Effects of acetazolamide and acid-base changes on biliary and pancreatic secretion

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Acetazolamide slightly augmented the bile flow but reduced the flow response of the pancreas to secretin. Normally, there was a characteristic flow-composition relationship such that an increase in the flow was accompanied by an increase in (HCO₃⁻) of both bile and pancreatic juice and by a fall in (Cl⁻) in pancreatic juice and an increase in bile (Cl⁻). Acetazolamide disrupted the normal flow-composition relationship in such a way that for a given flow (HCO₃⁻) was lowered whereas (Cl⁻) was elevated. When plasma (HCO₃⁻) was varied by inducing metabolic acidosis or alkalosis, it was observed that the flow and (HCO₃⁻) in bile and pancreatic juice were proportional to plasma (HCO₃⁻) even in the presence of acetazolamide. This apparent dependence of flow on plasma (HCO₃⁻) was much more distinct in pancreas than in bile.

Both bile and pancreatic juice contain bicarbonate. In both, the bicarbonate concentration increases as the flow rate increases, ultimately reaching a plateau at high secretory rates. This suggests that the formation of bicarbonate constitutes one of the most important steps in forming bile and pancreatic juice. Although the mechanism of bicarbonate formation in the liver and in the pancreas is not entirely understood, various investigators indicated that carbonic anhydrase seems to play an important role (1, 2, 4, 5, 8, 10). Moreover, the formation of pancreatic juice and the functions of carbonic anhydrase are greatly influenced by the acid-base changes (8). However, the effect of acid-base changes on the bile secretion has not been studied.

Hence the present investigation was undertaken to simultaneously compare the effects of acetazolamide and of acid-base changes on the biliary and the pancreatic secretion. On the basis of experimental results, differences between the liver and the pancreas in forming bicarbonate-rich solution are discussed.

METHODS

Experiments were carried out in 24 anesthetized dogs, each weighing approximately 10 kg. The dogs were anesthetized with sodium pentobarbital (27 mg/kg iv) after a 12- to 15-hr fast and an endotracheal tube inserted. A femoral artery was cannulated for blood sampling and a femoral vein for infusion. The common bile and the pancreatic ducts were cannulated and both cystic and accessory pancreatic ducts ligated. Metabolic acidosis was induced by intravenous infusion of 0.37 N HCl at a rate of 7 ml/min for 10 min and then 1-2 ml/min for the ensuing 3 hr; metabolic alkalosis was induced by infusion of 0.5 M NaHCO₃ in the same manner. A dual-syringe feeder (Modern Metalcraft) was used for continuous infusion. Occasional hemolysis was observed following HCl infusion.

From 40 to 50 min after the above surgical procedures, samples of hepatic bile, pancreatic juice, and arterial blood were collected under oil. Usually bile samples were collected every 10-40 min depending on the flow, pancreatic samples every 10 min, and blood samples every 20 min. Before administering any agent, one or two control bile samples were obtained and two successive doses of secretin (Vitrum) were administered intravenously 20 min apart. Twenty minutes after the administration of the second dose of secretin, one dose of Na acetazolamide (American Cyanamid Co.) was administered intravenously. Ten units of secretin was the usual dose, except after acetazolamide, when 20 units often was administered. The dosage of acetazolamide was 30-65 mg/kg except in the acidotic animals in which 25 mg/kg was given.

In three dogs, Na taurocholate was infused at a rate of 1.2 µM/min throughout the entire experimental period.
BICARBONATE IN BILE AND PANCREATIC JUICE

TABLE 1. Electrolyte compositions of plasma, hepatic bile, and pancreatic juice under various conditions

