Interaction of uncharged bile salt derivatives with the ileal bile salt transport system

Ralph Bundy,[1] Josephine Mauskopf, James T. Walker, and Leon Lack[2]

Department of Physiology and Pharmacology, Duke University Medical Center, Durham, NC 27710

Abstract Two series of uncharged conjugated bile salt derivatives, *N*-conjugates of ethanolamine and 3-amino-1,2-propanediol were studied for interaction with the ileal bile salt transport system. Evidence for interaction is threefold. *1*) In everted gut sac experiments more material was removed from the mucosal compartment when ileal sacs were used. *2*) These derivatives inhibited the in vitro transport of taurocholate. *3*) In vivo intestinal perfusion demonstrated greater absorption from ileum than from jejunum. Number three demonstrates that such interactions are followed by transmucosal movement. Their uphill transport was less than taurocholate transport. The Na^+ requirement for cholyl-3-amino-1,2-propanediol interaction with the system was greater than for taurocholate. This observation is similar to that previously observed with taurodehydrocholate, which had a greater Na^+ requirement for transport than taurocholate. Therefore removal of the anionic charge, as well as distortion of steroid shape, increases the Na^+ requirement for substrate interaction with the transport system. These observations support our hypothesis that this interaction involves two recognition components; one includes the steroid moiety, the other a coulombic interaction between the anionic bile salt and a cationic membrane site. Additionally the membrane would have an anionic group to accomodate the Na^+. Both factors (steroidal and coulombic) operate for optimal substrate attachment. Simultaneously the system's affinity for Na^+ increases and active transport then proceeds.

Supplementary key words substrate–transport system interactions

Previous structure–activity studies of the ileal bile salt transport system suggested that the single negative charge located on the side chain was necessary for maximal uphill transport and perhaps essential for any transport. Bile salts modified to bear a quaternary cationic charge in the side chain were not transported against their own concentrations by everted gut sacs; however, interaction with the transport system was evidenced by their ability to inhibit the active transport of conventional (anionic) bile salts, both in vitro and in vivo (1). When the charged state of the side chain was modified to bear both a positive and a negative charge and was tested for transport using ileal sacs, active transport was not observed. Once again, however, interactions with the transport system could be demonstrated by inhibition studies (2). Bile salts modified to bear to potential negative charges on the side chain were minimally transported (3), suggesting that the ileum transported those singly charged molecules that were in equilibrium with the doubly charged species. In agreement with this concept was the finding that lowering the pH of the medium enhanced the transport of these dibasic compounds both in vivo and in vitro. It was also found that these dibasic bile salt derivatives were more effective inhibitors of regular bile salts in media of lower pH (4, 5). These observations allowed us to speculate that the site of attachment of the regular substrate contains (in addition to a recognition component for the steroid moiety) a positive charge for coulombic interaction with the anionic substrate and an associated negative charge that could repel bile salt derivatives containing a double negative charge in the side chain region. We further speculated that this anionic site could interact with Na^+ ions (see reference 5).

These suggestions have prompted our preparation and study of uncharged bile salt analogues. It was anticipated that interaction of these uncharged compounds with the transport system could take place by virtue of the potential for interaction of the steroid portion of the substrate with the steroid recognition site of the membrane. Uphill translocation would be expected to be drastically curtailed, if not abolished, because the normal coulombic interaction between the anionic substrate and the hypothetical cationic region of the membrane cannot take place. In addition, the requirements of these interactions

Abbreviations: DMSO, dimethyl sulfoxide; cholyl NET, *N*-cholylethanolamine; cheno NET, *N*-chenodeoxycholylethanolamine; dehydrocholyl NET, *N*-dehydrocholylethanolamine; cholyl NPG, *N*-cholyl-3-amino-1,2-propanediol; cheno NPG, *N*-chenodeoxycholyl-3-amino-1,2-propanediol.

