Effect of Conjugated Dihydroxy Bile Salts on Electrolyte Transport in Rat Colon

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ABSTRACT The mechanism by which excess quantities of bile salts in the colon produce diarrhea is not known. Therefore, experiments were performed in which the effect of conjugated dihydroxy bile salts on ion transport was evaluated in the in vitro short-circuited rat colon. 2 mM glycochenodeoxycholic acid (GCDC), taurochenodeoxycholic acid (TCDC), or taurodeoxycholic acid caused a prompt increase in short-circuit current (I_{sc}) and electrical potential difference (PD). Similar results were obtained when theophylline was added. Removal of HCO_3 and Cl prevented the effects of both bile salts and theophylline. Pretreatment with theophylline blocked the increase in I_{sc} and PD produced by TCDC and pretreatment with either TCDC or GCDC inhibited the expected theophylline response. Na fluxes in the presence of both TCDC and theophylline demonstrated a decrease in net absorption; and TCDC decreased net Cl absorption and theophylline caused a reversal of net Cl absorption to net Cl secretion. It is proposed that the diarrhea associated with cholerheic enteropathy is produced by active anion secretion possibly mediated by cyclic AMP.

INTRODUCTION

The diarrhea associated with ileal disease or resection, cholerheic enteropathy (1, 2), probably results from bile salt interference with colonic ion transport since perfusion of human and canine large intestine with dihydroxy bile salts results in inhibition of water absorption and secretion of water and electrolytes (3, 4). In vitro studies were therefore initiated to evaluate further the precise mechanism of cholerheic enteropathy. The effect of conjugated bile salts on ion transport was evaluated in the short-circuited rat colon. The relationship between bile salts and certain aspects of the cyclic adenosine 3',5'-monophosphate (cyclic AMP)[1] system was also examined, since bile salts are detergents which stimulate adenyl cyclase (5), and since cyclic AMP has been implicated in small intestinal electrolyte secretion, especially in cholera (6–8). These studies suggest that cholerheic enteropathy is caused by bile salt stimulation of active anion secretion which may be mediated by cyclic AMP.

METHODS

Non-fasting male Sprague-Dawley rats weighing between 300 and 350 g were used in all experiments. Unidirectional transmural electrolyte fluxes were measured using the apparatus and methods initially described in the rabbit ileum by Schultz and Zalusky (9) as previously modified (10). Immediately after sacrifice, the colon was removed and flushed rapidly with a buffered electrolyte solution. The serosa and part of the muscular layer were carefully removed, and the remaining mucosa was mounted between two Lucite chambers and bathed with solutions of identical ionic composition maintained at 37°C. The exposed surface area was 1.13 cm^2. The spontaneous transmembrane electrical potential difference (PD) was monitored by balanced calomel electrodes attached to a direct reading potentiometer, and the tissue was continuously short-circuited using an automatic voltage clamp except for periods less than 30 s when the PD was being recorded. A positive PD reflects the serosal potential in reference to the mucosal and the sign of the short-circuit current (I_{sc}), the current necessary to nullify the PD, corresponds to the sign of the PD.

The composition of the Ringer electrolyte solution was, in millimoles, Na 140; Cl 119.8; HCO_3 25; HPO_4 2.4;

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[1] *Abbreviations used in this paper:* cyclic AMP, cyclic adenosine 3',5'-monophosphate; CDC, chenodeoxycholic acid; DB-cyclic AMP, N^6-2'0-dibutyryl-adenosine 3',5'-monophosphate, cyclic; GCDC, glycochenodeoxycholic acid; I_{sc}, short-circuit current; J^{Na}_{net}, J^{Cl}_{net}, net flux of Na and Cl, respectively; J^{Na}_{ms}, J^{Cl}_{ms}, unidirectional flux of Na and Cl, respectively, from mucosal to serosal side; J^{Na}_{sm}, J^{Cl}_{sm}, unidirectional flux of Na and Cl, respectively, from serosal to mucosal side; J^R, residual flux; PD, electrical potential difference; TCDC, taurochenodeoxycholic acid; TDC, taurodeoxycholic acid.

