Metabolism of 7β-alkyl chenodeoxycholic acid analogs and their effect on cholesterol metabolism in hamsters

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Abstract The metabolism of 7-ethyl- and 7-propyl-chenodeoxycholic acids was studied in hamsters. Both bile acid analogs were absorbed efficiently by the intestine and secreted into the bile at rates similar to those of chenodeoxycholic acid. After intraduodenal administration into bile fistula hamsters, the 7-alkyl analogs were present in bile as the glycine and taurine conjugates. The glycine/taurine ratios were: chenodeoxycholic acid, 1.9; 7-ethyl analog, 0.3; and 7-propyl analog, 0.2. After oral administration, during a 21-day feeding experiment, the ^{14}C-labeled analogs were recovered quantitatively in the feces. Chenodeoxycholic acid was largely 7-dehydroxylated to lithocholic acid in the intestinal tract. In contrast, the 7α-hydroxy group of the 7-alkyl bile acids was completely resistant to bacterial action. 7-Ethyl-chenodeoxycholic acid was transformed in part to a compound tentatively indentified as 7α-hydroxy-3-oxo-7β-ethyl-5β-cholanoic acid while 7-propyl-chenodeoxycholic acid was excreted unchanged. In the hamsters used, the 7-alkyl bile acid analogs did not inhibit the bacterial dehydroxylation of chenodeoxycholic acid. At the end of the 21-day feeding period, analysis of the gallbladder bile showed that 7-methyl-, 7-ethyl-, and 7-propyl-chenodeoxycholic acids accounted for 38, 31, and 12% of total bile acids, respectively. The 7-alkyl bile acids decreased cholesterol absorption; the 7-propyl analog caused significant decrease in serum and liver cholesterol concentration. These experiments demonstrate that the 7-ethyl- and 7-propyl chenodeoxycholic acids, just like the 7-methyl-analog, are absorbed by the intestine and participate in the enterohepatic circulation. In the hamster, introduction of a 7-alkyl group into chenodeoxycholic acid tends to lower cholesterol absorption and reduce tissue cholesterol levels. —**Une, M., K. Yamanaga, E. H. Mosbach, K. Tsujimura, and T. Hoshita.** Metabolism of 7β-alkyl chenodeoxycholic acid analogs and their effect on cholesterol metabolism in hamsters. *J. Lipid Res.* 1990. **31:** 1015–1021.

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The oral administration of chenodeoxycholic acid (CDC) is known to effect dissolution of cholesterol gallstones in humans (1). However, during passage through the intestinal tract, CDC is dehydroxylated by certain strains of anaerobic microorganisms to the potential hepatotoxin, lithocholic acid (LC) (2). We undertook, therefore, to develop new cholelitholytic agents that would be resistant to bacterial 7-dehydroxylation. We recently showed that 3α,7α-dihydroxy-7β-methyl-5β-cholanoic acid (7-methyl-CDC) is resistant to 7-dehydroxylation by the intestinal flora of hamster and prairie dog (3, 4). This CDC analog was effective both in preventing gallstone formation and in dissolving existing gallstones in the prairie dog (4). Our interest in the biochemistry of bile acid analogs led us to study the higher homologs of 7-methyl-CDC, namely, the 7-ethyl- and 7-propyl-compounds. Since these synthetic bile acids were likely to be more hydrophobic than CDC and 7-methyl-CDC, they were expected to differ from the latter in important respects.

This report describes the metabolism of 7-ethyl-CDC and 7-propyl-CDC in the hamster, with emphasis on intestinal absorption, hepatic and intestinal biotransformation, and resistance to bacterial 7-dehydroxylation. We further studied the effect of these compounds on the intestinal absorption of cholesterol and cholesterol concentration in serum and liver.

MATERIALS AND METHODS

Labeled compounds and reference compounds

[24-^{14}C]CDC (sp act 0.5 μCi/mg) was purchased from NEN Research Products (Boston, MA). [24-^{14}C]7-Ethyl-

Abbreviations: CDC, chenodeoxycholic acid; LC, lithocholic acid; TLC, thin-layer chromatography; GLC, gas-liquid chromatography; G/T, glycine/taurine ratio; UDC, ursodeoxycholic acid.

