Evidence Against Bicarbonate Reabsorption in the Ascending Limb, Particularly as Disclosed by Free-Water Clearance Studies[1]

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Bicarbonate reabsorption in the thick ascending limb of Henle's loop was examined by studies of free-water clearance (C_{H_2O}) and free-water reabsorption ($T^c_{H_2O}$). During maximal water diuresis in the dog, C_{H_2O}/GFR was taken as an index of sodium reabsorption in, and urine flow (V/GFR) as an index of delivery of filtrate to, this segment. Three different procedures—infusion of hypotonic sodium bicarbonate, infusion of a nonreabsorbable solute (hypotonic mannitol) and administration of an inhibitor of bicarbonate reabsorption (acetazolamide)—all increased C_{H_2O}/GFR per unit V/GFR to a similar extent, but less than that achieved with hypotonic saline infusion. This suggests that sodium bicarbonate is not reabsorbed in the ascending limb. Rather, it is the sodium chloride, swept out of the proximal tubule by osmotic diuresis due to nonreabsorbed mannitol or sodium bicarbonate, that is reabsorbed in the ascending limb thereby increasing C_{H_2O}, whereas the nonreabsorption of mannitol and sodium bicarbonate results in a depressed C_{H_2O} per unit V when compared with hypotonic saline. V/GFR is not a satisfactory index of delivery to the ascending limb during osmotic diuresis, since it includes water obligated by nonreabsorbable solutes. When a better index of delivery, the sum of the clearances of chloride (C_{Cl}) and free-water (C_{H_2O}) is used, hypotonic bicarbonate infusion, hypotonic mannitol infusion and acetazolamide administration increase C_{H_2O}/GFR per unit delivery to the same extent as does hypotonic saline infusion.

Studies in dogs and rats on $T^c_{H_2O}$ also indicate that sodium bicarbonate is an impermeant solute in the ascending limb. Osmotic diuresis due to sodium bicarbonate diuresis, produced either by inhibition of sodium bicarbonate reabsorption (acetazolamide, L-lysine monohydrochloride) or infusion of sodium bicarbonate, or mannitol diuresis both produced marked chloruresis and increased $T^c_{H_2O}$ to the same extent as did hypertonic saline infusion. If chloride excretion was almost eliminated by hemodialysis against a chloride-free dialysate (dogs) or prolonged feeding of a salt-free diet (rats), $T^c_{H_2O}$ formation was unimpaired if hypertonic saline was infused but virtually obliterated during mannitol or sodium bicarbonate diuresis. Sodium reabsorption in the ascending limb, therefore, appears to be dependent upon chloride as the accompanying anion.

At any given rate of bicarbonate excretion, more cloride is delivered out of the proximal tubule (as estimated from $C_{Cl} + C_{H_2O}$) with hypotonic sodium bicarbonate infusion than with acetazolamide administration. This suggests that magnitude of the chloruresis accompanying bicarbonate diuresis depends, not only on osmotic diuresis due to nonreabsorbed sodium bicarbonate, but also on the extent to which concomitant changes in effective extracellular volume influence overall sodium chloride reabsorption.

There is substantial evidence that the reabsorption of bicarbonate in both the proximal and distal nephron is mediated principally by the secretion of hydrogen ions (1–3). The maximal rate of hydrogen secretion, and consequently bicarbonate reabsorption, is dependent on an intact carbonic anhydrase enzyme system (2–4) and varies directly with the CO_2 tension of blood (4–7), mineralocorticoid activity

(8, 9), and the concentration of serum calcium (15) and inversely with effective extracellular volume (9–11), intracellular potassium (9, 12–14), and the plasma concentration of parathyroid hormone (15).

Micropuncture studies at the end of the accessible portion of the proximal convolution have demonstrated that normally about 90% of the filtered bicarbonate is reabsorbed in the rat (16) and about 75% in the dog (17). The regulatory influence of many of the factors listed previously—carbonic anhydrase activity (3), plasma CO_2 tension (18), effective extracellular volume (14, 18), intracellular potassium (13, 14), and parathyroid hormone (19)—have been demonstrated by micropuncture to be exerted to a major extent in the proximal tubule.

