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A COMPARATIVE STUDY ON THE EFFECTS OF DIFFERENT BILE SALTS ON MUCOSAL ATPase AND TRANSPORT IN THE RAT JEJUNUM IN VIVO

ERNESTO GUIRALDES*, SANATH P. LAMABADUSURIYA, JOSE E. J. OYESIKU, ANTHONY E. WHITFIELD and JOHN T. HARRIES

Institute of Child Health, Guilford Street, London WC1N 1EH (U.K.)

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SUMMARY

The effects of deoxycholate, taurocholate and cholate on transport and mucosal ATPase activity have been investigated in the rat jejunum in vivo using closed-loop and perfusion techniques.

In the closed-loops, 5 mM deoxycholate selectively inactivated $(Na^+ + K^+)$-ATPase, and net secretion of Na^+ induced by 2.5 mM deoxycholate was due to reduced lumen to plasma flux of the ion; deoxycholate (2.5 mM) produced marked inhibition of 3-*O*-methylglucose transport. Luminal disappearance rates of deoxycholate (60.5 ± 2.9 % per g wet wt of gut) greatly exceeded those of taurocholate (4.3 ± 1.0).

In the perfusion studies 1 mM deoxycholate induced net secretion of water, Na^+ and Cl^-, and inhibited active glucose transport; concomitantly "total" ATPase, $(Na^+ + K^+)$-ATPase, and Mg^{2+}-ATPase were inhibited. At higher concentrations (5 mM) deoxycholate stimulated Mg^{2+}-ATPase activity. Taurocholate and cholate at 1 mM had no effect on transport or $(Na^+ + K^+)$-ATPase. Mucosal lactase, sucrase and maltase activities were not affected by 1 mM deoxycholate, taurocholate or cholate.

These results suggest that deoxycholate inhibits sodium-coupled glucose transport by inhibition of $(Na^+ + K^+)$-ATPase at the lateral and basal membranes of the epithelial cell, rather than from an effect at the brush-border membrane level.

INTRODUCTION

The unconjugated dihydroxy bile salt deoxycholate has been shown to inhibit the in vivo small intestinal absorption of water, sodium and glucose both in the experimental animal and man [1–4], and has been implicated in the pathophysiological mechanisms responsible for certain types of diarrhoea [2, 5]. The mechanism(s) by which deoxycholate exerts these inhibitory effects on small intestinal transport, however, is highly speculative and requires further clarification.

* Present address: Hospital L. Calvo Mackenna, Santiago 9, Chile.

Active glucose transport is a sodium-dependent process, and coupling of these two solutes at a sodium-mediated carrier has been postulated [6–8]. Crane [9] has suggested that net transport of glucose against a concentration gradient is coupled to downhill movement of Na^+ into the cell, and that the Na^+ gradient is maintained by an energy dependent Na^+ pump located at the lateral cell membrane, energy being presumably derived from hydrolysis of ATP by ($Na^+ + K^+$)-ATPase. Of fundamental importance to this model is the energy source which drives the Na^+ pump and couples the active extrusion of intracellular sodium to the absorption of glucose against a concentration gradient across the brush border membrane. Radioautographic studies have localised this pump to the lateral membranes of rabbit small intestinal cells [10], and three independent groups of workers [11–13] have shown that approximately 85 % of rat and rabbit small intestinal mucosal ($Na^+ + K^+$)-ATPase is bound to the lateral and serosal membranes.

Since Skou [14] first reported ($Na^+ + K^+$)-ATPase activity in crab nerve evidence has been accumulating for the involvement of this membrane bound enzyme in active transport processes across a wide variety of biological membranes from several species [15–19].

In a limited number of in vitro studies deoxycholate has been shown to inhibit small intestinal ($Na^+ + K^+$)-ATPase in the experimental animal [20–22] but only one study has been reported on the effects of deoxycholate on ($Na^+ + K^+$)-ATPase in vivo [23]. In this study reversible inhibition of both jejunal ($Na^+ + K^+$)-ATPase and active arbutin transport was induced by feeding deoxycholate to rats.

In this paper we have investigated the simultaneous effects of unconjugated dihydroxy (deoxycholate), unconjugated trihydroxy (cholate) and conjugated trihydroxy (taurocholate) bile salts on mucosal ATPase activity and active sodium-coupled glucose absorption in rat jejunum in vivo, using two separate techniques.

