Deoxycholate 7α-hydroxylase in the hamster: substrate specificity and effect of phenobarbital

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Abstract In a recent publication, we reported that deoxycholic acid is 7α-hydroxylated to yield glycocholate or taurocholate in vivo in the hamster (1987. Kuroki et al. *Hepatology.* **7:** 229–234). In order to explore the possibility that amidation of free deoxycholic acid precedes the 7α-hydroxylation, we assayed 7α-hydroxylase activities of free and conjugated deoxycholates in vitro. 7α-Hydroxylase activities of glycodeoxycholate and taurodeoxycholate were 720 ± 132 and 640 ± 160 pmol/mg • min^{-1}, respectively. Activity of 7α-hydroxylation of free deoxycholate was very low (60 ± 20 pmol/mg • min^{-1}). After treatment with phenobarbital in a dose of 100 mg/kg per day for 6 days, 7α-hydroxylase activities of conjugated deoxycholates were decreased significantly (40%, $P < 0.01$, n = 8), whereas that of free deoxycholate was not significantly changed. In the rat, 7α-hydroxylase activities of conjugated deoxycholates were induced significantly (45% increase, $P < 0.05$, n = 5) by phenobarbital treatment in sharp contrast to the hamster. There were significant correlations between the 7α-hydroxylase activity of taurodeoxycholate and that of glycodeoxycholate both in the hamster and in the rat (hamsters: n = 16, $r = 0.98$, $P < 0.01$; rats: n = 10, $r = 0.82$, $P < 0.01$). These studies suggested that deoxycholic acid is 7α-hydroxylated after amidation with glycine or taurine in vivo and that the same enzyme may well catalyze the 7α-hydroxylation of glycodeoxycholate and taurodeoxycholate in the hamster. There was a species difference in the effect of phenobarbital treatment on 7α-hydroxylase activity of deoxycholates between the rat and the hamster.—**Yamashita, H., S. Kuroki, and F. Nakayama.** Deoxycholate 7α-hydroxylase in the hamster: substrate specificity and effect of phenobarbital. *J. Lipid Res.* 1989. **30:** 711–718.

Supplementary key words 7α-hydroxylation • bile acids • liver microsomes • cholic acid–deoxycholic acid ratio

Deoxycholic acid is a secondary bile acid formed by the microbial 7α-dehydroxylation of cholic acid during the enterohepatic circulation. Deoxycholic acid thus formed is absorbed efficiently by the intestine, extracted by the liver, rapidly conjugated with taurine or glycine in hepatocytes, and excreted into bile canaliculi and then to the duodenum to undergo the enterohepatic circulation (1, 2). Amidates of deoxycholate may serve as detergents to solubilize biliary and dietary lipids as those of primary bile acids. However, there seem to be some detrimental effects in that they may injure cell membranes and be toxic at least in some species (3–5). Further, deoxycholic acid may have some relevance to cholelithiasis (6) and act as promoter in colon carcinogenesis (7). Thus, we considered it worthwhile to investigate the metabolism of deoxycholic acid and the mechanisms of cell injury in order to find means to alleviate its toxicity or to prevent the formation of this harmful bile acid. Some animals, including man, are known to possess so-called detoxification mechanisms such as hydroxylation (3, 8–10), sulfation (11, 12), and glucuronidation (12, 13) of deoxycholic acid.

7α-Hydroxylation of deoxycholic acid was shown to occur in rats (8), mice (9), and prairie dogs (10), while in humans there is a very low capacity for deoxycholic acid 7α-hydroxylation (14, 15). This reaction was studied mainly in rats. It is catalyzed by enzyme(s) localized in the microsomal fraction of the liver, requires NADPH as a cofactor (16), and is stimulated by the administration of phenobarbital (16–19). We proposed that the hamster is a better experimental animal model to study cholesterol and bile acid metabolism (3, 20) because of the similarities in bile acid composition (20) and regulation of sterol synthesis (21) to those of humans. However, little is known about deoxycholate 7α-hydroxylation.

Recently, we showed that deoxycholic acid was 7α-hydroxylated to yield glycocholate or taurocholate when it was administered intravenously or intraduodenally to the hamster (3). We considered that amidation of free deoxycholic acid might precede the 7α-hydroxylation. This is based on the report that taurocholate was obtained efficiently from radiolabeled deoxycholic acid with a transient formation of taurodeoxycholate by rat liver slices and whole homogenates of rat liver (8), although there

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Abbreviations: DMES-imidazole, dimethylethylsilylimidazole; PHP-LH-20, piperidinohydroxypropyl Sephadex LH-20; GLC, gas-liquid chromatography; GLC-MS, gas-liquid chromatography-mass spectrometry.