[1] Dr. Ralph Bundy's present address is: Triplex Army Medical Center, Honolulu, Hawaii.

[2] Please send reprint requests to: Leon Lack, Box 3185, Duke University Medical Center, Durham, NC 27710.

for Na^+ ions would be expected to be greater than when the natural congeners were used, since the binding of natural bile salts and Na^+ ions appears to be cooperative (6). The present paper describes the results of such investigations.

MATERIALS

Cholic acid, dehydrocholic acid, chenodeoxycholic acid, and deoxycholic acid were purified by recrystallization prior to their being used to prepare conjugates. 24-^{14}C-Labeled unconjugated bile acids and [1,2-^{14}C]taurine were purchased from New England Nuclear Corporation, Boston, MA. EEDQ (*N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinone), ethanolamine, and 3-amino-1,2-propanediol were purchased from the Aldrich Chemical Company, Milwaukee, WI. The 3-amino-1,2-propanediol was purified prior to use by vacuum distillation. The analytical grade mixed bed resin AG 501-X8 was obtained from Bio Rad Laboratories, Richmond, CA.

Taurine-conjugated bile salts were prepared and purified by the procedure previously described (7). The procedure for conjugating bile acids to the nitrogen atom of ethanolamine and 3-amino-1,2-propanediol is described as follows. One mmol of the free bile acid and 1 mmol of ethanolamine or the amino propanediol together with 1.3 mmol of EEDQ were dissolved in 34 ml of 95% ethanol and boiled under reflux for 90 min. After adding an equal volume of water, sufficient 1 N HCl was added to bring the final acid concentration to 0.1 N. This solution was passed through a mixed bed ion exchange column (15 × 2 cm) that had previously been equilibrated with 50% ethanol. The resin was washed with two bed volumes of 50% ethanol and the washings were then combined with the original (neutral) effluent. Sufficient 1 N NaOH was added to give a final concentration of 0.1 N and the solution was passed through a second mixed bed ion exchange column in a similar manner. This procedure removes charged molecules originally present. The neutral alcoholic solution was evaporated to dryness with a rotary evaporator.

N-cholylethanolamine was dissolved in a minimum amount of hot ethanol and crystallized by the addition of excess ethyl acetate. *N*-chenodeoxycholylethanolamine and *N*-dehydrocholylethanolamine were recrystallized from hot ethyl acetate. *N*-cholyl-3-amino-1,2-propanediol was dissolved in a minimum amount of hot ethanol and crystallized by the addition of ether. *N*-chenodeoxycholyl-3-amino-1,2-propanediol was dissolved in a minimum amount of hot ethanol and crystallized by the addition of excess ethyl acetate.

The overall yields at this point were in excess of 60%. Final purification employed the reverse phase column chromatographic procedure of Norman (8) and utilized his solvent system designated as solvent system C (8). These materials were pure when tested by thin-layer chromatography following the procedure of Hofmann (9), employing solvent system 2. Infrared spectra were consistent with the presence of peptide bonds with absorption bands at 6.15 and 6.5 microns.

N-cholylethanolamine (cholyl NET) mp 250–253°C.
Theory: C, 69.14; H, 10.04; N, 3.1%.
Found: C, 68.25; H, 9.75; N, 3.0%.

N-chenodeoxycholylethanolamine (cheno NET) mp 95–98°C.
Theory: C, 71.68; H, 1041; N, 3.2%.
Found: C, 70.79; H, 1042; N, 3.4%.

N-dehydrocholylethanolamine (dehydrocholyl NET) mp 212–214°C.
Theory: C, 70.08; H, 8.82; N, 3.16%.
Found: C, 70.19; H, 9.50; N, 2.91%.

N-cholyl-3-amino-1,2-propanediol (cholyl NPG) mp 118–125°C.
Theory: C, 67.33; H, 9.84; N, 2.9%.
Found: C, 66.01; H, 9.77; N, 2.7%.

