Colonic Secretion of Water and Electrolytes Induced by Bile Acids: Perfusion Studies in Man

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ABSTRACT Each of the three major bile acids of man was tested for its influence on electrolyte and water absorption in the human colon. Transport from isotonic solutions, with or without added bile acids, was compared in 35 studies on 20 healthy volunteers by colonic perfusions under steady-state conditions. Electrolytes and water were always absorbed from control solutions, but dihydroxy bile acid solutions induced continuous secretion or inhibition of sodium, potassium, and water absorption, which was reversible. Deoxycholic acid caused consistent secretion at 3 mM concentrations, whereas chenodeoxycholic acid did not induce secretion until the concentration was 5 mM. The trihydroxy bile acid (cholic acid) produced no significant change in absorption at 10 mM. Inhibition of absorption was also induced by mixtures of the glycine or taurine conjugated bile acids. Secretion of sodium and chloride, induced by bile acid perfusion, was linearly correlated with secretion of water; potassium secretion was relatively constant regardless of the volume of secretion. These results establish a striking influence of bile acids on colonic absorptive activity, provide an explanation in part for the diarrhea that frequently accompanies ileal disease or resection, and imply that diarrhea should occur in other disease states that produce elevated concentrations of dihydroxy bile acids in the colonic lumen.

INTRODUCTION

In health, the major site of absorption of bile acids is the ileum (1, 2). Those bile acids not absorbed in the ileum pass into the colon, where further absorption is considered to occur (2, 3). In patients with ileal disease or resection, malabsorption and fecal wastage of bile acids lead to a compensatory increase in their hepatic synthesis (4); a new steady state is obtained, characterized by increased synthesis and increased passage of bile acids into the colon. Demonstration that bile acids block electrolyte and water transport in the isolated, perfused rat colon (5) led to the proposal (5, 6) that bile acids might have similar cathartic properties in man and, as a corollary, that the diarrhea frequently observed in such patients could be caused in part by the action of bile acids on the colonic cell.

This paper describes the influence of each of the three major bile acids present in human bile (7) on electrolyte and water movements in the human colon. Using the technique of colonic perfusion (8) previously modified and validated by us (9), we compared electrolyte and water transport from isotonic electrolyte solutions, with or without added bile acids, under steady-state conditions.

METHODS

Perfusion procedure. Experiments were performed on 20 healthy volunteers free of signs or symptoms of gastrointestinal disease, 10 of whom were studied on two occasions. A triple-lumen tube, with a terminal hole for perfusion and holes 20 and 40 cm proximal for sampling, was constructed from polyvinyl tubing (O.D. 3 mm). The tube was passed by mouth and monitored fluoroscopically until its distal hole reached the cecum. During the 24–72 hr required for small intestinal transit, subjects were fed a normal diet. On the day of the study, after the subject had fasted overnight, 154 mM sodium chloride was pumped from the distal tube opening in the cecum until collections from the rectum were free of solid feces. Test solutions were then perfused from this opening at 15 ml/min with an infusion pump (Harvard model 1201, Harvard Apparatus Co., Inc., Millis, Mass.). A rectal catheter (24 F) was inserted to several centimeters proximal to the anal verge, enabling continuous collections during the experiment. The proximal lumens of the tube were aspirated continuously to minimize contamination of the colon by ileal contents and to monitor the degree of reflux of perfusates from the cecum, which was

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trivial. When radioactivity concentrations in rectal samples indicated the achievement of a steady state (9), six sequential 10-min collections were taken and considered to be an experimental period. The time required to achieve steady-state conditions varied from 45-90 min and was usually 1 hr or longer. Steady-state conditions were verified by subsequent analysis of a nonabsorbable marker, polyethylene glycol (PEG).[1] In preliminary experiments, movement distally of the tube was sometimes induced by bile acid solutions; these studies were discarded, and only the studies in which the position of the tube was in the cecum at the end of the study are reported here. Because of these observations, the control solutions, which did not cause tube displacement, were usually perfused first.

Experimental design. Water and ion fluxes were compared under steady-state conditions in each subject during perfusion with a control electrolyte solution and with an identical solution containing bile acids in varied concentrations. In 25 experiments, two studies (one control and one bile acid perfusion) were carried out; 5 experiments featured three studies (one control and two bile acid studies). Each perfusion study was separated by an equilibration interval of at least 60 min, during which the next perfusate was employed and the achievement of a new steady state was verified. Perfusates containing individual free bile acids (cholic, chenodeoxycholic, or deoxycholic acid) were employed for 21 studies; perfusates with individual conjugated bile acids in varied concentrations were employed for 8 studies; and perfusates containing equimolar mixtures of taurine or glycine conjugated bile acids at 10 mM total concentration were employed in 6 studies.