The 10–25% of the filtered bicarbonate that normally escapes reabsorption in the proximal convolution must be reabsorbed more distally, since bladder urine is usually sufficiently acid to preclude the presence of any bicarbonate. It is not clear whether the distal tubule and collecting duct reabsorb all or only a portion of the bicarbonate delivered out of the proximal convolution. The fraction of bicarbonate that is reabsorbed in the distal segments appears to be stimulated by mineralocorticoid hormones (8, 9, 12), intracellular potassium deficiency (9, 12), and elevation of the distal transtubular potential difference (20).

The contribution of the thick ascending limb of Henle to bicarbonate reabsorption is uncertain. Two techniques have been employed to examine transport in this segment: (a) microperfusion of isolated segments of the thick ascending limb of the rabbit and (b) clearance studies, using free-water clearance (C_{H_2O}) and free-water reabsorption ($T^c_{H_2O}$) as rough approximations of sodium reabsorption in the ascending limb.

MICROPERFUSION OF ISOLATED SEGMENTS OF
RABBIT THICK ASCENDING LIMB (21–24)

It had been demonstrated by micropuncture in the rat (25–28), dog (29–30), and rhesus monkey (31) that the sodium concentration and osmolality in the early distal tubule are much lower than those of plasma. By contrast, in fluid obtained from the tip of Henle's loop in the rat, the concentration of sodium and osmolality is higher than that of plasma (32, 33). From these findings, the inference had been drawn that the thick ascending limb reabsorbs sodium salts far in excess of water. The inaccessibility of this segment to micropuncture techniques, however, prevented direct characterization of the nature of transport process.

Data derived from microperfusion of isolated segments of the thick ascending limb of the rabbit by two independent laboratories (21, 22) permitted the elaboration of an explanation for the micropuncture findings. Although there is some difference in details, the principal observations are the same in both studies.

It was shown that the thick ascending limb was virtually water-impermeable and had the capacity for outward solute transport. That the tubule was impermeable to the osmotic flow of water was demonstrated by raising the osmotic pressure of the bathing fluid with either raffinose or sodium chloride far above that of the perfusate; in neither instance did this osmotic gradient alter the difference between the rate of perfusion and collection.

To characterize the nature of the solute transport, an isosmolal ultrafiltrate of the rabbit serum used for the bath was perfused through the tubule, with the following results: (a) a potential difference of about +6 mV (lumen positive) was measured, (b) the concentration of sodium, chloride, and total solutes fell and (c) there was no

change in the concentration of a volume marker. The fact that the potential was positive constituted strong evidence for outward transport of chloride. Moreover, since the chloride transport was against an electrochemical gradient, it was presumably active. The hypothesis of active chloride transport was strengthened by the finding that the potential could be sharply reduced by cooling or adding ouabain to the bath. In accord with this proposal, it was further shown that removal of chloride from perfusate and bath by substitution with methyl sulfate reduced the potential toward zero, whereas removal of sodium and substitution with choline was still associated with a positive potential. In contrast to the strong evidence for active chloride transport, the reabsorption of sodium could be explained by passive forces, since the measured potential difference in a steady state was the same as the Nernst sodium equilibrium potential. From these observations, the conclusion was drawn that the thick ascending limb of Henle generates a dilute urine because it is water-impermeable and can reabsorb solute through the action of an active chloride pump, with sodium following passively.

It is not clear whether the entire reabsorption of sodium can be attributed to passive diffusion along the electrochemical gradient created by active chloride transport. Nor, indeed, is there a satisfactory explanation for the inhibition of active chloride transport by ouabain, which acts only on the bath surface, and furosemide (23, 24), which acts only on the luminal surface. These uncertainties do not detract from the basic observations establishing the presence of an active chloride transport system in a water-impermeable segment as the principal process of solute transport and urinary dilution.

If the thick ascending limb transports solute via an active chloride pump, with sodium following passively, the reabsorption of bicarbonate could not be accomplished by a sodium-dependent hydrogen secretory system analogous to that in the proximal tubule. Conceivably, some unique bicarbonate transport system might be present; alternatively, bicarbonate might be nonreabsorbable.