METHODS

All studies were performed on male Wistar rats weighing 200–350 g following a 12–18 h fast, tap water being allowed at libitum. The design of the closed-loop and perfusion techniques were as previously described and validated [2, 24]; in the closed-loop experiments a 25-min absorptive period was used unless indicated, and in the perfusion studies the infusate flow rate was 0.2 ml/min. In some of the perfusion studies mucosal potential difference (PD) was measured with a DC multimeter Type TM9B (Levell Electronics, High Barnet, Herts.). The luminal electrodes (Ag/AgCl electrodes built into 5 ml disposable syringes filled with normal saline) were inserted via a No. 12 needle into the inlet tubing near the point of entry into the perfused segment of jejunum. A similar serosal electrode was suspended in the peritoneal cavity, which was kept filled with normal saline. PD values varied little during the second hour of perfusion, and a mean value of measurements taken every 5 min was obtained for each experiment.

Composition of closed-loop and perfusion solutions

All solutions were isotonic (285 mosM/kg), buffered to pH 7.4 with phosphate (2.88 mM NaH_2PO_4, 12.12 mM Na_2HPO_4) and contained polyethylene glycol (3 g/l), KCl (4 mM), and glucose (2 or 20 mM) or 3-*O*-methylglucose (20 mM). The bile salt solutions contained varying concentrations of the sodium salts of deoxy-

cholate, cholate (Sigma London Chemical Company Ltd., Surrey) or taurocholate (Koch-Light Laboratories Ltd., Bucks.), and their purity was checked by thin-layer chromatography. Residual tonicity was made up with NaCl, the final Na^+ and Cl^- concentrations varying from 142–155 mM and 118–131 mM respectively. Deoxy-[^{14}C]cholate (International Chemical and Nuclear Corporation Tracerlab, Irvinne, Calif., U.S.A.) and [^{14}C]taurocholate and -cholate (Radiochemical Centre, Amersham, Bucks.) were added to solutions containing 1 mM deoxycholate, taurocholate or cholate in concentrations of 2.5 μCi/l; $^{22}Na^+$ (Radiochemical Centre, Amersham) was added in a concentration of 10 μCi/l.

Analytical methods

The initial solutions, effluent, and plasma were analysed for Na^+ by flame photometry, for Cl^- by coulometric titration, for glucose by the glucose oxidase method, for polyethylene glycol by the turbidometric method of Hyden [25], and for 3-*O*-methylglycose by the method of Somogyi [26]. For radioactive counting 0.5 ml of intestinal fluid was placed in 10 ml of scintillation fluid (1000 ml Toluene, 500 ml Triton X-100, containing 8 g PPO and 0.1 g POPOP per l) and counted in duplicate. ^{14}C-labelled tracers were counted in a Phillips automatic liquid scintillation analyser, and $^{22}Na^+$ in a gamma sample counter (GTL 300-500). The ^{14}C counts were obtained as dpm from computed quench curves whereas the $^{22}Na^+$ counts were uncorrected for quench or counting efficiency.

Mucosal ATPase assay

The assay was a modification of that reported by Quigley and Gotterer [11, 27] for rat small intestinal mucosa.

At the completion of the absorptive period of study the intact segment of intestine was gently rinsed with a cold (4 °C) solution of 5 mM EDTA in 0.9 % NaCl (pH 7.4–7.5). The animal was then killed, the segment of intestine removed and split longitudinally and washed in the 5 mM EDTA-saline solution. The mucosal surface was dried by gently blotting and a small amount of surface epithelium was scraped off with a spatula and weighed (25–50 mg). Mucosal scrapings were homogenised ($\times$30 strokes in PTFE homogeniser) in cold (4 °C) 5 mM EDTA (pH 7.4–7.5) as an 0.5 % solution. For assay of "total" ATPase, 2 ml Tris buffer (pH 7.1) containing 5 mM Na^+-free Tris · ATP (Sigma London Chemical Company Ltd., Surrey), 7.5 mM $MgCl_2$, 20 mM KCl, and 120 mM NaCl were incubated with 0.2 ml of the 0.5 % mucosal homogenate. For assay of Mg^{2+}-ATPase KCl and NaCl were excluded from the incubation medium.

