Metabolism of β-muricholic acid in the hamster and prairie dog

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Abstract The metabolism of β-muricholic acid was investigated in the prairie dog and the hamster. Intravenous infusion into bile fistula hamsters showed that β-muricholic acid was extracted by the liver and secreted into the bile (>85% in 1 h). Hepatic extraction of this compound and cholic acid in the prairie dog was not as rapid as in the hamster. In the bile of the prairie dog, most (93%) of the administered β-muricholic acid was present as the taurine conjugate. In the hamster, 28% of infused β-muricholic acid was secreted in unconjugated form, 43% as the taurine conjugate, and 22% as the glycine conjugate. In both species, the administered compound underwent little biotransformation. After intraduodenal injection of [6α-^{3}H]-labeled β-muricholic acid into bile fistula hamsters, the bile acid was rapidly secreted into the bile; more than 80% of the administered radioactivity was recovered in 3 h. In the prairie dog, biliary recovery after intraduodenal administration of either β-muricholic acid (43% in 3 h) or cholic acid (22% in 3 h) was slower than in the hamster. After intragastric administration, more than 80% of β-muricholic acid was recovered unchanged in feces of both animal species.—**Miki, S., E. H. Mosbach, B. I. Cohen, T. Mikami, R. Infante, N. Ayyad, and C. K. McSherry.** Metabolism of β-muricholic acid in the hamster and prairie dog. *J. Lipid Res.* 1993. **34:** 1709–1716.

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The 3,7-dihydroxy bile acids, chenodeoxycholic acid (CDCA) and ursodeoxycholic acid (UDCA), have been used clinically to dissolve cholesterol gallstones (1–4). With a hydrophobic bile acid, such as CDCA (5), gallstone dissolution probably proceeds largely via micelle formation (6). With the hydrophilic bile acid, UDCA, dissolution seems to proceed via a dual mechanism, namely, the formation of micelles and of liquid crystalline vesicles (7, 8). The success rates of bile acid therapy have not been consistently high (9). Moreover, CDCA and UDCA are 7-dehydroxylated to lithocholic acid (LCA) by the action of the intestinal microflora (10–12). This degradative pathway is responsible for most of the losses of CDCA and UDCA from the body (13). In addition, LCA is a potential hepatotoxin (14–16) and comutagen (17, 18). Therefore, there still exists a need to develop cholelitholytic agents that are more stable and more effective than CDCA and UDCA.

Previous studies dealt with the 3,6-dihydroxy bile acids, hyodeoxycholic acid (HDCA, 3α,6α-dihydroxy) and its 6β-epimer, murideoxycholic acid (MDCA, 3α,6β-dihydroxy) (19–23). These bile acids were resistant to bacterial dehydroxylation, but not to epimerization at the 6-position (20–24). HDCA and MDCA were effective in preventing the formation of cholesterol gallstones in animal models (21–23). Gallstone prevention with these highly hydrophilic bile acids is believed to occur via inhibition of the phase transition from vesicular cholesterol to crystalline cholesterol monohydrate (23). In the prairie dog (8) and hamster (24), HDCA and MDCA were more effective than UDCA, presumably because of their greater capacity to form a liquid crystalline phase (25). However, HDCA is not suitable for use in humans because this compound does not participate in the enterohepatic circulation: it is glucuronidated and excreted in urine (20, 26). As it has been shown that an active and highly specific UDP-glucuronyl transferase activity for 6α-hydroxy bile acids, but not for 6β-hydroxy bile acids, exists in human liver and kidney (27), the 6β-isomer

Abbreviations: CDCA, chenodeoxycholic acid; UDCA, ursodeoxycholic acid; LCA, lithocholic acid; HDCA, hyodeoxycholic acid; MDCA, murideoxycholic acid; β-MCA, β-muricholic acid; α-MCA, α-muricholic acid; HCA, hyocholic acid; CA, cholic acid; TLC, thin-layer chromatography.

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might be more suitable for use in humans. However, the relatively low solubility of the sodium and calcium salts of conjugated MDCA probably prohibits human application (28).

Sacquet et al. demonstrated that in humans, β-muricholic acid (β-MCA, 3α,6β,7β-trihydroxy-5β-cholanoic acid), a hydrophilic bile acid, was excreted into the bile as the glycine or taurine amidates. This bile acid formed no glucuronide (29), and was only slightly transformed by the human intestinal microflora (30). In vitro studies revealed that sodium β-MCA dissolved cholesterol essentially via the formation of lamellar liquid crystals; in dilute solutions of β-MCA the capacity of vesicles to solubilize cholesterol exceeded the capacity of mixed micelles of common bile salts to transport cholesterol (31).

It seemed possible, therefore, that β-MCA can function as a cholelitholytic agent, and studies of its effects in animal models were needed. The prairie dog and the golden Syrian hamster have been used successfully to test gallstone prevention and dissolution with various bile acids and bile acid analogues. Prior to such studies, it was necessary to investigate some of the biological properties of β-MCA in these species. The present study deals with the intestinal absorption, hepatic biotransformation, and bacterial modification of β-MCA in the prairie dog and the hamster.

MATERIALS AND METHODS

Labeled compounds and reference compounds

[6α-^{3}H]β-MCA (sp act 1.1 × 10^{7} dpm/mg) was prepared as described by Aranda et al. (32); [24-^{14}C]cholic acid (sp act 2.1 × 10^{5} dpm/mg) was purchased from NEN Research Products, Boston, MA. Radiochemical purity of the labeled compounds was greater than 99% as determined by radio thin-layer chromatography. Labeled β-MCA was diluted with unlabeled material to a specific activity of 1.0 × 10^{6} dpm/mg; labeled cholic acid (CA) was diluted with unlabeled CA to a specific activity of 9.8 × 10^{4} dpm/mg. Prior to administration, the compounds were dissolved in 1% aqueous $NaHCO_3$.