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CDC (sp act 0.4 μCi/mg) and [24-^{14}C]7-propyl-CDC (sp act 0.4 μCi/mg) were prepared from [24-^{14}C]CDC as described previously (5). Radiochemical purity of each labeled compound was higher than 98% as determined by radio-thin-layer chromatography. Unlabeled 7-alkyl bile acid analogs (7-methyl-CDC, 7 ethyl-CDC, and 7-propyl-CDC) were synthesized as described previously (5).

Experiments with bile fistula hamsters

Male golden Syrian hamsters (Hiroshima Experimental Animal Center, Hiroshima, Japan) weighing 125–135 g, were anesthetized with diethyl ether, and their bile ducts were cannulated with PE-10 polyethylene tubing (0.28 mm i.d.). A single dose of the labeled compound (0.5 mg, 0.2 μCi) was injected into the duodenum and bile samples were collected every hour for 12 h.

Experiments with intact animals

The hamsters (24 animals, weighing 125–135 g) were maintained for 10 days on a commercial rodent chow. The animals were divided into four groups of six animals each. Group 1 was fed rodent chow; group 2, 0.075% 7-methyl-CDC in chow; group 3, 0.075% 7-ethyl-CDC in chow; and group 4, 0.075% 7-propyl-CDC in chow. The animals were fed the different diets for a period of 21 days. On day 14, labeled 7-alkyl bile acids (1.0 mg, 0.4 μCi) or labeled CDC (1.0 mg, 0.5 μCi) were introduced directly into the stomach of two hamsters in each group by stomach tube. Feces were then collected for an additional 7 days. At the end of the 21-day period, the animals were anesthetized with diethyl ether and the bile was obtained by needle aspiration from the gallbladder.

In another experiment 24 hamsters weighing 125–135 g were divided into four groups (groups 5–8). The animals were fed rodent chow supplemented with 0.1% cholesterol (group 5). This diet was further supplemented with 0.1% 7-methyl-CDC (group 6), 0.1% 7-ethyl-CDC (group 7), and 0.1% 7-propyl-CDC (group 8), respectively. The animals were maintained on the different diets and water ad libitum for 12 days. [1α,2α(n)-^{3}H]Cholesterol (4.5 μCi) and [4-^{14}C]β-sitosterol (0.4 μCi) were orally administered on day 13 of the experiment to measure cholesterol absorption by method IV of Quintao, Grundy, and Ahrens (6–8). Feces were collected quantitatively on days 14, 15, and 16. The animals then were fed the experimental diets for an additional 8 days. At the end of the experiment (day 21), the animals were killed. Samples of blood and liver were obtained for the determination of cholesterol concentrations.

Determination of radioactivity

Radioactivity was determined with a liquid scintillation counter (Aloka LSC-3500, Tokyo, Japan) in a toluene-based scintillator. Corrections were made for background and quenching.

Thin-layer chromatography (TLC)

Silica gel 60 F_{254} (precoated aluminum sheets; thickness 0.2 mm; Merck) was used for TLC. The following solvent systems were used: system 1 (for conjugated bile acids), ethyl acetate–acetic acid–water 7:2:1 (v/v/v); system 2 (for unconjugated bile acid), isooctane–ethyl acetate–acetic acid 5:5:1 (v/v/v). The samples were applied as bands, and reference compounds were spotted simultaneously. After development of the plates, the bands and spots were made visible by spraying the plate with 10% phosphomolybdic acid in ethanol, followed by heating at 120°C. To locate the radioactivity on the plate, each TLC sheet was cut into 1-cm sections from origin to solvent front, and each section was then placed in a scintillation vial and counted.