H_2PO_4 0.4; K 5.2; Ca 1.2; Mg 1.2. In the HCO_3-free, Cl-free experiments, sodium isethionate, $CaSO_4$, and $MgSO_4$ were employed. When the Ringer's solution was used, the tissue was continuously oxygenated with 95% O_2-5% CO_2. 100% O_2 was employed in the HCO_3-free, Cl-free experiments. pH of these solutions was 7.4.

With animals of this size and weight, four or five pieces of colonic mucosa can usually be mounted in these chambers. Preliminary experiments comparing the most proximal and most distal segments of colonic mucosa failed to reveal any difference in I_{sc}, PD, J^{Na}_{net} or the response to the addition of either theophylline or bile salts. Therefore, four tissues from the same animal were studied simultaneously without regard to their in vivo location. In those experiments in which only short-circuit current and PD were monitored, the tissue was initially incubated in the appropriate solution in the absence of either theophylline or bile salts. After 20–25 min, at a time when the I_{sc} and PD had stabilized, a dihydroxy bile salt or theophylline was added at random to both the mucosal and serosal bathing solution of two or three of the four tissues. The I_{sc} was continually monitored; the PD at 5-min intervals. 35–40 min later, theophylline or a bile salt was added to all four tissues, and the I_{sc} and PD recorded for an additional 30 min. In a few experiments dibutyryl cyclic AMP (N^6-2'0-dibutyryladenosine 3',5'-monophosphate, cyclic, or DB-cyclic AMP) was added prior to the addition of bile salts. Although the I_{sc} was continuously monitored, the change in I_{sc} and PD was determined by the difference between the recorded peak value after addition of the substance in question and the value observed immediately prior to this addition.

Unidirectional simultaneous Na fluxes were determined on the same piece of tissue using ^{22}Na and ^{24}Na. Oppositely directed Cl fluxes were determined on adjacent pieces of tissue using ^{36}Cl. In these ion flux experiments, the tissue was initially mounted in Ringer's solution containing either bile salt or theophylline. After the appropriate isotope was added, a zero-time "hot" side sample was obtained and after 10 min a single 20 min flux period was determined. Preliminary experiments demonstrated that although bile salts increased tissue conductance, unidirectional fluxes were linear with respect to time for at least 40 min. The I_{sc} and PD for each flux period in these experiments were obtained by averaging I_{sc} and PD observed at the beginning and end of the flux period. The details of the counting procedure have been previously described (10). Results are expressed as $\mu eq/h \cdot cm^2$ and positive values represent net absorption; negative ones net secretion.

Glycochenodeoxycholic acid (GCDC), taurochenodeoxycholic acid (TCDC), unconjugated chenodeoxycholic acid (CDC), and taurodeoxycholic acid (TDC) were obtained from Calbiochem, (San Diego, Calif.); the conjugated bile salts were all greater than 98% chromatographically homogeneous when tested by thin-layer chromatography (11). ^{22}Na and ^{36}Cl were obtained from New England Nuclear Corp. (Boston, Mass.); ^{24}Na from Cambridge Nuclear Corp. (Billerica, Mass.); theophylline from the ICN Nutritional Biochemicals Div. (Cleveland, Ohio) and DB-cyclic AMP from the Boehringer Mannheim Corp. (New York).

All results are expressed as mean ±SEM. Standard statistical calculations were employed (12).

RESULTS

Electrical parameters. A stable I_{sc} and PD were observed for at least 50 min when rat colon was mounted in the HCO_3-Ringer solution in the absence of bile salts or theophylline, as demonstrated in Fig. 1 and 2. The addition of 2 mM glycochenodeoxycholic acid (GCDC) to both the mucosal and serosal side of the colonic mucosa bathed in Ringer's solution resulted in a prompt increase in both I_{sc} (Fig. 1) and PD. Since the effect of 2 mM GCDC was indistinguishable from that of 2 mM TCDC, the results of both bile salts were combined. After the addition of 2 mM conjugated chenodeoxycholic acid the mean increment in I_{sc} and PD was 107± 11 $\mu A/cm^2$ and 3.7±0.4 mV, respectively, in 19 tissues. 1 mM TCDC resulted in increases approximately 50% of those caused by 2 mM TCDC (42±5 $\mu A/cm^2$).