N - chenodeoxycholyl - 3 - amino - 1,2 - propanediol (cheno NPG) mp 100–103.5°C.
Theory: C, 69.64; H, 10.17; N, 3.0%.
Found: C, 68.51; H, 10.20; N, 2.8%.

TRANSPORT STUDIES

The everted gut sac preparation of Wilson and Wiseman (10) was used for all in vitro studies. These sacs were made from the small intestines taken from young, fasted guinea pigs of the Hartley Strain. Three major types of experiments were performed. *1*) To ascertain whether uncharged bile salt derivatives were actively transported by the ileal bile salt transport system, everted gut sacs 9 cm long were prepared from the midportion of each quarter of the small bowel. The concentration of ^{14}C-labeled bile salts and ^{14}C-labeled bile salt derivatives in the mucosal and serosal compartments at the end of the incubations were determined in a Beckman liquid scintillation counter Model LS 150 equipped with an external standard (Beckman Instruments, Fullerton, CA). Initially the substrate was present in equal concentrations in the serosal and mucosal compartments.

In this type of preparation, evidence for transport of solute against its concentration is the generation of a final serosal to mucosal concentration ratio in excess of unity. In addition, particularly when serosal to mucosal ratios in excess of unity were not encountered, interaction of these derivatives with the ileal system can be evidenced when significantly more material leaves the mucosal compartments in incubations employing distal gut sacs compared to those employing proximal tissues.

2) In determining whether any of the uncharged bile salt derivatives could inhibit the transport of regular substrates of the active bile salt transport system, gut sacs were prepared from the distal ileum only (segment 4). Each ileum provided four 9 cm sacs. Four animals were used for each experiment and the sacs were staggered in the previously described manner in order to obviate differences among animals (3). This procedure permits studying the inhibitory effectiveness at three concentrations.

In these experiments the substrate was present as the radioactive compound while the inhibitor contained no radioactive label. Inhibitions of active transport in such in vitro preparations can be demonstratd by depression of final serosal to mucosal concentration ratios. The degree of depression of bile salt removal from the mucosal compartment is used to quantitate inhibition and permits the determination of the relative order of inhibitory potency of these compounds. In these experiments one must consider the possibility of volume changes of the fluid remaining on the mucosal side. When such measurements were made in comparable incubations (11), volume changes were found to be sufficiently low to allow the assumption of constancy (10 ml) for all calculations.

In these experiments dimethyl sulfoxide (DMSO) was needed to solubilize the derivatives. The compounds were dissolved in DMSO and added to the incubation media to give the specified concentrations. The final concentrations of DMSO necessary to insure solubility varied (between 1% and 3%) with the specific derivative used and is specified in the descriptions of experimental conditions. All control solutions, i.e., those with taurocholate only, had the same amount of DMSO. Previous work (1) has demonstrated that DMSO is without effect on bile salt transport when studied under these in vitro conditions.

3) The effects of lowered Na^+ ion concentration in the incubation medium in altering the ability of the ileal gut sacs to remove substrate from the mucosal compartment was tested. In these experiments mannitol was used as the isosmotic replacement for sodium ions.

In vivo intestinal perfusion studies were performed with guinea pigs and employed the apparatus described by Heaton and Lack (12).

RESULTS

Fig. 1 depicts the results of a series of experiments whose purpose was to ascertain whether or not these uncharged bile salt analogues could be transported by the everted gut sacs. Each bar represents the mean of four incubations. With three of the compounds, cholyl NET, cheno NET, and cheno NPG, serosal to mucosal ratios in excess of unity were never observed. Ileal gut sac incubations with cholyl NPG demonstrated a real tendency to establish concentration gradients in excess of 1. Nevertheless, in all situations involving these uncharged compounds, significantly more material was taken up from the mucosal compartment when incubations were performed with tissue taken from the distal small bowel (Fig. 1). Furthermore, tissue uptake of these substances was significantly greater from the mucosal side than from the serosal side when incubations were carried out with the distal (ileal) gut sacs. In the case of cholyl NPG, the final concentrations

