# Bicarbonate Secretion by the Rat Colon: Effect of Intraluminal Chloride and Acetazolamide[1] (35001)

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In several species, absorption of sodium, chloride, and water by the colon is associated with concomitant secretion of bicarbonate (1–5). Mucosal exchange of bicarbonate for chloride has been proposed as an explanation (1–5), and bicarbonate secretion is augmented by the presence of chloride in the lumen (5). Moreover, the rat ileum, which also possesses a mechanism for bicarbonate secretion, transports bicarbonate against concentration gradients when chloride, but not another anion, is in the lumen (6, 7). Carbonic anhydrase, which is abundant in the mucosa of the colon (8, 9), has been implicated in anion transport (1), because drugs that inhibit carbonic anhydrase reduce bicarbonate secretion, and, simultaneously, sodium chloride and water absorption is reduced (1).

The present studies support the proposal that chloride and bicarbonate are exchanged across the mucosa of the colon, because bicarbonate was secreted in the presence of chloride but was absorbed when it was the only anion in the lumen or when chloride was replaced by other anions. In addition, we examined the effects on electrolyte and water transport of an inhibitor of carbonic anhydrase (acetazolamide) under two circumstances: one that favored anion exchange (chloride-containing perfusing solution) and the other when chloride-free solutions were used.

*Material and Methods.* The experiments compared net transport of electrolytes and water from the rat colon, perfused *in vivo* with solutions containing sodium and chloride, one or the other of these ions, or neither. Studies were performed on male Sprague–Dawley rats that weighed 200–300 g, were fasted overnight, and were lightly anesthetized with ether. Laparotomy was performed and polyvinyl catheters (OD 3 mm) were inserted through the cecum and ligated in the proximal colon (for infusion) and in the terminal ileum (for decompression). A similar catheter for collection of perfusates was passed from the anus into the sigmoid colon and ligated at the pelvic brim. Abdominal cannulae were led to the outside, the laparotomy was closed, a venous cannula was inserted in a tail vein, and animals were allowed to waken in a restraining cage. The colon was cleansed with isotonic (154 m*M*) sodium chloride and flushed gently with air.

The colon was perfused by continuous recirculation (1) of solutions at a rate of 1 ml/min (Harvard Infusion Pump, model 1203, Harvard Instruments, Cambridge, Mass.) from a reservoir that was sealed with mineral oil and maintained in a water bath at 37°. Test solutions (30 ml) were allowed to equilibrate by recirculation for 30 min before the initial sample (5 ml) was removed from the reservoir ("initial solution"). Absorption was then quantified by a further 60 min of perfusion, after which the colon was emptied as completely as possible to obtain the "final solution." Thereafter, the bowel was flushed several times with the second or subsequent test solution, and the perfusion was repeated. Samples were analyzed immediately or frozen at 4° in airtight containers. All perfusing solutions contained potassium and bicarbonate and were isosmolar with plasma that was withdrawn from the portal vein in preliminary studies on eight rats (Table I).

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TABLE I. Composition of Test Solutions.

| Test solution[a] | Sodium (meq/liter) | Potassium (meq/liter) | Chloride (meq/liter) | Bicarbonate (meq/liter) | Osmolality (mOsm/kg) | pH |
|---|---|---|---|---|---|---|
| Sodium chloride | 135 | 25 | 135 | 25 | 300 | 7.90 |
| Sodium isethionate | 135 | 25 | 0 | 25 | 301 | 7.85 |
| Sodium iodide | 135 | 25 | 0 | 25 | 300 | 8.30 |
| Choline chloride | 0 | 25 | 135 | 25 | 299 | 8.15 |
| Mannitol | 0 | 25 | 0 | 25 | 300 | 8.30 |
| Plasma[b] | 148.1 ± 1.5 | 4.2 ± 0.3 | 105.0 ± 0.9 | 17.6 ± 0.8 | 301 ± 1.4 | |

[a] All contained polyethylene glycol (PEG, mol wt 4000, Union Carbide, New York, N.Y.), 5 g/liter as nonabsorbable marker.