Unlabeled α-muricholic acid (α-MCA), β-MCA, and MDCA were synthesized as described previously (19, 33, 34). Hyocholic acid (HCA) and hyodeoxycholic acid (HDCA) were purchased from Sigma Chemical Co., St. Louis, MO. Glycine and taurine conjugates of β-MCA, CA, CDCA, and MDCA, were prepared by published procedures (35).

Experimental animals

Adult male prairie dogs, *Cynomys ludovicianus* (R-Zoo, Neshkoro, WI) ranging in weight between 779 and 1106 g, and male golden Syrian hamsters, *Mesocricetus auratus* (Sasco, Omaha, NE), weighing 97–117 g, were fed Purina rodent chow (Purina, St. Louis, MO) and water ad libitum. The animals were kept under a controlled 12-h light/dark cycle for 2 weeks prior to surgery.

Surgical procedures

All animals were operated on between 9 and 10 AM. The prairie dogs were anesthetized by intramuscular injection of 100 mg of ketamine (Ketaset, Bristol Labs, Syracuse, NY) and 20 mg of xylazine (Rompun, Haver Lockert, Shawnee, KS). The hamsters were anesthetized with 20 mg of ketamine. Intramuscular injections of ketamine (50 mg/dose for prairie dogs and 10 mg/dose for hamsters) were used to maintain anesthesia as required.

A polyethylene catheter (PE-90, 0.86 mm ID, for prairie dogs and PE-10, 0.28 mm ID, for hamsters, Clay Adams, Parsippany, NJ) was inserted into the left femoral vein, and 0.9% NaCl solution was infused at a rate of 2.9 ml/h (prairie dogs) or 1.5 ml/h (hamsters) using a Harvard syringe pump (Harvard Apparatus, Millis, MA). The abdomen was opened by a midline incision; the cystic duct was ligated with a hemostatic clip (Hemoclip, Edward Weck & Co., Inc., Research Triangle Park, NC); and an external biliary fistula was constructed using polyethylene tubing (PE-90 for prairie dogs, and PE-10 for hamsters). The urethra was ligated with a hemostatic clip to allow urine to accumulate in the bladder.

In the intravenous infusion studies, saline was infused into the femoral vein for 40 min prior to the administration of [6α-^{3}H]β-MCA (or [24-^{14}C]CA). The labeled compounds were then infused for 20 min at a dose of 1 μmol/min per kg; the infusion of saline was then resumed until the end of the experiment. Bile samples were collected at 20-min intervals in weighed tubes for a period of 340 min. At the end of the experiments, blood and urine were obtained by cardiac puncture and by aspiration from the urinary bladder, respectively, and the liver was excised. All biological specimens were stored at −20°C.

For experiments dealing with the intraduodenal administration of β-MCA, both the bile duct and femoral vein were cannulated as described above, and the labeled bile acid (20 μmol/kg) was injected as a bolus into the duodenum.

Intragastric administration

[6α-^{3}H]β-MCA (40 μmol/kg, sp act 1.0 × 10^{6} dpm/mg) was introduced directly into the stomach of intact prairie dogs and hamsters by stomach tube, and feces were collected for 24 h. Bile acids were extracted from the feces as reported previously (36).

Determination of radioactivity

Bile samples were collected from bile fistula animals at 20-min intervals. A 10-μl aliquot was removed for radioactivity determination. Aliquots of urine, serum, and an ethanolic fecal extract were checked for radioactivity.

Liver bile salts were analyzed according to the method of Yanagisawa et al. (37) and their radioactivity was measured. Radioactivity assay of all samples was carried out using Aquasol-2 (NEN Research Products, Boston, MA) in a Beckman LS 3801 liquid scintillation system (Beckman Instruments, Fullerton, CA). Corrections were made for background and quenching.

Thin-layer chromatography

Silica gel 60 F_{254} precoated TLC aluminum sheets (0.2-mm thickness; Merck, Darmstadt, Germany) were used for TLC analysis. The solvent systems were as follows: system 1, n-butanol–acetic acid–water 85:10:5 (v/v/v); system 2, benzene–isopropanol–acetic acid 30:10:1 (v/v/v); system 3, chloroform–acetone–methanol 70:25:5 (v/v/v); system 4, isooctane–isopropanol–acetic acid 30:10:1 (v/v/v). Aliquots of bile fractions from each animal, containing the highest radioactivity, and fecal extracts were applied as bands on the plates and reference bile salts were applied as spots at each side. After development (15 cm), bile salts were visualized by spraying the plates with 10% phosphomolybdic acid in ethanol followed by heating at 120°C for 1 min. To locate the radioactivity of chromatographed samples, each TLC plate was cut into 5-mm segments from origin to solvent front and each segment was put into a scintillation vial. The radioactive compounds were extracted with methanol and counted as described above.

Enzymatic hydrolysis

Bile (10 μl) was diluted with 2.0 ml of sodium acetate buffer (0.025 M, pH 5.6), 0.2 ml of a solution of 0.05 M ethylenediamine tetraacetic acid disodium salt, 0.2 ml of 0.1 M 2-mercaptoethanol solution, and 10 units of cholylglycine hydrolase from *Clostridium perfringens* (Sigma Chemical Co.) were added. The mixture was incubated at 37°C for 18 h. Bile salts were extracted with Sep-Pak C_{18} cartridges (Waters Associates, Milford, MA).

$NaBH_4$ treatment of fecal bile acids

Aliquots of fecal extracts were dissolved in 200 μl ethanol. To the solution at 0°C, 20 mg $NaBH_4$ (Sigma Chemical Co.) in 1 ml ethanol was added, and the mixture was kept overnight at room temperature. After the addition of saturated NH_4Cl solution (5 ml), the bile acids were extracted with Sep-Pak C_{18} cartridges and analyzed by TLC.