The Tris · ATP was always added to the medium on the day of the assay because of its tendency to slow spontaneous hydrolysis. Enzyme controls were run with all assays, i.e. 0.2 ml boiled homogenate and 0.2 ml Tris buffer substituted for 0.2 ml unboiled homogenate. All control and test solutions were run in duplicate and incubated at 37 °C for 30 min in a shaking water bath. The reaction was stopped with 0.25 ml of 50 % trichloroacetic acid and the tubes were immediately centrifuged at 3000 rev./min for 10 min. Two 0.5-ml aliquots of supernatant were taken from each tube for determination of P_i by the method of Fiske and SubbaRow [28]. For zero time P_i determinations 0.25 ml of 50 % trichloroacetic acid was added immediately prior to the incubation period and kept at 4 °C during the 30-min period. Release of

1 mol of P_i during the incubation period was taken to represent enzymatic hydrolysis of 1 mol of ATP. Homogenate protein was determined by the method of Lowry et al. [29] using bovine serum albumin as a standard. ATPase specific activity is expressed as μmol of P_i released per mg protein per h, and $(Na^+ + K^+)$-ATPase is calculated as the difference between "total" and Mg^{2+}-ATPase. All purchased reagents were of the highest purity.

1 mM ouabain produced 100 % inhibition of $(Na^+ + K^+)$-ATPase without affecting Mg^{2+}-ATPase, indicating that the $(Na^+ + K^+)$-ATPase assay represented the ouabain-sensitive component of "total" ATPase.

The coefficient of variation for 5 determinations performed on the same mucosal homogenate was 5 % for "total" ATPase, 7 % for Mg^{2+}-ATPase, and 8 % for $(Na^+ + K^+)$-ATPase. Enzyme activity remained unchanged following storage of homogenate at −20 °C for up to 2 weeks, the enzyme catalysed reaction was linear over a 90-min incubation period at 37 °C, and the relationship between reaction velocity and enzyme concentration was linear.

Reaction velocities exhibited Michaelis-Menten kinetics for "total" ATPase, $(Na^+ + K^+)$-ATPase, and Mg^{2+}-ATPase. Lineweaver-Burke plots gave K_m values of 0.50, 0.48 and 0.59 mM respectively for "total" ATPase, $(Na^+ + K^+)$-ATPase, and Mg^{2+}-ATPase.

1 and 2.5 mM deoxycholate produced a 75 % inhibition of $(Na^+ + K^+)$-ATPase and at higher concentrations (> 5 mM) the inhibition was complete.

Mucosal disaccharidase Assays

Mucosal scrapings were obtained immediately following the absorptive periods, as described for the ATPase assays, and snap-frozen for subsequent assay. Samples were taken from within 5 cm proximal and distal to the entry catheter, from the midpoint of the jejunal segment, and from 5 cm proximal to the exit catheter, and are designated sites 1, 2, 3 and 4 respectively; thus mucosal disaccharidase activity at site 1 served as a control for activities at other sites for each individual animal. Lactase, sucrase and maltase were assayed by a modification of the method of Plotkin and Isselbacher [30]. The mucosal tissue was washed briefly in ice-cold 150 mM KCl, blotted to remove excess liquid and weighed. Tissue homogenate (1 %) was prepared in ice-cold 150 mM KCl using an all-glass hand-operated homogeniser. Disaccharide solutions (56 mM) were prepared in 100 mM maleate buffer adjusted to pH 5.8 for sucrose, pH 5.3, for lactose, and pH 6.3 for maltose (a second pH optimum at 5.3 was not assayed). The assay mixture contained buffered substrate (20 μl), and tissue homogenate (20 μl for lactase; 5 μl for sucrase or maltase) in a final volume of 40 μl. Blank tubes contained 150 mM KCl in place of homogenate. After incubation at 37 °C for 60 min, water (200 μl) was added and the tubes were placed in a boiling water bath for 2 min, after which they were cooled in ice water. Glucose liberated in the reaction mixture was measured by the glucose oxidase method. The maltase assay mixture only was further diluted by the addition of 1.4 ml of water and, after mixing, a portion (200 μl) was removed for glucose assay. After incubation with glucose oxidase reagent (400 μl) at 37 °C for 30 min, 10 M HCl (400 μl) was added to each tube to stop the reaction and produce a stable colour. After standing at room temperature for 10 min absorbance was measured at 530 nm. Disaccharidase activities are expressed as μmol substrate hydrolysed per g wet wt of gut per min.