<table>
<thead>
<tr>
<th>Induced Change</th>
<th>No. of Dogs</th>
<th>Plasma</th>
<th></th>
<th>Hepatic Bile</th>
<th></th>
<th>Pancreatic Juice</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>pH</td>
<td>(HCO₃⁻)</td>
<td>pH</td>
<td>Flow</td>
<td>(HCO₃⁻)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>mEq/liter</td>
<td>mEq/liter</td>
<td>mEq/liter</td>
<td>mEq/liter</td>
<td>mEq/liter</td>
</tr>
<tr>
<td>Control</td>
<td>9</td>
<td>7.36</td>
<td>20</td>
<td>104</td>
<td>7.62</td>
<td>4.4±0.7</td>
</tr>
<tr>
<td>S, 10 U</td>
<td>9</td>
<td>7.36</td>
<td>20</td>
<td>104</td>
<td>7.70</td>
<td>7.6±0.9</td>
</tr>
<tr>
<td>A</td>
<td>3</td>
<td>7.26</td>
<td>16</td>
<td>7.34</td>
<td>7.5±1.0</td>
<td>45±1.2</td>
</tr>
<tr>
<td>A + S, 10 U</td>
<td>3</td>
<td>7.26</td>
<td>16</td>
<td>7.50</td>
<td>6.7±0.8</td>
<td>43±2.2</td>
</tr>
<tr>
<td>A + S, 20 U</td>
<td>3</td>
<td>7.26</td>
<td>16</td>
<td>7.50</td>
<td>6.7±0.8</td>
<td>43±2.2</td>
</tr>
</tbody>
</table>

A. Normal

B. Metabolic alkalosis

Control       | 3           | 7.47  | 38    | 98    | 7.86  | 8.9±2.4 | 95±5.5   | 51±7.2 |
| S, 10 U      | 3           | 7.47  | 38    | 98    | 7.77  | 7.4±2.3 | 73±5.0   | 64±9.7 |
| A + S, 10 U  | 3           | 7.42  | 35    | 90    | 7.77  | 8.5±2.3 | 82±6.4   | 59±5.5 |
| A + S, 20 U  | 3           | 7.41  | 38    | 90    | 7.76  | 8.4±0.6 | 84±6.4   | 50±1.8 |

C. Metabolic acidosis

Control       | 4           | 7.07  | 13    | 116   | 7.69  | 3.2±0.6 | 50±3.4   | 75±9.3 |
| S, 10 U      | 3           | 6.97  | 14    | 116   | 7.69  | 3.9±1.1 | 49±9.9   | 69±7.0 |
| A + S, 20 U  | 3           | 7.06  | 14    | 113   | 7.44  | 4.4±1.2 | 49±12.0  | 63±13.0 |

Abbreviations “S” and “A” are for secretin and acetazolamide, respectively. Values are means ± SE.

RESULTS

Normal response to secretin (Table 1). Before secretin the mean hepatic bile flow was 4.4 µl/kg per min. This was increased by 50-60% with a simultaneous increase in bicarbonate and chloride concentration and pH. The bicarbonate increase was double that of chloride.

There was a scanty basal secretion of pancreatic juice in this preparation. Secretin increased flow rate and bicarbonate but, unlike bile, the chloride concentration was reduced. When the flow rate anion relationships are plotted for bile and pancreatic juice, respectively, characteristic relationships as observed by Wheeler and Ramos (10) for bile and by Bro-Rasmussen et al. (3) for pancreatic juice were evident. The plateau is reached at

FIG. 1. Flow composition relationship of pancreatic juice after administration of acetazolamide. (Cross and open circles represent chloride and bicarbonate concentration, respectively. The three curves represent the normal relationship in absence of acetazolamide.)
juice electrolyte concentrations were studied (Fig. 3). For bile and pancreatic juice.

Effect of acetazolamide. After acetazolamide resting bile flow increased by 50%. Chloride concentration also increased by 50%, whereas the bicarbonate remained unaltered. At this point secretin did not further increase volume nor did it change the electrolyte concentrations. Evidently acetazolamide disrupts the usual flow-electrolyte relationships, as observed earlier by Wheeler and Ramos (10).