Calculations

The results are expressed as mean ± SEM. The significance of differences between the group means was calculated using one-way analysis of variance followed by Student's *t*-test (38).

RESULTS

^{3}H-labeled β-MCA, infused intravenously into bile fistula prairie dogs or hamsters, was extracted by the liver and secreted into the bile of both species. The biliary recovery of the labeled compound infused at a rate of 1 μmol/min per kg for 20 min is summarized in **Table 1.** In the prairie dog, 88.9% was recovered in bile during the 5-h collection period; radioactivity in the liver and urine accounted for 3.2% and 2.2%, respectively. In the hamster, 98.6% of the administered radioactivity was secreted in the bile during the same 5-h period and less than 0.5% was present in the liver or urine. Radioactivity in blood was not detected in either animal species.

Cumulative biliary excretion of radioactivity after intravenous infusion is shown in **Fig. 1.** In the hamster, β-MCA was rapidly excreted into bile and more than 85% of the radioactivity was recovered within 1 h. In contrast, this compound was extracted less rapidly by prairie dog liver; only 30% was recovered within 1 h. Biliary recovery of the radioactivity in the prairie dog was lower ($P < 0.05$) than in the hamster throughout the experimental period. It can be calculated from the data of Fig. 1, that the half times ($t_{1/2}$) of biliary recovery were 32 ± 1 min for the hamster and 83 ± 4 min for the prairie dog.

Similarly, after intraduodenal injection of 20 μmol/kg of ^{3}H-labeled β-MCA, the radioactivity was recovered more rapidly and efficiently in hamster bile as compared

TABLE 1. Recovery of radioactivity after intravenous infusion of ^{3}H-labeled β-MCA in prairie dogs and hamsters

Animals	No. of animals	Isotopic Recovery, %				
		Bile	Urine	Liver	Blood	Total
Prairie dog	3	88.9 ± 1.0	2.2 ± 0.4	3.2 ± 0.7	ND	94.2 ± 0.8
Hamster	3	98.6 ± 0.8	0.4 ± 0.1	0.3 ± 0.2	ND	99.3 ± 0.7

^{3}H-labeled β-MCA was infused intravenously in prairie dogs and hamsters at a dose of 1 μmol/min per kg for 20 min. Bile was collected for 5 h and, at the end of the experiment, urine, liver, and blood samples were obtained to determine their radioactivity. The values are expressed as mean ± SEM. ND, not detected.

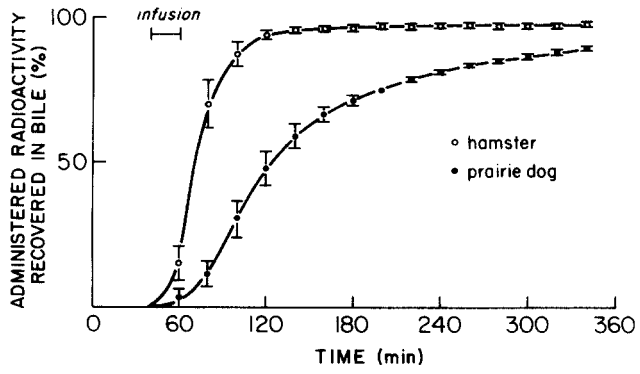

Fig. 1. Biliary secretion of radioactivity in bile fistula hamsters and prairie dogs after intravenous infusion of ^{3}H-labeled β-MCA. After a 40-min control period, the bile acid was infused at a dose of 1 μmol/min per kg for 20 min. Each value represents the average of three male animals.

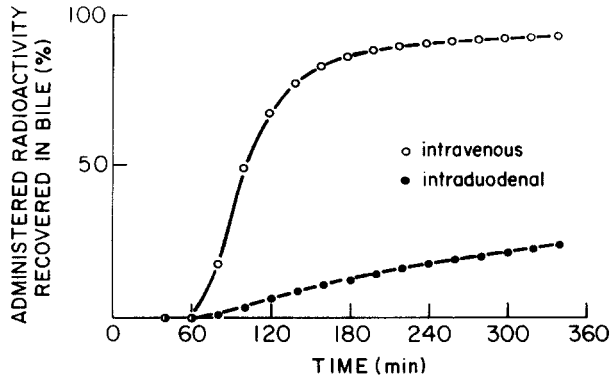

Fig. 3. Biliary secretion of radioactivity in bile fistula prairie dogs after intravenous infusion and intraduodenal administration of ^{14}C-labeled CA. After a 40-min control period, the bile acid was infused iv at a dose of 1 μmol/min per kg for 20 min. For intraduodenal administration, a bolus of labeled CA (20 μmol/kg) was given after a 40-min control period. Each value represents the average of two animals.

to the prairie dog (**Fig. 2**). In the hamster, 84% of the dose appeared in bile within 3 h, whereas only 43% of the radioactivity was recovered in the bile of the prairie dog within the same period. Biliary recovery of radioactivity was higher in the hamster than in the prairie dog in every period ($P < 0.05$). The half times ($t_{1/2}$) of biliary recovery calculated from the data of Fig. 2 were 87 ± 1 min and 230 ± 14 min for the hamster and the prairie dog, respectively. Little radioactivity (< 1%) appeared in urine in either species.

For comparison, [24-^{14}C]CA was administered either intravenously or intraduodenally into prairie dogs (iv 1 μmol/min per kg for 20 min; id 20 μmol/kg, bolus). The recovery of radioactivity is shown in **Fig. 3.** After intravenous infusion, ^{14}C-labeled CA was rapidly secreted into the bile so that 93.4% of the radioactivity was recovered during the 5-h collection period. In contrast, after intraduodenal injection of labeled CA, only 24.1% of the radioactivity was recovered during the same period; considerable amounts (45%) of the administered CA remained in the ileum.