[b] From portal vein, mean ± SE ($n = 8$).

The role of intraluminal chloride in net transport of water and electrolytes was tested by perfusing the colon of four rats successively with sodium chloride and sodium isethionate (sodium salt of hydroxyethylsulfonic acid) solutions, used in alternate sequence. The effect of sodium on net colonic transport was tested by a similar comparison between sodium chloride and choline chloride solutions in four rats. In an additional four animals, five test solutions were used in random order.

The effect of acetazolamide on water and electrolyte transport was tested in six rats. Each rat was perfused with sodium chloride and sodium isethionate solutions, used in alternate sequence, then 5 mg of acetazolamide (Diamox) was given by tail vein, and 30 min later, each rat was perfused with the same solutions to which 13.5 mg of acetazolamide had been added.

Sodium and potassium were measured by

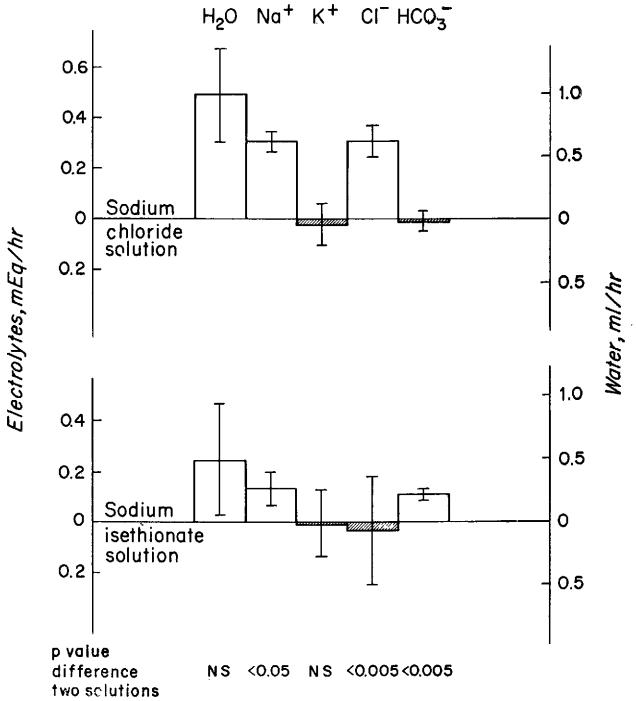


FIG. 1. Water and electrolyte transport from the whole colon of eight rats perfused with sodium chloride and sodium isethionate solutions, alternately. Mean ± 2 SE are shown. Hatched areas represent secretion.

TABLE II. Net (and SE) Transport of Water (ml/hr) and Electrolytes (μeq/hr) by Colon of Four Rats.

| Perfusing solution | Water | Sodium | Potassium | Chloride | Bicarbonate |
|---|---|---|---|---|---|
| Sodium iodide[a] | 0.21 ± 0.45 | 210 ± 50 | −20 ± 6 | [b] | 70 ± 10 |
| Mannitol[a] | −0.80 ± 0.13 | −60 ± 20 | −20 ± 6 | −110 ± 10 | 20 ± 10 |

[a] Minus (−) means secretion.

[b] Net transport of chloride not calculated.

flame photometry (IL model 650, Scientific Instruments), chloride was measured electrotitrametrically (Buchler Instruments, Fort Lee, N.J.), bicarbonate was measured manometrically (Natelson microgasometer, Scientific Instruments, Queens Village, N.Y.), polyethylene glycol (PEG) was measured turbidometrically (10), and osmolality was determined by freezing-point depression (Fiske Associates, Watertown, Mass.). Absorption of water and electrolytes per hour by the whole colon was calculated, relative to PEG, by the formulae:

$$\text{"Initial" volume } (V_I) = [30 \times (PEG_T/PEG_I)] - 5,$$

$$\text{final volume } (V_F) = V_I \times (PEG_I/PEG_F),$$

$$\text{net water transport} = V_I - V_F, \text{ and}$$

$$\text{net electrolyte transport} = V_IC_I - V_FC_F,$$

in which $PEG_T$ = concentration of PEG in test solution; and $PEG_I$, $C_I$ = "initial" concentrations, and $PEG_F$, $C_F$ = final concentrations of PEG and electrolytes, respectively.