In the pancreas also acetazolamide disrupts the flow-electrolyte relationship (Fig. 1), for the flow of juice is reduced, the chloride elevated, and the bicarbonate lowered to a greater extent than one would be led to expect if the usual relationship to volume held. Bicarbonate and chloride concentrations before and after acetazolamide were compared statistically. At a mean flow rate of 12.5 in the controls and 12.6 in the same animals after acetazolamide the HCO₃⁻ was 22.6 mEq/liter higher in the controls and the Cl⁻ was 17.1 mEq/liter lower. These differences are significant at the 1% level.

Effect of acid-base changes. Alkalosis was induced in three dogs and acidosis in four dogs (Table 1). Alkalosis increased the responsiveness of the bile flow to secretin and this was accompanied by increases in concentration of bicarbonate and chloride in bile. In alkalotic animals secretin still produced the expected volume increase even after acetazolamide. The carbonic anhydrase antagonist did not affect basal bile flow rate, although it did reduce bicarbonate and increase chloride slightly.

Similarly, in the pancreas, secretin produced slightly larger than usual responses in the alkalotic animal but the anion concentrations remained virtually unchanged. Unlike the bile, acetazolamide reduced the flow in response to secretin in the alkalotic animals by approximately 65% and a disruption of the flow-electrolyte pattern occurred, bicarbonate remaining higher and chloride lower than would be expected at comparable flow in normal animals.

In acidotic animals the response of bile flow and pancreatic flow to secretin was 50 and 75%, respectively, less than normal. In the case of the bile, for the observed flow rate, the bicarbonate was lower and the chloride higher than expected, whereas in pancreatic juice they were present in expected concentrations. After acetazolamide in acidotic animals 10 units of secretin had no effect on pancreatic secretion, whereas a very slight increase in bile flow and chloride concentration was observed.

It is clear therefore that plasma electrolytes influence the secretion of pancreatic juice and bile. In Fig. 2 this relationship is plotted using 10 units of secretin as stimulus. The dependence of pancreatic flow on plasma bicarbonate seems to be much closer than is the case with bile. The converse was the case when bile and pancreatic juice electrolyte concentrations were studied (Fig. 3). The regression line relating plasma bicarbonate to secreted bicarbonate concentration was much steeper for bile than for pancreatic juice. Because the bicarbonate increases with increasing flow to a plateau, the regression lines were plotted for flow rates below 10 μl/kg per min for bile and 15 μl/kg per min for pancreatic flow.

Effect of taurocholate infusion on bile. Sodium taurocholate was given by infusion to three dogs. Its effect was to double the rate of bile flow (Table 2). Although bicarbonate and chloride did rise, their increases were less than commensurate with the volume increase. The negatively charged taurocholate no doubt displaces the other anions. However, the basic responses to acetazolamide or secretin were quite similar to those observed earlier without taurocholate infusion (Table 1).

**DISCUSSION**

The pattern of changes relating bicarbonate and chloride concentrations to secretory volume in the case of both bile and pancreatic juice is well known. Acetazolamide, a carbonic anhydrase inhibitor, disrupts this well-known pattern of changes in both secretions. Our findings on hepatic bile are in agreement with those of Wheeler and Ramos (10). Dreiling and Janowitz (4, 5), who have studied the action of acetazolamide on pancreatic juice, have noted a disturbed pattern but, unlike
our findings, they have found a higher than usual bicarbonate concentration at any given volume, whereas our concentrations are lower than expected. In both our experiments and theirs the volume of juice was reduced. We have plotted the data of others (2, 7, 8) and find that the acetazolamide pattern which results is in agreement with ours. Further clarification of this disagreement is needed in future.