Although the GCDC and TCDC used in our experiments contained less than 1% unconjugated CDC, ad-

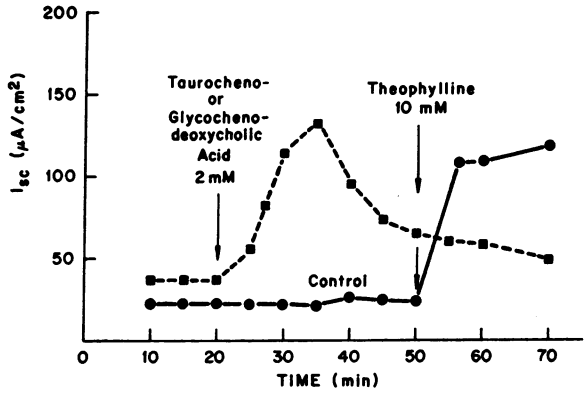

FIGURE 1 The effect of conjugated CDC and theophylline on I_{sc}. 2 mM GCDC or TCDC (■) was added at 20 min. 10 mM theophylline was added to both bile salt-treated and control tissue at 50 min.

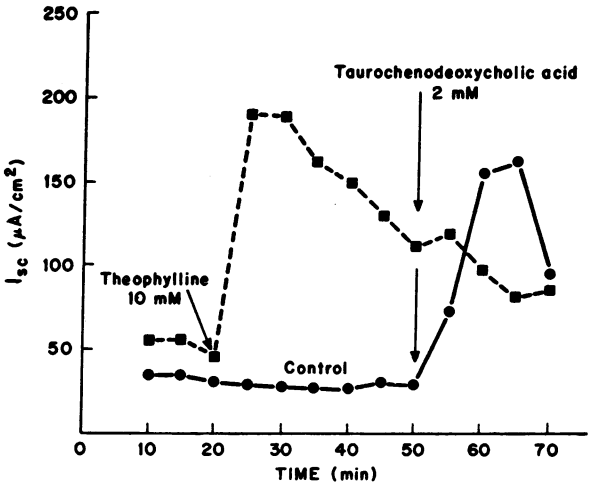

FIGURE 2 The effect of 10 mM theophylline on the TCDC-induced increase in I_{sc}. 10 mM theophylline (■) was added at 20 min, and 2 mM TCDC was added to both control (●) and theophylline-pretreated (■) tissues at 50 min.

TABLE I

*Effect of Bile Salts and Theophylline on the Increase in I_{sc} and PD Caused by (A) Theophylline and (B) Taurochenodeoxycholic Acid**

Pretreatment	No.	ΔI_{sc}	ΔPD
		$\mu A/cm^2$	mV
A. 10 mM Theophylline			
None	29	114±7	6.2±0.4
1 mM GCDC	2	30	1.7
2 mM GCDC or TCDC	12	6±3	0.1±0.1
1 mM TDC	4	79±24	3.4±1.2
2 mM TDC	4	0	0
B. 2 mM Taurochenodeoxycholic acid			
None	9	116±13	4.7±0.4
10 mM theophylline	8	12±4	0.6±0.3
0.5 mM DB-cyclic AMP	3	23±14	0.9±0.4

* In *A* bile salts were added to mucosal and serosal solution bathing colonic tissue at 20 min, and 30–35 min later 10 mM theophylline was added. In *B* theophylline or DB-cyclic AMP was added prior to the addition of TCDC. Increase in I_{sc} and PD calculated from the difference between the peak value after addition and value immediately prior to addition.

ditional experiments were performed to exclude the possibility that this observed effect was secondary to unconjugated CDC. 0.5 mM CDC was added to eight tissues, but only a minimal increment in I_{sc} (9±3 $\mu A/cm^2$) and PD (0.3±0.2 mV) was noted.

Similar to observations in rabbit and guinea pig ileum (8, 10), the addition of theophylline to both mucosa and serosa bathing solution also resulted in a prompt increase in both I_{sc} and PD. The mean increase in I_{sc} and PD after the addition of 10 mM theophylline to 29 pieces of tissue was 114±7 $\mu A/cm^2$ and 6.2±0.4 mV, respectively. Although the increase in I_{sc} promoted by 10 mM theophylline and 2 mM TCDC and GCDC were identical, the bile salts caused a significantly lower PD response than theophylline resulting in a higher conductance in the presence of bile salts.