The weight of the colon was determined in 15 animals (mean ± SE; 195 ± 20 mg) after the organ was dissected from attached tissue and dried to constant weight at 100°. Statistical analysis was by signed-rank sum test (11) for paired observations.

*Results. Absorption from solutions with or without chloride (Fig. 1 and Table II).* Bicarbonate was secreted and chloride was ab-

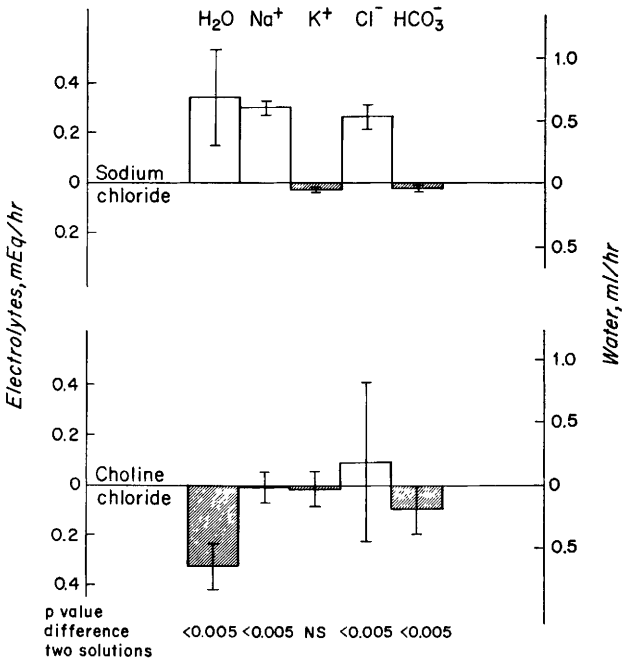


FIG. 2. Water and electrolyte transport from the whole colon of eight rats perfused with sodium chloride and choline chloride solutions, alternately. Mean ± 2 SE are shown. Hatched areas represent secretion.

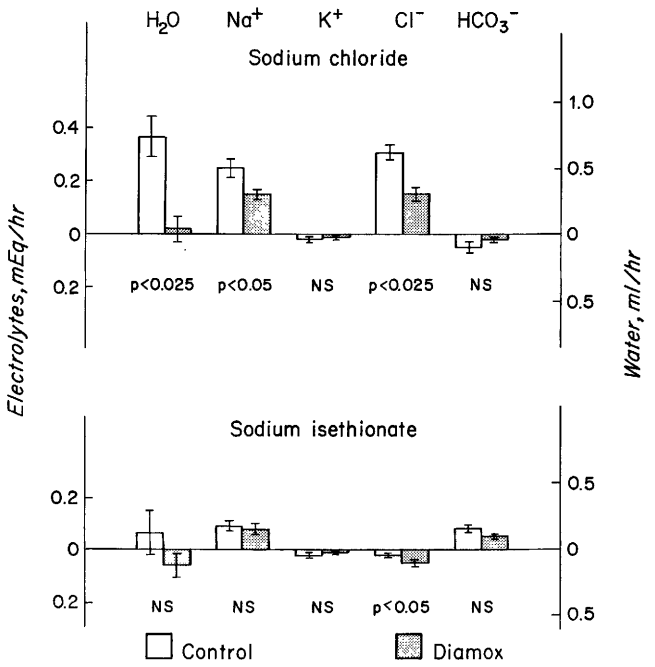


FIG. 3. Effect of acetazolamide (Diamox) on water and electrolyte transport by colon of six rats perfused with sodium chloride and sodium isethionate solutions. Mean ± 2 SE are shown.

sorbed when sodium chloride was perfused; bicarbonate was absorbed and chloride was secreted when sodium isethionate was perfused ($p<.005$). Replacement of chloride with iodide or perfusion with mannitol also resulted in absorption of bicarbonate (Table II).

