Effects of four taurine-conjugated bile acids on mucosal uptake and lymphatic absorption of cholesterol in the rat

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Abstract The importance of the bile acid structure on mucosal uptake and lymphatic absorption of cholesterol was investigated using four different taurine-conjugated bile acids. Pure synthetic conjugates of a trihydroxy bile acid, taurocholate, and three dihydroxy bile acids, tauroursodeoxycholate, taurochenodeoxycholate, and taurodeoxycholate were used to completely solubilize [^{14}C]cholesterol and polar lipids for steady rate intraduodenal infusion for 8 hr in bile fistula rats. Lymph output and esterification of [^{14}C]cholesterol and endogenous cholesterol were measured in hourly samples. A second group of bile fistula rats was given the same bile acids as the first group but without added cholesterol or other lipid, i.e., fasting lymph fistula group. Mucosal uptake of [^{14}C]cholesterol was studied using recovery of [^{14}C]cholesterol from lumen and mucosa after 1-hr infusions in conscious bile fistula rats. Lymph output of [^{14}C]cholesterol was promoted more rapidly with taurocholate than with the dihydroxy conjugates and [^{14}C]cholesterol output differed for the three groups given dihydroxy bile acids. The mass of cholesterol in lymph, measured chemically, varied in parallel with [^{14}C]cholesterol absorption. For fasting lymph, infusion of dihydroxy bile acids failed to produce a significant change in endogenous cholesterol output when compared with rats given saline only. Taurocholate infusion markedly increased endogenous cholesterol in lymph of fasted rats. Under all conditions where cholesterol output was stimulated, the increase could be accounted for mainly as esterified cholesterol. Mucosal uptake of [^{14}C]cholesterol during 1-hr infusions in conscious bile fistula rats was slower with the dihydroxy bile acids than with taurocholate. The results indicate the marked effect of the number and configuration of the hydroxyl groups on the solubilizing bile acid for cholesterol absorption.—**Watt, S. M., and W. J. Simmonds.** Effects of four taurine-conjugated bile acids on mucosal uptake and lymphatic absorption of cholesterol in the rat. *J. Lipid Res.* 1984. **25:** 448–455.

Supplementary key words cholesteryl ester • bile fistula • lipid infusion • lymph • taurocholate • tauroursodeoxycholate • taurochenodeoxycholate • taurodeoxycholate • small intestine • micelles

Bile salts are obligatory for cholesterol absorption (1). The first step in absorption is uptake from the intestinal lumen into absorptive cells. For this, micellar solubilizaton of cholesterol is necessary but not sufficient; the planar detergent structure of the bile salt molecule is critical (2).

Although both trihydroxy and dihydroxy bile salts are planar detergents, trihydroxy bile salts are more efficient in promoting cholesterol absorption (3, 4). The difference appears to be quantitative, not qualitative. It cannot be readily explained by differences in micellar-solubilizing efficiency (2) but can be correlated with the esterification of absorbed cholesterol and its transport in lymph (2, 5, 6).

Comparisons between the effects of different dihydroxy bile salts on cholesterol absorption have been stimulated by the use of chenodeoxycholic acid and ursodeoxycholic acid as agents for the dissolution of gallstones. So far, the evidence comes mainly from balance studies of cholesterol absorption in rats (7, 8), mice (9), and humans (10, 11) with intact bile flow and given supplementary bile acids. Some studies used unconjugated bile acids. This design makes it difficult to draw conclusions concerning the efficiency of a single conjugated bile acid in mediating cholesterol uptake from the micellar state in the lumen.

In the present study the absorption of cholesterol from micellar solution in four different taurine-conjugated bile acids has been compared under standardized conditions. Taurocholate (3α, 7α, and 12α hydroxyl groups) and three dihydroxy bile acids, taurodeoxycholate (3α, 12α hydroxyl groups), taurochenodeoxycholate (3α, 7α hydroxyl groups), and tauroursodeoxycholate (3α, 7β hydroxyl groups) were used. The use of 48-hr bile-diverted rats eliminated endogenous bile salts and biliary cholesterol from the lumen. The experimental design allowed some discrimination between effects on the initial uptake of cholesterol from the lumen and subsequent effects on intracellular handling of exogenous and endogenous cholesterol and its transfer to lymph. The findings suggest a considerable effect of the position, orientation, and

Abbreviations: TLC, thin-layer chromatography; TC, taurocholate; TCDC, taurochenodeoxycholate; TDC, taurodeoxycholate; TUDC, tauroursodeoxycholate.

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number of hydroxyl groups of the bile salt molecule on cholesterol absorption.