Analysis of biliary bile acids

Bile samples were hydrolyzed in 10% ethanolic KOH solution at 120°C for 3 h. The bile acids were extracted, methyl esterified, derivatized, and analyzed by gas–liquid chromatography (GLC) on columns of 3% OV-1 or 3% Poly I 110 as their methyl ester-trimethylsilyl ether derivatives as described previously (9).

Analysis of fecal bile acids

Bile acids were extracted from feces as reported previously (10). The radioactive compounds extracted were analyzed by radio-TLC and counted. $NaBH_4$ treatment of fecal bile acids was carried out according to the method reported previously (10).

Analysis of serum and liver cholesterol

Serum cholesterol was measured enzymatically using Monotest Cholesterol (Boehringer Mannheim GmbH, West Germany). Liver cholesterol was measured by GLC as previously described (4).

Calculations

Results are expressed as mean ± SEM. The significance of differences between various groups was calculated by Student's *t*-test (11).

RESULTS

[24-^{14}C]7-Ethyl-CDC, [24-^{14}C]7-propyl-CDC, and [24-^{14}C]CDC were administered intraduodenally into bile fistula hamsters in order to compare their intestinal absorption and hepatic metabolism. Fistula bile was collected hourly and analyzed for radioactivity. Recovery of the administered label is shown in **Fig. 1**. During the experiment, a remarkable change in bile flow was not detected. Each compound appeared rapidly in bile and more than 90% of the administered radioactivity was recovered within 8 h. Analysis of the bile acids by radio-

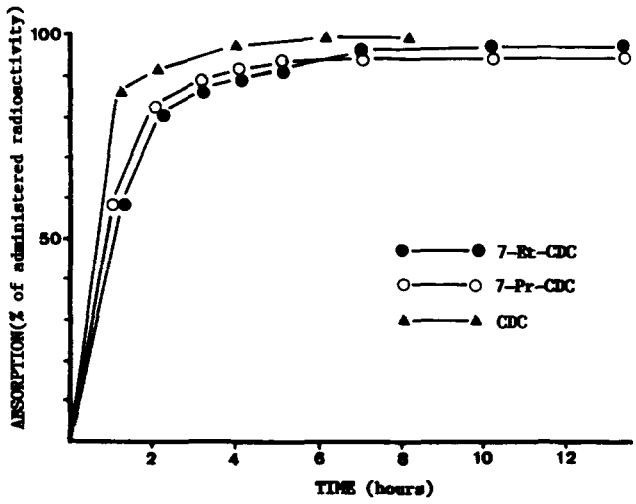

Fig. 1. Biliary excretion of radioactivity in bile fistula hamsters after intraduodenal administration of labeled chenodeoxycholic acid (▲), 7-ethyl-chenodeoxycholic acid (●), and 7-propyl-chenodeoxycholic acid (○). Each value represents the average of two hamsters.

TLC is shown in **Fig. 2**. 7-Ethyl-CDC (Fig. 2, left column) and 7-propyl-CDC (Fig. 2, a solid line in right column), like the naturally occurring CDC (Fig. 2, a broken line in right column), were conjugated completely with glycine and taurine; the G/T (glycine/taurine) ratios were 0.3, 0.2, and 1.9, respectively. These G/T ratios were estimated by TLC analysis of bile collected in the first 2 h, although the G/T ratio does not remain constant and probably decreased progressively during the experiment, as observed previously (3).

In the 7-alkyl bile acid feeding experiment, animals of each experimental goup were fed an average of 10 g of food per day. As calculated from food intake, each hamster received about 7.5 mg of each bile acid analog. In addition, all animals were healthy throughout the experiment period and gained similar amounts of weight. The biliary bile acid composition of intact hamsters fed 0.075% 7-alkyl bile acids for 21 days is shown in **Table 1**. In the strain of hamster studied, cholic acid and CDC were the major bile acids; small proportions of deoxycholic acid were also present. When 0.075% 7-methyl- or 7-ethyl- CDC was administered, these bile acid analogs became major biliary constituents, accounting for 38% and 31% of total bile acids, respectively. By comparison, only relatively small proportions of the 7-propyl analog were present (12%). The dietary administration of all three alkyl bile acids led to a significant diminution of the percentage of cholic acid in biliary bile acids.