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were no experimental data comparing the activity of 7α-hydroxylase of free deoxycholic acid with that of taurodeoxycholate in the microsomal fraction. We also speculated that glycodeoxycholate, rather than taurodeoxycholate, appeared to be the preferred substrate of the 7α-hydroxylase (3).

The present study was undertaken to establish optimal conditions for measuring the 7α-hydroxylation of free and conjugated deoxycholates by 7α-hydroxylase(s) of hamster liver microsomes. We investigated whether conjugated deoxycholates are 7α-hydroxylated more efficiently than the unconjugated form and whether taurodeoxycholate or glycodeoxycholate is the preferred substrate of the 7α-hydroxylase. In addition, we examined the effect of phenobarbital administration on the activity of deoxycholate 7α-hydroxylase and on the bile acid composition of gallbladder bile in the hamster. The effect was also studied in the rat and differences between the two species were examined.

MATERIALS AND METHODS

Reagents

All solvents were of analytical grade and were distilled before use. Sephadex LH-20 was purchased from Pharmacia Fine Chemicals AB (Uppsala, Sweden), and dimethylethylsilylimidazole (DMES-imidazole) and phenobarbital sodium salt were from Tokyo Kasei Kogyo (Tokyo, Japan). Piperidinohydroxypropyl Sephadex LH-20 (PHP-LH-20) was prepared according to the procedure described by Goto et al. (22). Cholylglycine hydrolase and 7α-hydroxysteroid dehydrogenase (EC 1.1.1.159) were obtained from Sigma Chemical Co., St. Louis, MO. NADPH was purchased from Kojin Co., Ltd (Tokyo, Japan). Sodium taurodeoxycholate, glycodeoxycholate, and nordeoxycholate were generous gifts from Dr. Erwin H. Mosbach, Beth Israel Medical Center, New York. Sodium glycodeoxycholate was further purified by chromatography on a PHP-LH-20 column (22) and recrystalized before use. Sodium deoxycholate was obtained from Nakarai Chemical Co., Ltd (Kyoto, Japan). 3α, from Nakarai Chemical Co., Ltd (Kyoto, Japan). 3α, 6α-Dihydroxy-5β-cholanoic acid (Canada Packers Ltd. Toronto, Canada), and 7-ketodeoxycholic acid (Steraloids, Inc., Wilton, NH) were obtained from the commercial sources as indicated. Bile acids used in this study were checked for purity by thin-layer chromatography on precoated silica gel G (thickness, 0.2 mm; Merck, Darmstadt, West Germany) using isopropanol- isooctane-dioxane-acetic acid 7:10:6:2 (v/v/v/v) (23) and n-butanol-acetic acid-water 10:1:1 (v/v/v) (24) as solvent systems, and by gas-liquid chromatography (GLC) as described below. Purities of the bile acids used in the study were better than 97%.

Animals

Female Golden Syrian hamsters, 6 weeks old with a mean weight of 104 ± 2 g, were purchased from Hamster Misaki (Kagawa, Japan) and divided into two groups. They were acclimated to the vivarium for at least 2 weeks at a temperature of 23°C and light periods from 8 AM to 8 PM. The animals had free access to water and Standard Powder Chow (Oriental Yeast Co., Ltd., Tokyo, Japan). Phenobarbital (100 mg/kg body weight per day), dissolved in 0.5 ml of saline, was administered intraperitoneally for 6 days. Saline was injected into control animals. The animals were fasted for 24 hr prior to the study. Two groups of animals were killed between 9 and 10 AM on the same day. Under ether anesthesia, the abdomen was opened, bile was aspirated with a 50-μl Hamilton Syringe, and stored at −20°C before processing. The liver was then excised, rinsed with ice-cold homogenizing solution (see below for composition), chilled on ice, and weighed.

Male Sprague-Dawley rats (196 ± 8 g) purchased from Charles River Japan Inc. (Kanagawa, Japan) were used. Environmental and experimental conditions were the same as those for hamsters.