Composition of perfusates. Bile acids were greater than 99 % pure by thin-layer and gas–liquid chromatography. Unconjugated cholic, chenodeoxycholic, and deoxycholic acids of high purity were obtained from commercial sources.[2] Cholic acid was recrystallized from absolute ethanol, then converted to its sodium salt and recrystallized from ethanol–diethyl ether. Chenodeoxycholic acid, after conversion to its sodium salt, was crystallized from *n*-propanol-ethyl acetate. Deoxycholic acid, after conversion to its sodium salt, was crystallized from *n*-propanol-ethyl acetate. Conjugated bile acids were synthesized and purified by solvent extraction and crystallization (10).

Electrolyte composition of perfusates (Table I) was designed to simulate ileostomy fluid (11). In solutions containing bile acids, the amount of chloride ion was reduced stoichiometrically to preserve isotonicity.

Analytic methods. The purity of bile acid was assessed by semiquantitative thin-layer chromatography (12) and quantitatively by gas chromatography of trifluoroacetates (13) or acetates (14). Sodium and potassium were measured by flame photometry (IL model 143, Instrumentation Laboratory, Lexington, Mass.), chloride electrotitrimetrically (Buchler Instruments, Fort Lee, N. J.), and bicarbonate manometrically (Natelson microgasometer, model 650, Scientific Industries, Inc., Queen Village, N. Y.). PEG was measured turbidimetrically at 650 mμ (15). ^{24}Na was counted in a sodium iodide well (dual-channel analyzer 600-059, Picker International Corp., White Plains, N. Y.) and ^{42}K in a plastic well (model LC-11, Hammer Electronic, Princeton, N. J.), utilizing differences in their emission spectra (16). PEG-^{14}C was counted using a dioxane-based cocktail by liquid–scintillation spectrometry (Liquimat, Picker International Corp., White Plains, N. Y.) with an efficiency of 65–75%. Rectal samples were examined by thin-layer chromatography for the presence of unconjugated bile acids caused by bacterial deconjugation during perfusion.

Calculations and statistical analysis. Net absorption of water (ml/min) and electrolytes (μEq/min) by the whole colon was calculated, relative to changes in the concentration of PEG, using standard formulae (17). Unidirectional movements of sodium and potassium were calculated by disappearance of isotope and changes in specific activity (18). Net water movement was evaluated using an analysis of variance technique which compared differences between subjects, differences between control and individual test solutions, and interactions (19). It was considered unnecessary to evaluate sodium movement by this technique, because sodium and water movements were so closely related ($r = 0.96$; see below). Linear regressions between electrolyte and water movements were calculated by the method of least squares, and comparisons were evaluated by appropriate statistical tests.

RESULTS

Unconjugated bile acids (Table II). Perfusion with control solutions always resulted in absorption of sodium, chloride, potassium, and water; bicarbonate transport was variable, but a small secretion usually occurred. Deoxycholic acid at 1 mM had no influence on water movement ($P > 0.05$). At 3 mM, deoxycholic acid caused a large and consistent secretion of water ($P < 0.01$), and secretion was still greater in one person when 6 mM was perfused. Chenodeoxycholic acid at 1 mM or 3 mM did not influence water movement consistently ($P > 0.05$). At 5 mM, fairly consistent but variable secretion of water occurred ($P \cong 0.05$); this was also observed in the single study at 10 mM. Cholic acid, in contrast to the dihydroxy acids, had no apparent influence on water and electrolyte movements at 10 mM ($P > 0.05$).

TABLE I
*Composition of Perfusates**

Perfusate	Control solution	Bile acid solutions
Sodium, *mEq/liter*	130 ±0.4	130 ±0.7
Potassium, *mEq/liter*	19.7 ±0.3	19.7 ±0.1
Chloride, *mEq/liter*	100 ±0.5	90–99‡
Bicarbonate, *mEq/liter*	48.0 ±0.3	47.8 ±0.2
pH	8.0 – 8.2	8.0 – 8.2
Osmolarity, *mOsm/kg*	276 ±1.5	279 ±1.0

* Mean ±SE of 30 control solutions and 35 bile acid solutions. All perfusates contained polyethylene glycol, 5 g/liter, and some contained ^{24}Na and ^{42}K.
‡ See text.

[1] *Abbreviations used in this paper:* GC, glycocholic acid; GCDC, glycochenodeoxycholic, GDC, glycodeoxycholic; PEG, polyethylene glycol; TDC, taurodeoxycholic.

