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Effect of Parenteral Acetazolamide on Intestinal Absorption of Salt and Water in Man (38933)

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Acetazolamide is known to have an inhibitory effect on absorption of sodium and chloride from small and large intestine of various species (1–5). This has been generally attributed to its inhibition of carbonic anhydrase activity. There have only been two published reports of the effect of acetazolamide on small intestinal absorption in man, from one group which added the agent to solutions perfusing jejunum and ileum of man and which contained significant amounts of bicarbonate (6, 7).

We have now studied the effect of intravenous acetazolamide administration on jejunal absorption in man. With perfusion of isotonic saline without augmented bicarbonate, inhibition of salt and water absorption occurred.

Method. Intestinal absorption was studied by triple lumen tube perfusion technique in the same manner as previously described (8). After an overnight fast, subjects were perfused after the infusion site of the tube had reached just beyond the ligament of Treitz. A mixing segment of 15 cm separated infusion from proximal collecting port and a study segment of 30 cm separated proximal and distal collecting ports. The proximal jejunum was infused at a rate of 9.5 ml per minute, using a Sigmamotor pump. At the time the infusion began, an intravenous infusion was also started with 5% glucose in water. The jejunal perfusion continued during a 1-hr equilibration period followed by a 1-hr study period. During the 2 hr, 500 ml of fluid was given intravenously. Then acetazolamide was added to another 500 ml of 5% glucose in water at a dose of 75 mg/kg body wt. This was given intravenously over the next 2 hr and the second hour of acetazolamide administration was considered the second study period. There were no untoward effects of this dosage.

Six volunteer patients with no gastrointestinal, metabolic, or renal abnormalities received a perfusion solution of isotonic saline, 154 mEq/liter. All perfusion solutions contained 1% polyethylene glycol (PEG) as a dilution indicator.

During study periods, proximal and distal aspirates were collected at a rate of 1 ml/min. Every 10 min, 1 ml was collected under oil. Sodium, chloride, and bicarbonate were measured by Technicon Autoanalyzer. The bicarbonate determination was performed on the fluid collected under oil. PEG was determined by a standard method (9). Net water, sodium, chloride, and bicarbonate flux were measured as previously described. Statistics were derived from the one-tailed comparison t test.

Results. Each of the six subjects absorbed less water during acetazolamide administration than during the first study period (Fig. 1, Table I). One subject, S. B., who had a low rate of water absorption in the control period, developed a net secretory state. The difference in means between first and second perfusion was significant, $P < .025$. Sodium absorption was reduced in five of six subjects and chloride absorption was reduced in all six during the acetazolamide part of the perfusion. Both results were also significant $P < .05$. Mean intraluminal sodium concentration was 144 mEq/liter in the study segment.

Bicarbonate concentration was low in the intestinal lumen during perfusion. Mean bicarbonate concentration at the proximal collecting port was 5.3 mEq/liter during the control perfusion and 5.6 mEq/liter during the acetazolamide perfusion. There was slight net secretion of bicarbonate in

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The Effect of Bile and of Sodium Taurocholate on the Epithelial Cell Dynamics of the Rat Small Intestine (38943)

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In animals and man, the small intestinal mucosa maintains a constant morphological appearance despite a very fast renewal rate. This is accomplished through the interplay of a basic dynamic process with a number of factors regulating enterocyte proliferation, differentiation, and loss. Physical trauma (1), gut flora (2), hormones (3), vitamin D (4), the state of nutrition (5), and the pattern of feeding (6) are some of the factors known to influence epithelial cell kinetics in the small intestine.

During the past decade, a large body of information has become available on the chemistry, physiology, and metabolism of bile and bile acids. However, their influence on intestinal mucosal cell turnover remains unexplored except for two reports showing mucosal hyperplasia following bile duct implantation into isolated ileal segments of rats (7) and increased turnover of ileal mucosal cells by cholic acid feeding in germ free mice (8). This study explores the regulatory role of bile and bile acids on epithelial cell dynamics in bile fistula rats after 2 days of biliary diversion and under a constant duodenal perfusion of Na taurocholate started with the creation of the bile fistula.