Measurement of microsomal deoxycholate 7α-hydroxylase activity

A liver homogenate, 20% (w/v), was prepared in 0.1 M potassium phosphate buffer, pH 7.4, containing 0.25 M sucrose, 2.5 mM neutralized EDTA, and 75 mM nicotinamide, in a Potter-Elvehjem homogenizer with a loosely fitting pestle (25). The homogenate was centrifuged at 800 *g* for 10 min and the supernatant was centrifuged at 20,000 *g* for 10 min. The microsomal fraction was obtained by centrifugation of 20,000 *g* supernatant fluid at 100,000 *g* for 1 h. The microsomal pellet was suspended in the homogenizing medium (about 2–3 mg of microsomal protein per ml) and 1 ml of the suspension was used in standard assays. A small aliquot was used for protein determination according to Lowry et al. (26). The enzymatic conversion was determined as follows. Each substrate, i.e., sodium salts of deoxycholate, taurodeoxycholate, or glycodeoxycholate, dissolved in 150 μl of distilled water was added to an incubation mixture containing, in a volume of 2.5 ml, potassium phosphate buffer, pH 7.4, 0.25 mmol; $MgCl_2$, 10 μmol; NADPH, 4 μmol; and 1 ml of the microsomal suspension (25). After 10 min of preincubation, incubations were started by addition of one of the substrates. Incubations were conducted at 37°C in air for 30 min; they were terminated by addition of four volumes of ethanol. Twenty μg of nordeoxycholic acid was added as an internal standard, and the precipitated protein was removed by centrifugation at 2,000 rpm for 15 min. An aliquot of the organic solvent layer was evaporated to dryness under reduced pressure. The resi-

due was hydrolyzed by Clostridial cholylglycine hydrolase (18, 27). After acidification to pH 1, the free bile acids were extracted three times with 4 ml each of ethyl acetate. The combined extracts were washed twice with 1 ml each of distilled water and evaporated to dryness. The free bile acids obtained were esterified with 5% ethanolic hydrochloric acid and silylated with DMES-imidazole. The samples were passed through a Sephadex LH-20 column to remove excess reagent as reported previously (28). The derivatives were analyzed by GLC using a 15 m × 0.2 mm i.d. fused silica capillary column (HiCap CBP1, Shimadzu) as described previously (29). Amounts of cholic acid formed were determined by peak area integration. An incubation mixture without substrate served as a blank. The ethyl ester-DMES ether derivatives of bile acids were also analyzed by gas-liquid chromatography-mass spectrometry (GLC-MS). Details for the equipment and operating conditions for GLC-MS were described in a previous publication (28). In order to elucidate the structure of the metabolite of deoxycholic acid, enzymatic oxidation was performed. The metabolite was treated with 7α-hydroxysteroid dehydrogenase from *Escherichia coli* (30). After extraction with ethyl acetate, the product was analyzed by capillary GLC-MS as its ethyl ester-DMES ether derivative as described above.

Analysis of bile acids in gallbladder bile

Gallbladder bile was deproteinized with 1 ml of isopropanol. An aliquot was hydrolyzed by Clostridial cholylglycine hydrolase (27). The hydrolyzate was acidified with HCl and extracted with ethyl acetate. The extracted free bile acids were derivatized as mentioned above. The resulting ethyl ester-DMES ether derivatives were analyzed by GLC (29). Nordeoxycholic acid was used as an internal standard. Relative retention times of ethyl ester-DMES ether derivatives of bile acids were as follows (deoxycholic acid, 1.00): cholesterol, 0.68; lithocholic acid, 0.74; nordeoxycholic acid, 0.77; chenodeoxycholic acid, 1.07; cholic acid, 1.39; ursocholic acid, 1.45; and 7-ketodeoxycholic acid, 1.50.

Statistical methods

Results were expressed as mean ± SD. The significance of differences between the group means was evaluated by Student's *t* test. Linear regression analysis was performed as described previously (31).