Calculations

In the closed loops absorption of fluid was calculated by a weighing technique previously described [2, 24] and bidirectional fluxes of $^{22}Na^+$ were calculated from the formula of Berger and Steele [31]. In the perfusion studies, absorption rates of water and solute were calculated from standard formulae [32] using polyethylene glycol as a non absorbable marker, and in each study a mean value was obtained from the three 20-min collection periods which followed a 60-min period of equilibration. The mean $\pm$S.E. percentage recovery in 6 animals perfused with control solution was 104 ± 3, and for a similar number perfused with 1 mM deoxycholate recoveries was 97 ± 4, and these differences were not statistically significant ($P = 0.10$). The significance of differences between mean values was assessed by the Student t test, and all values are expressed as mean ±1 S.E. throughout the paper.

RESULTS

Closed loops

Transport. 2.5 mM deoxycholate produced a highly significant ($p < 0.001$) inhibition of 3-*O*-methyl glucose transport (17.60 ± 2.71 μmol per 10 min per g wet wt of gut) compared with control values (37.47 ± 3.14).

Mucosal ATPase. Table I shows the effects of 5 mM deoxycholate on mucosal ATPase activities. There was a highly significant inhibition of "total" ATPase ($P < 0.005$) due to a selective inactivation of $(Na^+ + K^+)$-ATPase ($P < 0.0025$).

TABLE I

EFFECTS OF DEOXYCHOLATE ON MUCOSAL ATPase ACTIVITY IN CLOSED LOOPS

All initial solutions contained 20 mM glucose. Numbers of observations shown in parentheses. Enzyme activity is expressed as μmol of P_i hydrolysed per h per mg protein. Mean values $\pm$ 1 S.E.

Initial solutions	ATPase activity		
	"Total" ATPase	$(Na^+ + K^+)$-ATPase	Mg^{2+}-ATPase
Control (6)	15.25 ± 0.43	4.67 ± 0.73	10.67 ± 0.38
5 mM deoxycholate (5)	11.60 ± 0.58	1.50 ± 0.45	10.20 ± 0.85

Comparison of luminal disappearance of [^{14}C]deoxycholate and [^{14}C]taurocholate. In 17 animals a mean of $60.5\pm2.9\%$ of [^{14}C]deoxycholate per g wet wt of gut disappeared from the lumen during the 25-min absorptive period; by comparison in 6 animals the value for [^{14}C]taurocholate was 4.3 ± 1.0.

Bidirectional movements of Na^+ (Table II). Net secretion of Na^+ into the lumen induced by 2.5 mM deoxycholate ($P < 0.001$) was due to reduced lumen to plasma flux ($P < 0.001$), plasma to lumen fluxes remaining unaffected compared with control values.

Perfusions

Transport. The effects of deoxycholate, cholate and taurocholate on jejunal transport of fluid, electrolytes and glucose are shown in Table III. In 10 animals

TABLE II

BIDIRECTIONAL MOVEMENTS OF Na^+ IN CLOSED LOOPS

All initial solutions contained 20 mM glucose. Values in μequiv per g wet wt of gut per 25 min. $n = 6$ throughout. Negative sign indicates net secretion into intestinal lumen. Mean values $\pm$ 1 S.E.

Initial solutions	Net transport	Lumen → plasma flux	Plasma → lumen flux
Control	100.10 ± 8.50	437.50 ± 15.30	337.37 ± 10.70
2.5 mM deoxycholate	7.39 ± 9.50	347.10 ± 7.80	354.49 ± 14.20

TABLE III

EFFECTS OF DIFFERENT BILE SALTS ON WATER, ELECTROLYTE AND GLUCOSE TRANSPORT IN PERFUSION STUDIES

All initial solutions contained 2 mM glucose. Transport rates given as units/min per g wet wt of gut. Negative sign indicates net secretion into intestinal lumen. Numbers of observations shown in parentheses. Mean values $\pm$ 1 S.E.

