_3^- would, in effect, be competing with cellular processes for secreted H^+. Under these conditions progressive elevation of the concentration of filtered HCO_3^- could yield curves similar to those shown in Figure 5. Thus, similar

[2] Net secretion of H^+ during carbonic anhydrase inhibition could be influenced by intraluminal pH in several ways. Davies (8) has recently suggested that carbonic anhydrase is located on the luminal surface of the tubular cell; carbonic anhydrase inhibition could result in accumulation of H_2CO_3, thereby accelerating the back-diffusion of secreted H^+. Inhibition of carbonic anhydrase could also increase the back-diffusion of H^+ by increasing the permeability of the cell membrane to H^+ ions. Finally, inhibition of carbonic anhydrase could sensitize the mechanism for active H^+ transport to small changes in the pH of tubular urine.

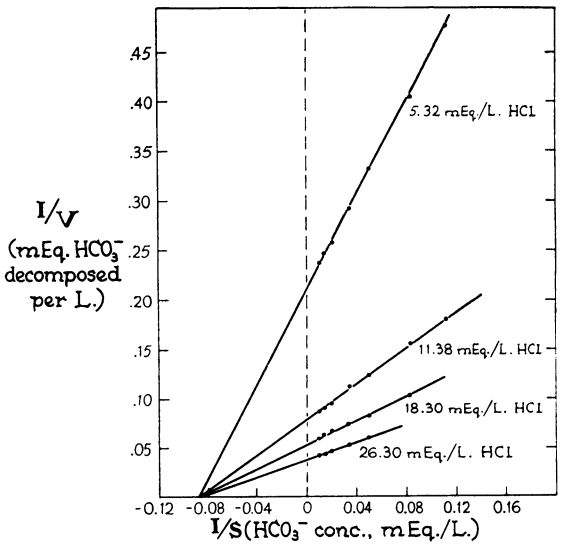

FIG. 5. RECIPROCAL PLOT OF BICARBONATE TITRATION EXPERIMENT

kinetics would be obtained regardless of whether Diamox® diminished the secretion of H^+ into the tubular fluid or decreased the number of enzyme sites available for interaction with filtered HCO_3^-.

DISCUSSION

The reabsorption of filtered HCO_3^- by the kidney in many respects resembles an active transport process involving enzyme-carrier molecules. Most of the criteria usually invoked to establish the existence of a specific carrier mecha-