After the 2 week feeding period with 0.075% of the unlabeled compounds in chow, labeled CDC, 7-ethyl-CDC, or 7-propyl-CDC were given intragastrically to the experimental groups. The cumulative fecal excretion of ^{14}C in feces is plotted in **Fig. 3**. The 7-alkyl bile acids were excreted into feces at about the same rate as CDC.

Radio-TLC analysis of the fecal bile acids after administration of labeled CDC, 7-ethyl-CDC, or 7-propyl-CDC is shown in **Fig. 4**. In every case, the radioactivity was present in the fraction representing the unconjugated bile acids. As evident from Fig. 4, LC (a broken line in both columns) was practically the sole metabolite of CDC in the hamsters fed the 7-alkyl bile acids. 7-Ethyl-CDC (a solid line in the left column) or 7-propyl-CDC (a solid line in the right column) were metabolized differently during passage through the intestinal tract. Radio-TLC of the fecal bile acids obtained from the latter (Fig. 4, right) suggested that almost all of the propyl analog was excreted unchanged. In contrast, radio-TLC of the fecal extract after the administration of labeled 7-ethyl-CDC (Fig. 4, left) revealed three major radioactive components. One component was unmetabolized 7-ethyl-CDC, accounting

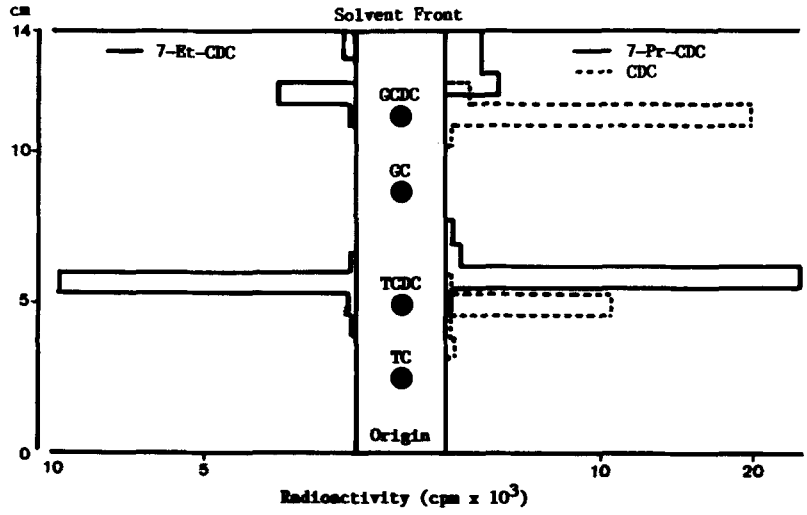

Fig. 2. TLC analysis of radioactivity in bile recovered after intraduodenal administration of labeled 7-ethyl-chenodeoxycholic acid (left), and chenodeoxycholic acid and 7-propyl-chenodeoxycholic acid (right). Solvent system: ethyl acetate–acetic acid–water 7:2:1 (v/v/v); TC, taurocholate; TCDC, taurochenodeoxycholate; GC, glycocholate; GCDC, glycochenodeoxycholate. Metabolites of 7-ethyl-chenodeoxycholic acid (7-ethyl-CDC) are shown with a solid line in left column. Metabolites of chenodeoxycholic acid (CDC) and 7-propyl-chenodeoxycholic acid (7-propyl-CDC) with a broken line and a solid line in right column, respectively.