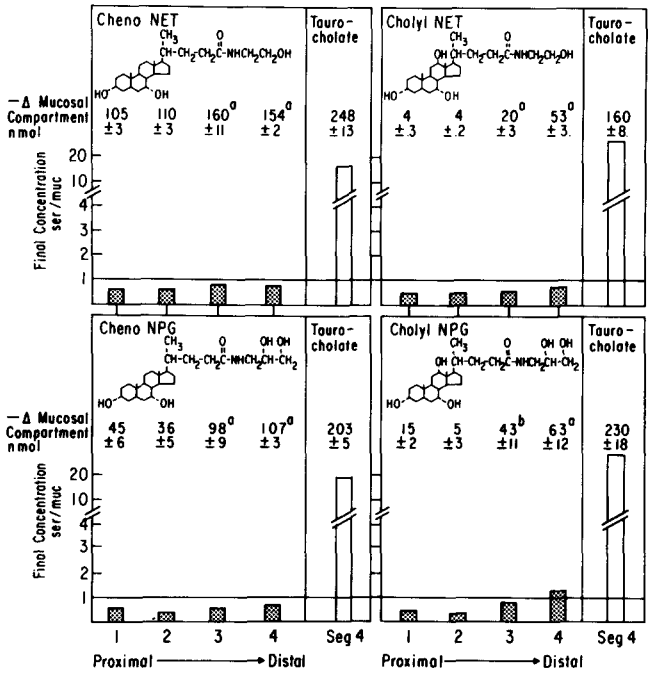

Fig. 1. Incubations of everted gut sacs from different regions of the small bowel with uncharged bile salt derivatives. Initial concentration 35 nmol per ml in both the serosal and mucosal compartments. Each bar represents the average of four sacs. Also shown is the amount ± SEM of material removed from the mucosal compartment. DMSO was present as follows: cheno NET, cholyl NET, and their respective controls, 3%; cheno NPG, cholyl NPG, and their respective controls, 1%. Time of incubation 90 min, temperature 37°C, gas phase 95% O_2, 5% CO_2.

in serosal compartments were greater than those that were present initially.

Also shown, for the purpose of comparison, is the transport of sodium taurocholate by ileal tissue obtained from the fourth quarter of the small bowel adjacent to the region used to prepare those sacs numbered 4.

These substances did not undergo chemical modification by the mucosal cells of the ileum. This was determined as follows. Ileal gut sacs were incubated with the various compounds labeled with ^{14}C at the 24-position of the steroid moiety. Initially substrate was present only in the mucosal compartment. At the end of the 90 min incubation periods, the fluid from the serosal compartments was lyophilized and taken up in a volume of ethanol equal to the initial volume, filtered, and then reduced to approximately 1/10 volume. These were analyzed by thin-layer chromatography. In all cases (cholyl NET, cholyl NPG, cheno NET, cheno NPG) radioactivity migrated as the original substance.

Table 1 presents the data from experiments designed to ascertain whether these compounds can inhibit the active transport of taurocholate. It can be seen that the ethanolamine, as well as the amino propyl conjugates of cholic acid and chenodeoxycholate, do have the propensity for such inhibition. The triketo analogue of cholyl NET, i.e., dehydrocholyl NET, is without such effect. The dihydroxylated compounds are better inhibitors than their respective analogues with three hydroxy groups.

The data of **Table 2** demonstrate that cholyl NPG has no significant inhibitory effect on the transport of glucose by jejunal tissue. However, ileal transport of glucose is compromised somewhat by the presence of this bile salt analogue, although to a lesser extent than the transport of taurocholate (compare with Table 1, experiment 4).