Although bicarbonate transport in the thick ascending limb has not been fully examined, early studies (22) disclose that in the steady state, the potential difference appears to be lower, and the sodium concentration higher in tubules with bicarbonate-containing perfusate than in those with bicarbonate-free perfusate. This has been interpreted to mean (22) that bicarbonate may be reabsorbed poorly, if at all, in this segment.

BICARBONATE REABSORPTION IN THE ASCENDING LIMB EXAMINED BY FREE-WATER CLEARANCE AND FREE-WATER REABSORPTION

To examine the function of the ascending limb, we (34–37) have made use of the assumption that during maximal water diuresis (suppression of antidiuretic hormone), free-water clearance is a rough index of sodium reabsorption in this segment. The justification for this assumption hinges in large part on the micropuncture findings that fluid at the bend of Henle's loop is hypertonic and emerges into the distal tubule markedly hypotonic, which indicates that the bulk of sodium delivered out of the proximal tubule is ordinarily reabsorbed in the ascending limb. The generation of free water results from continued sodium chloride reabsorption (after tubular fluid has been rendered isotonic in the inner medullary segment) in the medullary and cortical segments of the ascending limb. To be sure, additional free water may be generated by solute reabsorption in excess of water at more distal sites, but the
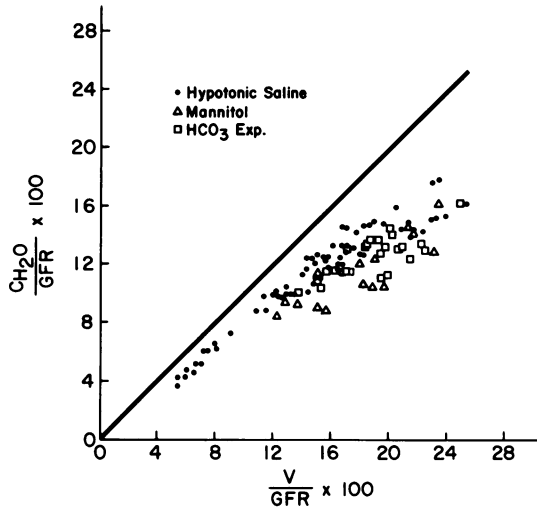
FIG. 1. The method of expanding extracellular volume and inducing and maintaining water diuresis is described in previous papers (34–36). Hypotonic saline was injected as a 0.45% solution at a sustaining rate of 10 ml/min greater than V. Hypotonic sodium was infused in a concentration of 75 mequiv/liter at a sustaining rate of 2 ml/min greater than V. The sustaining infusion for hypotonic mannitol was at a concentration of 150 mM/liter.

amount is small in comparison with the thick ascending limb. The free water generated by sodium reabsorption is largely excreted into the urine because the ascending limb is always virtually water-impermeable (as indicated by microperfusion studies) and the remainder of the nephron is relatively water-impermeable during maximal water diuresis.

C_{H_2O} may change as a result of either alterations in delivery of filtrate to the ascending limb from more proximal sites or because of a change in its intrinsic reabsorptive capacity. For this reason, it is useful to express C_{H_2O} as a function of delivery of filtrate. We have used urine flow (V) for this purpose (34), since there is very little fluid loss from tubular urine beyond the beginning of the ascending limb (the ascending limb itself is always water-impermeable, and the distal tubule and collecting duct relatively so during maximal ·vater diuresis).

The use of V to approximate delivery of filtrate to the ascending limb is based on the tacit assumption that the solutes contained in the urine are reabsorbable and therefore constitute substrates for free-water formation. If, however, a solution of hypotonic mannitol is infused, V will increase, but part of the increase in V is obligated by mannitol, which is not a substrate for free-water formation. Under such circumstances, V is not a suitable index of delivery, since, to the extent that nonreabsorbable solutes are present, it does not express the delivery of substrates for free-water formation. This problem is particularly pertinent to the use of C_{H_2O} for the purpose of examining whether bicarbonate is reabsorbed in the ascending limb.