TABLE 1. Effect of dietary bile acids on gallbladder bile acid composition in hamsters

Bile Acid	Group 1 Control (n = 6)	Group 2 7-Methyl-Chenodeoxycholic Acid (n = 6)	Group 3 7-Ethyl-Chenodeoxycholic Acid (n = 6)	Group 4 7-Propyl-Chenodeoxycholic Acid (n = 6)
		% of bile acids		
Cholic acid	60.1 ± 2.7	28.5 ± 1.1	24.1 ± 1.3	38.4 ± 7.9
Chenodeoxycholic acid	33.1 ± 3.1	27.6 ± 0.8	40.5 ± 2.2	34.3 ± 4.5
Deoxycholic acid	6.8 ± 0.8	5.9 ± 0.4	4.8 ± 0.4	15.0 ± 1.1
7-Alkyl-chenodeoxycholic acid		37.9 ± 1.0	30.6 ± 2.1	12.3 ± 3.7

The 7-alkyl bile acids (0.075%) were fed in a chow diet for 21 days (see Materials and Methods).

for about 30% of total radioactivity. The major metabolite had a somewhat greater mobility than the parent compound. After treatment of the fecal bile acids with sodium borohydride, the area corresponding to the unknown compound decreased while that of 7-ethyl-CDC increased (**Fig. 5**), suggesting conversion of a ketonic metabolite to the hydroxy-compound. The third radioactive component accounted for about 15% of the total radioactivity and was more polar than 7-ethyl-CDC. It was not investigated further. No radioactivity was found corresponding to 7-ethyl-LC, the 7-dehydroxylation product of 7-ethyl-CDC.

In order to study the effect of the alkyl bile acids on cholesterol absorption, animals were fed chow plus 0.1% cholesterol supplemented with 0.1% of one of the following bile acid analogs: 7-methyl-CDC, 7-ethyl-CDC, and 7-propyl-CDC. Though the liver function test or histology was not studied in detail at the end of the experiment, hepatomegaly or fatty liver was not macroscopically observed. Cholesterol absorption was calculated on the basis of administered [^{14}C]β-sitosterol and [^{3}H]cholesterol recovered in feces [method IV of Quintao et al. (8)] as modified by Cohen et al. (7). The results are shown in **Table 2**. In all alkyl CDC-fed groups, cholesterol absorption tended to decrease compared to controls. **Table 3** shows the concentration of serum and liver cholesterol. The administration of 7-propyl-CDC caused a significant decrease in serum and liver cholesterol concentration, while 7-methyl-CDC produced a significant increase in comparison with the controls. There was no difference in fecal cholesterol output among any of groups (not shown in the table).

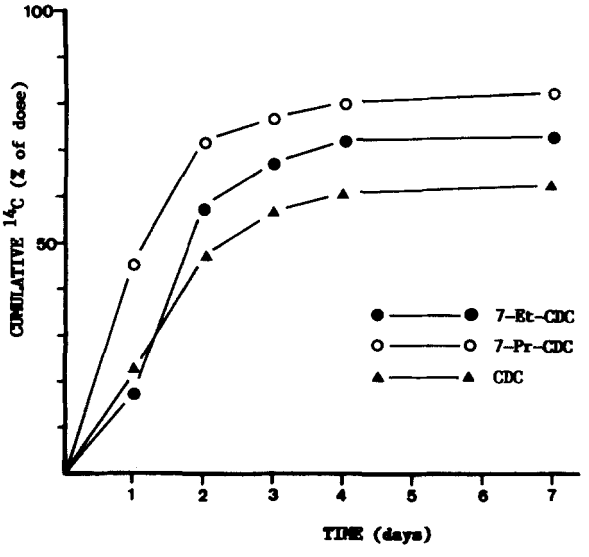

Fig. 3. Fecal excretion of radioactivity after intragastric administration of labeled chenodeoxycholic acid (▲), 7-ethyl-chenodeoxycholic acid (●), and 7-propyl-chenodeoxycholic acid (○) in hamsters. Each value represents the average of two hamsters.

