7-Methyl bile acids: 7 β-methyl-cholic acid inhibits bacterial 7-dehydroxylation of cholic acid and chenodeoxycholic acid in the hamster

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Abstract The effect of dietary 7β-methyl-cholic acid [0.075% in rodent chow (6.4 mg/animal per day)] on cholesterol and bile acid metabolism was studied and compared with that of cholic acid in the hamster. Following oral administration of 7β-methyl-cholic acid for 3 weeks, the glycine-conjugated bile acid analog became a major constituent of gallbladder bile. Biliary cholic acid concentration decreased significantly, while that of chenodeoxycholic acid remained unchanged. Serum and liver cholesterol levels were increased by dietary 7β-methyl-cholic acid and by cholic acid. Hepatic microsomal HMG-CoA reductase activity was inhibited (30% of the control value) by both bile acids; cholesterol 7α-hydroxylase activity was not affected. In chow controls and cholic acid-fed animals, bacterial 7-dehydroxylation of [^{14}C]chenodeoxycholic acid and [^{14}C]cholic acid was nearly complete. In contrast, dietary 7β-methyl-cholic acid effectively prevented the 7-dehydroxylation of the two primary bile acids. These results show that dietary 7β-methyl-cholic acid is preserved in the enterohepatic circulation and has an effect on serum and liver cholesterol concentrations similar to those produced by the naturally occurring cholic acid. 7β-Methyl-cholic acid is an efficient inhibitor of the bacterial 7-dehydroxylation of the primary bile acids in the hamster.—**Kuroki, S., E. H. Mosbach, B. I. Cohen, R. J. Stenger, and C. K. McSherry.** 7-Methyl bile acids: 7β-methyl-cholic acid inhibits bacterial 7-dehydroxylation of cholic acid and chenodeoxycholic acid in the hamster. *J. Lipid Res.* 1987. **28:** 856–863.

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The steroidal 7α-hydroxyl group plays an important role in cholesterol-bile acid metabolism. Thus, the 7α-hydroxylation of cholesterol is the initial and rate-limiting step of bile acid synthesis (1), and the primary bile acids of most mammalian species, chenodeoxycholic acid (CDCA) and cholic acid (CA), possess a 7α-hydroxyl group. Both bile acids participate significantly in the solubilization and absorption of lipids in the intestinal tract (2). 7-Dehydroxylation of these primary bile acids to form lithocholic acid (LCA) and deoxycholic acid (DCA), respectively, is the major pathway for the excretion of bile acids into feces (3). In addition, certain bile acids that do not have a 7-hydroxyl group are considered to be hepatotoxic and carcinogenic (3–5).

Recent studies have focused on the in vivo inhibition of the 7-dehydroxylation of bile acids, because this might result in prolonged enterohepatic circulation of physiologically valuable bile acids and reduce the concentration of the potentially toxic secondary bile acids. Hatono et al. (6) and Kimura et al. (7) synthesized N-methyl-glycine (sarcosine)-conjugated CDCA and ursodeoxycholic acid (UDCA) and reported that these compounds are resistant to bacterial 7-dehydroxylation. Some antibiotics are known to reduce the number of intestinal bacteria involved in the 7-dehydroxylation of bile acids (8, 9).