Figure 1, which is derived from the data of Rosin and his associates (35), displays the relations of C_{H_2O} to V in three groups of dogs infused with either hypotonic saline (solid circles), hypotonic sodium bicarbonate (squares), or hypotonic mannitol (triangles). It is evident that the infusion of hypotonic bicarbonate resulted in an increase of C_{H_2O}/GFR as V/GFR increased. However, the increase in C_{H_2O}/GFR per unit V/GFR was less than that achieved with hypotonic saline infusion.

These findings could be interpreted to mean that sodium bicarbonate was reabsorbed in the ascending limb but to a lesser extent than sodium chloride. In consequence, C_{H_2O}/GFR would increase with bicarbonate infusion but not as much as when hypotonic saline was infused. However, Fig. 1 also displays the influence of hypotonic mannitol infusion, which increases C_{H_2O}/GFR per unit V/GFR to the same extent as does sodium bicarbonate infusion, yet mannitol is a nonreabsorbable solute. This suggests that the increase in C_{H_2O} with both sodium bicarbonate and mannitol may be the result of accelerated delivery of sodium chloride out of the proximal tubule as a result of an osmotic diuresis. This increased delivery of sodium chloride could then be responsible for the increased C_{H_2O}/GFR in both circumstances.

This hypothesis is strengthened by the finding that acetazolamide administration also increases C_{H_2O}/GFR per unit V/GFR (Fig. 2). Here, too, the increase is less than that achieved with hypotonic saline but similar to that noted after sodium bicarbonate and mannitol. The fact that acetazolamide and sodium bicarbonate infusion produce the same relative depression of C_{H_2O} per unit delivery (as compared with hypotonic saline) suggests that acetazolamide does not exert its effect by inhibiting bicarbonate reabsorption in the ascending limb. The data are more consonant with the interpretation that acetazolamide inhibits proximal bicarbonate reabsorption, thereby resulting in increased delivery of sodium bicarbonate and sodium chloride to the ascending limb, where the reabsorption of the latter results in increased C_{H_2O}, but the nonreabsorption of the former results in a depressed level of C_{H_2O} per unit delivery.

If the hypothesis is valid that sodium bicarbonate behaves as an impermeant solute in the ascending limb, it follows that V is not a satisfactory index of delivery, since it includes the water obligated by nonreabsorbable solutes. To correct for the presence of mannitol, the use of the term $(C_{Na} + C_{H_2O})/GFR$ as an index of delivery has been suggested (38). Such an expression might be satisfactory when mannitol is the loading solute, but it would not correct for the copious excretion of sodium bi-
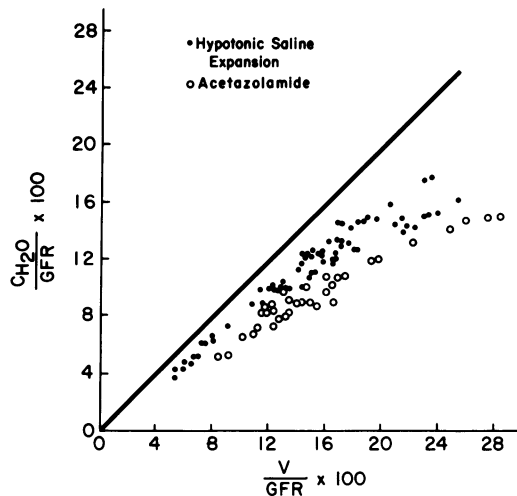