Fig. 2 demonstrates that when cholyl NPG and cheno NPG are injected via a mesenteric vein into guinea pigs the material is extracted from the blood by the liver and excreted into the bile. Although

TABLE 1. Inhibition of in vitro transport of taurocholate by uncharged bile salt derivatives

Expt No.	Initial Concentration μmol/ml		Substrate Transported		
	Substrate	Inhibitor	Final Serosal/Mucosal Ratio	μmol Transported Mean ± SEM[a]	% Control
1	Taurocholate	Cholyl NET			
	0.37	0	6.3	1.67 ± 0.19	100
	0.37	0.055	5.7	1.27 ± 0.24[b]	76
	0.37	0.11	3.7	1.15 ± 0.20[c]	67
	0.37	0.22	3.6	1.09 ± 0.11[c]	65
2	Taurocholate	Cheno NET			
	0.37	0	5.3	1.66 ± 0.19	100
	0.37	0.055	3.9	1.33 ± 0.20[c]	80
	0.37	0.11	3.1	1.08 ± 0.17[d]	65
	0.37	0.22	2.2	0.73 ± 0.14[d]	44
3	Taurocholate	Dehydrocholyl NET			
	0.37	0	7.3	2.10 ± 0.09	100
	0.37	0.055	6.2	1.96 ± 0.15[ns]	93
	0.37	0.11	5.8	1.72 ± 0.25[ns]	82
	0.37	0.22	6.3	1.98 ± 0.12[ns]	94
4	Taurocholate	Cholyl NPG			
	0.37	0	5.5	1.70 ± 0.16	100
	0.37	0.22	4.8	1.50 ± 0.14[ns]	88
	0.37	0.44	3.8	1.30 ± 0.07[b]	76
	0.37	0.83	2.5	0.80 ± 0.11[b]	47
5	Taurocholate	Cheno NPG			
	0.37	0	5.4	1.76 ± 0.16	100
	0.37	0.054	3.6	1.27 ± 0.11[c]	72
	0.37	0.11	3.3	1.22 ± 0.09[c]	69
	0.37	0.214	2.2	0.69 ± 0.05[c]	39

[a] Each value is the mean ± SEM of four gut sacs. Incubations were for 60 min at 37°C. Initial volumes: mucosal fluid 10 ml, serosal fluid 1.5 ml. DMSO present: experiment 1, 1%; experiment 2, 3%; experiment 4, 1%; experiment 5, 3%.

[b,c,d] Significantly different from control incubations: *b*, $P < 0.05$; *c*, $P < 0.02$; *d*, $P < 0.001$; paired *t* test.

[ns] Not significantly different from control.

TABLE 2. Effect of *N*-cholyl-3-amino-1,2-propanediol on the in vitro transport of glucose

Expt No.	Tissue	Initial Concentration		Substrate Transported Mean ± SEM[a]	% Control Activity
		Substrate Glucose	Inhibitor Cholyl NPG		
		μmol/ml		*μmol transported*	
1	Jejunum	5.5	0	20.4 ± 1.9	100
		5.5	0.21	21.9 ± 2.4 ns	108
		5.5	0.42	20.0 ± 2.1 ns	98
		5.5	0.84	18.6 ± 1.5 ns	91
2	Ileum	5.5	0	27.1 ± 1.7	100
		5.5	0.21	23.4 ± 2.2 ns	86
		5.5	0.42	22.8 ± 1.7[b]	84
		5.5	0.84	16.8 ± 0.87[c]	62

[a] Each value is the mean ± SEM of four gut sacs. Incubations were for 60 min at 37°C. Initial volume of mucosal fluid 10 ml, serosal fluid 1.5 ml, DMSO present, final concentration 1%.