Abbreviations: HMG-CoA, hydroxymethylglutaryl-coenzyme A; TLC, thin-layer chromatography; GLC, gas–liquid chromatography; GLC–MS, gas–liquid chromatography–mass spectrometry; SIM, selective ion monitoring; DMES, dimethylethylsilyl; TMS, trimethylsilyl. Trivial and systematic names for the bile acids referred to are: LCA, lithocholic acid (3α-hydroxy-5β-cholanoic acid); CDCA, chenodeoxycholic acid (3α,7α-dihydroxy-5β-cholanoic acid); UDCA, ursodeoxycholic acid (3α,7β-dihydroxy-5β-cholanoic acid); HDA, hyodeoxycholic acid (3α,6α-dihydroxy-5β-cholanoic acid); DCA, deoxycholic acid (3α,12α-dihydroxy-5β-cholanoic acid); CA, cholic acid (3α,7α,12α-trihydroxy-5β-cholanoic acid); UCA, ursocholic acid (3α,7β,12α-trihydroxy-5β-cholanoic acid); α-muricholic acid (3α,6β,7α-trihydroxy-5β-cholanoic acid); β-muricholic acid (3α,6β,7β-trihydroxy-5β-cholanoic acid); 7-ketolithocholic acid (3α-hydroxy-7-oxo-5β-cholanoic acid); 7-ketodeoxycholic acid (3α,12α-dihydroxy-7-oxo-5β-cholanoic acid); 7-Me-LCA, 7ξ-methyl-lithocholic acid (3α-hydroxy-7ξ-methyl-5β-cholanoic acid); 7-Me-CDCA, 7β-methyl-chenodeoxycholic acid (3α,7α-dihydroxy-7β-methyl-5β-cholanoic acid); 7-Me-UDCA, 7α-methyl-ursodeoxycholic acid (3α,7β-dihydroxy-7α-methyl-5β-cholanoic acid); 7-Me-DCA, 7ξ-methyl-deoxycholic acid) (3α,12α-dihydroxy-7ξ-methyl-5β-cholanoic acid); 7-Me-CA, 7β-methyl-cholic acid (3α,7α,12α-trihydroxy-7β-methyl-5β-cholanoic acid); 7-Me-UCA, 7α-methyl-ursocholic acid (3α,7β,12α-trihydroxy-7α-methyl-5β-cholanoic acid).

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Our laboratory has prepared analogs of the primary bile acids and of 7β-hydroxy-cholanoic acids with a methyl group at the 7-position, such as, 7β-methyl-chenodeoxycholic acid (7-Me-CDCA), 7α-methyl-ursodeoxycholic acid (7-Me-UDCA), 7β-methyl-cholic acid (7-Me-CA), and 7α-methyl-ursocholic acid (7-Me-UCA) (10, 11). It was hypothesized that these 7-methyl bile acids were not only resistant to bacterial 7-dehydroxylation but would also serve as inhibitors of the 7-dehydroxylation of the naturally occurring primary bile acids. In previous experiments, we have shown that in the hamster these 7-methyl bile acids are absorbed by the small intestine, efficiently extracted by the liver, largely conjugated with glycine and taurine, and excreted into bile as efficiently as the primary bile acids (12, 13). 7-Me-CDCA, 7-Me-UDCA, 7-Me-CA, and 7-Me-UCA were found to be resistant to bacterial 7-dehydroxylation (12, 13). 7-Me-CDCA competitively inhibited the 7-dehydroxylation of CDCA in vitro (14).

This report describes the effects of dietary CA and 7-Me-CA on cholesterol and bile acid metabolism and the inhibition of the bacterial 7-dehydroxylation of CDCA and CA by 7-Me-CA in the hamster.

MATERIALS AND METHODS

Chemicals

3α,7α,12α-Trihydroxy-7β-methyl-5β-cholanoic acid was synthesized and purified by recrystallizations from ethyl acetate as described previously (11). Cholic acid (Inolex, Park Forest South, IL) was purified by crystallization from methanol. 3α-Hydroxy-7-oxo-5β-cholanoic acid and 3α,12α-dihydroxy-7-oxo-5β-cholanoic acid were synthesized as reported by Fieser and Rajagopalan (15, 16). Methyl 3-oxo-5β-cholanoate, methyl 7α-hydroxy-3-oxo-5β-cholanoate, methyl 12α-hydroxy-3-oxo-5β-cholanoate, and methyl 7α,12α-dihydroxy-3-oxo-5β-cholanoate were synthesized by oxidation with silver carbonate on Celite as described by Tserng (17). α-Muricholic acid (3α,6β,7α-trihydroxy-5β-cholanoic acid) and β-muricholic acid (3α,6β,7β-trihydroxy-5β-cholanoic acid) were generous gifts of Dr. W. H. Elliott. Other reference bile salts (free and amidated) were purchased or synthesized in this laboratory as mentioned elsewhere (10, 11, 18).

