CHARACTERISTICS OF RENAL BICARBONATE REABSORPTION IN MAN *

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The reabsorption of filtered HCO₃⁻ by the kidney is thought to result not from the active reabsorption of HCO₃⁻ ions but rather from the secretion of cellular H⁺ in exchange for tubular Na⁺ (1, 2). The secreted H⁺ reacts with filtered HCO₃⁻ to form H₂CO₃ which then decomposes to CO₂ and H₂O, thereby effecting the reabsorption of NaHCO₃. The enzyme carbonic anhydrase, the inhibition of which blocks HCO₃⁻ reabsorption, is thought to maintain an adequate supply of H⁺ for secretion by accelerating the hydration of CO₂ within the renal cells. In support of this theory is the fact that HCO₃⁻ reabsorption varies linearly with plasma pCO₂ (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 HCO₃⁻ reabsorption during severe metabolic acidosis they found that at a given dose of Diamox® the reciprocals of HCO₃⁻ reabsorption and of plasma HCO₃⁻ 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 HCO₃⁻ was reabsorbed by some mechanism in which cellular carbonic anhydrase was the enzyme and filtered HCO₃⁻ (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 HCO₃⁻, the effect of Diamox® on the relationship between HCO₃⁻ reabsorption and plasma pCO₂ was studied in normal subjects and in subjects with pre-existing metabolic acidosis. During the course of these experiments, certain relations between HCO₃⁻ reabsorption, plasma HCO₃⁻ concentration and plasma pCO₂ became apparent which suggested that CO₂⁻³ rather than filtered HCO₃⁻ constituted the substrate involved in HCO₃⁻ 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 CO₂ in oxygen.
3) Metabolic acidosis—by oral ingestion of a total of 20 to 25 Gm. of NH₄Cl during the 24 hours preceding the

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experiment. In several studies chronic administration of 15 Gm. NH₄Cl 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 NH₄Cl.

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 NH₄Cl acidosis was associated with the same response as acute NH₄Cl 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 HCO₃⁻ 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 [HCO₃⁻] and pCO₂ are plotted. It is noteworthy that metabolic acido-
sis was accompanied by only a slight decrease in plasma pCO₂ despite a marked depression in plasma concentration of HCO₃⁻. In the other experimental states the plasma [HCO₃⁻] was related to pCO₂ in a roughly linear fashion.

Following the administration of Diamox®, HCO₃⁻ reabsorption was linearly related to plasma pCO₂ (Figure 2). The regression equation describing this relationship is Y = 0.61 + 0.028 X. Brazeau and Gilman (3), Relman, Etten and Schwartz (4) and Dorman, Sullivan and Pitts (5) have previously shown that HCO₃⁻ reabsorption was linearly related to plasma pCO₂ in the presence of normal carbonic anhydrase activity. Thus, inhibition of carbonic anhydrase does not disturb this linear relationship.

The administration of Diamox® during NH₄Cl acidosis resulted in moderate HCO₃⁻ excretion (200 to 300 µEq. per minute) despite the reduced concentration of HCO₃⁻ in plasma. As seen in Figure 2 the values for HCO₃⁻ reabsorption are somewhat skewed below the regression line. Although this skewed distribution is not striking, it is conceivable that the reduced concentration of HCO₃⁻ in glomerular filtrate during NH₄Cl acidosis partially limited the reabsorption of HCO₃⁻ despite the excretion of moderate amounts of HCO₃⁻ into the urine.

Schwartz and associates (6) have reported that at a given dose of Diamox® HCO₃⁻ reabsorption was approximately proportional to plasma HCO₃⁻ concentration. In the present experiments this same proportionality was also apparent (Figure 3). However, at approximately the same plasma [HCO₃⁻], the reabsorption of HCO₃⁻ was much less during respiratory alkalosis than during metabolic acidosis (Figure 3). In respiratory alkalosis and metabolic acidosis, plasma [HCO₃⁻] (and the filtered load of HCO₃⁻) were comparably depressed, yet during respiratory alkalosis 30 to 40 per cent of the filtered HCO₃⁻ was excreted while in metabolic acidosis only 7 to 15 per cent of the filtered HCO₃⁻ was excreted. This difference in HCO₃⁻ reabsorption between respiratory alkalosis and metabolic acidosis was probably the consequence of the fact that in respiratory alkalosis plasma pCO₂ falls more than [HCO₃⁻] does, whereas in metabolic acidosis the depression in plasma [HCO₃⁻] is far greater than any decrease in plasma pCO₂ (Figure 1). These observations suggest that during HCO₃⁻ diuresis plasma pCO₂ is a more important determinant of HCO₃⁻ reabsorption than is the concentration of HCO₃⁻.

To examine whether the correlation between plasma [HCO₃⁻] and HCO₃⁻ reabsorption in a reciprocal plot establishes the existence of an interaction between filtered HCO₃⁻ and cellular carbonic anhydrase in the course of HCO₃⁻ reab-

![Fig. 1. Effect of Acute Changes in Acid-Base Balance on Plasma pCO₂ and HCO₃⁻ Concentration](image-url)
The plasma pCO₂ 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.