Results were either not significantly different from control values; [b] significantly different from controls by $P < 0.05$; [c] significantly different from controls by $P < 0.02$; values obtained using the paired Student *t* test.

greater than 80% of the radioactivity is recoverable from the bile, thin-layer chromatographic analysis of the bile indicates that more than half of the radioactivity migrates as a band more polar than the original material, indicating some degree of hepatic alteration. Nevertheless, these observations did allow us to use the guinea pig as an in vivo model to ascertain the degree of absorption of such compounds from the proximal and distal regions of the small bowel. **Fig. 3** depicts four such representative experiments in which cholyl NPG and cheno NPG were perfused through proximal and distal segments of the small bowel.

Transmucosal movement of the radioactive substrate was monitored by measuring the total amount of radioactive material recovered in the bile via a common bile duct fistula. By this criterion, significantly more material was absorbed from the distal region than from the proximal small gut. **Table 3** summarizes the results of 20 such in vivo perfusion experiments. The results were given as nanomoles of substrate absorbed per gram dry weight of the perfused intestinal segment. Absorption from the distal region is 4 to 25-fold greater than is absorption from the proximal region.

Table 4, which summarizes the results of 48 ileal everted gut sac incubations, demonstrates that the removal of cholyl NPG from the mucosal space is dependent on the presence of Na^+ ions. Tissue uptake of cholyl NPG is significantly more dependent on the presence of Na^+ than is the comparable uptake of taurocholate.

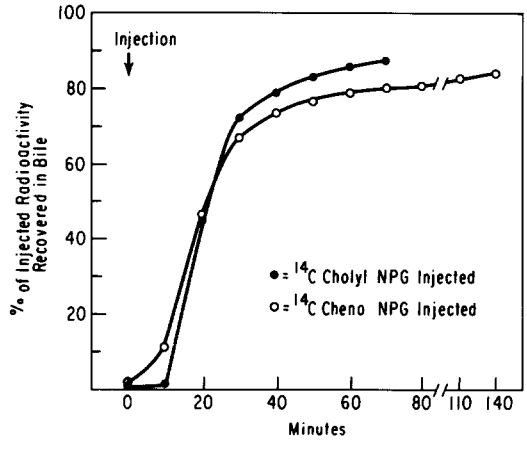

Fig. 2. Hepatic clearance of radioactivity following injection of [^{14}C]cholyl NPG and [^{14}C]cheno NPG in the mesenteric vein of guinea pigs with a common bile duct fistula. 0.4 μmol [^{14}C]cholyl NPG injected, 0.25 μmol cheno NPG injected.

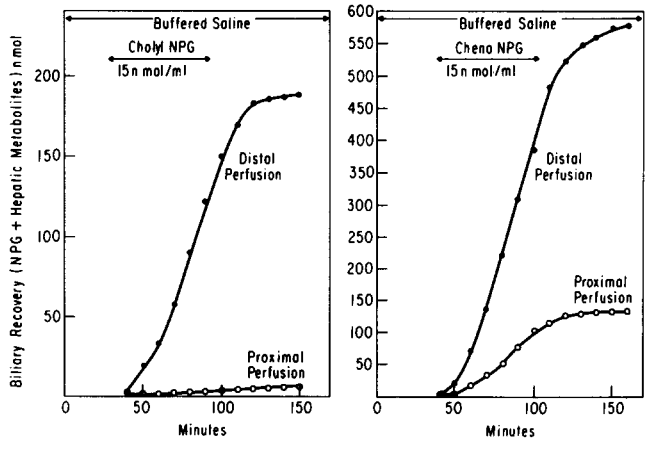

Fig. 3. Recovery of cheno NPG and cholyl NPG and their respective hepatic metabolites following perfusion through distal and proximal regions of the small bowel. Perfusion rate was 3.0 ml per min. Saline was buffered with 0.01 M sodium phosphate buffer, pH 7.