[24-^{14}C]Cholic acid and [24-^{14}C]chenodeoxycholic acid were purchased from NEN Products (Boston, MA) and diluted with the unlabeled bile acids to a specific activity of 1 μCi/mg. Radiochemical purity of each labeled compound was better than 99% as determined by radio-thin-layer chromatography (described below).

Experimental design

Male golden Syrian hamsters (*Mesocricetus auratus,* Harlan Sprague-Dawley, Indianapolis, IN), weighing 119 ± 13 g, were quarantined for 2 weeks and divided into three groups (ten animals each). The animals were fed Purina rodent laboratory chow (control, group 1), Purina rodent chow containing 0.075% cholic acid (CA, group 2), or Purina rodent chow with 0.075% 7β-methyl-cholic acid (7-Me-CA, group 3) (Teklad, Madison, WI) for 3 weeks. Food and water were given ad libitum.

At the end of 2 weeks (on day 14 of the experimental period) the animals were anesthesized by intramuscular injection of ketamine hydrochloride (10 mg). The three groups were subdivided (five animals/group, group 1a, 1b; 2a, 2b; 3a, 3b) for the radioisotope study. Either ^{14}C-labeled CDCA (2 μCi/animal) or CA (2 μCi/animal) dissolved in 1% $NaHCO_3$ solution was given by stomach tube to the five animals in each group, so that the CDCA pool of five animals (groups 1a, 2a, 3a) or the CA pool of another five animals (groups 1b, 2b, 3b) in each group was radiolabeled. Feces were collected daily for 7 days during the third week and were stored at −20°C until analyzed.

On day 21, the animals were anesthesized by intramuscular injection of ketamine hydrochloride (10 mg), and kept under anesthesia with diethyl ether. The abdomen and the chest were opened. Blood was obtained by cardiac puncture, bile was aspirated with a 50-μl microsyringe (Hamilton Co., Reno, NE) and the liver was resected.

Analytical techniques

Analysis of gallbladder bile acids. Conjugation profiles were examined by semiquantitative TLC based on the method reported by Kibe, Kuramoto, and Hoshita (19). Silica gel 60 F_{254} precoated TLC aluminum sheets (200 μm thickness, Merck, Darmstadt, West Germany) were used. Gallbladder bile samples were applied as small spots and the plates were developed in n-butanol–acetic acid–water 85:10:5 (v/v/v). Bile salts were visualized by spraying the plate with 10% phosphomolybdic acid in ethanol followed by heating. Conjugation profiles were judged by the densities of the bile salt spots in comparison with spots of known amounts of standards.

For the determination of biliary bile acid composition, bile samples (5 μl) were hydrolyzed with cholylglycine hydrolase (Sigma Chemical, St. Louis, MO) and deconjugated bile acids were analyzed by gas–liquid chromatography (GLC) as their methyl ester-dimethylethylsilyl (DMES) ether derivatives (13, 20) on 3% QF-1 (2 mm × 6 ft columns). Ten μg of nordeoxycholic acid (3α,12α-dihydroxy-24-nor-5β-cholan-23-oic acid, Steraloids, Inc., Wilton, NH) and 5α-cholestane (5 μg) were used as internal standards. The identification of biliary bile acids was confirmed by using a second derivative (methyl ester-trimethylsilyl ether) and other stationary phases (3% SE-30, 2 mm × 6 ft column and 3% SP-2250, 2 mm × 6 ft column).

Gas–liquid chromatography–mass spectrometry (GLC-MS) was performed as described previously (13). Both individual mass spectrograms and selected ion monitoring (SIM) were

used for positive identification of metabolites of 7β-methyl-cholic acid. On SIM, 7-methyl-3,7-dihydroxy structures gave characteristic fragment ions (m/e 143 and 257 for TMS derivatives (11) and m/e 157 and 285 for DMES derivatives); derivatives of 7ξ-methyl-deoxycholic acid gave m/e 269 as the base peak (11).