[2] Cholic acid (Matheson, Coleman and Bell, East Rutherford, N. J.); chenodeoxycholic acid (Tupman-Thurlow Co., New York, N. Y.); and deoxycholic acid (T. Schuchardt GmbH, Munich, Germany).

TABLE II

Effect of Unconjugated Bile Acids on Water and Electrolyte Transport in the Human Colon

Study	Bile acid	Concentration	Net transport of water (ml/min) and electrolytes (μEq/min) by whole colon									
			Control solution					Bile acid solution				
			H_2O	Na	K	Cl	HCO_3	H_2O	Na	K	Cl	HCO_3
		mM										
1*	Deoxycholic	1.0	1.83 ±0.20	258 ±15	36 ±3	383 ±17	−44 ±6‡	1.99 ±0.10	294 ±7	47 ±2	390 ±74	−32 ±5
2*	Deoxycholic	1.0	2.42 ±0.10	327 ±10	64 ±35	407 ±14	15 ±4	2.56 ±0.20	331 ±37	85 ±67	427 ±27	37 ±9
3*	Deoxycholic	1.0	1.22 ±0.02	160 ±12	0 ±3	198 ±10	−76 ±8	1.24 ±0.20	132 ±24	29 ±1	268 ±19	−99 ±8
4*	Deoxycholic	3.0	1.22 ±0.02	160 ±12	0 ±3	198 ±10	−76 ±8	−0.52 ±0.10	−29 ±13	−85 ±7	28 ±5	−121 ±15
5	Deoxycholic	3.0	2.2 ±0.10	293 ±18	62 ±4	326 ±22	−2 ±8	−0.33 ±0.10	6 ±17	−78 ±15	82 ±6	−94 ±10
6	Deoxycholic	3.0	0.89 ±0.16	97 ±22	44 ±2	177 ±13	−30 ±8	−0.28 ±0.10	−31 ±15	−8 ±4	74 ±16	−106 ±16
7*	Deoxycholic	3.0	1.83 ±0.20	258 ±15	36 ±3	383 ±17	−44 ±6	−0.61 ±0.10	0 ±15	−73 ±5	84 ±10	−145 ±12
8*	Deoxycholic	6.0	2.42 ±0.10	327 ±10	64 ±35	407 ±14	15 ±4	−2.78 ±0.20	−356 ±25	−85 ±11	−248 ±29	−141 ±7
9	Chenodeoxycholic	1.0	1.64 ±0.10	221 ±12	41 ±2	301 ±10	−28 ±7	1.79 ±0.10	227 ±15	44 ±3	283 ±13	−23 ±10
10	Chenodeoxycholic	1.0	0.96 ±0.05	143 ±25	63 ±12	311 ±3	16 ±1	1.61 ±0.07	202 ±14	80 ±2	349 ±10	34 ±2
11	Chenodeoxycholic	1.0	1.91 ±0.10	279 ±12	57 ±2	348 ±11	−10 ±7	2.79 ±0.20	387 ±22	96 ±4	498 ±23	27 ±9
12	Chenodeoxycholic	3.0	2.08 ±0.20	299 ±36	16 ±2	367 ±37	−59 ±9	2.77 ±0.20	363 ±33	44 ±8	450 ±23	−39 ±11
13	Chenodeoxycholic	3.0	2.10 ±0.10	298 ±18	63 ±4	402 ±17	18 ±6	0.64 ±0.05	108 ±8	−9 ±4	244 ±11	−68 ±36
14	Chenodeoxycholic	3.0	0.65 ±0.01	74 ±1	44 ±16	206 ±1	−77 ±2	0.47 ±0.10	80 ±9	−18 ±6	184 ±9	−123 ±3
15	Chenodeoxycholic	5.0	3.72 ±0.10	565 ±25	33 ±2	492 ±18	52 ±11	−0.92 ±0.05	−95 ±8	−18 ±9	−87 ±5	−85 ±5
16	Chenodeoxycholic	5.0	1.38 ±0.01	162 ±20	49 ±2	296 ±4	−41 ±5	−2.96 ±0.40	−310 ±46	−47 ±5	−214 ±42	−90 ±7
17	Chenodeoxycholic	5.0	1.05 ±0.01	148 ±7	43 ±7	217 ±7	—	−0.51 ±0.05	− 29 ±3	−44 ±0.4	−6 ±5	−99 ±2
18	Chenodeoxycholic	10.0	1.68 ±0.10	304 ±14	−20 ±5	285 ±3	2 ±10	−3.58 ±0.30	−442 ±39	−33 ±29	−402 ±33	−82 ±28
19	Cholic	10.0	2.10 ±0.20	277 ±24	55 ±3	321 ±20	26 ±6	1.96 ±0.40	269 ±49	37 ±17	242 ±38	−12 ±7
20	Cholic	10.0	0.50 ±0.05	52 ±12	9 ±1	120 ±8	−58 ±10	0.72 ±0.05	102 ±8	6 ±1	132 ±5	−34 ±7
21	Cholic	10.0	0.76 ±0.10	160 ±10	19 ±2	255 ±15	—	0.56 ±0.05	160 ±16	15 ±5	234 ±24	—

* Subjects were perfused with two bile acid solutions on the same day.