FIG. 2. A sustaining solution was prepared to permit acetazolamide infusion at a rate of 20 mg kg^{-1} hour^{-1}. Simultaneously, sodium bicarbonate was infused in a concentration of 75 mM/liter at a rate estimated to replace urinary losses and thereby prevent a fall in plasma bicarbonate.
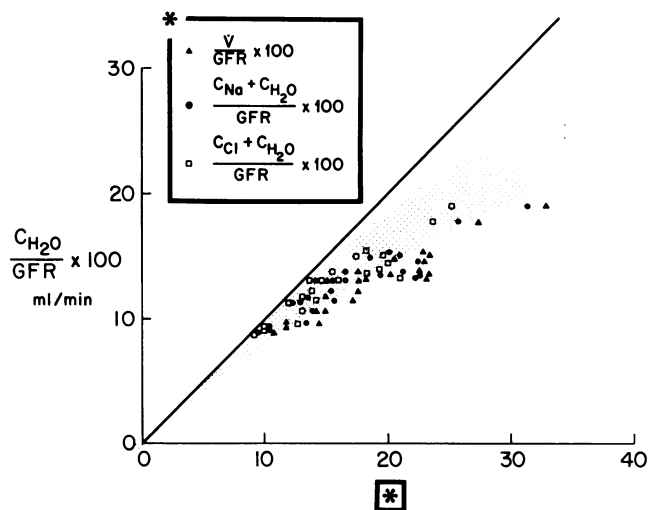
FIG. 3. Fractional free water clearance as a function of delivery of fractional sodium, chloride, or urine volume during hypotonic bicarbonate infusions.

carbonate, either during sodium bicarbonate loading or acetazolamide adminis-
tration. Moreover, to the extent that mannitol sweeps sodium bicarbonate out of the
proximal tubule, the use of $(C_{Na} + C_{H_2O})/GFR$ as an index of delivery would not
wholly correct for nonreabsorbable solute. If the inferential arguments are valid
that sodium chloride is the substrate for free-water reabsorption in the ascending
limb, the best expression of delivery should be $(C_{Cl} + C_{H_2O})/GFR$.

In Fig. 3 the bicarbonate data, and in Fig. 4 the acetazolamide data, are plotted
using three indices of delivery: V/GFR, $(C_{Na} + C_{H_2O})/GFR$, and $(C_{Cl} + C_{H_2O})/GFR$. In both figures the stippled area indicates the relation of free water clearance
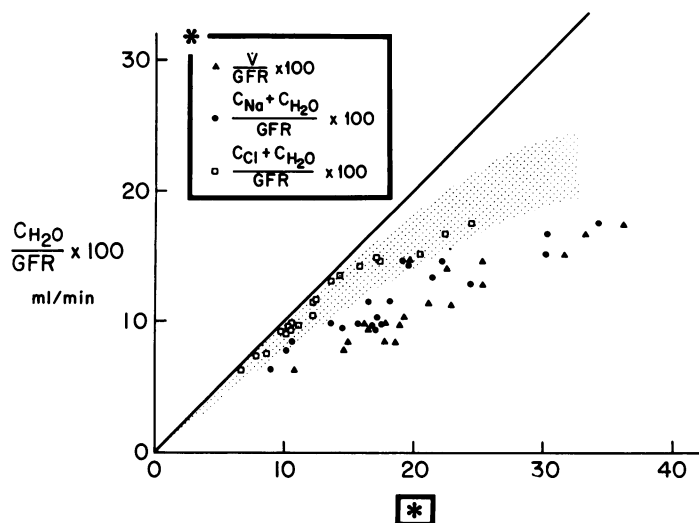

FIG. 4. Fractional free water clearance as a function of delivery of fractional sodium, chloride, or urine volume during acetazolamide.

to delivery when hypotonic saline is given. All three ways of plotting delivery yield the same results for the data from hypotonic saline loading.

Figure 3 discloses that C_{H_2O} per unit delivery is less with hypotonic bicarbonate than with hypotonic saline when the delivery term is V/GFR or $(C_{Na} + C_{H_2O})/GFR$. However, when delivery is plotted as $(C_{Cl} + C_{H_2O})/GFR$, the relation of C_{H_2O} to delivery is completely normalized, so that all points fall within the stippled area.

Essentially the same results are forthcoming for the data obtained during acetazolamide administration (Fig. 4), but the effect of correcting for nonreabsorbable solutes by using $(C_{Cl} + C_{H_2O})/GFR$ as an index of delivery is more striking. This is probably the consequence of the greater excretion of chloride per unit bicarbonate excretion during hypotonic bicarbonate infusions than with acetazolamide administration, a point that will be discussed in the following.