DISCUSSION

After intraduodenal administration of labeled 7-ethyl-CDC and 7-propyl-CDC into bile fistula hamsters, the recovery of label was very rapid and resembled that of CDC. This suggests that the bile acid analogs tested were absorbed from the intestine, extracted efficiently from the liver, and secreted rapidly into the bile, very much like the naturally occurring bile acid, CDC. TLC analysis revealed that all administered bile acids, CDC as well as its analogs, were completely conjugated with taurine or glycine during a single passage through the liver. However, the conjugation pattern of the analogs differed from that of CDC. The latter was predominantly conjugated with glycine (G/T ratio, 1.9), whereas the ratios for 7-ethyl-CDC and 7-propyl-CDC, 0.3 and 0.2, respectively, indicated predominant conjugation with taurine. These results are similar to those obtained previously with 7-methyl-CDC where a G/T ratio of 0.4 was observed (3). The difference in the conjugation pattern between CDC and its alkyl analogs may be explained perhaps by the difference in substrate specificity of the enzyme(s) catalyzing the conjugation reaction.

In the control group, the biliary bile acids consisted mainly of cholic acid and CDC (Table 1). The feeding of 7-alkyl bile acids increased the proportion of the administered analogs in the bile largely at the expense of

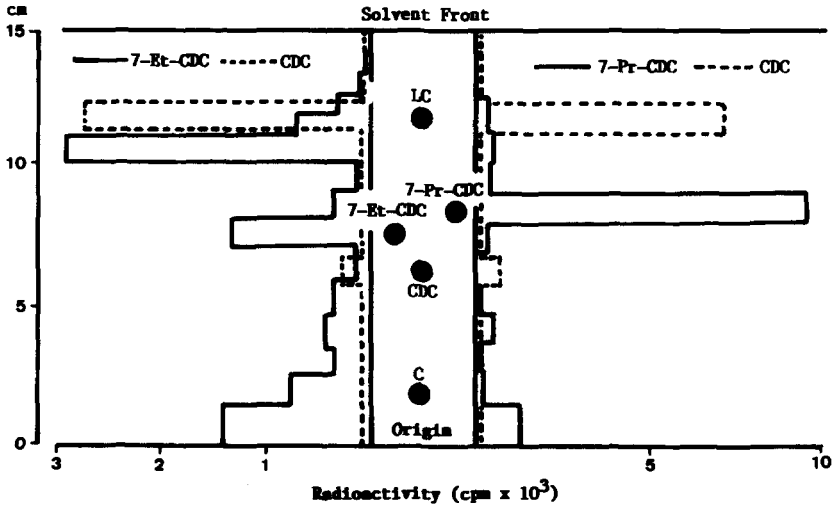

Fig. 4. Radio TLC analysis of fecal bile acids after intragastric administration of labeled bile acids in hamsters fed 7-ethyl-chenodeoxycholic acid (left) and 7-propyl-chenodeoxycholic acid (right). Solvent system: isooctane–ethyl acetate–acetic acid 5:5:1 (v/v/v); LC, lithocholic acid; CDC, chenodeoxycholic acid; C, cholic acid; 7-Pr-CDC, 7-propyl-chenodeoxycholic acid; 7-Et-CDC, 7-ethyl-chenodeoxycholic acid. Fecal metabolites of labeled chenodeoxycholic acid (broken line) and labeled 7-ethyl-chenodoexycholic acid (solid line) in hamsters fed 7-ethyl-chenodeoxycholic acid are shown in left column. Fecal metabolites of labeled chenodeoxycholic acid (broken line) and labeled 7-propyl-chenodeoxycholic acid (solid line) in hamsters fed 7-propyl-chenodeoxycholic acid are shown in right column.

cholic acid. This finding suggests that the 7-alkyl bile acids participate in the enterohepatic circulation. The relatively low proportion of 7-alkyl-CDC in bile is similar to the case of ursodeoxycholic acid (UDC) feeding, in which UDC accounted for 20–40% in total biliary bile acid in hamsters (12, 13). One possible explanation could be that UDC feeding does not suppress endogeneous bile acid synthesis. Another could be due to the limited absorption. In the present study, since 7-alkyl-CDC was absorbed efficiently as CDC, it is assumed that the relatively low proportion of 7-alkyl-CDC resulted in suppression of endogeneous bile acid synthesis. An especially low proportion of 7-propyl-CDC in bile is considered to suppress endogeneous bile acid synthesis to a lesser extent than 7-methyl-CDC and 7-ethyl-CDC.