RESULTS

Incubation with free and conjugated deoxycholates

Free and conjugated deoxycholates were 7α-hydroxylated to give cholic acid when incubated with hamster liver microsomes fortified with NADPH (**Fig. 1**). After enzymatic hydrolysis, the reaction product was analyzed by combined capillary gas-liquid chromatography and mass spectrometry as ethyl ester-DMES ether derivative. The retention time and mass spectrum of the derivative were completely identical with those of authentic cholic acid. In order to exclude the possibility that the metabolite was one of 6-hydroxylated isomers, enzymatic 7α-dehydrogenation was carried out and the reaction product was analyzed by capillary GLC-MS. The metabolite as well as authentic cholic acid were stereospecifically and quantitatively oxidized to 7-ketodeoxycholic acid. 6-Hydroxylated bile acids, such as 3α, 6α-dihydroxy-5β-cholanoic acid and 3α, 6β-dihydroxy-5β-cholanoic acid, were not oxidized under the conditions used. Thus, we concluded that the metabolite of deoxycholic acid produced by hamster liver microsomes was cholic acid. No other products were detected under the standard assay conditions. The reaction rates of 7α-hydroxylation of conjugated deoxycholates were linear with time up to 60 min (**Fig. 2**) and with microsomal protein up to at least 3 mg (**Fig. 3**). The effects of substrate concentration and NADPH concentration on the rates of the reaction are shown in **Fig. 4** and **Fig. 5**, respectively. We chose 450 nmol of substrate concentration, 30 min of reaction time, 4 μmol of NADPH, and 2-3 mg of microsomal protein in 2.5 ml of the incubation mixture as the standard assay conditions. The rates of 7α-hydroxylation of taurodeoxycholate and glycodeoxycholate were similar under varying incubation conditions. The rate of 7α-hydroxylation of free deoxycholate was extremely low compared with those of amidated deoxycholates.

Effect of the phenobarbital treatment

The effects of the phenobarbital administration on body weight, liver size, microsomal protein concentration, and the activity of 7α-hydroxylation of each substrate are summarized in **Table 1.** No significant differences were found among the groups in the initial and the final body weights. The liver weight and the amount of microsomal protein were increased significantly by phenobarbital treatment. 7α-Hydroxylation of conjugated deoxycholates was inhibited significantly by phenobarbital treatment (40% decrease), whereas that of free deoxycholate was not significantly changed. There was a highly significant correlation (slope = 1.027, intercept = 49.1, n = 16, $r = 0.98$, $P < 0.01$) between the 7α-hydroxylase activity of glycodeoxycholate and that of taurodeoxycholate both in control and phenobarbital-treated animals (**Fig. 6**).

In the rat, 7α-hydroxylase activities of free deoxycholate, glycodeoxycholate, and taurodeoxycholate were 26 ± 5, 107 ± 18, and 150 ± 29 pmol/mg • min $^{-1}$ respectively, in the control group (n = 5) and 30 ± 18,