The initial [HCO₃⁻] which ranged from 93.96 to 8.97 mEq per L. Next, 0.1 ml of 2.5 N HCl was added to each funnel. Again the samples were aerated for 15 minutes with 8.23 per cent CO₂ and 3 ml aliquots were once more removed for HCO₃⁻ determinations. An additional 0.1 ml of 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 HCO₃⁻ concentration and the HCO₃⁻ concentration after each increment of acid represents the quantity of HCO₃⁻ dissipated by the addition of HCl.

It is clear from Table II that the quantity of HCO₃⁻ decomposed is not equivalent to the quantity of acid added, but more nearly approximates it at the higher initial HCO₃⁻ concentrations. The data in Table II are plotted in Figure 4, where the initial [HCO₃⁻] concentrations are plotted along the horizontal axis and the quantities of HCO₃⁻ 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 HCO₃⁻ decomposed for each quantity of acid added is related to the initial [HCO₃⁻] in a curvilinear fashion. If the reciprocal of the decomposed HCO₃⁻ (1/V) is plotted against the reciprocal of the initial HCO₃⁻ 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 HCO₃⁻ by the secretion of H⁺ into
RENAL BICARBONATE REABSORPTION

2.8 - 2.6 - 2.4 - 2.2 - 0

2.0    0

dO  L AcO

1.8

1.6

1.4

1.2

1.0

0.8

0.6

0.4

0.2

0

Plasma \([\text{HCO}_3^-]\) mEq./100 ml.

Fig. 3. Relation of Bicarbonate Reabsorption to Plasma \([\text{HCO}_3^-]\) 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 \([\text{HCO}_3^-]\) concentration and \([\text{HCO}_3^-]\) reabsorption during the administration of Diamox® could arise from competition between bicarbonate and nonbicarbonate buffer systems for secreted \(\text{H}^+\). The competing buffering action, however, did not necessarily originate in the glomerular filtrate, but could, instead, have arisen as

<table>
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<tr>
<th>Initial ([\text{HCO}_3^-])*</th>
<th>([\text{HCO}_3^-]) decomposed</th>
<th>([\text{HCO}_3^-]) decomposed</th>
<th>([\text{HCO}_3^-]) decomposed</th>
<th>([\text{HCO}_3^-]) decomposed</th>
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<tr>
<td>([\text{HCO}_3^-]) sol. + 0.25 mEq. HCl (5.32 mEq./L.)</td>
<td>([\text{HCO}_3^-]) sol. + 0.25 mEq. HCl (5.32 mEq./L.)</td>
<td>([\text{HCO}_3^-]) sol. + 0.25 mEq. HCl (5.32 mEq./L.)</td>
<td>([\text{HCO}_3^-]) sol. + 0.25 mEq. HCl (5.32 mEq./L.)</td>
<td>([\text{HCO}_3^-]) sol. + 0.25 mEq. HCl (5.32 mEq./L.)</td>
</tr>
<tr>
<td>93.96</td>
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<td>4.21</td>
<td>82.74</td>
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<td>6.88</td>
<td>2.08</td>
<td>3.43</td>
<td>5.53</td>
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* After equilibration with 8.23 per cent CO₂.
† All solutions contained 15 mMoles per L. sodium phosphate pH 7.4.
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, then filtered HCO₃⁻ would, in effect, be competing with cellular processes for secreted H⁺. Under these conditions progressive elevation of the concentration of filtered HCO₃⁻ could yield curves similar to those shown in Figure 5. Thus, similar 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 HCO₃⁻.

**DISCUSSION**

The reabsorption of filtered HCO₃⁻ 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-
nism, *i.e.* saturation, competitive inhibition, specific inhibition, and kinetics characteristic of an enzyme-substrate interaction (9), have been demonstrated for the reabsorption of HCO₃⁻. First, Pitts and Lotspeich (10) have shown that in normal animals elevation of the filtered load of HCO₃⁻ saturated the tubular reabsorptive mechanism at a HCO₃⁻ 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 HCO₃⁻ Tm, suggesting competition between Cl⁻ and HCO₃⁻ for carrier sites. Third, carbonic anhydrase inhibitors specifically block HCO₃⁻ reabsorption without impairing other metabolic processes of renal tissue. Finally, Schwartz and co-workers (6) have found that the relationship between HCO₃⁻ reabsorption and the concentration of plasma HCO₃⁻ 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 HCO₃⁻ from the tubular lumen.

According to a more widely accepted theory (1, 2), the primary process in HCO₃⁻ reabsorption is the secretion of cellular H⁺ in exchange for tubular Na⁺. The secreted H⁺ reacts with HCO₃⁻ to form H₂CO₃ which then decomposes to CO₂ and H₂O. According to this theory the H⁺ involved in HCO₃⁻ reabsorption arises from the hydration of CO₂ catalyzed by carbonic anhydrase.