Material and Methods. [^{3}]*thymidine incorporation into DNA.* A bile fistula was established by inserting a cannula (Clay Adams PE-10) in the common duct 5 mm distal to the bifurcation of the hepatic ducts in 10 male Sprague–Dawley rats weighing between 150 and 200 g. A similar number of animals were sham operated. The animals were placed in restraining cages and offered a 5% sucrose solution containing 40 and 20 mEq/liter of Na and K, respectively. After 48 hr, [^{3}H]thymidine (New England Nuclear, 6.7 Ci/mmole) was injected iv at a dose of 1 μCi/g body wt. One hour later, the animals were sacrificed. Sections for autoradiography were taken 1 cm distal to the ligament of Treitz and 2 cm proximal to the ileocecal junction. After fixation in 10% formol–saline, the tissue embedded in paraffin, was cut at a thickness of 2.5 μm, colored with the standard Feulgen method, dipped in Eastman Kodak NTB_2 emulsion, and kept for 48 hr at 4° in a light tight box under a nitrogen atmosphere. The slides were then developed in Eastman Kodak D-170, fixed in hypo, washed in distilled water, and mounted. Background grains were negligible. If a cell had four grains or more over its nucleus, it was counted as labeled. The cellularity of crypts and the labeling index was calculated under oil immersion in eight crypts from each of two sections taken from the proximal and distal small intestine. The rest of the small intestine was rapidly removed and measured by attaching a constant weight to its distal end. Its mucosa was removed with a glass slide and weighed. The protein concentration was determined by the method of Lowry (9). The DNA content of the mucosa was extracted with cold TCA and ethanol at room temperature followed by hydrolysis in hot TCA according to the method of Schneider (10). The DNA was then estimated by the colorimetric method of Burton (11) as modified by Croft and Lubran (12). Highly polymerized calf thymus DNA (Sigma type 1, organic phosphorus content 6.1%) was used as standard. [^{3}H]thymidine activity in aliquots of DNA extracts was expressed as cpm $\times$ 10^3/mg of mucosal DNA.

DNA loss by cell exfoliation. Two groups of eight animals were studied 48 hr after a biliary diversion or a sham operation. Segments of polyvinyl chloride tubing 8F and 10F were tied separately at the duodenojejunal flexure and at the terminal ileum. The small intestine with the cannulae was returned to the peritoneal cavity and normal saline, warmed to 37°, was infused through

most of the subjects and no significant change during acetazolamide administration (Table I).

Discussion. It has been previously demonstrated that acetazolamide inhibits net sodium, chloride, and water absorption from ileum and colon of rat and dog *in vivo* (1–3) and rat and rabbit ileum *in vitro* (4, 5). In the human, Turnberg *et al.* (6) using a triple lumen perfusion technique similar to the one used in this study, observed a decrease in bicarbonate absorption in the jejunum when acetazolamide was added directly to a perfusate containing bicarbonate, 70 mEq/liter. On the basis of extensive studies, they postulated a hydrogen secretion—sodium absorption exchange with subsequent hydrogen bicarbonate reaction to form carbonic acid with release of diffusible carbon dioxide. By inactivating carbonic anhydrase, the exchange would be interrupted. Without hydrogen secretion, bicarbonate could not be converted to carbon dioxide and hence could not be absorbed. The same group observed depression of sodium and chloride absorption from the ileum by acetazolamide (7). The exchange scheme postulated for the ileum was different as perfusion data suggested both bicarbonate and hydrogen were secreted in exchange for chloride and sodium, respectively. Interference with secretion, therefore, would reduce chloride and sodium absorption.

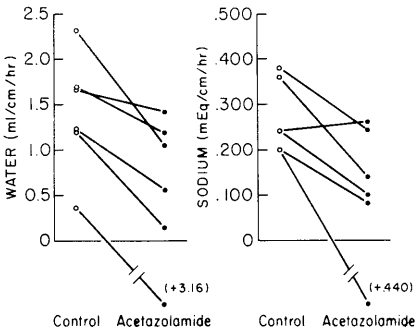

FIG. 1. Water and sodium absorption during control (○) and acetazolamide (○) perfusion. + represents net secretion into the intestinal lumen.

Our findings confirm the inhibitory effect of acetazolamide on jejunal absorption. In a previous study (10) we found no significant variation in water and electrolyte absorption over a 4-hr period so our results were not affected by the sequences of perfusions. Our method differed somewhat from that of Turnberg *et al.* First we administered the agent parenterally by intravenous infusion rather than intraluminally. We used the same high dosage shown in a previous study to effectively inhibit gastric acid secretion (11). More importantly, the bicarbonate concentration intraluminally was of a much lower magnitude. The mean bicarbonate concentration at the proximal collecting port was 5.3 mEq/liter. Using a perfusion system with saline, Fordtran *et al.* (12) found bicarbonate concentration to be 2.1 mEq/liter and Phillips and Summerskill (13) recorded an equilibration concentration for bicarbonate in human jejunum at 6.0 mEq/liter so our results are comparable. Turnberg *et al.* (6) noted bicarbonate absorption with an intraluminal

TABLE I. NET TRANSPORT OF WATER, SODIUM, CHLORIDE, AND BICARBONATE DURING PERFUSIONS.