MATERIALS AND METHODS

In the main experiments, intraduodenally infused lymph fistula bile-diverted rats were used to measure lymph output and esterification of exogenous and endogenous cholesterol during steady rate infusions.

A second group of fasted lymph fistula rats was used to examine the effect of infusion of a test bile salt on lymph absorption of endogenous cholesterol only. A third group of bile-diverted rats, without lymph fistula, was used to compare the distribution of intraduodenally infused labeled cholesterol after 1-hr infusions.

Animal methods

Cannulations of the abdominal thoracic lymph duct were performed under ether anesthesia on 200-g male rats of an inbred Wistar strain fasted overnight, as described previously (2). Bile fistula rats had the bile duct cannulated with a silicone cannula tipped with polyethylene tubing (i.d. 0.28 mm). For infusions, a silicone tube was placed through a small incision in the fundus of the stomach into the duodenum with the tip secured near the entrance of the common bile duct. After transfer to restraining cages kept at 30°C, rats were infused with 150 mM NaCl, 4 mM KCl, and 10 mM glucose at 3 ml/hr for at least 48 hr before being given a test infusate at the same rate.

Operated rats without lymph fistula were maintained in the same way, but were infused at 1.5 ml/hr (to prevent fluid overload) until the test infusion was given at 3 ml/hr. After the appropriate infusion time, these rats were anesthetized, given a cardiac puncture, and the digestive tract was ligated to separate the stomach, upper small intestine, lower small intestine, and caecum. All succeeding steps were carried out over ice. Lumen contents were washed from each segment with 10 mM bile salts to make a volume of 15 ml. The scraped mucosa from upper and lower small intestine was homogenized and made up to volume for lipid extraction, chemical assays, and counting.

Preparation of infusates

Infusates were made up in phosphate-buffered saline (6.75 mM Na_2HPO_4, 16.5 mM NaH_2PO_4, 115 mM NaCl, 15 mM KCl, pH 6.4) containing 10 mM glucose and 1 mM $CaCl_2$. Appropriate aliquots of lipids were taken from stocks made up in chloroform, the solvent was evaporated off under N_2, and the required weight of the test bile salt mixed in a small volume of warm buffer was added to the evaporated lipid residue. The solution was gently sonicated in a Branson sonifier, with further additions of buffer and intermittent sonications until the final volume was attained. Optically clear micellar solutions were obtained with each of the bile salts used.

The lipid composition of the cholesterol-containing infusates was 3 mM monoolein, 6 mM oleic acid, 10 mM bile salts, and 0.13 mM [^{14}C]cholesterol, unless otherwise stated. In some experiments bile acids were infused without addition of other lipids to the phosphate-buffered saline. These rats were referred to as fasted rats.

Analytical

Lipids were extracted from duplicate aliquots of lymph, intestinal contents, and homogenized mucosal suspensions by the method of Blankenhorn and Ahrens (12). The efficiency of this method was found to vary less than 3% when compared with duplicate aliquots extracted by the method of Bligh and Dyer (13). Aliquots of lipid extracts were taken for counting, assay of total cholesterol, and TLC. TLC was carried out on 0.25-mm layers of silica gel G (Merck, Darmstadt, Germany) on prewashed plates developed in hexane–diethyl ether–glacial acetic acid 70:30:2 (v/v/v). Free and esterified cholesterol were visualized with iodine vapour against standards run on the same plate, removed from the plate by suction (14), and eluted with chloroform–methanol 2:1 (v/v). Aliquots from each sample were taken for saponification prior to cholesterol assay and also for counting.

Cholesterol determinations were carried out by the method of Zlatkis and Zak (15). All standards, blanks, and lipid extracts were saponified (16) before cholesterol assays were done. Protein in mucosal homogenates was determined according to Lowry et al. (17).

Radioactivity was determined using one of two different scintillant mixtures. After evaporation of solvents, lipid residues were mixed with 10 ml of a mixture containing 2,5-phenyloxazole, 4 g/l, and 1,4-bis-2(4-methyl-5-phenyloxazolyl-benzene), 0.05 g/l in toluene. To some aqueous samples, not exceeding 0.3 ml in volume, was added 10 ml of a water-miscible scintillant "Aquasolv" (Beckman, Fullerton, CA). Samples were counted in a Nuclear Chicago Isocap 300 counter with quenching corrected by the external standard channels ratio method.