Other lipid analyses. Biliary phospholipid concentration was determined as previously reported (21). Serum and liver total cholesterol concentrations were analyzed by GLC, using the TMS ether derivatives (22).

HMG-CoA reductase and cholesterol 7α-hydroxylase activities. Liver microsomes were prepared as described previously (23) and hepatic microsomal HMG-CoA reductase and cholesterol 7α-hydroxylase activites (24, 25) were expressed as pmol/mg protein per min. Microsomal protein concentrations were measured according to Lowry et al. (26).

Analysis of fecal bile acid metabolites. Fecal samples were lyophilized and ground to a powder. To 1-g portions of each fecal sample there was added 20 ml of 1 N NaOH in 90% ethanol and the mixture was refluxed for 1 hr. Removal of neutral steroids and extraction of bile acids were carried out as described by Grundy, Ahrens, and Miettinen (27). The chloroform–methanol extract was dried under a stream of nitrogen and dissolved in 1 ml of acetone. One aliquot was used for radio-TLC of the free acids (benzene–isopropanol–acetic acid 30:10:1, v/v/v). A second aliquot was methylated with diazomethane and used for radio-TLC of the methyl esters (benzene–acetone 1:1, v/v). A third aliquot was treated with $NaBH_4$ in ethanol (13), esterified with diazomethane, and analyzed by radio-TLC (benzene–acetone 1:1, v/v). Suitable standards were run concurrently. After development and spraying, the plates were cut into 2-mm segments and analyzed for radioactivity in Aquasol-2 (NEN Products, Boston, MA) with a Beckman LS 8000 liquid scintillation system (Beckman Instruments, Fullerton, CA). Corrections were made for background and quenching.

Histological examination. Liver specimens were fixed in Millonig's buffered formalin and embedded in paraffin. Sections were stained with hematoxylin and eosin and examined by the pathologist (R. J. S.) without knowledge of the previous dietary history of the animals.

Calculations. The results are expressed as mean ± SEM. The significance of differences among the groups' means was determined by one-way analysis of variance followed by Student's *t*-test (28). Percent cholesterol saturation of bile was calculated using a personal computer program described previously (29).

RESULTS

General

The food consumption of the experimental animals averaged 8.5 ± 0.9 g of food per animal per day. This means that the animals of the CA and 7-Me-CA groups ingested about 6.4 mg of bile acid per day. This dose was about 1.4 times the endogenous bile acid synthesis in normal hamsters (30). There was no significant weight gain in any group during the experiment.

Biliary bile acids

On the basis of semi-quantitative TLC, the bile acids in the control group were conjugated mainly with taurine; glycine conjugation predominated in the bile acid-fed animals. Unconjugated bile salts amounted to less than 5% of total bile acids in all groups. Metabolites with R_f values corresponding to bile acid glucuronides or sulfates were not detected.

The biliary bile acid composition of all animals in the three groups is summarized in **Table 1.** CA and CDCA were the major bile acids in the control group; small proportions of secondary bile acids were also detected. CA feeding increased the percentage of CA and decreased that of CDCA. When 7-Me-CA was fed, this bile acid accumulated in gallbladder bile; in this group biliary CA decreased significantly. The proportion of CDCA was unchanged. As a result, the ratio of CA/CDCA decreased from 2.28 (control group) to 0.39 (7-Me-CA group). Metabolites of 7-Me-CA were not detected by TLC, GLC, and GLC–MS.

Biliary lipids

The biliary lipid concentrations and the percent cholesterol saturation of bile for all animals in the three groups are listed in **Table 2**. Bile samples of the control animals had very low cholesterol concentrations and the cholesterol saturation was low. Dietary CA or 7-Me-CA had no effect on the biliary lipid concentrations or cholesterol saturation.