Initial solutions	Water (μl)	Glucose (μmol)	Na^+ (μequiv)	Cl^- (μequiv)
Control (7)	44.5 ± 5.5	0.25 ± 0.02	6.24 ± 0.69	6.00 ± 0.95
1 mM deoxycholate (5)	−11.8 ± 2.1	0.17 ± 0.03	−0.58 ± 0.36	1.04 ± 0.35
1 mM taurocholate (6)	48.3 ± 9.9	0.22 ± 0.03	7.13 ± 1.45	5.33 ± 1.24
Control (6)	31.0 ± 3.2	0.34 ± 0.01	4.44 ± 0.57	2.19 ± 0.46
1 mM cholate (5)	18.5 ± 6.7	0.38 ± 0.02	4.39 ± 1.29	0.99 ± 1.16

plasma glucose was determined on material obtained by cardiac puncture immediately after the perfusion studies, and the mean value was 3.85 ± 0.35 mmol/l. Absorption of glucose from the initial solutions containing a 2-mM concentration was thus assumed to be an active process. The cholate perfusion studies were performed several months after the deoxycholate and taurocholate experiments and, because of seasonal variations in biological processes [3], two sets of control studies were performed.

1 mM deoxycholate induced net secretion of water ($P < 0.001$), Na^+ ($P < 0.001$) and Cl^- ($P < 0.001$) transport and inhibited active glucose absorption ($P < 0.05$), whilst the other two bile salts had no effect on transport.

Mucosal ATPase. Table IV shows the effects of deoxycholate, cholate and taurocholate on mucosal ATPase activities; enzyme determinations were performed in the same animals from which the transport data shown in Table III was obtained.

1 mM deoxycholate produced a highly significant inhibition of "total" ATPase ($P < 0.0005$), $(Na^+ + K^+)$-ATPase ($P < 0.0005$), and Mg^{2+}-ATPase ($P < 0.0025$). Although there was no statistical difference between the inhibition of $(Na^+ + K^+)$-ATPase produced by 1 and 5 mM deoxycholate, in 2 of the 6 animals studied the higher concentration of bile salt produced a 100% inhibition of enzyme activity. "Total" ATPase activity was no different to control values in the 5 mM deoxycholate perfusions because of the stimulation ($P < 0.0125$) of Mg^{2+}-ATPase observed at this higher concentration of bile salt. Except for inhibition ($P < 0.025$) of Mg^{2+}-ATPase by 1 mM taurocholate, the other two bile salts had no effect on enzyme activities.

TABLE IV

EFFECTS OF DIFFERENT BILE SALTS ON MUCOSAL ATPase ACTIVITY IN PERFUSION STUDIES

Enzyme activity expressed as μmol of P_i hydrolysed per h per mg protein. Numbers of observations shown in parentheses. Mean value $\pm$ 1 S.E.

Initial solutions	ATPase activity		
	"Total" ATPase	$(Na^+ + K^+)$-ATPase	Mg^{2+}-ATPase
Control (7)	13.00±1.64	6.22±1.13	6.79±0.74
5 mM deoxycholate* (6)	12.50±2.00	1.59±0.69	11.50±1.86
Control** (7)	13.36±1.35	5.22±0.76	8.15±0.70
1 mM deoxycholate** (10)	7.90±0.50	2.35±0.27	5.55±0.40
1 mM taurocholate** (6)	11.00±0.34	4.92±0.42	6.09±0.24
Control** (5)	14.70±0.75	5.40±0.29	9.30±0.65
1 mM cholate** (5)	13.40±1.34	5.30±1.00	8.10±0.56

* Initial solutions contained 20 mM glucose.
** Initial solutions contained 2 mM glucose.

There was no difference in ATPase activities between initial control solutions containing 20 mM glucose compared with those containing 2 mM glucose.

Luminal disappearance of deoxy[^{14}C]cholate, cholate and taurocholate. Mean disappearance rates of deoxy[^{14}C]cholate, cholate and taurocholate from the intestinal lumen were 50.0±3.6, 30.0±4.0, and 17.7±5.8 % g wet wt gut per min in 12, 5 and 4 animals respectively. The differences between disappearance rates of deoxycholate and taurocholate ($P < 0.001$), and deoxycholate and cholate ($P < 0.01$) were significant.

TABLE V

EFFECT OF DIFFERENT BILE SALTS ON MUCOSAL DISACCHARIDASE ACTIVITY IN PERFUSION STUDIES

All initial solutions contained 2 mM glucose and 1 mM bile salt. Enzyme activity is expressed as μmol disaccharide hydrolysed per min per g wet wt of gut. Enzyme assay sites are as described in methodology section.