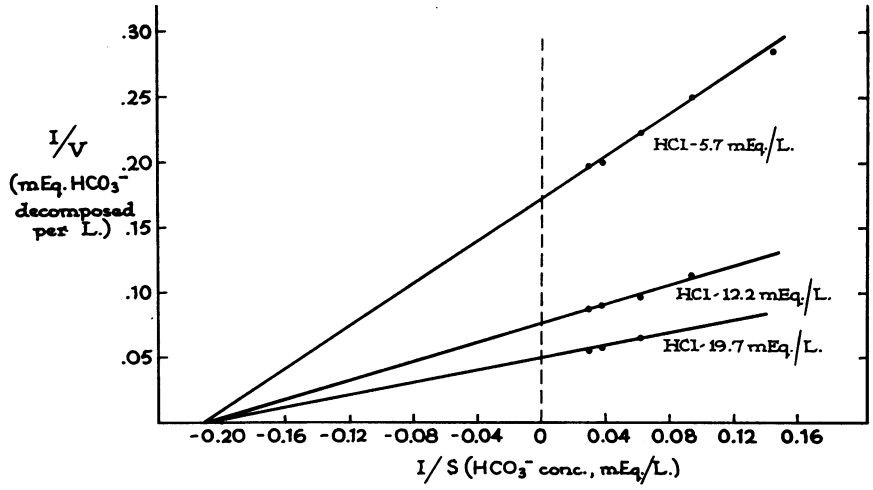

FIG. 6. RELATIONSHIP BETWEEN BICARBONATE CONCENTRATION AND DECOMPOSITION OF BICARBONATE BY ADDITION OF HYDROCHLORIC ACID

nism, *i.e.* saturation, competitive inhibition, specific inhibition, and kinetics characteristic of an enzyme-substrate interaction (9), have been demonstrated for the reabsorption of HCO_3^-. First, Pitts and Lotspeich (10) have shown that in normal animals elevation of the filtered load of HCO_3^- saturated the tubular reabsorptive mechanism at a HCO_3^- Tm of approximately 2.6 mEq. per 100 ml. glomerular filtrate. Second, Hilton and his associates (11) have presented evidence that in acute respiratory acidosis the administration of NaCl depressed the HCO_3^- Tm, suggesting competition between Cl^- and HCO_3^- for carrier sites. Third, carbonic anhydrase inhibitors specifically block HCO_3^- reabsorption without impairing other metabolic processes of renal tissue. Finally, Schwartz and co-workers (6) have found that the relationship between HCO_3^- reabsorption and the concentration of plasma HCO_3^- during partial inhibition of carbonic anhydrase with Diamox® was typical of substrate-enzyme-inhibitor kinetics. In the light of these data, the possibility exists, at least on theoretical grounds, that the formation of a bicarbonate-carbonic anhydrase complex mediates the active removal of HCO_3^- from the tubular lumen.

According to a more widely accepted theory (1, 2), the primary process in HCO_3^- reabsorption is the secretion of cellular H^+ in exchange for tubular Na^+. The secreted H^+ reacts with HCO_3^- to form H_2CO_3 which then decomposes to CO_2 and H_2O. According to this theory the H^+ involved in HCO_3^- reabsorption arises from the hydration of CO_2 catalyzed by carbonic anhydrase.

Two of the criteria listed as evidence supporting the theory of active HCO_3^- reabsorption, *i.e.* saturation and specific inhibition with Diamox®, however, are equally consistent with the theory of H^+ secretion. So, too, the enhanced excretion of HCO_3^- following NaCl loads (11) is not conclusive evidence of competitive inhibition, inasmuch as this effect could equally well result from a limitation on Na^+ reabsorption. The question then arises of whether the demonstration of typical substrate-enzyme-inhibtor kinetics (6) establishes the existence of active HCO_3^- reabsorption involving carbonic anhydrase as the carrier molecule, or whether these same kinetics are compatible with the theory that HCO_3^- is reabsorbed as the passive consequence of H^+ secretion. The results of our *in vitro* experiments showed that a linear relationship between the reciprocals of HCO_3^- reabsorption and plasma $[HCO_3^-]$ was nonspecific, equally compatible with either of the above mentioned theories of HCO_3^- reabsorption.

While the results of these *in vitro* studies do not exclude the existence of active HCO_3^- reabsorption involving carbonic anhydrase as the carrier molecule, other lines of evidence render it unlikely. First, the elevated urinary CO_2 tensions observed during $NaHCO_3$ infusions (10) could not be explained if active transport were the sole mechanism for HCO_3^- reabsorption. Second, the linear relationship between plasma pCO_2 and HCO_3^- reabsorption (3–5) would be difficult to explain if the reabsorption of HCO_3^- were accomplished solely by an interaction between cellular carbonic anhydrase and filtered HCO_3^-. To explain this effect it would be necessary to propose that the activity of carbonic anhydrase varied linearly with pCO_2. Studies in this laboratory by Carter, Seldin and Teng (12), however, have shown that the activity of carbonic anhydrase in rat kidney was not measurably increased in chronic respiratory acidosis. While it is possible that CO_2 increases the *in vivo* activity of the enzyme in such a way that no change is detectable by an *in vitro* assay, Roughton and Booth (13) have demonstrated that lowering pH actually depressed the activity of carbonic anhydrase. Thus, intracellular acidosis accompanying respiratory acidosis would be expected to inhibit rather than accelerate HCO_3^- reabsorption. The linear relation between plasma pCO_2 and HCO_3^- reabsorption, therefore, supports the theory that HCO_3^- is reabsorbed as the passive consequence of H^+ secretion rather than the active removal of HCO_3^- ions from the tubule lumen.