Replacement of chloride by isethionate resulted in decreased absorption of sodium ($p<.05$) and water (Fig. 1); replacement of chloride with iodide did not significantly alter the transport of sodium and water (Table II). Sodium and water were secreted into mannitol solutions. Potassium was secreted equally into all test solutions.

*Absorption from solutions with or without sodium (Fig. 2).* Bicarbonate secretion was increased and chloride absorption was reduced ($p<.005$) when choline chloride solutions were used. Sodium and water were secreted ($p<.005$) into choline chloride solutions; secretion of potassium was unaltered.

*Effect of acetazolamide.* Sodium chloride solutions. Acetazolamide significantly reduced absorption of sodium ($p<.05$), chloride ($p<.025$), and water ($p<.025$); potassium transport was unaltered (Fig. 3). Bicarbonate secretion was greatly reduced by acetazolamide in three animals, but in three the effect of the drug was minimal. The concentration of bicarbonate in the initial solution (approximately 25 meq/liter) increased during perfusion of the sodium chloride solution (mean ± SE) 3.12 ± 0.8 meq/liter (Table

TABLE III. Change in Concentration[a] of Bicarbonate (meq/liter) During Perfusion of Colon of Six Rats.

| Sodium chloride solution | | Sodium isethionate solution | |
|---|---|---|---|
| Control | After acetazolamide | Control | After acetazolamide |
| +5.5 | +1.4 | −2.5 | −3.3 |
| +4.7 | +0.9 | −2.5 | −2.8 |
| +3.6 | +1.1 | −3.3 | −4.3 |
| +2.6 | +0.7 | −3.9 | −2.4 |
| +1.6 | +0.8 | −4.6 | −3.2 |
| +0.7 | 0 | −4.9 | −1.6 |

[a] Positive values indicate increase in concentration during perfusion; negative values indicate decrease.

TABLE IV. Change in Osmolality During Absorption by Rat Colon.

| Solutions (each, $n = 8$) | Osmolality, mOsm/kg | | | | Significance of differences | |
|---|---|---|---|---|---|---|
| | Test | Initial[a] | Final[a] | Change | Initial-final | Between solutions |
| Sodium chloride | 300 | 303.6 | 289.5 | —14.1 | <.005 | |
| | | | | | | <.025 |
| Sodium isethionate | 301 | 307.3 | 300.0 | — 7.3 | <.025 | |
| Sodium chloride | 300 | 301.0 | 285.0 | —16.0 | <.005 | |
| | | | | | | <.005 |
| Choline chloride | 299 | 305.5 | 299.0 | — 6.5 | <.005 | |

[a] See text.

III); when acetazolamide was used, this increase was less, 0.8 ± 0.2 meq/liter ($p<.025$).

Sodium isethionate solutions. Acetazolamide had an inconsistent effect on net transport, although chloride secretion was increased slightly (Fig. 3). During perfusion with isethionate, the concentration of bicarbonate decreased to similar extent during perfusion with control solutions (—3.62 ± 0.5 meq/liter), and after acetazolamide (—2.93 ± 0.4 meq/liter).

*Changes in osmolality during perfusion (Table IV).* The osmolality of test solutions increased slightly during the 30-min period of equilibration; thereafter, osmolality always decreased during perfusion. The decrease in osmolality was significantly greater for sodium chloride solutions than for chloride-free or sodium-free solutions.

*Discussion.* Bicarbonate was secreted when the rat colon contained chloride but was absorbed when the constituent anion was iodide or isethionate or when mannitol was perfused. All test solutions contained concentrations of bicarbonate, which were greater that those of portal blood, thus favoring bicarbonate absorption by simple diffusion, particularly since the pH of mucosal fluids was greater than that of portal blood. Therefore, chloride facilitated the movement of bicarbonate into the lumen against a chemical gradient. By contrast, the presence of sodium was not essential for bicarbonate secretion; greater amounts of bicarbonate were secreted into solutions containing choline chloride than into those containing sodium chloride. The effect of water flow (solvent drag) was not a major determinant of bicarbonate transport; bicarbonate was secreted when water was absorbed (sodium chloride solutions) and bicarbonate was absorbed when water was secreted (mannitol solutions). However, bulk water flow may have modified the extent of bicarbonate transport, augmenting its secretion into choline chloride solutions and reducing its absorption from mannitol solutions.