Materials

[4-^{14}C]Cholesterol (Radiochemical Centre, Amersham) was repurified by TLC before use. Unlabeled oleic acid, glycerol-1-mono-oleate, and cholesterol were all purchased as high purity grade (>99% pure) from Nu-Chek-Prep Inc., Elysian, MN, and used as supplied. Each of the taurine-conjugated bile acids was prepared by the method of Lack et al. (18) in this laboratory. In addition generous gifts of taurochenodeoxycholate and tauroursodeoxycholate were received from Dr. A. F. Hofmann,

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University of California School of Medicine, San Diego, CA. All bile acids moved as one spot on TLC plates developed in propionic acid–isoamylacetate–water–n-propanol 15:20:5:10 (v/v/v/v). *o*-Phthalaldehyde was purchased from Sigma, St. Louis, MO. All chemicals and solvents were of analytical grade except ethanol which was redistilled.

Statistical analysis

The results are expressed as mean values and standard error of the means. Student's *t*-test was used to determine the significance of differences. An analysis of variance was also performed on the data for 1-hr mucosal uptake (four bile acid treatments, six animals per block). This led to the same statistical conclusions.

RESULTS

Lymphatic absorption of labeled micellar cholesterol

Four groups of bile fistula rats were given [^{14}C]-cholesterol by intraduodenal infusion continuously for 8 hr. For each group a different taurine-conjugated bile acid was used, at a concentration of 10 mM, to completely solubilize the lipid. The volume infused and the lipid compositions were the same for all groups: 0.13 mM cholesterol, 6 mM oleic acid, and 3 mM monoolein. With sodium taurocholate (TC) lymphatic output of labeled cholesterol at 8 hr was much greater than with the dihydroxy conjugates (**Fig. 1**). The pattern of labeled cholesterol output differed for the three groups given dihydroxy conjugates. Taurodeoxycholate (TDC) and taurochenodeoxycholate (TCDC) gave cholesterol output into lymph at a rate similar to TC for the first 4 hr. Thereafter the output increased slowly with TDC, whereas with TCDC it declined to a rate less than half that with TDC and less than one-fifth that with TC. With tauroursodeoxycholate (TUDC) the output increased steadily throughout, but much more slowly than with the other bile salts. These differences in patterns were found consistently. The declining output from the fifth hour onwards with TCDC was associated with distention of the small intestine with fluid, found at 8 hr. Bile flow also became slower and, in some rats, ceased. Pure synthetic TCDC was used and no unconjugated bile acid was found on TLC.

Lymphatic transport of mass cholesterol

Mass transport of cholesterol, measured chemically, includes labeled exogenous cholesterol absorbed from the lumen and unlabeled endogenous cholesterol from a variety of sources. The changes in mass transport during cholesterol infusion were large compared to the amounts calculated from radioactivity collected and the specific activity of the infusate (compare Fig. 1 and Fig. 2). This

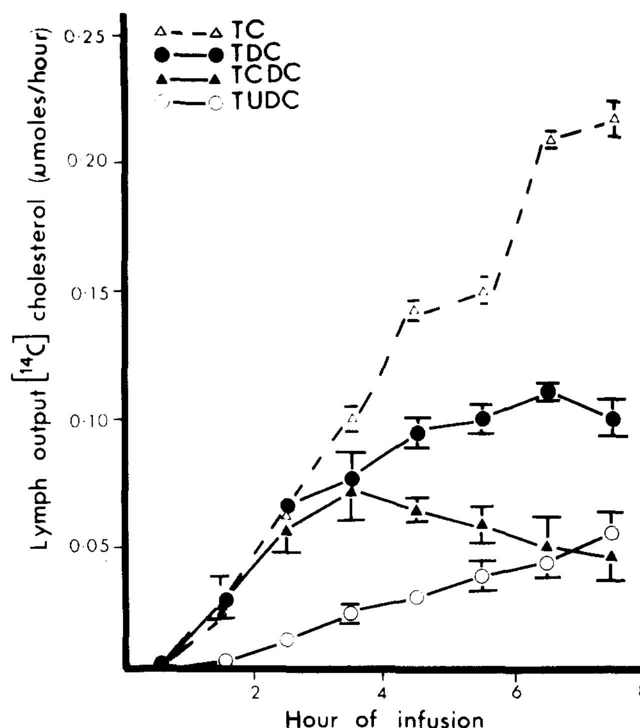

Fig. 1. The effect of different bile acids on lymph output of infused [^{14}C]cholesterol. Bile-diverted rats were infused intraduodenally at 3 ml/hr with 6 mM oleic acid, 3 mM monoolein, and 0.125 mM [^{14}C]cholesterol. The lipids were completely solubilized in the appropriate bile acid (10 mM) in phosphate-buffered saline, pH 6.4. Hourly lymph output of [^{14}C]cholesterol is shown for four rats given taurocholate (TC), △----- △; three rats given tauroursodeoxycholate (TUDC), ○ —— ○; three rats given taurochenodeoxycholate (TCDC), ▲ —— ▲; and four rats given taurodeoxycholate (TDC), ● —— ●. Values are means ± SEM.

indicated considerable dilution of exogenous labeled cholesterol with unlabeled endogenous cholesterol at one or more sites en route to the lymph.