Alkalosis and acidosis clearly influence the secretion of hepatic bile and pancreatic juice. In general the bicarbonate concentrations of both secretions were proportional to those in plasma when compared at a given flow rate. This relationship remained after acetazolamide, thus a fraction of the secreted bicarbonate is not formed by carbonic anhydrase catalysis. In this we agree with Rawls et al. (8). They showed further that the relationship is between secreted bicarbonate and plasma bicarbonate and not with plasma pH directly. Why bile bicarbonate followed plasma bicarbonate more closely than did pancreatic bicarbonate is not clear, but seems to be related to the fact that change in plasma bicarbonate changes the flow rate of pancreatic juice much more profoundly than that of bile. Acetazolamide depressed somewhat the pancreatic flow rate and bicarbonate in acidosis but to a very much smaller extent than in normal animals. This again illustrates the dual origin of pancreatic bicarbonate. The relationship between bile flow and plasma bicarbonate was unaltered by acetazolamide. The slight or absent effect of secretin on bile and pancreatic juice in acidic animals after acetazolamide is consistent with the view that the primary action of secretin is on bicarbonate secretion.

Although the site of bicarbonate secretion in pancreatic system is not known, it is considered to be located in the duct cell (1, 5). In order to account for the known behavior of anion secretion in pancreatic juice, Lim et al. (6) proposed the "admixture" concept and Janowitz and Dreiling (5) the "exchange" notion. Our data also suggest that there are perhaps two isotonic pancreatic secretions, one high in Cl- and containing HCO₃⁻ dependent on plasma HCO₃⁻ and the other high in HCO₃⁻ produced by carbonic anhydrase and low in Cl-. The characteristic flow-composition relationship can be easily visualized if the high HCO₃⁻ secretion is added by the duct cells as the secretory rate rises. However, the disruption of this characteristic relationship by acetazolamide (Fig. 1) indicates that at one stage of pancreatic secretion HCO₃⁻ seems to be secreted in exchange for Cl-. Janowitz and Dreiling (5) have felt that there is carbonic anhydrase-dependent reabsorption of HCO₃⁻ in the ducts in exchange for Cl-. The latter view is based on their observation that acetazolamide decreased flow rate whereas (HCO₃⁻) remained unchanged. Although the biliary system seems to share some of the secretory characteristics with the pancreas, the increase in bile flow by acetazolamide complicates the picture beyond any speculation.

ADDENDUM

Since this manuscript was submitted for publication, we learned of a paper by T. H. Maren, A. C. Ellison, S. K. Feller, and W. B.

### Table 2. Effects of various agents on flow, anionic composition, and pH of bile in Na-taurocholate-infused dogs (7.5 μM/min)

<table>
<thead>
<tr>
<th>Agents and Dosage</th>
<th>Flow, μl/min per kg</th>
<th>HCO₃⁻, mEq/liter</th>
<th>Cl⁻, mEq/liter</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8.3±0.1</td>
<td>45.9±2.25</td>
<td>54.3±3.48</td>
<td>7.62±0.024</td>
</tr>
<tr>
<td>Secretin, 10 U</td>
<td>13.6±1.4</td>
<td>31.3±1.3</td>
<td>70.6±0.56</td>
<td>7.71±0.052</td>
</tr>
<tr>
<td>Acetazolamide, 65 mg/kg</td>
<td>12.4±1.4</td>
<td>31.5±2.86</td>
<td>69.8±1.20</td>
<td>7.79±0.071</td>
</tr>
<tr>
<td>Acetazolamide, 65 mg/kg + secretin, 10 U</td>
<td>9.0±1.7</td>
<td>35.3±1.29</td>
<td>99.5±3.48</td>
<td>7.53±0.021</td>
</tr>
<tr>
<td>Acetazolamide + secretin, 20 U</td>
<td>15.5±1.8</td>
<td>36.3±1.06</td>
<td>93.0±2.12</td>
<td>7.58±0.021</td>
</tr>
</tbody>
</table>

Values are means ± SE and represent average of experiments on three dogs.
Graham: Effect of sulfonamides on hepatic carbonic anhydrase, which is being submitted for publication. These authors also studied the effects of various carbonic anhydrase inhibitors as well as of acid-base changes on the bile secretion and obtained data similar to ours.

REFERENCES


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