TABLE 3. Intestinal absorption of uncharged bile salt derivatives from different regions of the small bowel of guinea pigs[a]

Substrate	Expt No.	nmol Absorbed per g Dry Weight of Perfused Intestine Proximal Perfusion	Expt No.	nmol Absorbed per g Dry Weight of Perfused Intestine Distal Perfusion
Cholyl NPG	1	7.6	2	217.5
	3	15.7	4	391.2
	5	14.0	6	364.4
		12.4 ± 2.5		324.4 ± 54[b]
Cheno NPG	7	166.5	8	837.1
	9	177.8	10	847.0
	11	166.6	12	753.5
		170.3 ± 3.8		812.5 ± 30[b]
Cholyl NET	13	19.1	14	148.9
	15	9.0	16	130.1
	17	12.8	18	271.2
	19	23.0	20	240.6
		16.0 ± 3.1		197.7 ± 34[b]

[a] Absorption is assessed by the amount of material (substrate + hepatic metabolites) appearing in the bile following intestinal perfusion. Conditions are those described in Fig. 3. The absorption means ± SEM of each substrate are also shown.

[b] Absorption from distal region is significantly different from proximal absorption $P < 0.003$.

DISCUSSION

The in vitro and in vivo experiments were designed to ascertain whether natural bile salts, modified in a manner to remove the anionic charge on the side chain, retain some propensity for interaction with the ileal bile salt transport system. Three lines of evidence indicate that this does indeed occur. The demonstration that everted gut sacs taken from the ileum remove significantly more neutral bile salt derivatives than do jejunal sacs would indicate that some interaction between these substances and the transport system takes place. The in vivo studies demonstrating that greater absorption takes place from the ileal region are also in accord with the idea that interaction takes place, and that such interaction allows for a specific transmucosal movement of these substances. Finally, these neutral materials have the capacity to inhibit the in vitro active transport of taurocholate, suggesting that the site of interaction is the same one occupied during the transport of the naturally occurring substrate. It might also be noted that the ability of the dihydroxy derivative to inhibit is greater than that of its respective trihydroxylated compound, while the triketo compound did not exhibit inhibitory potential. In this regard these compounds demonstrate the same relative structure–activity relationship that was observed in mutual inhibition studies employing conventional bile salts (3) (12).

TABLE 4. Sodium requirements for transport of cholyl NPG by ileal gut sacs[a]

Expt No.	Substrate	Initial Concentration	Na	K	nmol[a] Transported Mean ± SEM	% Inhibition
		nmol/ml	*mEq/l*	*mEq/l*		
1	Cholyl NPG	31	145	8.8	77 ± 6	0
		31	90	8.5	61 ± 3[c]	21
		31	60	8.5	43 ± 4[d]	44
		31	30	8.5	9.2 ± 2[d]	88
2	Taurocholate	28	145	8.0	209 ± 11	0
		28	80	8.0	208 ± 2[ns]	1
		28	55	7.0	194 ± 5[ns]	7
		28	28	7.0	161 ± 10[c]	23

[a] Each value for experiment 1 represents the mean of 12 gut sacs. The values for experiment 2 represent the mean of 4 gut sacs. Incubations were for 90 min at 37°C.

[b] Removed from mucosal compartment.

[c] Different from control, P 0.05.

[d] Different from control P 0.001.

In addition, the pattern of inhibition of glucose transport (Table 2) by one of these compounds, cholyl NPG, follows a pattern similar to that observed for the inhibition of glucose transport by the naturally occurring anionic bile salts. Previous work demonstrated that the jejunal transport of glucose was not inhibited by bile salts. However, it was shown that ileal glucose transport was significantly compromised by these substances. The difference in sensitivity of the two regions of the intestine is probably attributable to the fact that these substances are concentrated within the cells that transport them and elicit an intracellular detergent effect (3, 12).