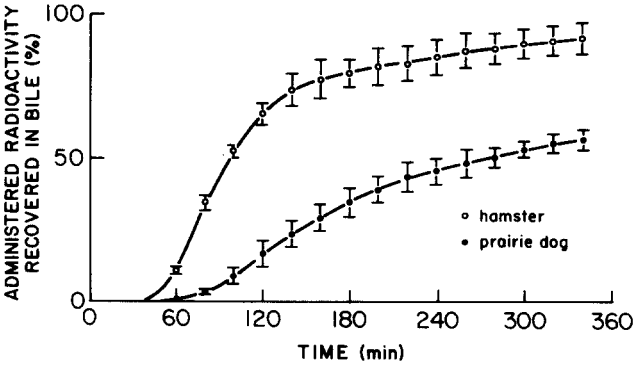

Fig. 2. Biliary secretion of radioactivity in bile fistula hamsters and prairie dogs after intraduodenal administration of ^{3}H-labeled β-MCA. A bolus (20 μmol/kg) of the bile acid was given after a 40-min control period. Each value represents the average of three animals.

Fig. 4 shows the qualitative analysis by radio-TLC of the bile collected after intravenous infusion of [^{3}H]β-MCA, and **Table 2** summarizes the conjugation profiles of this compound after intravenous infusion or intraduodenal injection into prairie dog and hamster. In the prairie dog, regardless of the route of administration, most (> 92%) of the β-MCA was conjugated with taurine during passage through the liver; conjugation with glycine was not detected. Less than 5% of the dose was secreted in the unconjugated form. In the hamster, some of the infused β-MCA was conjugated with either taurine (43%) or glycine (22%). However, a considerable amount (28%) of this bile acid was secreted into bile without prior conjugation.

Cholylglycine hydrolase treatment of the bile samples of the prairie dog and the hamster, followed by radio-TLC, revealed a single peak of radioactivity at the R_f value of unconjugated β-MCA (**Fig. 5**). Methyl esterification of the bile samples with diazomethane followed by radio-TLC gave a single peak of radioactivity in either group identical with β-MCA methyl ester. These data indicate that during passage through the liver there was no biotransformation of β-MCA and no radioactivity was associated with compounds having the mobility of glucuronides or sulfates.

After intragastric administration of 40 μmol/kg of labeled β-MCA, feces were collected for 24 h. Fecal excretion of radioactivity was 7.9 ± 2.1% and 8.9 ± 2.4% for the prairie dog and the hamster, respectively. Radio-TLC analysis of the fecal metabolites of the labeled β-MCA is shown in **Fig. 6.** In the prairie dog, 84.2 ± 2.0% of the recovered β-MCA remained unchanged. The less polar metabolites had R_f values consistent with those of CDCA (6.1 ± 2.3%) and LCA (1.7 ± 1.1%); they were inert to $NaBH_4$ reduction. 7-Dehydroxylated metabolites of β-MCA, namely, MDCA and its 6α-epimer, HDCA, were

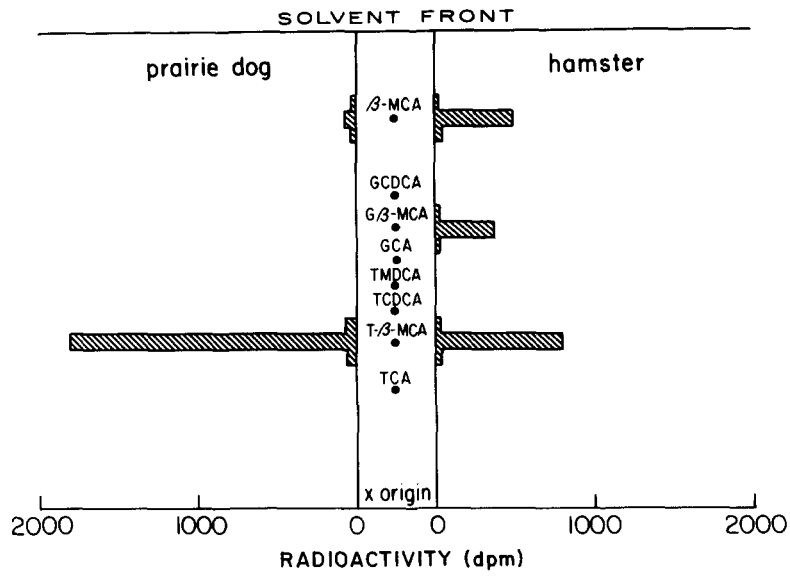

Fig. 4. Thin-layer chromatographic analysis of radioactivity of bile samples of bile fistula prairie dogs and hamsters infused intravenously with labeled β-MCA. The bile samples were analyzed by solvent system 1 (n-butanol–acetic acid–water 85:10:5, v/v/v) before hydrolysis. Reference compounds were as follows: TCA, taurocholate; Tβ-MCA, tauro-β-muricholate; TCDCA, taurochenodeoxycholate; TMDCA, tauromurideoxycholate; GCA, glycocholate; Gβ-MCA, glyco-β-muricholate; GCDCA, glycochenodeoxycholate; β-MCA, β-muricholate.

not found. In the hamster, 80.1 ± 1.4% of the recovered fecal β-MCA was unchanged. There was no radioactivity in the band corresponding to di- or monohydroxy bile acids. The metabolites more polar than β-MCA amounted to 18.6 ± 2.0%, which were stable toward $NaBH_4$ reduction and cholylglycine hydrolase treatment, suggesting that they are neither ketonic nor aminoacyl amidated compounds. Because of the small amounts of these metabolites in the biological samples, the structures of these compounds were not established.