The basal cholesterol output, before lipid infusion, agreed closely for all four groups (**Fig. 2**). The effects of the four bile acids on output of mass cholesterol were consistent with the effects on absorbed labeled cholesterol. There were some differences in detail. For example, output of radioactivity increased steeply with taurocholate between 4th and 8th hr (Fig. 1) whereas mass output did not (Fig. 2). This could be attributed to increased labeling of the mixture exported into lymph. Nevertheless, for both mass and radioactive cholesterol, transport was greatest with TC; for dihydroxy bile acids, transport was greater for TDC than for TUDC, while for TCDC transport was similar to TDC at 4 hr and then fell off.

Esterification of lymph cholesterol

It is well known that absorbed cholesterol is predominantly esterified before transport into lymph (5). In the present experiments the mass output of esterified cholesterol determined chemically was much larger than the output of esterified labeled cholesterol from the infusate.

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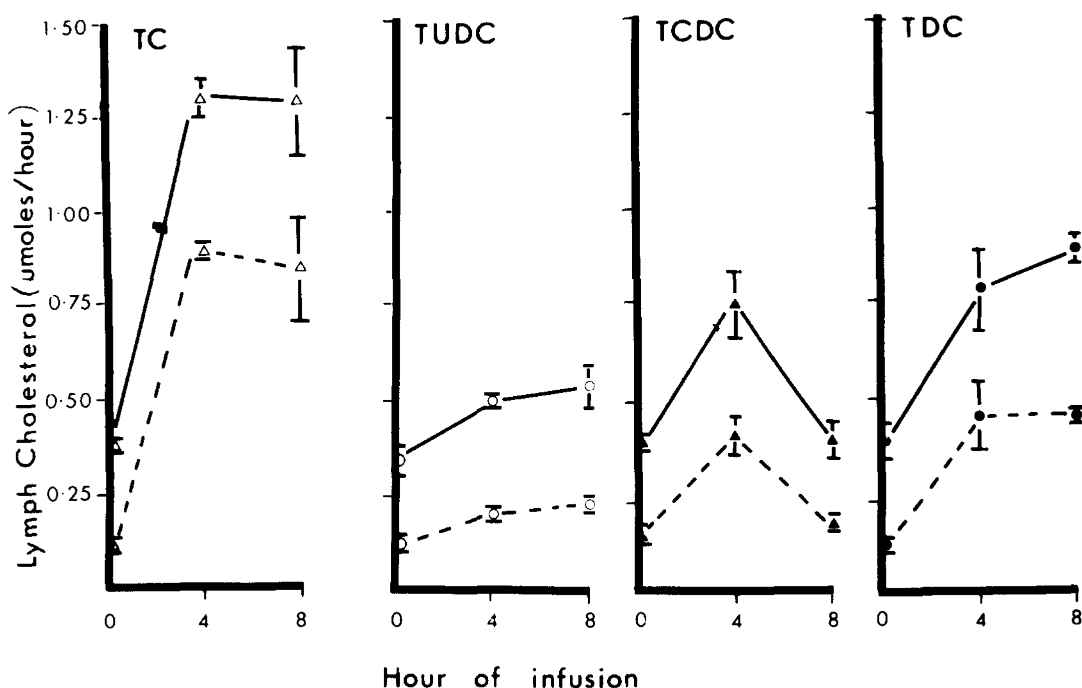

Fig. 2. The effect of different bile acids on lymph output of mass cholesterol during infusion of [^{14}C]cholesterol. Bile-diverted rats infused at 3 ml/hr with 6 mM oleic acid, 3 mM monoolein, and 0.125 mM [^{14}C]cholesterol. Lipids were completely solubilized in the test bile acid (10 mM) in phosphate-buffered saline, pH 6.4. Total mass cholesterol (——) and mass cholesterol appearing in the esterified form (-----) are shown for lymph samples collected at 0, 4, and 8 hr of infusion with taurocholate, TC (△), three rats; tauroursodeoxycholate, TUDC (○), four rats; taurochenodeoxycholate, TCDC (▲), three rats; and taurodeoxycholate, TDC (●), four rats. Values are means ± SEM.