Serum and liver cholesterol concentrations

The effect of bile acid feeding on serum and liver cholesterol concentrations is shown in **Table 3**. Both bile acids increased the cholesterol levels; 7-Me-CA seemed to be more potent in this respect than CA, but the difference was not significant.

Microsomal enzymes

Feeding of both bile acids decreased the activity of hepatic microsomal HMG-CoA reductase to approximately one-third of the control value (Table 3). Cholesterol 7α-hydroxylase activity was unchanged.

Inhibition of 7-dehydroxylation of CDCA and CA

Results of radio-TLC analyses of fecal metabolites of ^{14}C-labeled CDCA and CA are summarized in **Table 4** and **Table 5.** In the control and CA-fed hamsters, administered ^{14}C-labeled CDCA was largely 7-dehydroxylated to LCA while ^{14}C-labeled CA was metabolized to DCA. Some minor metabolites, such as 3-ketocholanoic acid, 7-keto-

TABLE 1. Effect of dietary cholic acid and 7β-methyl-cholic acid on gallbladder bile acid composition in hamsters[a]

	Group		
	1 Control (n = 10)	2 Cholic Acid (n = 10)	3 7β-Methyl-Cholic Acid (n = 10)
Bile acid composition (%)			
Cholic acid	63.3 ± 3.0[b]	70.8 ± 1.5	13.7 ± 1.1[c]
7β-Methyl-cholic acid			47.4 ± 1.7[c]
Chenodeoxycholic acid	30.1 ± 2.6	21.6 ± 1.1[c]	37.2 ± 2.1
Deoxycholic acid	2.9 ± 0.4	3.9 ± 0.8	0.8 ± 0.1[c]
7-Ketodeoxycholic acid	2.7 ± 0.4	3.0 ± 0.2	0.5 ± 0.1[c]
7-Ketolithocholic acid	0.4 ± 0.2	0.2 ± 0.0	0.4 ± 0.1
Lithocholic acid	0.6 ± 0.1	0.5 ± 0.1	0 ± 0[c]
Ratio: cholic/chenodeoxycholic	2.28 ± 0.27	3.35 ± 0.22[c]	0.39 ± 0.05[c]

The absolute concentration of total bile acid in gallbladder bile was as follows: control, 74.2 ± 4.0 mM; cholic acid, 79.9 ± 8.5 mM; 7β-methyl-cholic acid, 66.7 ± 3.9 mM.

[a]Cholic acid or 7β-methyl-cholic acid (0.075% in rodent chow) was fed for 21 days. Gallbladder bile was analyzed by GLC of the methyl ester-dimethylethylsilyl ether derivatives after enzymatic hydrolysis.

[b]Mean ± SEM.

[c]Differs significantly from the control ($P < 0.05$).

lithocholic acid, α-muricholic acid (from CDCA), and 12α-hydroxy-3-oxo-5β-cholanoic acid (from CA), were also detected. In the 7-Me-CA-fed animals, however, most of the administered ^{14}C-labeled CDCA was recovered unchanged and considerable amounts of 7-ketolithocholic acid were also detected (Table 4). At the same time the proportion of LCA decreased dramatically. Similarly, the 7-dehydroxylation of ^{14}C-labeled CA was inhibited by dietary 7-Me-CA (Table 5); 72% of CA was recovered unchanged and only 19% was 7-dehydroxylated to DCA.

Histological observations

The liver sections disclosed no appreciable differences between the bile acid-treated and the control hamsters.

DISCUSSION

Theoretically, there exist at least three different ways in which the bacterial 7-dehydroxylation of bile acids may be inhibited or prevented. First, the number of bacteria that carry out the 7-dehydroxylation can be reduced by administration of bactericidal or bacteriostatic compounds, such as lincomycin (8), ampicillin (9), and bile acid oxazolines (31). Second, because the 7-dehydroxylation is thought to occur only after bacterial deconjugation of amidated bile acids (3), compounds that are resistant to deconjugation should be resistant to 7-dehydroxylation (7, 14). Third, introduction of a protective group in the bile acid nucleus on or near the 7 position might render the bile acid analog resistant to enzymatic dehydroxylation (12–14). Such 7-substituted compounds, e.g., 7-methyl-substituted CDCA, UDCA, CA, and UCA, could conceivably serve as inhibitors of the enzymatic 7-dehydroxylation reaction (14).