DISCUSSION

The prairie dog and the hamster have been used extensively as animal models of cholesterol cholelithiasis. Before carrying out studies of gallstone prevention with β-MCA in these models, it seemed desirable to investigate certain properties of this compound, such as hepatic and intestinal transport and biotransformation.

When β-MCA was infused intravenously into prairie dogs or hamsters, it was largely secreted into the bile and only small amounts of this hydrophilic bile acid appeared in the urine of either species (Table 1). Biliary excretion of radioactivity after intravenous and intraduodenal administration of labeled β-MCA was more rapid in the hamster than in the prairie dog (Figs. 1 and 2). In the prairie dog, the biliary recovery of β-MCA resembled that of CA. The relatively hydrophobic bile acid, CA, was secreted more slowly after intraduodenal injection than after intravenous infusion (Figs. 1–3). The slow rate of intestinal absorption of β-MCA after intraduodenal injection into the prairie dog is not specifically a property of β-MCA but is shared by CA, the major bile acid of the prairie dog.

The proportion of any given organic compound excreted in bile varies widely among different species (39). Differences in liver weight per kg body weight (prairie dog, 20 ± 3; hamster, 46 ± 4 g/kg, in the present experiments), bile flow (prairie dog, 18 ± 2; hamster, 46 ± 3

TABLE 2. Conjugation profiles of β-MCA after intravenous (iv) or intraduodenal (id) administration into bile fistula prairie dogs and hamsters

Animals		No. of animals	Conjugation Profile			
			Unconjugated	Taurine	Glycine	G/T Ratio
Prairie dog	(iv)	3	4.9 ± 0.4	92.5 ± 0.8	0	0
	(id)	3	2.1 ± 1.1	95.5 ± 2.8	0	0
Hamster	(iv)	3	27.9 ± 3.5	43.3 ± 1.8	22.2 ± 4.7	0.52 ± 0.12
	(id)	3	21.4 ± 3.5	36.9 ± 4.9	35.9 ± 2.2	1.02 ± 0.19

Bile samples were analyzed by thin-layer chromatography using solvent system 1: n-butanol–acetic acid–water 85:10:5 (v/v/v), followed by liquid scintillation counting. The values are expressed as mean ± SEM.

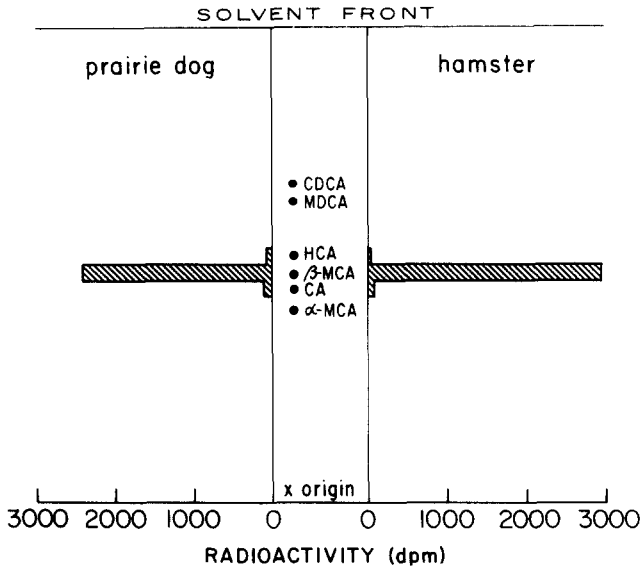

Fig. 5. Thin-layer chromatographic analysis of radioactivity from enzymatically hydrolyzed bile samples of bile fistula prairie dogs and hamsters infused intravenously with labeled β-MCA. The samples were analyzed by solvent system 2 (benzene–isopropanol–acetic acid 30:10:1, v/v/v). Reference compounds are as follows: α-MCA, α-muricholic acid; CA, cholic acid; β-MCA, β-muricholic acid; HCA, hyocholic acid; MDCA, murideoxycholic acid; CDCA, chenodeoxycholic acid.

μl/min per kg), total bile acid output, hepatic blood flow, or the number of bile acid binding sites on liver surface membranes (40) may explain the differences in biliary recovery between these animal species. It has been demonstrated that hepatic extraction is more dependent on the affinity of the bile acids for the carrier system than the capacity with which they can be transported. However, the present experiments were not designed to define the reasons for the species difference.

Montet et al. (31) reported that the equilibrium precipitation pH of a saturated solution of β-MCA was 7.9, about one pH unit higher than that obtained for CDCA. Thus, at the normal upper intestinal pH (pH 5.5 to 6.5) (41), most of the β-MCA should be protonated. In vitro studies revealed that more than 80% of β-MCA existed in crystalline form within the range of bile salt concentration found in intestinal content (5–10 mM) (31). Therefore, this unconjugated bile acid should be absorbed by passive nonionic diffusion across the small intestine and colon (42). When conjugated with glycine or taurine, β-MCA should be absorbed via the active transport system of the terminal ileum (43).