In another series of experiments 10 mM theophylline was added to tissue previously exposed to TCDC and TCDC was added to tissue previously exposed to theophylline. Fig. 2 demonstrates that a significant inhibition in the expected bile salt induced increase in I_{sc} was observed when theophylline had been added to the colonic mucosa 30 min previously. 2 mM TCDC increased I_{sc} by 116±13 $\mu A/cm^2$ in nine tissues, but if theophylline was present, the increase in I_{sc} caused by 2 mM TCDC was only 12±4 $\mu A/cm^2$. Similarly, presence of 2 mM TCDC or 2 mM GCDC inhibited the usual increase in I_{sc} observed with 10 mM theophylline (Fig. 1 and Table I). Further, the addition of 0.5 mM DB-cyclic AMP, which also increased I_{sc} and PD when added alone, inhibited the augmentation of I_{sc} and UD caused by 2 mM TCDC When 0.5 mM DB-cyclic AMP is present, the increase in I_{sc} caused by 2 mM TCDC is only 23±14 $\mu A/cm^2$.

The addition of 2 mM TDC resulted in similar increases in I_{sc} and PD and also completely abolished the increase in I_{sc} and PD observed after the addition of 10 mM theophylline. 1 mM TDC only minimally inhibited the theophylline-induced I_{sc} augmentation (Table I).

Anion substitution. Our results, in which theophylline blocks the exepected effect of bile salts, are strikingly similar to that described by Field, Fromm, Al-Awqati, and Greenough in rabbit ileum with cholera enterotoxin and theophylline (6). Since both theophylline and cholera enterotoxin are not effective in at HCO_3-free, Cl-free media in rabbit ileum (13 and footnote 2), further studies were performed in which bile salts and theophylline were added to a HCO_3-free, Cl-free solution. Under these conditions, both TCDC and theophylline produced minimal increases in either I_{sc} or PD. In the Ringer's solution theophylline induced an increase in I_{sc} of 114±7 $\mu A/cm^2$ compared with an increase of 10±1 $\mu A/cm^2$ when added to a HCO_3-free, Cl-free solution. Intermediate in-

[2] Powell, D. W., H. J. Binder, and P. F. Curran. Active electrolyte secretion stimulated by choleragen in rabbit ileum in vitro. Submitted for publication.

TABLE II

*Effect of Bicarbonate and Chloride on the Bile Salt and Theophylline-induced Increases of PD and I_{sc}**

Solution	Addition of 2 mM GCDC or TCDC			Addition of 10 mM theophylline		
	No.	ΔI_{sc}	ΔPD	No.	ΔI_{sc}	ΔPD
		$\mu A/cm^2$	mV		$\mu A/cm^2$	mV
Ringer (HCO_3, Cl containing)	19	107±11	3.7±0.4	29	114±7	6.2±0.4
HCO_3 free, Cl containing	6	59±9	4.0±0.6	—	—	—
Cl free, HCO_3 containing	5	52±4	2.5±0.3	9	46±6	2.6±0.5
HCO_3 free, Cl free	9	9±2	0.3±0.1	8	10±1	0.4±0.1

* Mean ±SEM. Bile salts and theophylline were added at 20–25 min. Increase in I_{sc} and PD calculated from the difference between the peak value after addition and value immediately prior to addition. All solutions contained 140 meq/liter Na.

TABLE III

*Effect of 10 mM Theophylline and 2 mM Conjugated Chenodeoxycholic Acid on Unidirectional and Net Ion Fluxes, I_{sc} and Conductance across Rat Colon**

	Na fluxes				Cl fluxes						
	No.‡	J_{ms}	J_{sm}	J_{net}	No.‡	J_{ms}	J_{sm}	J_{net}	I_{sc}	J^R	G
Control	16	12.6±0.7	8.6±0.8	4.0±0.4	9	13.6±0.6	11.2±0.7	2.4±0.9	1.6±0.1	0.0	12.9
GCDC	5	17.1±0.7	15.1±0.8	2.0±0.5	—	—	—	—	2.8±0.4	—	30.3
TCDC	6	13.4±0.6	11.4±0.5	2.0±0.4	7	15.9±0.6	16.1±0.5	−0.2±0.9	3.6±0.1	1.4	21.3
Theophylline	8	9.1±0.5	8.9±0.5	0.2±0.3	9	10.1±0.6	11.8±0.8	−1.7±0.9	3.8±0.1	1.9	14.2

Mean ±SEM. Fluxes and I_{sc} expressed as μeq/h·cm²; G represents tissue conductance and is expressed as mmho/cm².
Residual flux or J^R calculated from $[= I_{sc} - (J^{Na}_{net} - J^{Cl}_{net})]$ and most likely represents bicarbonate secretion (10, 14).
Positive values represent net absorption, negative ones net secretion.
* Unidirectional ion fluxes determined with ^{22}Na, ^{24}Na, and ^{36}Cl as described in text.
‡ Number of tissues or tissue pairs studied.

crements were observed when either a HCO_3-free, Cl-containing solution or a Cl-free, HCO_3-containing solution was employed (Table II).