‡ Minus signs throughout table indicate secretion; mean ±SE for sequential periods in each study.

TABLE III
Bile Acid Concentrations During Colonic Perfusion

Study Number	Bile acid	Concentration, mM: Test solution	Concentration, mM: Rectal samples*	Net water transport
1	Deoxycholic	1.00	0.76	Absorption
2	Deoxycholic	1.00	0.57	Absorption
3	Deoxycholic	1.00	0.79	Absorption
Mean		1.00	0.71	
4	Deoxycholic	3.00	2.30	Secretion
5	Deoxycholic	3.00	2.70	Secretion
6	Deoxycholic	3.00	2.16	Secretion
7	Deoxycholic	3.00	2.49	Secretion
Mean		3.00	2.41	
8	Deoxycholic	6.00	4.69	Secretion
9	Chenodeoxycholic	1.00	0.77	Absorption
10	Chenodeoxycholic	1.00	0.34	Absorption
11	Chenodeoxycholic	1.00	0.51	Absorption
Mean		1.00	0.54	
12	Chenodeoxycholic	3.00	1.35	Absorption
13	Chenodeoxycholic	3.00	1.69	Decreased absorption
14	Chenodeoxycholic	3.00	1.44	Absorption
Mean		3.00	1.49	
15	Chenodeoxycholic	5.00	4.54	Secretion
16	Chenodeoxycholic	5.00	3.67	Secretion
17	Chenodeoxycholic	5.00	4.09	Secretion
Mean		5.00	4.07	
18	Chenodeoxycholic	10.00	7.06	Secretion
19	Cholic	10.00	5.60	Absorption
	Cholic	10.00	10.00	Absorption
	Cholic	10.00	9.84	Absorption
Mean		10.00	8.48	

* Mean of six collection periods.

Bile acid concentrations in test solutions (Table III) decreased in 18 of 19 studies during perfusion owing to bile acid absorption and, in some studies, water secretion. The pH of solutions did not change significantly during perfusion, an increase or decrease of 0.1–0.2 unit occurring in most studies.

Mixtures of conjugated bile acids. Equimolar mixtures of bile acid conjugates, whether conjugated with glycine or taurine, inhibited absorption and caused consistent secretion of water ($P \cong 0.01$), sodium, and potassium (Fig. 1). Bicarbonate secretion, which occurred in control studies, increased further ($P \cong 0.035$), and chloride absorption was inhibited ($P \cong 0.01$). The degree to which individual electrolyte transport was altered by these bile acid mixtures was similar to that observed with the free bile acids. However, the response observed was greater than that induced by 3 mM deoxycholic acid, consistent with the dihydroxy bile acid conjugates of the mixture acting additively.

When the bile acid solution was perfused first (studies 32, 34, and 35), the inhibitory effect on absorption was reversed partially or completely by the control solution within 30–60 min (Table IV, Fig. 2). Unidirectional fluxes of sodium and potassium were calculated for these experiments (Fig. 3). Sodium flux into the lumen (exsorption) was increased by perfusions that contained bile acids ($P < 0.05$), and an inconsistent change ($P > 0.05$) in sodium movement from the lumen into the circulation (insorption) was observed. Potassium ion behaved similarly.

Individual conjugated bile acids. A limited number of experiments examined the effect of individual conjugated bile acids. These studies confirmed the results obtained with mixtures of conjugates in showing that secretion can be induced by conjugated bile acids. Further, the secretion produced by 3 mM and 6 mM deoxycholic acid, whether conjugated with glycine or taurine, suggested that nuclear configuration rather than the ionic group on the side chain (conjugate), is the structural determinant of secretory activity. Thin-layer chromatography of rectal collections obtained during perfusions with conjugated bile acids showed no free bile acids. From this observation and the observed rates of absorption of free bile acids, it could be concluded that deconjugation did not occur during perfusions of conjugated bile acids.