The present experiment has clearly shown that the dietary 7-Me-CA (0.075% in chow or 6.4 mg/animal per day) inhibited the bacterial 7-dehydroxylation of the primary bile acids, CDCA and CA. This study was designed to achieve a steady state by feeding either rodent chow, chow plus CA, or chow plus 7-Me-CA for 2 weeks followed by

TABLE 2. Effect of dietary cholic acid and 7β-methyl-cholic acid on biliary lipids in hamsters

	Group		
	1 Control (n = 10)	2 Cholic Acid (n = 10)	3 7β-Methyl-Cholic Acid (n = 10)
Bile acids (mol %)	91.3 ± 1.8[a]	92.6 ± 0.8	89.8 ± 1.2
Phospholipid (mol %)	7.6 ± 1.6	6.3 ± 0.7	8.9 ± 1.2
Cholesterol (mol %)	1.1 ± 0.2	1.1 ± 0.1	1.3 ± 0.1
Total lipid (g/dl)	4.19 ± 0.39	4.67 ± 0.52	3.66 ± 0.32
Cholesterol saturation (%)	36 ± 4	37 ± 4	40 ± 3

[a]Mean ± SEM.

TABLE 3. Effect of dietary cholic acid and 7β-methyl-cholic acid on serum and liver cholesterol concentrations and activities of hepatic microsomal enzymes in hamsters

	Group		
	1 Control (n = 10)	2 Cholic Acid (n = 10)	3 7β-Methyl-Cholic Acid (n = 10)
Serum cholesterol (mg/dl)	102 ± 7	129 ± 10[a]	145 ± 5[a]
Liver cholesterol (mg/g)	2.56 ± 0.26	3.76 ± 0.60	4.51 ± 0.37[a]
HMG-CoA reductase	137 ± 38	40 ± 11[a]	42 ± 8[a]
Cholesterol 7α-hydroxylase	6.6 ± 1.3	7.8 ± 1.5	7.3 ± 2.7

Enzyme activities: pmol/min per mg of protein. All data are expressed as mean ± SEM.
[a]Significantly different from control value ($P < 0.05$).

^{14}C-labeling of the primary bile acid pools. Radio-TLC analyses of radioactive metabolites in feces showed that in the control and CA groups bacterial 7-dehydroxylation of CDCA and CA was nearly complete and that LCA and DCA were major fecal metabolites (Tables 4 and 5). The results were consistent with those of previous publications (3, 12, 13). However, dietary 7-Me-CA inhibited the 7-dehydroxylation of both CDCA and CA. This effect probably represents competitive inhibition on the basis of a previous in vitro study with 7-Me-CDCA (14); 7-Me-CDCA competitively inhibited the bacterial 7-dehydroxylation of CA but did not affect the growth of the bacteria themselves. However, the possibility remains that 7-Me-CA inhibited bacterial growth during prolonged intense exposure of the large intestine to the bile acid analog. The extent of the inhibition of bacterial 7-dehydroxylation (expressed as the percentage of metabolites without 7-hydroxyl group) of CDCA or CA was approximately 80% (Tables 4 and 5).

Since 7-Me-CA structurally resembles CA, it could conceivably bind to the active center of the bacterial bile acid 7-dehydroxylase; presumably, the enzyme is unable to break the C–O bond because the 7β-methyl group interferes. The results of the present study further suggest that CA and CDCA are 7-dehydroxylated by the same enzyme, because the 7-dehydroxylation of CDCA and CA was inhibited to a similar extent by 7-Me-CA (Tables 4 and 5).