Uphill transport, as evidenced by the ability of ileal tissue to develop a serosal to mucosal concentration ratio in excess of unity, was seriously compromised when the negative charge was removed. Evidence for such uphill transport was observed in only one instance, that for cholyl NPG, and this was only a small fraction of that observed with the natural analogue, taurocholate. Our previous structure–activity studies implicating a critical role of the anionic group of the bile salt substrate for uphill transport would appear to require modification, in that at least one uncharged substance can also be transported in this manner but at a drastically reduced rate. When transport of this substance was studied at lower Na^+ ion concentrations, it was found that cholyl NPG was relatively more dependent on the presence of Na^+ ions than was taurocholate (Table 4).

Thus it would appear that removal of the anionic sulfonate radical increased the requirements of Na^+ ions for interaction with the bile salt system. This complements our previous analogous studies of the sodium requirements of the transport system for the uphill transport of taurodehydrocholate (6), which was also quantitatively more dependent on the Na^+ ion concentration than taurocholate. Taurodehydrocholate can be considered as taurocholate modified in such a manner as to have marked distortion of the steroid moiety. Cholyl NPG, on the other hand, is a derivative in which the steroid portion remains unchanged from that of taurocholate but differs in the absence of a negative charge. The fact that interactions of both these derivatives with the transporting system are more dependent on sodium than the natural analogue points to the fact that both the steroid shape and the negative charge operate together for maximal attachment to the system, and with such optimal interaction, the interaction with sodium ions is enhanced. It would also appear that these two factors, simultaneous and optimal sodium ion and bile salt substrate interaction, are required for the uphill transport process.

This work was supproted by research grant AM-09582 from the National Institutes of Health, and in part by a grant (RR-30) from the General Clinical Research Centers Program of the Division of Research Sources, National Institutes of Health.

Manuscript received 19 July 1976 and accepted 17 January 1977.

REFERENCES

1. Firpi, A., J. T. Walker, and L. Lack. 1973. Interactions of cationic bile salt derivatives with the ileal bile salt transport system. *J. Lipid Res.* **16:** 379–385.
2. Singletary, W. V. Jr., J. T. Walker, and L. Lack. 1972. Ileal transport of bile acids conjugated with norleucine and lysine. *Biochim. Biophys. Acta.* **266:** 238–245.
3. Lack, L., and I. M. Weiner. 1966. Intestinal bile salt transport: Structure–activity relationships and other properties. *Amer. J. Physiol.* **210:** 1142–1152.
4. Lack, L., and I. M. Weiner. 1967. The ileal bile salt transport system: effect of the charged state of the substrate on activity. *Biochim. Biophys. Acta.* **135:** 1065–1068.
5. Lack, L., J. T. Walker, and G. Singletary. 1970. Ileal bile salt transport: in vivo studies of effect of substrate ionization on activity. *Amer. J. Physiol.* **219:** 487–490.
6. Gallagher, K., J. Mauskopf, J. T. Walker, and L. Lack. 1976 Ionic requirements for the active ileal bile salt transport system. *J. Lipid Res.* **17:** 572–577.
7. Lack, L., F. O. Dorrity, J. T. Walker, and G. Singletary. 1973. Synthesis of conjugated bile acids by means of a peptide coupling reagent. *J. Lipid Res.* **14:** 367–370.
8. Norman, A. 1953. Separation of conjugated bile acids by partition chromatography. Bile acids and steroids. *Acta Chem. Scand.* **7:** 1413–1419.
9. Hofmann, A. F. 1962. Thin-layer adsorption chromatography of free and conjugated bile acids on silicic acid. *J. Lipid Res.* **3:** 127–128.
10. Wilson, T. H., and G. Wiseman. 1954. The use of sacs of everted small intestine for the study of the transference of substances from the mucosal to the serosal surface. *J. Physiol., London.* **123:** 116–125.
11. Lack, L., and I. M. Weiner. 1961. In vitro absorption of bile salts by small intestine of rats and guinea pigs. *Amer. J. Physiol.* **200:** 313–317.
12. Heaton, K. W., and L. Lack. 1968. Ileal bile salt transport: mutual inhibition in an in vivo system. *Amer. J. Physiol.* **214:** 585–590.