Electrolyte flux studies. Since the increase in I_{sc} may represent an increase in either cation absorption or an increase in anion secretion in addition to other possibilities, Na and Cl fluxes were performed in the presence of conjugated CDC and theophylline. Table III demonstrates that net Na absorption was observed in control tissues (4.0±0.4 μeq/h·cm²). The presence of either 2 mM GCDC or TCDC resulted in a decrease in net Na absorption. J^{Na}_{net} was 2.0±0.4 μeq/h·cm² when 2 mM TCDC was present. Analysis of the unidirectional fluxes revealed that TCDC caused an increase in J^{Na}_{sm}. However, as previously noted, a significant increase in conductance also occurred. J^{Na}_{net} was also diminished in the presence of 10 mM theophylline. In the theophylline experiments, the significant change in unidirectional fluxes was a decrease in J^{Na}_{ms}, while J^{Na}_{sm} did not differ from controls (Table III).

Cl transport was also determined in additional experiments. Net Cl absorption was demonstrated in control tissues and a significant inhibition of net Cl absorption was observed in the presence of TCDC and net Cl secretion was noted in the presence of theophylline. Net Cl transport was 2.4±0.9 μeq/h·cm² in control tissues. With 2 mM TCDC and 10 mM theophylline net Cl transport was −0.2±0.9 and −1.7±0.9 μeq/h·cm², respectively. Similar to the unidirectional Na fluxes, theophylline decreased J^{Cl}_{ms} but did not appear to alter J^{Cl}_{sm} and TCDC increased J^{Cl}_{sm} more than J^{Cl}_{ms}.

The unaccounted ionic flux or the residual flux, J^R, can be calculated by $J^R = I_{sc} - (J^{Na}_{net} - J^{Cl}_{net})$. Previous studies in guinea pig and rabbit ileum (10, 14) have suggested that the residual flux represents HCO_3 secretion. Table III demonstrates that both TCDC and theophylline increase J^R or HCO_3 secretion.[3]

[3] Although the term bicarbonate secretion is used, this is not meant to indicate actual bicarbonate transport from the serosa to the mucosa. The actual mechanism of mucosal alkalinization is not known and bicarbonate secretion is compatible with H^+ absorption, OH^- secretion or tissue production of H^+ and HCO_3^-, with their extrusion across the serosal and mucosal borders respectively.

DISCUSSION

Forth, Rummel, and Glasner provided the first experimental evidence that bile salts affect colonic transport when they demonstrated that bile salts placed in isolated loops of the rat large intestine caused a decrease in water and Na absorption (15). They further showed that dihydroxy bile salts were more active than trihydroxy bile salts and that unconjugated bile salts, but not conjugated forms, produced this defect. In 1967, Hofmann proposed that the diarrhea associated with ileal disease and resection was related to increased quantities of bile salts entering the colon and named this entity cholerheic enteropathy (1). Hofmann and associates (2–4, 16–20) have since delineated its pathophysiology and therapy while examining both normal bile salt metabolism and other metabolic consequences of ileal dysfunction. Mekhjian, Phillips, and Hofmann observed net secretion of salt and water into the large intestine in man during perfusion with both conjugated and unconjugated chenodeoxycholic acid and deoxycholic acid but not with cholic acid (3). Although these in vivo studies clearly demonstrated that dihydroxy bile salts will induce secretion, these investigators neither established nor proposed a mechanism to explain this secretory phenomenon. Since more than one mechanism can produce net secretion, which has recently been observed in several pathologic and physiologic conditions in the small intestine (21), the present in vitro experiments were performed in an attempt to clarify the pathogenesis of cholerheic enteropathy.