Subject	Water Control ml/cm/hr	Water Acet. ml/cm/hr	Sodium Control mEq/cm/hr	Sodium Acet. mEq/cm/hr	Chloride Control mEq/cm/hr	Chloride Acet. mEq/cm/hr	Bicarbonate Control mEq/cm/hr	Bicarbonate Acet. mEq/cm/hr
Saline perfusions								
T.A.	2.32	1.04	.360	.140	.640	.160	+.062[a]	.017
M.S.	1.68	1.18	.380	.240	.180	.140	.006	+.003
L.C.	1.22	0.56	.200	.080	.260	.210	+.065	+.340
J.V.	1.18	0.14	.240	.110	.288	.120	+.039	+.047
C.M.	1.66	1.42	.240	.260	.260	.212	+.0002	+.006
S.B.	0.36	+3.16	.200	+.440	.360	+.540	+.204	+.187
Mean ± SE	1.40	0.18	.270	.063	.331	.049	+.061	+.092
	±.30	±.63	±.030	±.097	±.061	±.108	±.029	±.064

[a] —+ indicates net secretion into lumen.

concentration of 3 mEq/liter or greater while Phillips *et al.* (13) reported bicarbonate secretion at concentration below 5 mEq/liter.

We found an apparent slight net secretion of bicarbonate in five of six subjects during control and acetazolamide perfusions with no significant effect of the drug. The lack of net bicarbonate absorption during the control period probably was related to the very low intraluminal bicarbonate concentration creating a diffusion gradient. Using a similar saline perfusion system with low bicarbonate intraluminally, Turnberg *et al.* (6) attributed sodium and water absorption to passive chloride transport down a concentration gradient, not to a sodium–hydrogen exchange. Since there may still have been some hydrogen secretion occurring, the reduction in salt and water absorption may have been related to carbonic anhydrase inhibition during acetazolamide administration. However this should have produced a greater apparent effect on net bicarbonate secretion. In the absence of specific pCO_2 and pH measurements, statements concerning hydrogen secretion can be only speculative. There is some evidence that acetazolamide may effect ATPase activity (14) and it has been suggested that the drug may act at a site in the brush border at a site other than carbonic anhydrase (15). Whichever explanation is applicable to our results, we have shown that the effect can occur in the presence of very low intraluminal bicarbonate concentration.

Summary. Acetazolamide was administered intravenously during jejunal perfusion of isotonic saline in six subjects. Bicarbonate was present in very low concentration intraluminally and there was net bicarbonate secretion during control and acetazolamide perfusions. Acetazolamide significantly inhibited sodium chloride, and water absorption. As this occurred in the absence of an effect on net bicarbonate secretion, it may have been due to an action other than carbonic anhydrase inhibition.

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1. Parsons, D. S., *Quart. J. Exp. Physiol. Cog. Med. Sci.* **41**, 410 (1956).
2. Kinney, V. R., and Code, C. F., *Amer. J. Physiol.* **207**, 998 (1964).
3. Phillips, S. F. and Schmalz, P. F., *Proc. Soc. Exp. Biol. Med.* **135**, 116 (1970).
4. Frizzell, R. A., Nellams, H. N., Rose, R. C., Markscheid-Kaspi, L., and Schultz, S. G., *Amer. J. Phiol.* **224**, 328 (1973).
5. Strombeck, D. R., and Ingraham, R. C., *Proc. Soc. Exp. Biol. Med.* **139**, 383 (1972).
6. Turnberg, L. A., Fordtran, J. S., Carter, N. W., and Rector, F. C., Jr., *J. Clin. Invest.* **49**, 548 (1970).
7. Turnberg, L. A., Bieberdorf, F. A., Morawski, S. G., and Fordtran, J. S., *J. Clin. Invest.* **49**, 557 (1970).
8. Gerson, C. D., Cohen, N., Hepner, G. W., Brown, N., Herbert, V., and Janowitz, H. D., *Gastroenterology* **61**, 224 (1971).
9. Malawer, S. J., and Powell, D. W., *Gastroenterology* **53**, 250 (1967).
10. Gerson, C. D., Hepner, G. W., Brown, N., Cohen, N., Herbert, V., and Janowitz, H. D., *Gastroenterology* **63**, 246 (1972).
11. Janowitz, H. D., Dreiling, D. A., Rolbin, H. L., and Hollander, F., *Gastroenterology* **33**, 378 (1957).
12. Fordtran, J. S., Rector, F. C., Jr., and Carter, N. W., *J. Clin. Invest.* **47**, 884 (1968).
13. Phillips, S. F., and Summerskill, W. H. J., *J. Lab. Clin. Med.* **70**, 686 (1967).
14. Hepner, G. W., Aledort, L. M., Gerson, C. D., Cohen, N., Herbert, V., and Janowitz, H. D., *Clin. Res.* **18**, 382 (1970).
15. Schultz, S. G., and Zalusky, R., *J. Gen. Physiol.* **47**, 1043 (1964).

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