Regardless of the route of administration, most of the β-MCA was conjugated exclusively with taurine in the liver of the prairie dog (Table 2); in hepatic bile the natural bile acids, CA and CDCA, are also secreted mainly as the taurine conjugates. [A recent study in the prairie dog reported that most of the biliary CDCA was in the unconjugated form (44). We have been unable to confirm this finding in our animals.] These facts indicate that the enzyme system that catalyzes conjugation of bile acids in the prairie dog liver has a high affinity for taurine and a poor affinity for glycine. In the hamster, β-MCA was not completely conjugated with glycine and taurine and considerable amounts of β-MCA were secreted in bile in unconjugated form (Table 2). When similar amounts (50 μg/min for 20 min) of CA were infused into the hamster, more than 95% of the bile acid was amidated with glycine and taurine (45). Bile acid amidation is mediated by two enzymes: cholanoyl-CoA ligase which forms the CoA thioester of the bile acid, and cholanoyl-CoA amino acid transferase which transfers the bile acid moiety of the CoA bile acid thioester to glycine or taurine. In the hamster, β-MCA might not be a good substrate for

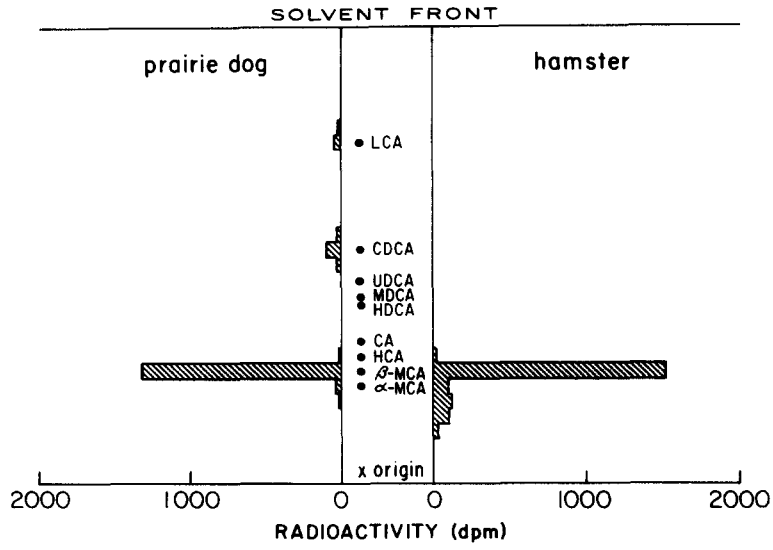

Fig. 6. Thin-layer chromatographic analysis of fecal metabolites of a prairie dog and a hamster after intragastric administration of labeled β-MCA (40 μmol/kg). The samples were analyzed by solvent system 4 (isooctane–isopropanol–acetic acid 30:10:1, v/v/v). Reference compounds are as follows: α-MCA, α-muricholic acid; β-MCA, β-muricholic acid; HCA, hyocholic acid; CA, cholic acid; HDCA, hyodeoxycholic acid; MDCA, murideoxycholic acid; UDCA, ursodeoxycholic acid; CDCA, chenodeoxycholic acid; LCA, lithocholic acid.

these enzymes. More definitive enzymatic studies are required to verify this point. Apparently, the major proportion (> 95%) of bile acids are secreted in the amidated form if they are sufficiently lipophilic (45). Such bile acids can be reabsorbed in the biliary ductules so that their proportion in hepatic bile is well below that of canalicular bile.

After intragastric administration of labeled β-MCA, its fecal metabolites were analyzed by radio-TLC (Fig. 6). In the prairie dog, more than 80% of β-MCA remained unchanged. The 7β-dehydroxylated metabolite of β-MCA, MDCA, and its 6α-epimer, HDCA, were not detected. However, a small amount of radioactive CDCA and a trace amount of radioactive LCA were found in the feces. These results suggest that β-MCA is resistant to bacterial 7β-dehydroxylation, but a small portion of this compound is 6β-dehydroxylated to UDCA, and its 7β-hydroxyl group is almost completely epimerized to form CDCA. It is well known that there is a potential interconversion between CDCA and UDCA by hepatic/bacterial interaction (46, 47). In the hamster, more than 80% of β-MCA was recovered unchanged in the feces; there was no detectable 6β- or 7β-dehydroxylation. In the intestine of both species, the presence of a 6β-hydroxyl group seems to protect β-MCA against bacterial 7β-dehydroxylation as has been reported for the human intestine (29). In contrast, when HCA, a 6α-hydroxy bile acid, was fed to hamsters, a considerable proportion of this bile acid was dehydroxylated to form HDCA (25). The polar metabolites of β-MCA in hamster feces were thought to be neither sulfated nor glucuronidated on the basis of TLC mobilities (48, 49). They are probably hydroxylation products of β-MCA, but further studies are needed for positive identification.

It is concluded that in the hamster, β-MCA was absorbed in the intestine, completely extracted by the liver, and secreted into the bile in the form of unconjugated β-MCA and taurine or glycine conjugates. In the prairie dog, this compound was predominantly conjugated with taurine and appeared more slowly in bile than in the case of the hamster regardless of the route of administration. Large proportions (> 80%) of recovered β-MCA were found unchanged in the feces of both animal species, indicating that β-MCA is relatively resistant to bacterial 6β- and 7β-dehydroxylation. This bile acid may be of interest as a potential cholelitholytic agent; studies of its effects on cholesterol gallstones and cholesterol metabolism in animal models are in progress.