Two of the criteria listed as evidence supporting the theory of active HCO₃⁻ 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 HCO₃⁻ 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-inhibitor kinetics (6) establishes the existence of active HCO₃⁻ reabsorption involving carbonic anhydrase as the carrier molecule, or whether these same kinetics are compatible with the theory that HCO₃⁻ 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 HCO₃⁻ reabsorption and plasma [HCO₃⁻] was nonspecific, equally compatible with either of the above mentioned theories of HCO₃⁻ reabsorption.

While the results of these *in vitro* studies do not exclude the existence of active HCO₃⁻ reabsorption involving carbonic anhydrase as the carrier molecule, other lines of evidence render it unlikely. First, the elevated urinary CO₂ tensions observed during NaHCO₃ infusions (10) could not be explained if active transport were the sole mechanism for HCO₃⁻ reabsorption. Second, the linear relationship between plasma pH and HCO₃⁻ reabsorption (3–5) would be difficult to explain if the reabsorption of HCO₃⁻ were accomplished solely by an interaction between cellular carbonic anhydrase and filtered HCO₃⁻. To explain this effect it would be necessary to propose that the activity of carbonic anhydrase varied linearly with pH. 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 CO₂ 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 HCO₃⁻ reabsorption. The linear relation between plasma pCO₂ and HCO₃⁻ reabsorption, therefore, supports the theory that HCO₃⁻ is reabsorbed as the passive consequence of H⁺ secretion rather than the active removal of HCO₃⁻ ions from the tubule lumen.

The failure of complete carbonic anhydrase inhibition to abolish HCO₃⁻ reabsorption (14) raises the question of whether there might be a second mechanism for the reabsorption of HCO₃⁻ not involving the secretion of H⁺. The results of the present studies, however, indicate that following the administration of 250 mg. Diamox® intravenously HCO₃⁻ reabsorption is still linearly related to plasma pCO₂, despite marked inhibition of carbonic anhydrase activity.⁸ This suggests that

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⁸ Counihan, Evans and Milne (15) have shown that 90 to 95 per cent of the maximally achievable inhibitory
all HCO₃⁻ reabsorption results from the secretion of H⁺ and that the uncatalyzed hydration of CO₂ is an important source of H⁺ for the Na⁺–H⁺ exchange process.

The demonstration that the uncatalyzed hydration of CO₂ constitutes an important source of H⁺ for the reabsorption of HCO₃⁻ affords an explanation for the failure of Diamox® to produce maximum HCO₃⁻ diuresis during metabolic acidosis. It was shown in Figure 1 that the concentration of plasma HCO₃⁻ was depressed in NH₄Cl acidosis without a commensurate decrease in plasma pCO₂. Therefore, under these circumstances, the quantity of HCO₃⁻ filtered was reduced, but the capacity of the uncatalyzed hydration of CO₂ to effect the reabsorption of HCO₃⁻ remained comparatively unchanged. Thus, when the plasma HCO₃⁻ concentration fell in metabolic acidosis to the point where the filtration of HCO₃⁻ was equal to the capacity of the uncatalyzed hydration of CO₂ to reabsorb HCO₃⁻, Diamox® no longer produced a HCO₃⁻ diuresis. Following the administration of 250 mg. Diamox® the reabsorption of HCO₃⁻ at a plasma pCO₂ of 35 mm. Hg (the average pCO₂ during metabolic acidosis) was approximately 1.5 mEq. per 100 ml. glomerular filtration rate (GFR) (Figure 2), presumably due to the uncatalyzed hydration of CO₂ and any residual uninhibited carbonic anhydrase. Therefore, in man given this amount of Diamox® reabsorption of HCO₃⁻ would be virtually complete when the plasma HCO₃⁻ 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 HCO₃⁻ concentration to 15 mEq. per L. by hyperventilation drastically diminished the capacity of the uncatalyzed hydration of CO₂ to reabsorb HCO₃⁻, and hence Diamox® would be expected to produce marked HCO₃⁻ diuresis despite the smaller quantity of filtered HCO₃⁻.

SUMMARY

The pattern of renal HCO₃⁻ 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® HCO₃⁻ reabsorption varied linearly with plasma pCO₂, as described by the regression equation Y = 0.61 + 0.028 X. This linear relationship in the presence of marked inhibition of carbonic anhydrase indicated that the uncatalyzed hydration of CO₂ was an important source of H⁺ for the reabsorption of HCO₃⁻. The failure of Diamox® to produce marked HCO₃⁻ diuresis during metabolic acidosis was attributable to nearly complete reabsorption of the small filtered load via the uncatalyzed hydration of CO₂.

In vitro studies, in which varying amounts of HCl were added to phosphate-buffered HCO₃⁻ solutions, disclosed that the linear relationship between the reciprocals of HCO₃⁻ reabsorption and plasma HCO₃⁻, used as evidence for the active reabsorption of HCO₃⁻, was equally compatible with the theory that HCO₃⁻ is reabsorbed as the passive consequence of the secretion of H⁺.

REFERENCES

1. Pitts, R. F., and Alexander, R. S. The nature of the renal tubular mechanism for acidifying the urine. Amer. J. Physiol. 1945, 144, 239.


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.