The esterified fraction of unlabeled cholesterol (30–40%) was low in lymph before infusion of micellar cholesterol. To estimate the effect of infused bile salts and cholesterol on esterification, the basal (preinfusion) hourly outputs of free and esterified cholesterol were subtracted from the chemically determined values for the 4th and 8th hr. Treatment of the data in this way showed that percentage esterification was much the same for excess mass cholesterol as it was for the labeled infused component (**Table 1**). Subtraction of the preabsorptive unlabeled outputs led to very similar specific activities of the free and esterified cholesterol moieties in absorptive lymph. That is, labeled and unlabeled molecules seemed to be handled in the same way during esterification and transport into lymph. On the other hand, without correction for preabsorptive contributions of endogenous cholesterol, percentage esterification was considerably lower for mass than for labeled molecules and the specific activity of ester cholesterol was considerably greater than that of the unesterified fraction. That is, the uncorrected data suggest preferential esterification of newly absorbed cholesterol.

The lymphatic output of unesterified cholesterol was increased by infusion of micellar cholesterol in all four groups but, due to increased esterification, it was the ester fraction which accounted for most of the differentiation in total cholesterol output between groups (Fig. 2, Table 1). The efficiency of esterification (fraction esterified) was greater for the trihydroxy bile acid than for the dihydroxy bile acids. The mean percentage esterified, measured by radioactivity or by mass, in 8th-hr lymph (Table 1) was significantly higher with TC when compared separately with TUDC, TCDC, and TDC (P for t tests, respectively, <0.01, <0.01, and <0.05).

Effect of bile salts on fasting lymph cholesterol

The same bile salts (TUDC, TCDC, TDC, and TC) were given without cholesterol or other lipids to another four groups of fasting rats. The concentration of bile salts (10 mM), the volume infused (3 ml/hr), and the inorganic composition of the infusate (phosphate-buffered saline, pH 6.4) were the same as before.

There was no appreciable change in mass cholesterol output in lymph with TUDC. With the other two dihydroxy bile salts there was a small increase by the fourth hour with a subsequent decline. These changes were statistically insignificant compared with preinfusion values or values for rats given saline alone. The esterified fraction remained at the low preinfusion values, about 0.3, in all groups (**Fig. 3**).

On the other hand, taurocholate infusion without added cholesterol or other lipid produced a marked increase both in total output and in esterification of lymph cholesterol. In this group of rats, total cholesterol output increased from 0.36 to 0.91 μmol/hr during the 8-hr

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TABLE 1. Lymph cholesterol output, esterification, and specific activity during infusion with different bile acids

	4-Hr Infusion Time				8-Hr Infusion Time			
	TUDC	TCDC	TDC	TC	TUDC	TCDC	TDC	TC
Free cholesterol, nmol/hr								
^{14}C-Labeled	8	16	21	18	18	21	29	42
Mass (corrected)[a]	62	122	80	244	78	−8	176	251
Cholesteryl ester[b], nmol/hr								
^{14}C-Labeled	16	54	57	83	36	26	73	174
Mass (corrected)[a]	86	269	332	775	114	34	337	731
Cholesteryl ester (%)[c]								
^{14}C-Labeled	67	78	73	84	67	56	72	80
Mass (corrected)[a]	58	68	81	77	60	71	66	74
Mass (uncorrected)[d]	40	54	57	67	43	44	51	66
Specific activity[e]								
^{14}C/Mass (corrected)[a]								
Free cholesterol	0.13	0.13	0.26	0.07	0.23	?	0.16	0.17
Cholesteryl ester	0.18	0.20	0.17	0.11	0.32	0.77	0.22	0.21
^{14}C/Mass (uncorrected)[d]								
Free cholesterol	0.026	0.045	0.027	0.042	0.058	0.095	0.065	0.096
Cholesteryl ester	0.077	0.130	0.130	0.107	0.157	0.149	0.159	0.206

These values are calculated from the experiments shown in Fig. 1 and Fig. 2. Rats were infused with micellar [^{14}C]cholesterol and polar lipids solubilized in the four different bile acids in phosphate buffer, pH 6.4. TUDC, tauroursodeoxycholate; TCDC, taurochenodeoxycholate; TDC, taurodeoxycholate; TC, taurocholate.