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REFERENCES

1. Danzinger, R. G., A. F. Hofmann, L. J. Schoenfield, and J. L. Thistle. 1972. Dissolution of cholesterol gallstones by chenodeoxycholic acid. *N. Engl. J. Med.* **286:** 1–8.
2. Nakagawa, S., I. Makino, T. Ishizaki, and I. Dohi. 1977. Dissolution of cholesterol gallstones by ursodeoxycholic acid. *Lancet.* **II:** 367–369.
3. Bateson, M. C., P. E. Ross, J. Murison, and A. D. Bouchier. 1978. Comparison of fixed doses of chenodeoxycholic acid for gallstone dissolution. *Lancet.* **I:** 1112–1114.
4. Roda, E., F. Bazzoli, A. M. M. Labate, G. Mazzolla, A. Roda, C. Sama, D. Festi, R. Aldini, F. Taroni, and L. Barbara. 1982. Ursodeoxycholic acid vs. chenodeoxycholic acid as cholesterol gallstone-dissolving agents: a comparative randomized study. *Hepatology.* **2:** 804–810.
5. Hofmann, A. F. 1984. Medical treatment of cholesterol gallstones by bile desaturating agents. *Hepatology.* **4:** 199S–208S.
6. Park, Y-H., H. Igimi, and M. C. Carey. 1984. Dissolution of human cholesterol gallstones in simulated chenodeoxycholate-rich and ursodeoxycholate-rich biles. An in vitro study of dissolution rates and mechanisms. *Gastroenterology.* **87:** 150–158.
7. Salvioli, G., H. Igimi, and M. C. Carey. 1983. Cholesterol gallstone dissolution in bile. Dissolution kinetics of crystalline cholesterol monohydrate by conjugated chenodeoxycholate-lecithin and conjugated ursodeoxycholate-lecithin mixtures: dissimilar phase equilibria and dissolution mechanisms. *J. Lipid Res.* **24:** 701–720.
8. Cohen, B. I., E. H. Mosbach, C. K. McSherry, B. Rzigalinski, and S. Kuroki. 1986. A hydrophilic bile acid effects partial dissolution of cholesterol gallstones in the prairie dog. *Lipids.* **21:** 575–579.
9. Schoenfield, L. J., J. M. Lachin, The Steering Committee, and The National Cooperative Gallstone Study Group. 1981. Chenodiol (chenodeoxycholic acid) for dissolution of gallstones. The National Cooperative Gallstone Study. A controlled trial of efficacy and safety. *Ann. Int. Med.* **95:** 257–282.
10. Danielsson, H., P. Eneroth, K. Hellström, S. Lindstedt, and J. Sjövall. 1963. On the turnover and excretory products of cholic and chenodeoxycholic acid in man. *J. Biol. Chem.* **238:** 2299–2304.
11. Fedorowski, T., G. Salen, A. Colallio, G. S. Tint, E. H. Mosbach, and J. C. Hall. 1977. Metabolism of ursodeoxycholic acid in man. *Gastroenterology.* **77:** 1131–1137.
12. Bazzoli, F., H. Fromm, R. P. Serva, R. F. Sembrat, and S. Ceryak. 1982. Comparative formation of lithocholic acid from chenodeoxycholic and ursodeoxycholic acids in the colon. *Gastroenterology.* **83:** 753–760.
13. Mosbach, E. H. 1983. Approaches to the development of new cholelitholytic agents. *In* Bile Acids and Cholesterol in Health and Disease. G. Paumgartner, A. Stiehl, and W. Gerok, editors. MTP Press, Lancaster, U.K. 11–20.
14. Palmer, R. H. 1972. Bile acids, liver injury, and liver disease. *Arch. Int. Med.* **130:** 606–617.
15. Sarva, R. P., H. Fromm, S. Farivar, R. F. Sembrat, H. Mendelow, H. Shinozuka, and S. K. Wolfson. 1980. Comparison of the effects between ursodeoxycholic and chenodeoxycholic acids on liver function and structure and on bile acid composition in the rhesus monkey. *Gastroenterology.* **79:** 629–636.
16. Miyai, K., N. B. Javitt, N. Gochman, H. M. Jones, and D.