Sodium, chloride, bicarbonate, and water relationships (Fig. 4). The linear regression ($r = 0.96$; $P < 0.001$) between water and sodium secretion induced by bile acids indicates secretion of a solution having a relatively constant concentration of sodium (135 ±8 mM SD). A close correlation was also found between induced-water and chloride secretion ($r = 0.91$; $P < 0.001$), and an equivocal correlation was observed for water and bicarbonate movements ($r = 0.48$; $P = 0.05$). With lesser rates of water secretion, bicarbonate was the only anion secreted; secretion of water greater than 1 ml/min was accompanied by nearly stoichiometric secretion of chloride in addition.

During control perfusions, absorptions of sodium and chloride were closely correlated with that of water ($r =$ 0.94 and 0.86, respectively), and net transport of bicarbonate was also related to that of water ($r = 0.79$). The sodium concentration calculated for the fluid absorbed (145 ±10 mM SD) did not differ significantly from that secreted. Comparison of linear regressions between sodium, chloride, or bicarbonate and water in the absorbed and secreted fluids indicated these did not differ significantly ($P > 0.05$) in either slope or intercept.

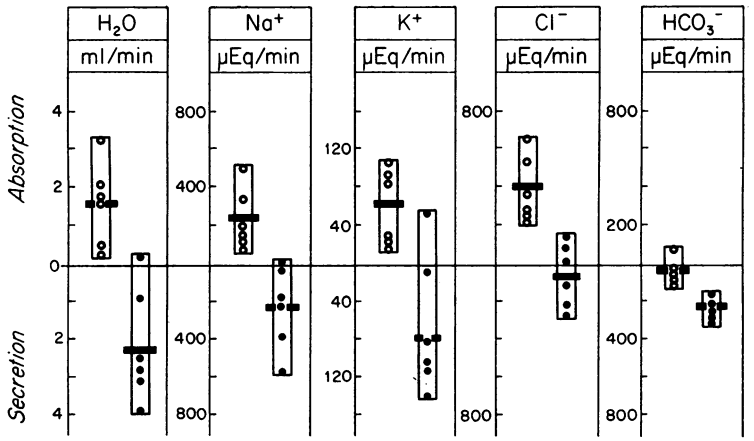

FIGURE 1 Effect of equimolar (10 mM total) mixtures of glycine or taurine conjugated bile acids on water and electrolyte transport. Each point represents a single study, that is, the mean of six sequential 10-min periods. Open symbols represent control studies, and closed symbols represent bile acid studies.

Potassium and water relationships. Secretion of potassium did not increase in proportion to sodium and water secretion induced by bile acid solutions ($r = 0.34$) (Fig. 4). When control solutions were perfused, potassium absorption occurred uniformly, but its magnitude was unrelated to that of water absorption ($r = 0.20$). The relatively constant absorption of potassium in control tests, and the relatively constant secretion of potassium when water was secreted were reflected in a difference ($P < 0.05$) of relatively constant magnitude for potassium movement between absorptive and secretory states.

Lack of PEG absorption. Calculation of water secretion in all studies was based on the assumption that PEG was a valid marker for bile acid perfusates. To test this, the absorption of PEG-^{14}C (New England Nuclear Corp., Boston, Mass.), 10 μc/liter (found to be of uniform molecular weight of about 4000 by gel-permeation chromatography on Sephadex G 50) was examined during bile acid as well as saline perfusions in two studies in which water secretion was induced. Recovery in rectal collections was 98.0 ±1.4% (SE) for saline perfusates and 96.8 ±2.1% (SE) for bile acid perfusions. No radioactivity was detected in urine during these studies.

DISCUSSION

Structure activity relationships. Our experiments show that the prevalent dihydroxy bile acids, whether free or conjugated, inhibit absorption and induce reversible secretion of water and sodium ions; this response is dependent on concentration. Response was re-

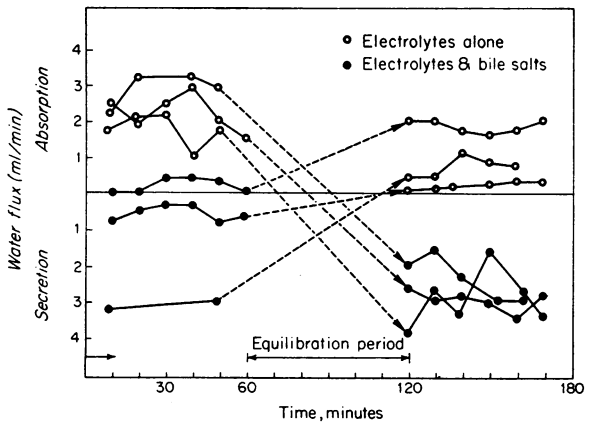

FIGURE 2 Influence of order of perfusion on net water transport during perfusion of control or 10 mM mixtures of conjugated bile acids showing reversibility of induced secretion. Each point represents one 10 min sample.