The bile acid analog did not inhibit bacterial *dehydrogenation:* considerable amounts of the primary bile acids were transformed into ketonic derivatives (7-ketolithocholic acid and 7-ketodeoxycholic acid) instead of undergoing 7-dehydroxylation. If these oxo-acids are absorbed, they are probably reduced by hepatic enzyme(s) to the original 7α-hydroxy-bile acids (32). This hypothesis was supported by the analysis of gallbladder bile which showed little secondary bile acids in bile (Table 1). A similar increase of ketonic metabolites in feces was reported when the 7-dehydroxyla-

TABLE 4. Effect of dietary cholic acid and 7β-methyl-cholic acid on bacterial transformation of [^{14}C]chenodeoxycholic acid in hamsters

	Group		
	1a Control (n = 5)	2a Cholic Acid (n = 5)	3a 7β-Methyl-Cholic Acid (n = 5)
		(%)	
Fecal metabolites of [^{14}C]chenodeoxycholic acid			
Chenodeoxycholic acid	6.0 ± 0.8[a]	4.9 ± 1.2	55.2 ± 4.1[b]
7-Ketolithocholic acid	1.0 ± 1.4	1.2 ± 0.5	14.1 ± 2.8[b]
Lithocholic acid	84.2 ± 5.1	80.0 ± 2.0	13.8 ± 1.7[b]
3-Ketocholanoic acid	5.3 ± 4.0	12.4 ± 1.6[b]	9.1 ± 2.9
α-Muricholic acid[c]	3.5 ± 0.6	1.5 ± 1.1[b]	3.3 ± 0.4
β-Muricholic acid[c]	N.D.[d]	N.D.	2.4 ± 0.5
Unknown[e]	N.D.	N.D.	2.1 ± 1.8
Bacterial 7-dehydroxylation of chenodeoxycholic acid[f]	89.5 ± 3.9	92.4 ± 1.6	22.9 ± 4.0[b]

The group designations (1a, 2a, 3a) are defined in Experimental Design. The data are based on radio-TLC analysis of fecal bile acids.
[a]Mean ± SEM.
[b]Significantly different from the control ($P < 0.05$).
[c]Identification was based on the mobility on TLC and is tentative.
[d]Not detected.
[e]TLC mobility was nearly identical with that of cholic acid.
[f]Expressed as the percentages of radioactive metabolites without 7-hydroxyl group.

TABLE 5. Effect of dietary cholic acid and 7β-methyl-cholic acid on bacterial transformation of [^{14}C]cholic acid in hamsters

	Group		
	1b Control (n = 5)	2b Cholic Acid (n = 5)	3b 7β-Methyl-Cholic Acid (n = 5)
		(%)	
Fecal metabolites of [^{14}C]cholic acid			
Cholic acid	2.2 ± 1.1[a]	2.7 ± 0.8	71.6 ± 1.9[b]
7-Ketodeoxycholic acid	N.D.[c]	N.D.	9.2 ± 1.0[b]
Deoxycholic acid	94.3 ± 2.4	93.6 ± 0.6	19.2 ± 2.8[b]
12α-Hydroxy-3-oxo-cholanoic acid	3.5 ± 1.4	3.7 ± 0.4	N.D.[b]
Bacterial 7-dehydroxylation of cholic acid[d]	97.8 ± 1.1	97.3 ± 0.8	19.2 ± 2.8[b]

The group designations (1b, 2b, 3b) are defined in Experimental Design. The data are based on radio-TLC analysis of fecal bile acids.

[a] Mean ± SEM.

[b] Significantly different from the control ($P < 0.05$).

[c] Not detected.

[d] Expressed as the percentages of radioactive metabolites without 7-hydroxyl group.

tion of CDCA and UDCA was inhibited by conjugation of CDCA and UDCA with sarcosine (N-methylglycine) (6, 7). It is suggested that normally, under the anaerobic conditions prevailing in the large intestine, 7-dehydroxylation is so effective that dehydrogenation of CDCA and CA is less pronounced. Under aerobic conditions (33) or when 7-dehydroxylation was inhibited (7, 12, 13), oxidation of bile acids might become pronounced.