Baker. 1982. Hepatotoxicity of bile acids in rabbits. Ursodeoxycholic acid is less toxic than chenodeoxycholic acid. *Lab. Invest.* **46:** 428–437.
17. Reddy, B. S., and K. Watanabe. 1979. Effect of cancer metabolites and promoting effect of lithocholic acid in colon carcinogenesis in germ-free and conventional F344 rats. *Cancer Res.* **39:** 1521–1524.
18. Turjman, N., and P. P. Nair. 1981. Nature of tissue bound lithocholic acid and its implications in the role of bile acids in carcinogenesis. *Cancer Res.* **41:** 3761–3763.
19. Parquet, M., V. Legrand-Defretin, M. Riottot, A. Karpouza, and C. Lutton. 1990. Metabolism and effects on biliary lipid secretion of murocholic acid in the hamster. *J. Hepatol.* **11:** 111–119.
20. Sacquet, E., M. Parquet, M. Riottot, A. Raizman, P. Jarrige, C. Huguet, and R. Infante. 1983. Intestinal absorption, excretion, and biotransformation of hyodeoxycholic acid in man. *J. Lipid Res.* **24:** 604–613.
21. Dam, H., I. Prange, and E. Sondergaard. 1972. Alimentary production of gallstones in hamsters. 24. Influence of orally ingested chenodeoxycholic acid and hyodeoxycholic acid on formation of gallstones. *Z. Ernährungswiss.* **11:** 80–94.
22. Wheeler, H. O. 1973. Biliary excretion of bile acids, lecithin, and cholesterol in hamsters with gallstones. *Gastroenterology.* **65:** 92–103.
23. Singhal, A. K., B. I. Cohen, E. H. Mosbach, M. Une, R. J. Stenger, C. K. McSherry, P. May-Donath, and T. Palaia. 1984. Prevention of cholesterol-induced gallstones by hyodeoxycholic acid in the prairie dog. *J. Lipid Res.* **25:** 539–549.
24. Cohen, B. I., N. Matoba, E. H. Mosbach, N. Ayyad, K. Hakam, S. O. Suh, and C. K. McSherry. 1990. Bile acids substituted in the 6 position prevent cholesterol gallstone formation in the hamster. *Gastroenterology.* **98:** 397–405.
25. Singhal, A. K., B. I. Cohen, J. Finver-Sadowsky, C. K. McSherry, and E. H. Mosbach. 1984. Role of hydrophilic bile acids and of sterols on cholelithiasis in the hamster. *J. Lipid Res.* **25:** 564–570.
26. Almé, B., A. Norden, and J. Sjövall. 1978. Glucuronides of unconjugated 6-hydroxylated bile acids in urine of a patient with malabsorption. *Clin. Chim. Acta.* **86:** 251–259.
27. Parquet, M., M. Pessah, E. Sacquet, C. Salvat, and A. Raizman. 1988. Effective glucuronidation of 6α-hydroxylated bile acids by human hepatic and renal microsomes. *Eur. J. Biochem.* **171:** 329–334.
28. Cohen, B. I., N. Ayyad, E. H. Mosbach, C. K. McSherry, N. Matoba, A. F. Hofmann, H-T. Ton-Nu, Y. Peng, C. D. Schteingart, and R. J. Stenger. 1991. Replacement of cholesterol gallstones by murideoxycholyl taurine gallstones in prairie dogs fed murideoxycholic acid. *Hepatology.* **14:** 158–168.
29. Sacquet, E. C., M. Parquet, M. Riottot, A. Raizman, B. Nordlinger, and R. Infante. 1985. Metabolism of β-muricholic acid in man. *Steroids.* **45:** 411–426.
30. Sacquet, E. C., D. E. Gadelle, M. J. Riottot, and P. M. Raibaud. 1984. Absence of transformation of β-muricholic acid by human microflora implanted in the digestive tracts of germfree male rats. *Appl. Environ. Microbiol.* **45:** 1167–1168.
31. Montet, J-C., M. Parquet, E. Sacquet, A-M. Montet, R. Infante, and J. Amic. 1987. β-Muricholic acid: potentiometric and cholesterol-dissolving properties. *Biochim. Biophys. Acta.* **918:** 1–10.
32. Aranda, A., M. Bertranne, M. Fétizon, and R. Infante. 1992. Synthesis of 6α-tritiated β-muricholic acid for use as a molecular probe. *Steroids.* **57:** 205–207.
33. Hsia, S. L., J. T. Matschiner, T. A. Mahowald, W. H. Elliott, E. A. Doisy, Jr., S. A. Thayer, and E. A. Doisy. 1957. Bile acids. VI. The structure and synthesis of acid II. *J. Biol. Chem.* **226:** 667–671.
34. Hsia, S. L., J. T. Matschiner, T. A. Mahowald, W. H. Elliott, E. A. Doisy, Jr., S. A. Thayer, and E. A. Doisy. 1957. Bile acids. V. Chemical studies on new bile acids from the rat and the hog. *J. Biol. Chem.* **225:** 811–823.
35. Tserng, K-Y., D. L. Hachey, and P. D. Klein. 1977. An improved procedure for the synthesis of glycine and taurine conjugates of bile acids. *J. Lipid Res.* **18:** 404–407.
36. Cohen, B. I., R. F. Raicht, G. Salen, and E. H. Mosbach. 1975. An improved method for the isolation, quantitation, and identification of bile acids in rat feces. *Anal. Biochem.* **64:** 567–577.
37. Yanagisawa, J., M. Itoh, M. Ishibashi, H. Miyazaki, and F. Nakayama. 1980. Microanalysis of bile acid in human liver tissue by selected ion monitoring. *Anal. Biochem.* **104:** 75–86.
38. Snedecor, G. W., and W. G. Cochran. 1946. Statistical Methods. Iowa State University Press, Ames, IA. 56–81; 182–184.
39. Klaassen, C. D., and J. B. Watkins, III. 1984. Mechanisms of bile formation, hepatic uptake, and biliary excretion. *Pharmacol. Rev.* **36:** 1–67.
40. Accatino, L., and F. R. Simon. 1976. Identification and characterization of a bile acid receptor in isolated liver surface membranes. *J. Clin. Invest.* **57:** 496–508.
41. Carey, M. C. 1982. The enterohepatic circulation. *In* The Liver: Biology and Pathology. I. Arias, H. Popper, D. Schachter, and D. A. Shafritz, editors. Raven Press, New York. 429–465.
42. Dietschy, J. M. 1968. Mechanisms for the intestinal absorption of bile acids. *J. Lipid Res.* **9:** 297–309.
43. Lack, L., and I. M. Weiner. 1966. Intestinal bile salt transport: structure activity relationships and other properties. *Am. J. Physiol.* **210:** 1142–1152.
44. Broughton, G., II, A. Tseng, R. Fitzgibbons, Jr., A. F. Fishkin, and E. L. Rongone. 1990. The quantitative and qualitative analysis for biliary lipids in the prairie dog, *Cynomys ludovicianus*. *Comp. Biochem. Physiol.* **97B:** 521–526.
45. Kuroki, S., E. H. Mosbach, B. I. Cohen, and C. K. McSherry. 1987. Metabolism of the bile acid analogues, 7β-methyl-cholic acid and 7α-methyl-ursocholic acid. *Gastroenterology.* **92:** 876–884.
46. Fromm, H., R. P. Sarva, and F. Bazzoli. 1983. Formation of ursodeoxycholic acid from chenodeoxycholic acid in the human colon: studies of the role of 7-ketolithocholic acid as an intermediate. *J. Lipid Res.* **24:** 841–853.
47. Salen, G., G. S. Tint, B. Eliav, N. Deering, and E. H. Mosbach. 1974. Increased formation of ursodeoxycholic acid in patients treated with chenodeoxycholic acid. *J. Clin. Invest.* **53:** 612–621.
48. Parmentier, G., and H. Eyssen. 1978. Thin-layer chromatography of bile acid sulfates. *J. Chromatogr.* **152:** 285–289.
49. Back, P., and D. V. Bowen. 1976. Chemical synthesis and characterization of glucuronic acid coupled mono-, di- and trihydroxy bile acids. *Hoppe-Seyler's Z. Physiol. Chem.* **357:** 219–224.