The naturally occurring conjugated C_{24} bile acids are largely hydrolyzed by the intestinal flora during passage through the intestinal tract. As a consequence, only trace amounts of conjugated C_{24} bile acids are found in the feces. Similarly, in the case of the 7-alkyl bile acids, only negligible amounts of conjugated alkyl bile acids were excreted in the feces. Apparently, the presence of the 7-alkyl groups does not interfere with bacterial deconjugation and suggests that the bacterial enzymes that act upon the naturally occurring conjugates can also hydrolyze the 7-alkyl bile acid conjugates.

The present study was based upon the hypothesis that 7-ethyl- and 7-propyl-CDC, like 7-methyl-CDC, are more resistant to bacterial 7-dehydroxylation than CDC. It was initially thought that 7-methyl-CDC is partially dehy-

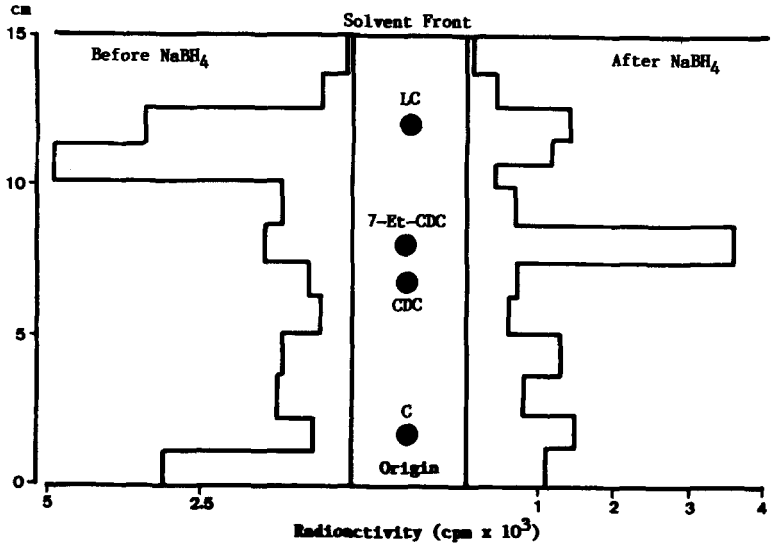

Fig. 5. Radio TLC analysis of fecal metabolites after intragastric administration of labeled 7-ethyl-chenodeoxycholic acid. The fecal bile acids were chromatographed before (left) and after $NaBH_4$ reduction (right). Solvent system, see Fig. 4; LC, lithocholic acid; CDC, chenodeoxycholic acid; C, cholic acid; 7-Et-CDC, 7-ethyl-chenodeoxycholic acid.

TABLE 2. Effect of dietary bile acids on intestinal cholesterol absorption

Group	Administered Bile Acid	Cholesterol Absorption	[^{3}H]Cholesterol/ [^{14}C]β-Sitosterol
		%	
5	control	61.6 ± 7.3	4.68 ± 1.85
6	7-methyl-CDC	58.5 ± 4.1	4.89 ± 0.88
7	7-ethyl-CDC	45.3 ± 4.1[a]	6.53 ± 0.99
8	7-propyl-CDC	49.7 ± 4.5[b]	6.02 ± 1.08

The hamsters were fed a diet containing 0.1% cholesterol plus 0.1% 7-alkyl bile acids. Cholesterol absorption was measured by the method of Quintao et al. (8).

[a]Differs from controls, $P > 0.06$.