The biliary bile acids in the control group consisted mainly of CA and CDCA (Table 1). Secondary bile acids were minor constituents. In the CA group, the proportion of CA increased and that of CDCA decreased, but these changes were not significant. In the CA group DCA did not increase significantly. Hamsters have an active hepatic DCA 7α-hydroxylase activity which reconverts the secondary bile acid to CA (34). This explains, at least in part, why biliary DCA concentration did not increase even though there was an increase in the production of DCA in the large intestine (Table 5). However, hamsters purchased from commercial suppliers are not genetically homogeneous and it is conceivable that hamsters from other sources might give different results (35). It certainly must be considered that the proportion of biliary DCA varies as a function of the concentration of CA in the diet (30).

7-Me-CA feeding increased the proportion of this analog in bile to nearly 50% of total bile acids. This finding and previous observations (12, 13) lead to the conclusion that the 7-methylcholanoic acid participates actively in the enterohepatic circulation. In the present, relatively long-term feeding experiments, metabolites of 7-methyl-CA could not be detected in bile by TLC, GLC, or GLC-MS, though considerable amounts of keto-derivatives were found in feces in a previous study with a single dose of the analog (13). These ketonic compounds, if present, can reach the liver via passive diffusion through the colonic mucosa (36) and can then be reduced by the liver to hydroxy acids (37).

In this feeding experiment, biliary 7-Me-CA was found mainly conjugated with glycine and to a lesser extent with taurine. Unconjugated 7-Me-CA amounted to less than 5% of the total as judged by semi-quantitative TLC analysis. In contrast, following the intravenous infusion of 7-Me-CA, considerable proportions (39%) of unconjugated 7-Me-CA were recovered in fistula bile (13). It is suggested that 7-Me-CA is inherently more resistant to hepatic enzymatic amidation than the naturally occurring CA, but that the percentage of the conjugated analog increased during repeated passage through the liver.

The effects of dietary CA and 7-Me-CA on hepatic microsomal HMG-CoA reductase were almost identical. Both bile acids inhibited the enzyme activity by 70%. However, under the conditions employed it is not possible to state whether we are dealing with a direct effect of the bile acids on the enzyme or whether it was mediated via the increased hepatic cholesterol concentrations caused by increased cholesterol absorption (Table 3).

Dietary 7-Me-CA as well as CA exerted no significant effect on the activity of hepatic cholesterol 7α-hydroxylase, the rate-limiting enzyme of bile acid biosynthesis. A similar effect of bile acid feeding on this enzyme has been found in a previous study in the hamster (38). There is no clear-cut explanation for this finding, but it brings into focus the question of rate-control of bile acid synthesis in nonmurine species. Perhaps, hamsters on rodent chow have such a low basal 7α-hydroxylase activity (38) that addition of bile acid to this diet does not produce any additional inhibitory effect.

7-Me-CA feeding changed the ratio of biliary CA/CDCA from 2.28 to 0.39 (Table 1). It is known that dietary CA inhibits bile acid 12α-hydroxylase activity (30, 39) and that there is a positive correlation between the activity of steroid 12α-hydroxylase and the CA/CDCA ratio (30). It seems likely that administration of 7-Me-CA inhibited the

steroid 12α-hydroxylase activity because of the structural resemblance to CA.

Both bile acids increased the levels of serum and liver cholesterol significantly. This result is consistent with those of previous reports dealing with the effects of CA (2, 22) and is considered to be due to an increase in cholesterol absorption (2). 7-Me-CA may act as the naturally occurring CA in this respect. A cholesterol absorption study may be necessary to clarify this point.

In conclusion, dietary 7-Me-CA inhibited the bacterial 7-dehydroxylation of both CDCA and CA to a similar extent. Glycine-conjugated 7-Me-CA became a major biliary bile acid and a proportion of CA decreased significantly. CA and 7-Me-CA have similar effects on the activities of HMG-CoA reductase and cholesterol 7α-hydroxylase and on serum and liver cholesterol levels.

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