Our studies performed in vitro demonstrate that the effect of bile salts are similar to those observed in vivo. That is, net Na absorption is inhibited and net anion secretion is observed; dihydroxy bile salts are more ef-

fective than trihydroxy bile salts.[4] These results demonstrate that the conjugated dihydroxy bile salts increase the I_{sc} which most likely reflects active anion secretion. Evidence to support the role of HCO_3 or Cl or both is provided by (*a*) the failure to observe a substantial effect of bile salts in a HCO_3-free, Cl-free media and (*b*) the inhibition of Cl absorption by flux measurements and the suggestion of HCO_3 secretion by calculation of an increase in residual flux.[5]

Initial inspection of the unidirectional fluxes suggest that theophylline and bile salts alter these fluxes in different ways (Table III). It must be emphasized, however, that a significant increase in tissue conductance is observed in the presence of TCDC. A change in conductance will affect both unidirectional fluxes equally. Therefore, after correction for the increase in conductance,[6] the predominant change caused by TCDC is a decrease in both J^{Na}_{ms} and J^{Cl}_{ms} with little alteration in either J^{Na}_{sm} or J^{Cl}_{sm} similar to that observed with theophylline.

These in vitro results are qualitatively similar to experiments previously reported in which the effect of bile salts on colonic electrolyte movement was studied in vivo. In the dog, Mekhjian and Phillips observed that dihydroxy bile salts inhibited the absorption of water, sodium, chloride, potassium, and bicarbonate (4). However, in perfusion studies in man, these same investigations demonstrated that bile salts converted the absorption of water, sodium, and chloride to secretion and caused an increase in HCO_3 secretion (3). These differences are probably quantitative, not qualitative, and may represent species differences in respect either to the response to the perfusion of bile salts or to the nature of electrolyte movement under control conditions. That is, in the dog absorption of Na, Cl, and HCO_3 was observed during control perfusions and in man absorption of Na and Cl but secretion of HCO_3 occurred. It has been previously emphasized that the composition of electrolyte secretion produced by cholera enterotoxin in the jejunum and ileum reflects, in part, the characteristics of electrolyte movement normally observed in these two locations.

Although the evidence is indirect and circumstantial at best, the most interesting results are those suggesting that the effect of bile salts may be mediated by cyclic AMP. Most of the effects of bile salts are strikingly similar to those produced by theophylline. These similarities can be summarized: (*a*) both bile salts and theophylline produce comparable increments in I_{sc} and both increase the PD; (*b*) a markedly impaired effect is produced by either agent in a HCO_3-free, Cl-free media; (*c*) both agents produce qualitatively similar effects on Na, Cl, and residual flux measurements (Table III); and (*d*) prior administration of either bile salts or theophylline inhibits the expected increase in I_{sc} produced by the other agent (Figs. 1 and 2 and Table I).

Our results in rat colon are also similar to the effect of cholera enterotoxin, theophylline, cyclic AMP, and prostaglandins that have been observed in rabbit ileum when identical methodology has been employed (6, 8, 22, and footnote 2). In rabbit ileum, cholera enterotoxin, theophylline, cyclic AMP, and prostaglandins all increase I_{sc} and PD, decrease J^{Na}_{net}, reverse net Cl absorption so that net Cl secretion is observed, but do not alter I_{sc} or PD when HCO_3 and Cl are absent from the solution. This suggested relationship between cholera enterotoxin and cyclic AMP has been confirmed by the demonstration of the activation of intestinal adenyl cyclase by cholera enterotoxin (23–26).

Although we have not as yet studied the effect of bile salts on colonic adenyl cyclase, we propose that bile salts produce net colonic secretion in cholerheic enteropathy by stimulating adenyl cyclase. In other systems detergents, including bile salts, stimulate adenyl cyclase (5, 27). Triton X-100 and Na dodecyl sulfate have been demonstrated to stimulate adenyl cyclase in rat cerebral cortex; Oye and Sutherland observed in turkey erythrocyte ghosts that deoxycholic acid increased adenyl cyclase activity (5). However, proof of this hypothesis awaits actual demonstration of bile salt stimulation of adenyl cyclase and cyclic AMP content in colonic mucosa.