[a] Corrected for basal output, i.e., values are for free or esterified mass cholesterol in lymph in excess of the 0-hr value for that fraction.
[b] As nmol cholesterol appearing in the esterified form.
[c] Cholesterol in the esterified form as % of the total cholesterol for that fraction.
[d] Values are not corrected for 0-hr output.
[e] Specific activity as nmol [^{14}C]cholesterol/nmol mass cholesterol relative to infusate taken as 1.0.

infusion, i.e., equivalent to about 2.4 μmol of extra lymph cholesterol in 8 hr. The increase was mainly in esterified cholesterol, an increased output of about 1.7 μmol in 8 hr.

Mucosal uptake

Output of labeled cholesterol in lymph during steady duodenal infusion is the end result of a sequence of events.

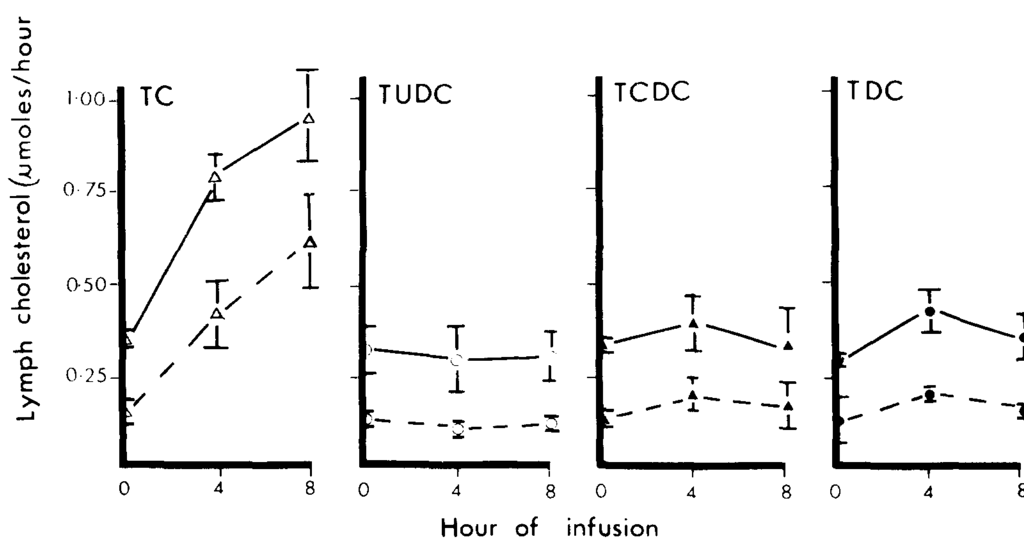

Fig. 3. Lymph output of mass cholesterol in fasted rats infused with four different bile acids. Rats were infused intraduodenally at 3 ml/hr with a given bile acid (10 mM) in phosphate-buffered saline, pH 6.4. Total cholesterol (——) and cholesterol in the esterified form (-----) are shown for at least three rats in each group. Bile acids infused were taurocholate, TC, (△); tauroursodeoxycholate, TUDC, (○); taurochenodeoxycholate, TCDC (▲); and taurodeoxycholate, TDC, (●). Values are means ± SEM.

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An attempt was made to evaluate the role of the first step, uptake from the lumen, in mediating the differences between bile acids.

In the previous experiments, little or no translocation of labeled cholesterol from mucosa to lymph occurred during the first hour of infusion. Differences in mucosal content should then reflect mainly uptake from the lumen.

The recovery of labeled cholesterol in lumen and mucosa was measured in a series of rats after perfusing micellar solutions in different bile acids for 1 hr. Six rats were used for each bile acid tested. These rats had intact lymphatics but otherwise were comparable with the lymph fistula series, having chronic biliary and duodenal cannulae and being kept in restraint cages. The post-operative care and test perfusates were the same as for the lymph fistula rats.

With the exception of the group that received TCDC, 90% or more of the labeled cholesterol was recovered from lumen plus wall (**Table 2**). Some of the infused label (about 6%) was also taken up by the plastic duodenal cannular tubing (19). Most of the label in the wall was in the mucosa of the upper half of the small intestine. No explanation was found for the consistently lower total recovery with TCDC (about 75%).

Mucosal uptake by the whole small intestine was faster from TC than from dihydroxy bile acids. The difference after 1 hr was statistically significant for TCDC ($P < 0.01$) and TUDC ($P < 0.001$). Uptake from TUDC was slower than from the other dihydroxy bile acids tested ($P < 0.05$ for TCDC and $P < 0.02$ for TDC). Significant differences were obtained for uptake by the proximal halves of small intestine. No statistically significant differences between groups were found for uptake by distal halves nor for comparisons of proximal or total uptake between TDC and TCDC.