Initial solutions and enzyme assay sites		Disaccharidase activity		
		Lactase	Maltase	Sucrase
Site 1	Control (7)	1.77±0.38	27.56±4.39	6.87±2.09
	Deoxycholate (5)	1.66±0.39	30.12±4.20	7.40±1.43
	Taurocholate (6)	1.30±0.29	26.53±2.72	6.70±0.73
Site 2	Control (6)	2.02±0.17	29.80±3.14	7.30±1.18
	Deoxycholate (6)	1.35±0.43	23.83±4.01	6.32±1.12
	Taurocholate (6)	1.23±0.34	28.18±3.13	7.10±1.05
Site 3	Control (6)	2.02±0.15	33.18±4.17	9.43±0.84
	Deoxycholate (6)	1.25±0.26	27.65±3.88	7.58±1.18
	Taurocholate (6)	1.45±0.44	35.60±4.25	8.63±1.29
Site 4	Control (6)	2.22±0.19	32.78±3.82	9.43±1.79
	Deoxycholate (6)	2.15±0.44	34.48±0.99	8.45±0.43
	Taurocholate (6)	1.25±0.36	29.17±3.73	7.15±0.97

Mucosal disaccharidases. The effects of 1 mM deoxycholate and taurocholate on the 3 disaccharidase activities at the 4 different jejunal sites are shown in Table V. The difference between lactase activities in control and deoxycholate perfused animals at site 3 was just significant ($0.02 < P < 0.05$), as was the difference between lactase activities in control and taurocholate perfused animals at site 4 ($0.02 < P < 0.05$): there was no difference between values obtained from any of the other sites compared with control values. It is unlikely that these 2 differences are due to bile salt induced brush border enzyme inhibition, since inhibition would also have been expected at more proximal sites where higher intraluminal bile salt concentrations would be present. For example, in 6 perfusion studies the concentration of deoxy[^{14}C]cholate in the final effluent solution was 1901.0 ± 62.4 compared with a concentration of 3936.0 dpm/ml in the initial solution ($P < 0.001$).

Potential difference (PD). In 5 studies with control solution containing 20 mM glucose PD was 5.5 ± 0.4 mV, compared with values of 3.2 ± 0.2 in 3 studies with control solution not containing glucose ($P < 0.01$). In 7 studies 1 mM deoxycholate (with 20 mM glucose) significantly reduced ($P < 0.01$) PD to 3.8 ± 0.3 mV. Since there was no difference ($P > 0.1$) in PD between controls without glucose and 1 mM deoxycholate, the inhibitory effect of this concentration of deoxycholate may have been due to a glucose-dependent component of transmural PD.

DISCUSSION

Using both closed-loop and perfusion techniques deoxycholate was shown to inhibit jejunal ($Na^+ + K^+$)-ATPase, and in the closed-loop studies the inhibition was selective, Mg^{2+}-ATPase being unaffected; in this model the selective action of deoxycholate was analogous to the in vitro effects of the metabolic inhibitor ouabain. In the perfusion studies low (1 mM) concentration of deoxycholate inhibited both ($Na^+ + K^+$)-ATPase and Mg^{2+}-ATPase, whereas at higher (5 mM) concentrations Mg^{2+}-ATPase was stimulated. Hepner and Hofmann [22] showed 5 mM deoxycholate to stimulate Mg^{2+}-ATPase of rat small intestine in vitro, but no similar effect has previously been reported for any biological tissue in vivo.

Since approximately 85 % of the cellular pool of ($Na^+ + K^+$)-ATPase is bound to the lateral and serosal membranes and has a K_i for ouabain which is four times greater than the brush border enzyme [11, 12], it seems likely that the predominant fraction of inhibited ($Na^+ + K^+$)-ATPase in our studies was lateral and serosal membrane bound. This is in keeping with the observations of Hepner and Hofmann [22] who found deoxycholate had no effect on brush border ($Na^+ + K^+$)-ATPase but inhibited the enzyme in a membrane fraction of rat mucosal homogenate; the same workers were also unable to demonstrate an effect by 5 mM deoxycholate on brush border alkaline phosphatase. Similarly we found deoxycholate to have no definitive effect on brush border disaccharidases.