The failure of complete carbonic anhydrase inhibition to abolish HCO_3^- reabsorption (14) raises the question of whether there might be a second mechanism for the reabsorption of HCO_3^- not involving the secretion of H^+. The results of the present studies, however, indicate that following the administration of 250 mg. Diamox® intravenously HCO_3^- reabsorption is still linearly related to plasma pCO_2, despite marked inhibition of carbonic anhydrase activity.[3] This suggests that

[3] Counihan, Evans and Milne (15) have shown that 90 to 95 per cent of the maximally achievable inhibitory

all HCO_3^- reabsorption results from the secretion of H^+ and that the uncatalyzed hydration of CO_2 is an important source of H^+ for the Na^+–H^+ exchange process.

The demonstration that the uncatalyzed hydration of CO_2 constitutes an important source of H^+ for the reabsorption of HCO_3^- affords an explanation for the failure of Diamox® to produce maximum HCO_3^- diuresis during metabolic acidosis. It was shown in Figure 1 that the concentration of plasma HCO_3^- was depressed in NH_4Cl acidosis without a commensurate decrease in plasma pCO_2. Therefore, under these circumstances, the quantity of HCO_3^- filtered was reduced, but the capacity of the uncatalyzed hydration of CO_2 to effect the reabsorption of HCO_3^- remained comparatively unchanged. Thus, when the plasma HCO_3^- concentration fell in metabolic acidosis to the point where the filtration of HCO_3^- was equal to the capacity of the uncatalyzed hydration of CO_2 to reabsorb HCO_3^-, Diamox® no longer produced a HCO_3^- diuresis. Following the administration of 250 mg. Diamox® the reabsorption of HCO_3^- at a plasma pCO_2 of 35 mm. Hg (the average pCO_2 during metabolic acidosis) was approximately 1.5 mEq. per 100 ml. glomerular filtration rate (GFR) (Figure 2), presumably due to the uncatalyzed hydration of CO_2 and any residual uninhibited carbonic anhydrase. Therefore, in man given this amount of Diamox® reabsorption of HCO_3^- would be virtually complete when the plasma HCO_3^- concentration fell below about 15 mEq. per L., a level similar to that obtained in man by others (15). On the other hand, depression of the plasma HCO_3^- concentration to 15 mEq. per L. by hyperventilation drastically diminished the capacity of the uncatalyzed hydration of CO_2 to reabsorb HCO_3^-, and hence Diamox® would be expected to produce marked HCO_3^- diuresis despite the smaller quantity of filtered HCO_3^-.

SUMMARY

The pattern of renal HCO_3^- reabsorption during various alterations in acid-base balance was examined in 13 normal subjects with and without the administration of Diamox®. Following the administration of Diamox® HCO_3^- reabsorption varied linearly with plasma pCO_2, as described by the regression equation $Y = 0.61 + 0.028X$. This linear relationship in the presence of marked inhibition of carbonic anhydrase indicated that the uncatalyzed hydration of CO_2 was an important source of H^+ for the reabsorption of HCO_3^-. The failure of Diamox® to produce marked HCO_3^- diuresis during metabolic acidosis was attributable to nearly complete reabsorption of the small filtered load via the uncatalyzed hydration of CO_2.

In vitro studies, in which varying amounts of HCl were added to phosphate-buffered HCO_3^- solutions, disclosed that the linear relationship between the reciprocals of HCO_3^- reabsorption and plasma HCO_3^-, used as evidence for the active reabsorption of HCO_3^-, was equally compatible with the theory that HCO_3^- is reabsorbed as the passive consequence of the secretion of H^+.

effects of Diamox® are obtained in man by the intravenous injection of 250 mg. of the drug.

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CORRECTION

On page 1279 of the paper "Evidence that a humoral agent stimulates the adrenal cortex to secrete aldosterone in experimental secondary hyperaldosteronism" by N. A. Yankopoulos, J. O. Davis, B. Kliman and R. E. Peterson (J. clin. Invest. 1959, **38**, 1278), credit for the double isotope derivative method is incorrectly attributed. The double isotope derivative procedure for analysis of aldosterone was developed by Kliman and Peterson. An editorial error resulted in the insertion of Davis and Yankopoulos in the citation.