Studies in both dogs (39) and rats (40) on free-water reabsorption lead to the same conclusions that sodium bicarbonate behaves as an impermeant solute in the ascending limb and that sodium transport in this segment is specifically dependent on chloride as the accompanying anion. In the dog, hypertonic mannitol infusion and a variety of procedures designed to increase distal bicarbonate delivery by different mechanisms—hypertonic sodium bicarbonate infusion, acetazolamide administration, and L-lysine monohydrochloride administration—all gave the same increase in free-water reabsorption $(T^c H_2O)$ per unit osmolar clearance (Cosm) as did hypertonic saline. In all instances urinary chloride excretion was copious. The fact that infusion of an impermeant solute (mannitol), injection of inhibitors of bicarbonate reabsorption (acetazolamide, L-lysine monohydrochloride), and infusion of sodium bicarbonate all produced marked chloruresis suggested that the capacity to produce maximal increases in $T^c H_2O$ is dependent on increased delivery of sodium chloride out of the proximal tubule in association with an osmotic diuresis due to mannitol or sodium bicarbonate (whether the latter results from infusion or inhibition of reabsorption).

To test this hypothesis, chloride excretion was reduced virtually to zero by hemodialyzing dogs against a chloride-free dialysate. This resulted in alkalosis and hypochloremia comparable to that produced by hypertonic sodium bicarbonate infusion but without extracellular volume expansion. Infusion of hypertonic sodium bicarbonate after dialysis disclosed in all dogs virtually a complete obliteration of the capacity to form $T^c H_2O$. That this was not the consequence of some disturbance inherent to the dialyzing procedure is indicated by the normal increase in $T^c H_2O$ that followed infusion of hypertonic saline into dialyzed dogs. These studies, therefore, are in accord with the interpretation of the free-water clearance data that sodium chloride, but not sodium bicarbonate, is reabsorbed in the ascending limb.

Similar results were obtained in rats (40) where infusions of hypertonic bicarbonate and hypertonic mannitol could both be shown to generate less $T^c H_2O$ than did hypertonic saline at any given osmolar clearance. When rats were chloride-depleted by prolonged feeding of a salt-free diet, the capacity to form $T^c H_2O$ was unimpaired when hypertonic saline was infused but virtually obliterated with infusions of hypertonic sodium bicarbonate or mannitol. Indeed, in chloride-depleted rats infusion of these latter two solutes sometimes actually resulted in the excretion of free water. It seems reasonable to interpret such findings as evidence for low rates of solute (chloride) reabsorption in the ascending limb, resulting in a reduction in medullary tonicity; this, in turn, would lead to negative $T^c H_2O$ in a setting where osmotic diuresis was sweeping hypotonic urine rapidly through the medullary

collecting duct. By contrast, at the same osmolar clearance, infusion of hypertonic saline can generate $T^c_{H_2O}$ because marked sodium chloride reabsorption in the ascending limb elevates medullary tonicity, thereby furnishing a steeper gradient for the abstraction of free water from the collecting duct.

In the aggregate, then, the data derived from clearance studies, where free-water clearance and free-water reabsorption are used as approximations of sodium reabsorption in the ascending limb, constitute strong evidence that bicarbonate is not significantly reabsorbed in this segment; sodium reabsorption is therefore dependent upon chloride as the accompanying anion.

RELATION OF BICARBONATE TO CHLORIDE EXCRETION

The finding that bicarbonate is nonreabsorbable in the ascending limb may provide an explanation for the varied pattern of chloride excretion associated with bicarbonate diuresis.

To examine this issue, the effect of bicarbonate diuresis on chloride excretion was examined during maximal water diuresis under two different circumstances (Fig. 5): (a) acetazolamide administration and (b) sodium bicarbonate infusion. The sum of C_{Cl} and C_{H_2O}, plotted along the vertical axis, is taken to represent sodium chloride delivered out of the proximal tubule that has been reabsorbed in the ascending limb (C_{H_2O}) or excreted into the urine (C_{Cl}). Care was taken during the acetazolamide studies to avoid expanding extracellular volume by infusing only sufficient bicarbonate to maintain the serum concentration at a normal value. The data indicate that the sum of C_{Cl} and C_{H_2O} is greater at any given level of bicarbonate excretion with sodium bicarbonate infusion than with acetazolamide administration.