DISCUSSION

The concept that differences in the number and disposition of hydroxyl groups on the bile acid molecule might influence its role in mediating cholesterol absorption is not new (2–10). However, to demonstrate a specific effect of molecular structure on the absorptive process, it is necessary to control variables such as the load of cholesterol, both endogenous and exogenous, presented for mucosal uptake and the degree of micellar solubilization in luminal contents. Comparisons between bile acids are difficult to interpret when single gastric test meals containing labeled cholesterol and a large dose of bile acid are given to animals with an intact bile flow (3, 4, 7, 8, 10, 11). Factors such as rate of stomach emptying, partition of cholesterol between oil and micellar phase, contributions of biliary cholesterol, lecithin, and bile salts to luminal contents, and cellular effects of unconjugated bile acids may contribute to observed differences.

In the present study, these complicating factors were controlled or evaded as far as possible: by the administration of pure synthetic taurine conjugates, by steady intraduodenal infusion of a standard dose of cholesterol, polar lipid, and bile salts as a micellar solution, and by the use of unanesthetized rats with chronic (48 hr) bile fistulae. The overall absorptive process was studied by lymphatic output of labeled exogenous and unlabeled endogenous cholesterol during 8 hr steady perfusion. The first step in the process, viz. uptake from the lumen, was studied by measuring mucosal content after 1 hr infusion, during which time labeled cholesterol was taken up from the lumen but not appreciably transported into lymph. In general, the results confirmed earlier reports that taurocholate promoted absorption more efficiently than the dihydroxy bile acids taurodeoxycholate, taurocheno-

TABLE 2. Effect of individual bile acids on the regional distribution of infused [^{14}C]cholesterol

	Recovery of [^{14}C]Cholesterol (% of total infused)						
	Lumen[a]			Wall of Small Intestine			
		Small Intestine		Mucosa			
Bile Acid	Stomach	Upper	Lower	Upper	Lower	Muscle Layer[b]	Total Recovery[b]
TUDC (6)	5.6 ± 2.1	11.7 ± 2.2	28.7 ± 5.1	33.2 ± 6.4	11.3 ± 2.3		90.5 ± 3.6
TCDC (6)	3.2 ± 0.7	4.1 ± 0.5	4.1 ± 1.1	56.0 ± 2.7	4.6 ± 1.7		72.4 ± 3.0
TDC (6)	3.7 ± 1.6	6.9 ± 1.1	5.5 ± 1.7	62.5 ± 5.3	5.4 ± 2.0	5.4 ± 1.0	89.9 ± 6.7
TC (6)	4.5 ± 1.5	4.7 ± 1.1	3.9 ± 1.5	73.1 ± 5.5	5.6 ± 2.2		91.7 ± 2.6

Bile fistula rats were infused intraduodenally at 3 ml/hr for 1 hr with 6 mM oleic acid, 3 mM monoolein, 0.125 mM [^{14}C]cholesterol, solubilized in the appropriate bile acid (10 mM), in phosphate buffer, pH 6.4. Values are [^{14}C]cholesterol recovered in each fraction as percent of total infused (means ± SEM) for the number of rats in parentheses. TUDC, tauroursodeoxycholate; TCDC, taurochenodeoxycholate; TDC, taurodeoxycholate; TC, taurocholate.

[a] No detectable recovery of isotope in caecum contents.

[b] Recovery of isotope in the muscle layer was measured in only three of the six rats given TUDC, TCDC, and TC, respectively. After mucosal scraping recovery of isotope in muscle as percent of total isotope infused for three rats per group was TUDC, 11.3 ± 2.3; TCDC, 4.7 ± 0.7; and TC, 10.3 ± 2.2. Values are means ± SEM.

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deoxycholate, and tauroursodeoxycholate, and that dihydroxy bile acids differed in their effectiveness. The reasons for the differences are still not clear but certain features of the results merit brief discussion.

Mucosal labeling after 1 hr perfusion (Table 2) provides quite good evidence that the differences observed in lymphatic absorption were mediated, at least partly, at the stage of uptake from the lumen. Uptake was greatest with taurocholate, least with tauroursodeoxycholate, and intermediate with taurodeoxycholate and taurochenodeoxycholate. This was also the order observed for lymphatic output of labeled cholesterol at the end of the first 4 hr of an 8-hr perfusion (Fig. 1).