Together, these findings are suggestive of a specific role of luminal chloride in the secretion of bicarbonate by the rat colon, as has been suggested previously by perfusion studies in man (5), in which the presence of chloride in the lumen provoked secretion of bicarbonate. The mechanisms by which the colon absorbs chloride and secretes bicarbonate are uncertain (12). Chloride absorption is considered to be by passive diffusion, explicable by the high negative mucosal electric potential recorded from the colon (2, 13, 14). It follows that bicarbonate secretion should involve an active mechanism because transport occurs against electric and chemical forces. However, the simultaneous and opposed transport of these anions precludes precise definition of which (or both) of these movements is determined or effected by active mechanisms (12). In the rat ileum, chloride can be absorbed against electrochemical gradients (7), and active lumen-to-blood flux of chloride is necessary for bicarbonate secretion to occur (6, 7).

Acetazolamide, in doses calculated to inhibit carbonic anhydrase completely (15), re-

duced the absorption of sodium, chloride, and water and the secretion of bicarbonate during perfusion with sodium chloride solutions. Although acetazolamide did not reduce the net secretion of bicarbonate significantly in these studies, the increase in bicarbonate concentration that developed during control studies was significantly reduced by acetazolamide.

These responses of the colon to inhibition of carbonic anhydrase, observed previously (1), are suggestive of the participation of carbonic anhydrase in the mechanisms for both sodium transport and anion exchange. Specifically, carbonic anhydrase could provide mucosal cells with bicarbonate ions, which are than secreted concomitant with and perhaps coupled to, the absorption of chloride. Further, as Parsons (1) has proposed, anion exchange in the distal bowel may provide energy for sodium absorption. Concentrations of carbonic anhydrase in the colon are high (8, 9), approximating those in erythrocytes and gastric mucosa where the enzyme is believed to be metabolically important. However, evidence linking carbonic anhydrase with electrolyte transport relies largely on studies utilizing inhibitors of the enzyme, and nonspecific effects of these agents have been proposed (16), particularly since they modify transport in the frog cornea (17), a tissue devoid of carbonic anhydrase activity. However, the failure of acetazolamide to alter water, cation, or anion transport in a chloride-free environment argues against a nonspecific effect of this drug.

Potassium was always secreted against a concentration gradient, and its transport was independent of the movement of water or other electrolytes. Potassium transport by the rat colon may involve an active secretory process (13), also postulated for the dog (18), and, if so, this mechanism is presumably insensitive to carbonic anhydrase inhibitors. During absorption and secretion, osmolality of the colonic contents decreased. The rat colon absorbs a fluid that is hypertonic, relative to plasma (19), and the greater change in osmolality, which occurred when sodium chloride was perfused, suggests that both sodium and chloride contribute to the hypertonicity of the absorbed fluid.

*Summary.* To examine the conditions under which bicarbonate is secreted by the colon, rats were perfused by a recirculation technique using solutions containing sodium and chloride, one or other of these ions, or neither. All solutions contained bicarbonate in concentrations greater than those in portal venous blood. In 12 animals, bicarbonate was secreted when chloride was a constitutent of the luminal contents, but bicarbonate was absorbed when chloride was excluded from perfusing solutions. Acetazolamide reduced the secretion of bicarbonate and the absorption of sodium, chloride, and water when sodium chloride solutions were used, but the drug had no effect on water and electrolyte transport when a chloride-free solution was used. These results suggest that carbonic anhydrase has a role in the exchange of bicarbonate and chloride across the mucosa of the colon and, further, they imply anion exchange may be related to the mechanism of sodium and water transport in the colon.

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