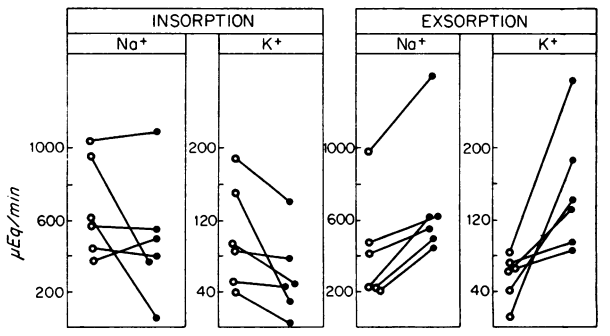

FIGURE 3 Influence of 10 mM mixtures of conjugated bile acids on unidirectional movement of cations from lumen to blood (insorption) and from blood to lumen (exsorption). Each point represents one study. Open symbols represent control studies, and closed symbols represent bile acid studies.

TABLE IV

Effect of Conjugated Bile Acids on Water and Electrolyte Transport in the Human Colon

Study number	Bile acid*	Concentration	Net transport of water (*ml/min*) and electrolytes (*μEq/min*)									
			Control solution					Bile acid solution				
			H_2O	Na	K	Cl	HCO_3	H_2O	Na	K	Cl	HCO_3
		mM										
22	GDC	1.0	2.10 ±0.1	310 ±18	32 ±5	360 ±20	−4 ±5¶	2.10 ±0.1	262 ±10	71 ±3	479 ±10	−60 ±11
23‡	GDC	3.0	1.60 ±0.1	203 ±16	7 ±4	298 ±10	−35 ±3	−0.85 ±0.1	−91 ±17	−70 ±7	65 ±13	−166 ±8
24	GDC	6.0	1.42 ±0.1	177 ±25	13 ±4	294 ±17	−21 ±4	−1.24 ±0.1	−155 ±20	−74 ±4	−26 ±9	−126 ±13
25	TDC	3.0	1.55 ±0.2	215 ±34	37 ±3	288 ±29	−40 ±8	−0.61 ±0.1	−52 ±3	−49 ±7	81 ±17	−153 ±11
26‡	GCDC	3.0	2.00 ±0.2	321 ±37	33 ±4	366 ±18	4 ±1	2.27 ±0.2	309 ±28	78 ±5	427 ±18	−26 ±6
27‡	GC	5.0	2.00 ±0.2	321 ±37	33 ±4	366 ±18	4 ±1	1.57 ±0.1	208 ±14	77 ±2	359 ±9	−87 ±3
28‡	GC	10.0	1.60 ±0.1	203 ±16	7 ±4	298 ±10	−35 ±3	0.55 ±0.1	86 ±15	−24 ±4	200 ±26	−65 ±9
29	GC	10.0	1.41 ±0.1	176 ±22	13 ±4	293 ±15	−21 ±5	0.85 ±0.1	98 ±18	0 ±4	213 ±15	−59 ±4
30	Mixture of	10.0	3.31 ±0.5	506 ±54	98 ±11	674 ±49	61 ±16	−2.50 ±0.3	−156 ±29	−107 ±18	−21 ±8	−218 ±34
31	glycine	10.0	1.64 ±0.2	175 ±40	89 ±6	302 ±21	−35 ±14	−2.90 ±0.4	−297 ±39	−125 ±21	−141 ±29	−325 ±58
32§	conjugates	10.0	0.72 ±0.1	76 ±25	106 ±2	282 ±19	−68 ±6	−3.90 ±0.2	−576 ±17	−61 ±0.1	−282 ±5	−313 ±14
33	Mixture of	10.0	2.13 ±0.2	192 ±47	27 ±4	474 ±36	—	−3.10 ±0.1	−393 ±38	−142 ±72	−225 ±80	—
34§	taurine	10.0	1.84 ±0.1	334 ±18	24 ±3	380 ±28	−28 ±16	0.20 ±0.1	15 ±15	−10 ±4	135 ±18	−102 ±19
35§	conjugates	10.0	0.40 ±0.1	133 ±20	22 ±3	240 ±12	−78 ±6	−0.57 ±0.1	0 ±20	−61 ±38	146 ±19	−152 ±20

* GDC, glycodeoxycholic; TDC, taurodeoxycholic; GCDC, glycochenodeoxycholic; GC, glycocholic acids.

‡ Subjects were perfused with two bile acid solutions on the same day.