The differences in uptake cannot be ascribed simply to the degree of solubilization, which was complete for the micellar perfusates given. Nevertheless, it must be recognized that the conditions at the absorptive surface might have differed considerably from those in the perfusate. The small amount of labeled cholesterol in the perfusate would have mixed with endogenous cholesterol in the lumen. Also the polar lipid which acted as a cosolubilizer in the mixed micelles is absorbed more rapidly than cholesterol (20). The efficiency of solubilization at the absorbing surface might be a limiting factor rather than the completeness of solubilization in the perfusate. It has been found that tauroursodeoxycholate, the least effective in promoting absorption, is a poor solubilizer of cholesterol in mixed micelles, relative to taurocholate or taurochenodeoxycholate (21).

It is more difficult to identify differences in later stages of absorption which might be attributable to the type of bile acid in the lumen. Absorbed cholesterol is transported into lymph mainly as esterified cholesterol in lipid transport particles, chylomicrons, and very low density lipoproteins. The preeminence of taurocholate in mediating cholesterol absorption has been ascribed to enhancement of cholesterol esterification (5). In the present experiments the efficiency of esterification (percentage esterified) of lymphatic cholesterol both labeled and endogenous, was greater for taurocholate than for dihydroxy bile acids, confirming earlier reports (2, 6). There were also differences between dihydroxy bile acids. Whether these reflect effects of bile acids on mucosal esterification or dependence of mucosal esterification on the influx of absorbed cholesterol is uncertain, since there was an association between percentage esterified, rate of output in lymph, and rate of uptake from the lumen.

In fasting bile fistula animals the lymphatic output of endogenous cholesterol and percentage esterified were increased by perfusion of taurocholate alone, whereas the effect of dihydroxy bile acids was statistically insignificant. This suggested an effect at least of taurocholate on the unlabeled pool of mucosal cholesterol. However, one cannot exclude the possibility that influx from a luminal pool of endogenous cholesterol was the stimulating factor.

Finally, there were differences between bile acids in the pattern of lymphatic output of both labeled and unlabeled cholesterol, as well as differences when labeled and unlabeled outputs were compared for a given bile acid. Regarding the latter, it is well recognized that labeled exogenous molecules mix with relative large pools of endogenous cholesterol in lumen and mucosa and are further diluted in lymph by cholesterol-containing lipoproteins derived from plasma. Thus, after a single dose of labeled cholesterol, the pattern of lymphatic output of label is influenced by mixing with unlabeled pools and may diverge considerably from the pattern of mass output (22). To a lesser extent, this also occurs during steady perfusion of [^{14}C]cholesterol (Figs. 1 and 2).

Mixing of labeled and unlabeled pools could also account in large part for the high specific activity of cholesteryl ester relative to unesterified cholesterol. This has been attributed to preferential esterification of absorbed labeled cholesterol. However, there is a steady basal (preinfusion) output of cholesterol in lymph. If the basal output, in which the fraction of esterified cholesterol is low, is subtracted from mass outputs of unesterified and esterified cholesterol during absorption, the differences in specific activity are considerably reduced (Table 1).

Pool mixing would not explain the decline in output of both labeled and mass cholesterol in the second 4 hr of the 8-hr perfusion of micellar cholesterol in rats given taurochenodeoxycholate. It was not seen with other dihydroxy bile acids and probably reflects a toxic effect. The possibility of contamination by the presence of a significant amount of unconjugated chenodeoxycholate was excluded by TLC. Adventitious impurities seem unlikely since a number of batches of synthetic conjugate were tested. An indirect effect is possible: for example, dilution of micellar solution by intestinal secretion stimulated by taurochenodeoxycholate (23). Whatever the explanation, it must be recognized that differences between bile acids may be related to interference with cholesterol absorption as well as to promotion by solubilization or other mechanisms.

Lymph was collected from the thoracic duct in these studies. This method provides a more complete collection of lymph, but at the cost of contamination with hepatic lymph. Cannulation of the main mesenteric lymph duct enables a complete collection of intestinal lymph, but problems may arise due to presence of accessory ducts. Contamination of mesenteric lymph by hepatic lymph can be excluded only if cross connections are successfully ligated. In the present study thoracic duct lymph appeared to be reflecting changes of intestinal origin, e.g., changes in lymph mass cholesterol although larger, were consistent

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with those observed in isotopic cholesterol after infusion into the lumen. In the rabbit, large changes in hepatic cholesterol turnover do not appear to be reflected in hepatic lymph (24).

Finally, we appreciate the inescapable difficulties in comparing solubilization of cholesterol in the different bile acids. The solutions were optically clear but there appears to be no satisfactory measure of intermicellar concentration of cholesterol. We found no gross differences between the four micellar solutions in partitioning of labeled cholesterol into a fixed nonpolar phase (polyethylene discs). The total concentration of cholesterol was the same in all four bile salt solutions and it appeared to be completely solubilized in all four solutions.

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