Additional support for the concept that bile salts alter ion movement secondary to an increase in cyclic AMP is provided by comparison of the action of theophylline and cyclic AMP with that of bile salts on ion influx across the brush border of rabbit ileum (28, 29). Frizzell, Schultz, and co-workers demonstrated that both theophylline and cyclic AMP inhibited Na entry into the epithelial cell and that Na entry was also inhibited by the presence of taurodeoxycholic acid (28, 29). Although Frizzell and Schultz proposed other mechanisms to explain their results with taurodeoxycholic acid, the similarity of both the effect of cyclic AMP and TDC on Na

[4] The addition of 2 mM taurocholic acid does not significantly increase either PD or I_{sc}; nor does it alter the normal "theophylline-response" (Binder, H. J., unpublished observations).

[5] A positive residual flux represents either cations moving from mucosa to serosa or anions moving from serosa to mucosa. Previous studies (10, 15) have suggested that the residual flux most likely represents HCO_3 secretion. Since actual determination of bicarbonate fluxes with $HC^{14}O_3$ was not technically feasible, demonstration of active anion secretion is provided by calculation of a bile salt induced increase in residual flux.

[6] TCDC caused a 65% increase in tissue conductance. To determine the effect of bile salts on unidirectional fluxes independent of those changes secondary to the increase in conductance, each unidirectional flux was decreased by 65%.

influx and the inhibition of net Na absorption observed with theophylline and cyclic AMP in rabbit ileum and with TCDC in rat colon suggest that TDC in rabbit ileum may be affecting Na entry, via the cyclic AMP system. Frizzell and Schultz also demonstrated that TDC removed significant amounts of protein from the mucosal surface and these observations may, in part, account for the bile salt-induced increase in tissue conductance.

That these bile salt effects were induced by the conjugated compound, not by the unconjugated form, is supported by the absence of significant unconjugated bile salts in our conjugated bile salt preparations and the failure to observe any effects with small amounts of unconjugated CDC. Additional experiments employing higher concentrations of unconjugated bile salts were not performed because of the known cytotoxic properties of unconjugated bile salts in in vitro systems and of the requirement of calcium in this in vitro system to maintain adequate membrane integrity.

Although explanations related to other surface active properties of bile salts have not been excluded, these studies are compatible with the hypothesis that excess bile salts in the colon, as observed in cholerheic enteropathy, decrease Na and Cl absorption and stimulate HCO_3 secretion. Exploration of additional therapeutic approaches might include methods to interfere with either bile salt membrane interaction or with the mechanism by which cyclic AMP induces ion secretion. Additional information concerning both absorption and secretion will probably be required first.