§ Bile acid solution was perfused first, followed by control solution.

¶ Minus signs throughout table indicate secretion; mean ±SE for sequential periods in each study.

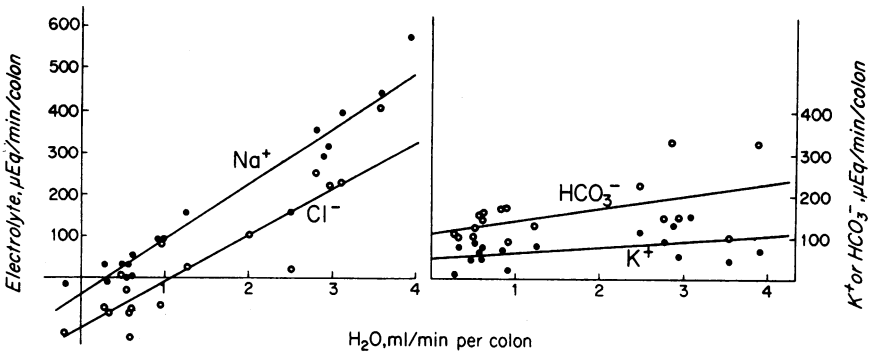

FIGURE 4 Linear regressions between electrolyte and water secretion induced by perfusion of bile acids. Sodium (closed symbols) and chloride (open symbols) movements (*left*) are closely correlated with water. Bicarbonate (open symbols) and potassium (closed symbols) movements (*right*) are poorly related to water movement.

lated to intraluminal concentration and not to the amount of bile acids absorbed. Bile acid absorption (20) will be detailed in a separate report, but the lack of response to trihydroxy acids cannot be explained by lack of absorption, since cholic acid or its conjugates were always absorbed. In addition, the absorption rate of chenodeoxycholic acid at 3 mM (24.3 μmoles/min; mean of three studies) was much greater than that at 5 mM (9.0 μmoles/min), yet water secretion occurred only at the higher concentration. The absorption rate of deoxycholic acid at 3 mM (9.1 μmoles/min) was similar to that of chenodeoxycholic acid at 1 mM (7.9 μmoles/min), yet only the former induced water secretion. Together, these results suggest that intraluminal concentration is a more important determinant of water secretion than bile acid absorption and, further, that the magnitude of response is influenced by molecular structure of the bile acid nucleus (Fig. 5).

The major fecal bile acids in man are deoxycholic acid (from bacterial 7 α-dehydroxylation of cholic acid) and lithocholic acid (from bacterial 7 α-dehydroxylation of chenodeoxycholic acid) (7). Lithocholic acid, whether free or conjugated, is insoluble at body temperature (21), and we could not have studied its effect on water and electrolyte movement in the manner described. We chose not to prepare micellar solutions of lithocholic acid (using 9 moles of taurocholate per mole of lithocholic acid) (22) because of the potential cytotoxicity of lithocholic acid (23).

The secretory effect of dihydroxy acids, whether free or conjugated, and the absence of any secretory effect of trihydroxy acids, has also been observed in our laboratory for the dog (24). Forth, Rummel, and Glasner (5) have reported that deoxycholic acid inhibits water and sodium absorption in ligated loops of rat colon.

Mechanism of action of bile acids. Perfusion techniques by their nature cannot define either the mechanism or the site of action of a compound. Present views of water and electrolyte absorption by the colon include active sodium transport as a primary event that is coupled with passive absorption of chloride and results in osmotic forces for passive water absorption (25). In addition, an unspecified mechanism for bicarbonate/chloride exchange is present. The biochemical basis of active sodium transport by the colon is poorly understood, but energy may be provided by hydrolysis of ATP by a membrane-bound Na-ATPase (26), stimulated by sodium and potassium ions and present in colonic mucosa of the toad (27). Bile acid anions are surface-active molecules, dihydroxy greater than trihydroxy (28), and could impair active sodium transport by altering enzyme configuration, by interfering competitively with the binding of phosphate, or by altering lipid-protein interactions as cellular membranes. Inhibition of sodium transport should predictably reduce