[b]Differs from controls, $P < 0.1$.

droxylated in the hamster (3), but subsequent experiments did not confirm this conclusion [M. Une et al., unpublished observations and (4)]. The compound originally identified as 7-methyl-LC was 7α-hydroxy-3-oxo-7β-methyl-5β-cholanoic acid. A similar problem did not arise in the case of 7-propyl-CDC which was largely excreted unchanged. However, after the administration of labeled 7-ethyl-CDC, analysis by radio-TLC revealed the existence of a major transformation product (60% of total radioactivity) plus unmetabolized material (30%). The major metabolite was tentatively identified as the 3-oxo derivative of 7-ethyl-CDC, namely 7α-hydroxy-3-oxo-7β-ethyl-5β-cholanoic acid, since $NaBH_4$ reduction caused its transformation to the original 7-ethyl-CDC. These findings strongly suggest, therefore, that all three 7-alkyl-CDC analogs studied so far are completely resistant to bacterial 7-dehydroxylation.

Recently Kuroki et al. (14) reported that dietary 7-methyl-cholic acid (0.075%, the same dose as used in the present study) inhibited the bacterial 7-dehydroxylation of cholic acid and CDC in the hamster. However, we found in the present study that CDC was efficiently converted to LC in the presence of 7-ethyl-CDC or 7-propyl-CDC. This suggests that the inhibitory effect of the 7-alkyl bile acids on bacterial 7-dehydroxylation of the naturally occurring bile acids may be a function of the chain-length of the 7-alkyl group. In any case, the observation that CDC was dehydroxylated to LC in the present study in the presence of 7-ethyl- or 7-propyl-CDC suggests that these compounds are resistant to 7-dehydroxylation by virtue of the steric hindrance exerted by the 7-alkyl group and not because they exert an antibiotic effect upon the intestinal bacterial flora (14).

TABLE 3. Effect of dietary bile acids on tissue cholesterol concentration

Group	Administered Bile Acids	Serum	Liver
		mg/dl	*mg/g*
5	control	161 ± 18	10.5 ± 2.3
6	7-methyl-CDC	226 ± 19[a]	10.0 ± 0.4
7	7-ethyl-CDC	186 ± 7	6.4 ± 0.9
8	7-propyl-CDC	99 ± 6[b]	3.0 ± 1.0[b]

The 7-alkyl bile acids (0.1%) were fed in a chow diet containing 0.1% added cholesterol for 21 days. Serum cholesterol was measured enzymatically, liver cholesterol by GLC (see Materials and Methods).

[a]Differs from controls, $P < 0.05$.

[b]Differs from controls, $P < 0.02$.

7-Ethyl-CDC and 7-propyl-CDC are more hydrophobic than CDC (R_f values on reversed phase RP-18 TLC; solvent system, methanol–water 8:2; CDC, 0.20; 7-methyl-CDC, 0.16; 7-ethyl-CDC, 0.12; 7-propyl-CDC, 0.09). Based on their hydrophobicity, these bile acids might be assumed to be more hepatotoxic than CDC. As shown in the present study, however, these bile acid analogs had the advantage of not being metabolized to more hydrophobic and more hepatotoxic monohydroxy bile acids.

Recently, Matoba et al. (6) reported that the administration of 7-methyl-CDC decreased cholesterol absorption in hamsters in comparison with a control group and CDC-fed group, and caused a significant decrease in serum cholesterol level. In the present study, 7-methyl-CDC produced a similar effect but the difference from the control group was not significant. The disparity between the two studies may be due, at least in part, to the fact that the Charles River hamsters used in the earlier study (6) had a greater basal cholesterol absorption (71%) than the Hiroshima hamsters (62%). In the previous study, the oral administration of 7-methyl-CDC produced a significant decrease in serum cholesterol concentration, while in the present study an increase was observed. The reason for this difference is not obvious but may be related to the fact that hamsters from entirely different sources were used. In any case, the present study showed a progressive decrease in tissue cholesterol levels as the chain-length of the 7-alkyl group was increased.

In summary, the bile acid analogs 7-ethyl-CDC and 7-propyl-CDC are metabolized in the hamster in a manner closely resembling that of the naturally occurring CDC, with the exception that 7-dehydroxylation of the 7-alkyl analogs was abolished. The introduction of a relatively small 7-alkyl group into the CDC molecule did not interfere with the participation of the analogs in the enterohepatic circulation.

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