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REFERENCES

1. Hofmann, A. F. 1967. The syndrome of ileal disease and the broken enterohepatic circulation: cholerheic enteropathy. *Gastroenterology.* **52**: 752.
2. Hofmann, A. F., and J. R. Poley. 1969. Cholestyramine treatment of diarrhea associated with ileal resection. *N. Engl. J. Med.* **281**: 397.
3. Mekhjian, H. S., S. F. Phillips, and A. F. Hofmann. 1971. Colonic secretion of water and electrolytes induced by bile acids: perfusion studies in man. *J. Clin. Invest.* **50**: 1569.
4. Mekhjian, H. S., and S. F. Phillips. 1970. Perfusion of the canine colon with unconjugated bile acids: effect on water and electrolyte transport, morphology, and bile acid absorption. *Gastroenterology.* **59**: 120.
5. Oye, I., and E. W. Sutherland. 1966. The effect of epinephrine and other agents on adenyl cyclase in the cell membrane of avian erythrocytes. *Biochim. Biophys. Acta.* **127**: 347.
6. Field, M., D. Fromm, Q. Al-Awqati, and W. B. Greenough, III. 1972. Effect of cholera enterotoxin on ion transport across isolated ileal mucosa. *J. Clin. Invest.* **51**: 796.
7. Field, M. 1971. Intestinal secretion: Effect of cyclic AMP and its role in cholera. *N. Engl. J. Med.* **284**: 1137.
8. Field, M. 1971. Ion transport in rabbit ileal mucosa. II. Effects of cyclic 3',5'-AMP. *Am. J. Physiol.* **221**: 992.
9. Schultz, S. G., and R. Zalusky. 1964. Ion transport in isolated rabbit ileum. I. Short-circuit current and Na fluxes. *J. Gen. Physiol.* **47**: 567.
10. Powell, D. W., H. J. Binder, and P. F. Curran. 1972. Electrolyte secretion by the guinea pig ileum in vitro. *Am. J. Physiol.* **223**: 531.
11. Hofmann, A. F. 1962. Thin-layer absorption chromatography of free and conjugated bile acids on silicic acid. *J. Lipid Res.* **3**: 127.
12. Snedecor, G. W., and W. G. Cochran. 1967. Statistical Methods. Iowa State University Press, Ames. 6th edition.
13. Field, M., G. R. Plotkin, and W. Silen. 1968. Effects of vasopressin, theophylline and cyclic adenosine monophosphate on short-circuit current across isolated rabbit ileal mucosa. *Nature (Lond.).* **217**: 469.
14. Field, M., D. Fromm, and I. McColl. 1971. Ion transport in rabbit ileal mucosa. I. Na and Cl fluxes and short-circuit current. *Am. J. Physiol.* **220**: 1388.
15. Forth, W., W. Rummel, and H. Glasner. 1966. Zur resorptionshemmenden Wirkung von Gullensauren. *Naunyn Schmiedebergs. Arch. Exp. Pathol.* **254**: 364.
16. Fromm, H., and A. F. Hofmann. 1971. Breath test for altered bile-acid metabolism. *Lancet.* **2**: 621.
17. Hofmann, A. F., and J. R. Poley. 1972. Role of bile acid malabsorption in pathogenesis of diarrhea and steatorrhea in patients with ileal resection. I. Response to cholestyramine or replacement of dietary long chain triglycerides by medium chain triglycerides. *Gastroenterology.* **62**: 918.
18. Hepner, G. W., A. F. Hofmann, and P. J. Thomas. 1972. Metabolism of steroid and amino acid moieties of conjugated bile acids in man. I. Cholylglycine. *J. Clin. Invest.* **51**: 1889.
19. Hepner, G. W., A. F. Hofmann, and P. J. Thomas. 1972. Metabolism of steroid and amino acid moieties of conjugated bile acids in man. II. Glycine-conjugated dihydroxy bile acids. *J. Clin. Invest.* **51**: 1898.
20. Smith, L. H., H. Fromm, and A. F. Hofmann. 1972. Acquired hyperoxaluria, nephrolithiasis and intestinal disease. *N. Engl. J. Med.* **286**: 1371.
21. Hendrix, T. R., and T. M. Bayless. 1970. Digestion: intestinal secretion. *Annu. Rev. Physiol.* **32**: 139.
22. Al-Awqati, Q., and W. B. Greenough, III. 1972. Prostaglandins inhibit intestinal sodium transport. *Nat. New Biol.* **238**: 26.
23. Schaefer, D. E., W. D. Lust, R. Sircar, and N. D. Goldberg. 1970. Elevated concentration of adenosine 3',5'-cyclic monophosphate in intestinal mucosa after treatment with cholera toxin. *Proc. Natl. Acad. Sci. U. S. A.* **67**: 851.
24. Sharp, G. W. G., and S. Hynie. 1971. Stimulation of intestinal adenyl cyclase by cholera toxin. *Nature (Lond.).* **229**: 266.

25. Kimberg, D. Y., M. Field, J. Johnson, A. Henderson, and E. Gershon. 1971. Stimulation of intestinal mucosal adenyl cyclase by cholera enterotoxin and prostaglandins. *J. Clin. Invest.* **50**: 1218.
26. Parkinson, D. K., H. Ebel, D. R. DiBona, and G. W. G. Sharp. 1972. Localization of the action of cholera toxin on adenyl cyclase in mucosal epithelial cells of rabbit small intestine. *J. Clin. Invest.* **51**: 2292.
27. Perkins, J. P., and M. M. Moore. 1971. Adenyl cyclase of rat cerebral cortex. Activation of sodium fluoride and detergents. *J. Biol. Chem.* **246**: 62.
28. Frizzell, R. A., H. N. Nellans, I. Mark-Scheid-Kasp, and S. G. Schultz. 1972. Effect of cyclic-AMP on coupled NaCl influx across brush border of rabbit ileum. *Fed. Proc.* **31**: 279. (Abstr.)
29. Frizzell, R. A., and S. G. Schultz. 1970. Effect of bile salts on transport across brush border of rabbit ileum. *Biochim. Biophys. Acta.* **211**: 589.