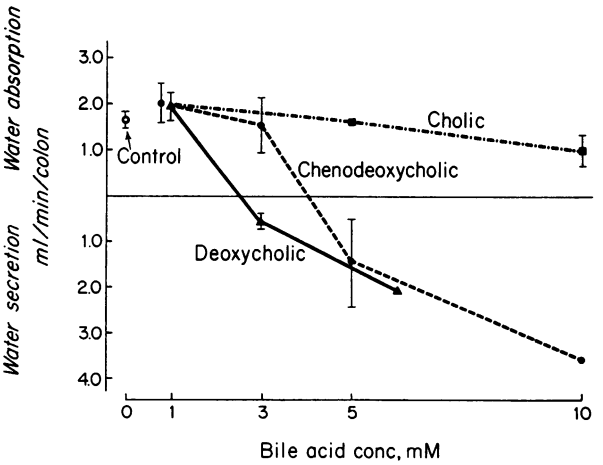

FIGURE 5 Effect of different concentrations of bile acids on absorption of water. Results from all studies with a given individual bile acid are included; mean (±SE) has been calculated from perfusions with glycine and taurine conjugates, as well as unconjugated bile acid.

coupled absorption of chloride and, in the absence of solute transport and osmotic gradients so generated, water. The effects on potassium and bicarbonate are less readily defined but may involve the effect of solvent drag, alterations of the mucosal potential, or the mechanism of chloride-bicarbonate exchange, which may also be enzyme mediated. An intracellular effect in addition to the proposed membrane effect cannot be excluded. However, effects of bile acids were not related to their absorption and, although morphologic studies were not carried out, the effect was reversible. In dogs, similar concentrations of bile acids inhibit water and electrolyte absorption but cause no morphologic change (24). Mucus secretion from goblet cells was induced by bile acids in the dog (24) but was independent of an effect on electrolyte transport. The volume of mucus recovered from the rectum in some of the present studies was too small to explain secretion of water and sodium observed; however, colonic mucus, which is rich in potassium (29), may have contributed to secretion of potassium.

Composition of induced secretion. In our studies, sodium and chloride secretions were linearly correlated with secretion of water; moreover, water, sodium, and chloride movements were similarly interrelated whether absorption or secretion occurred. When greater than 1 ml/min of water was secreted, sodium and chloride moved into the colon in linear correlation with water, as has been shown to be induced by hypertonic colonic contents (30) or observed in diarrheal conditions (31). Conversely, potassium secretion was relatively constant, regardless of the volume of induced secretion (30, 31). Thus, various intraluminal agents appear to induce identical patterns of colonic secretion. Our observations, which indicate that bile acids are potent inducers of sodium and water secretions in the colon, complement the recent report of Billich and Levitan (30), who concluded that the main forces influencing water movement across the colon were osmotic gradients and sodium transport.

Clinical implications. Our data suggest a possible mechanism for diarrhea in disease states in which elevated concentrations of dihydroxy bile acids occur in the colonic lumen. These circumstances apply to patients with ileal resection and could be relevant to other causes of ileal dysfunction; however, in such patients, diarrhea may be aggravated by the passage of increased amounts of water into the colon and the consequences of associated resection of the proximal colon. In four patients with bile acid malabsorption proved by increased turnover of labeled taurocholate, the concentrations of chenodeoxycholic and deoxycholic acids in the aqueous supernatant of stools ultracentrifuged for 2 hr (50 rotor, 105,000 *g*) were determined by gas-liquid chromatography. Ranges of concentration in multiple samples were (mM): for chenodeoxycholic acid, patient 1, 0.9–2.2; patient 2, 4.1–5.0; patient 3, 0.2–1.2; and patient 4, 0.9–1.9, and for deoxycholic acid, patient 1, 0.4–1.4; patient 2, 0.10–0.13; patient 3, 0–0.5; and patient 4, 0.4–0.5. The diarrhea of these patients responded to the oral administration of a bile acid–binding resin (32, 33), concomitantly with a striking decrease in the concentration of bile acids in the fecal supernate.[3] Such patients, in contrast to normal subjects, may have little deoxycholic acid in their feces (34), presumably because of reduced bacterial 7 α-dehydroxylation, and in these patients the colonic cell is exposed chiefly to chenodeoxycholic acid and cholic acid. If bile acids are responsible for diarrhea in such patients, chenodeoxycholic acid should be the causal agent. If bile acids are determinants of fecal water in health, however, deoxycholic acid would be most important in this respect since lithocholic acid is presumably present only in particulate form.

The greater potency of deoxycholic acid than of cholic acid suggests that bacterial transformations of bile acids may critically influence the effect of bile acids on secretion of water by the colon. Many varieties of bile acids are normally formed by bacterial action in the colon (35, 36), but no information is available on the relationship between structure and secretory activity. Finally, other substances such as fatty acids or their bacterial metabolites, hydroxy fatty acids, could be present in increased concentrations in patients with malabsorption, and should be tested for their ability to cause secretion of water and electrolytes by the colon and contribute to diarrhea. Lastly, these studies provide a possible basis of action for a number of anionic cathartics as also suggested by Forth et al. (5).

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