We would interpret these data to mean that at least two factors determine the influence of bicarbonate on chloride excretion: (a) the osmotic diuresis produced by nonreabsorbable sodium bicarbonate and (b) the change in extracellular volume, which influences overall proximal tubular reabsorption.

Infusion of sodium bicarbonate expands extracellular volume, thereby suppressing overall proximal tubular reabsorption, at the same time that it produces an osmotic diuresis owing to nonreabsorbed sodium bicarbonate. The net effect is the delivery of large amounts of sodium chloride out of the proximal tubule, subsequently to be partially reabsorbed in the ascending limb and partially excreted into the urine. By contrast acetazolamide produces a copious bicarbonate diuresis, but since extracellular volume is not overexpanded, there is considerably less chloruresis.
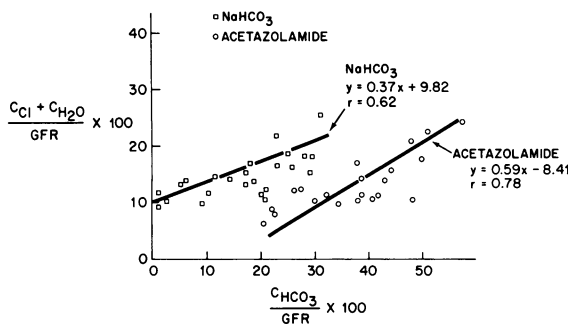
FIG. 5. Influence of bicarbonate clearance produced by bicarbonate infusion or acetazolamide on chloride delivery to the loop during water diuresis.

It thus appears that bicarbonate diuresis can readily be produced by exceeding proximal reabsorptive capacity, since the ascending limb reabsorbs bicarbonate poorly or not at all and the distal nephron has a low capacity for bicarbonate reabsorption. The accompanying chloruresis may vary greatly, however. Since the ascending limb has a great capacity for chloride transport, a modest increase in chloride delivery may be completely reabsorbed, whereas bicarbonate may be excreted into the urine. These findings may explain the pattern of bicarbonate and chloride excretion found in vomiting.

When vomiting is persistent, the very rapid rise in the serum bicarbonate is associated with the urinary excretion of large amounts of bicarbonate, accompanied by sodium and potassium, but almost no chloride (41–43). We (9) have termed this a disequilibrium state. When vomiting has stopped without the deficits having been replenished, an alkalosis exists, but the urine contains little bicarbonate, sodium, potassium, or chloride. This is the equilibrium state of vomiting (9).

The equilibrium state can be reasonably explained by assuming that with the cessation of vomiting the continued, though transient, excretion of bicarbonate results in a reduction in the filtered load (serum bicarbonate falls slightly) and increased bicarbonate reabsorptive capacity (extracellular volume contracts because of sodium bicarbonate excretion). In consequence, enhanced tubular reabsorption is sufficient to reclaim more or less completely the reduced filtered load. In the absence of bicarbonate diuresis, the urine contains little sodium, potassium, or chloride.

The disequilibrium state is more difficult to explain. In the presence of an appreciable bicarbonate diuresis, the urine, though containing plentiful amounts of sodium and potassium, is nevertheless chloride-free. It seems reasonable to attribute the bicarbonate diuresis to the marked increase in filtered bicarbonate, which exceeds even the enhanced proximal bicarbonate reabsorptive capacity that results from contracted extracellular volume and potassium deficiency (9, 12–14). The increment in bicarbonate leaving the proximal tubule, since it cannot be reabsorbed in the ascending limb, floods the distal nephron, overcoming its limited ability to reabsorb bicarbonate, and spills into the urine. There is no accompanying chloruresis because contracted extracellular volume partially counteracts the effects of osmotic diuresis (nonreabsorbable sodium bicarbonate), thus limiting the delivery of chloride to the ascending limb. Since the capacity of this segment for active chloride transport is very great, it may reabsorb the bulk of the chloride delivered to it, thereby rendering the urine chloride-free.

The chloruresis, therefore, that accompanies bicarbonate diuresis may be great (sodium bicarbonate infusion), modest (acetazolamide administration), or totally absent (disequilibrium state of vomiting), depending on factors that control overall sodium chloride reabsorption.

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