In the perfusion studies, 1 mM deoxycholate induced net secretion of water, Na^+ and Cl^-, and inhibited active glucose transport whilst simultaneously inactivating mucosal ($Na^+ + K^+$)-ATPase. Since the reduced rate of luminal disappearance of glucose induced by deoxycholate might have reflected changes in the rate of glucose metabolism rather than changes in transport capability, the effects of the bile salt on luminal disappearance of a non-metabolizable sugar which shares the same transport

system as glucose were examined. Deoxycholate produced a highly significant inhibition of 3-*O*-methylglucose disappearance indicating an effect on the transport system. The transmural potential difference which exists across the small intestine is considered to be largely dependent on the electrogenic transport of sodium, and this process is closely linked to active sugar and amino acid absorption [33]. The finding that 1 mM deoxycholate induced net secretion of Na^+ yet only partially inhibited the transmural potential difference may indicate that in rat jejunum maintenance of the potential difference is not completely dependent on Na^+. This concentration (1 mM) of deoxycholate did not produce mucosal abnormalities on light microscopy which is in agreement with previous reports in the rat [24, 34] and hamster [3] jejunum; Shiner [34] was also unable to demonstrate convincing changes on electron microscopy. In contrast to deoxycholate, neither cholate nor its taurine conjugate had an effect on jejunal transport or mucosal $(Na^+ + K^+)$-ATPase.

At least two explanations exist for the observed inhibition of sodium and glucose transport by 1 mM deoxycholate. Firstly, there is an effect on the coupling of these two solutes at the carrier level of the brush border membrane. The concentration of deoxycholate used produces no epithelial cell abnormalities on light or electron microscopy, and had no effect on brush border marker enzymes. Secondly, like ouabain deoxycholate may act as a metabolic inhibitor inactivating $(Na^+ + K^+)$-ATPase and the "sodium pump", dissipating the sodium gradient, and inhibiting active sodium-dependent solute transport. The findings of reduced lumen to plasma Na^+ fluxes, and normal plasma to lumen fluxes are consistent with deoxycholate inhibition of active sodium extrusion out of the cell, and similar flux data have been reported by Mekhjian and Phillips [35] in canine colon perfused with 10 mM deoxycholate or chenodeoxycholate.

Inhibition of sodium-coupled transport by inactivation of $(Na^+ + K^+)$-ATPase presupposes cellular penetration of the inactivating substance. Absorption of bile salts in the jejunum is an entirely passive process, and since passive non-ionic diffusion is considerably more rapid than ionic diffusion [36] intraluminal H^+ ion concentration will be an important determinant of the relative rates of bile salt absorption. In the present study the pH of the perfused solutions was 7.4, and at this pH a proportion of the deoxycholate and cholate molecules are unionised, whereas taurocholate which has a much lower p*K*a (2.0) is highly ionised. Thus, there were large differences in luminal disappearance rates of deoxy[^{11}C]cholate compared with the taurocholate isotope. Disappearance rates of cholate were significantly less than those of deoxycholate and these differences may reflect the greater polarity of the trihydroxy bile salt. The finding that cholate and taurocholate had no effect on $(Na^+ + K^+)$-ATPase may therefore be due to their relatively impaired capacity to penetrate the cell, or alternatively enzyme inhibition may require a molecular specificity from the inhibiting substance. Okishio and Nair [37] showed that within the cell relatively more deoxycholate is bound to organelle membranes compared with other bile salts, and a greater affinity for deoxycholate binding to the membrane-bound $(Na^+ + K^+)$-ATPase may be another factor involved in the selective action of deoxycholate observed in the present study. The location(s) of deoxycholate-induced inhibition of sodium and glucose transport requires further clarification, but our results suggest that inactivation of membrane bound $(Na^+ + K^+)$-ATPase may be important, and that an effect on coupling of the two solutes at the carrier site is less likely.

The results of the present study suggest that the inhibitory effects of deoxycholate on active jejunal transport processes are related to the concomitant inactivation of $(Na^+ + K^+)$-ATPase. $(Na^+ + K^+)$-ATPase may well play a fundamental role in the physiology of intestinal transport, and inhibitory substances may be important in the pathophysiology of certain types of diarrhoea.

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