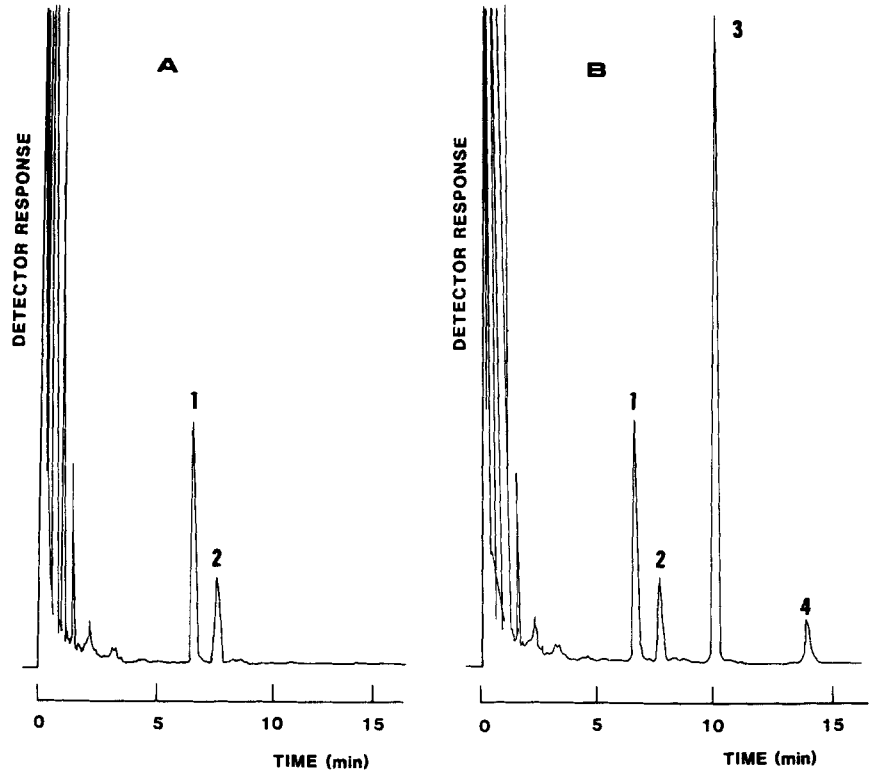

Fig. 1. Representative gas chromatograms of ethyl ester-dimethylethylsilyl ether derivatives of bile acids extracted from the standard incubation mixtures with (B) or without (A, blank) substrate, taurodeoxycholate. 1, Cholesterol; 2, internal standard; 3, deoxycholic acid; 4, cholic acid. Note that few endogenous bile acids are seen in the blank (A).

171 ± 38, and 200 ± 31 pmol/mg • min^{-1} in phenobarbital-treated animals (n = 5). Activities of 7α-hydroxylase of conjugated deoxycholates were enhanced significantly by phenobarbital ($P < 0.05$). A significant correlation between the activity of 7α-hydroxylase of glycodeoxycholate and that of taurodeoxycholate was also found in the rat (slope = 0.921, intercept = −22.1, n = 10, $r = 0.82$, $P < 0.01$). However, the slope for rats was smaller than that for hamsters.

Bile acid composition of gallbladder bile

The bile acid composition in gallbladder bile in control and phenobarbital-treated hamsters are shown in Table 1. The major bile acids in both groups were cholic acid and chenodeoxycholic acid. Deoxycholic acid, lithocholic acid, and 7-ketodeoxycholic acid were also detected in the bile as minor components. 6-Hydroxylated bile acids, which are usually seen in rats and mice, were not detected in the hamster bile. The proportions of cholic acid, deoxycholic acid, and 7-ketodeoxycholic acid were higher in control than in phenobarbital-treated hamsters, while those of chenodeoxycholic acid and lithocholic acid were lower in control than in phenobarbital-treated groups. The ratio of cholic acid to deoxycholic acid was decreased by 30% by phenobarbital treatment.

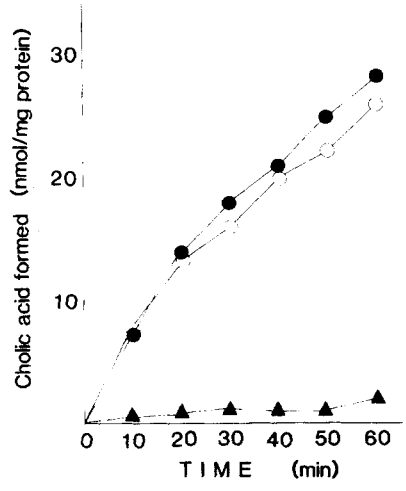

Fig. 2. Time course of 7α-hydroxylations of free deoxycholate (▲), glycodeoxycholate (○), and taurodeoxycholate (●). Standard assay conditions, except that incubation time varied from 10 to 60 min.

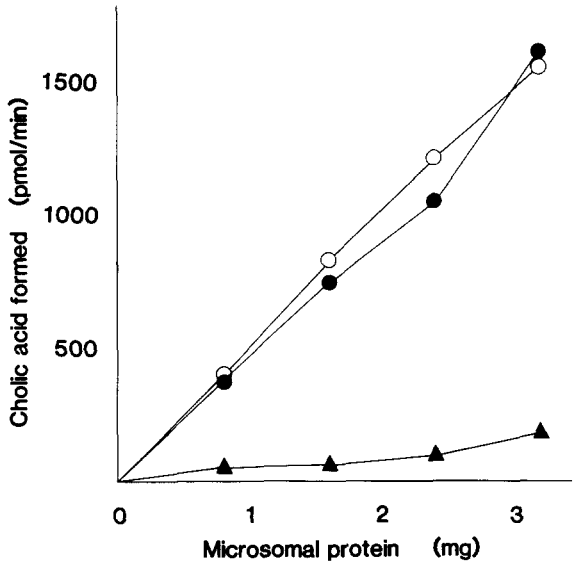

Fig. 3. Dependence of 7α-hydroxylase activities of free deoxycholate (▲), glycodeoxycholate (○), and taurodeoxycholate (●) on the amount of microsomal fraction (mg of protein). Standard assay conditions were used.

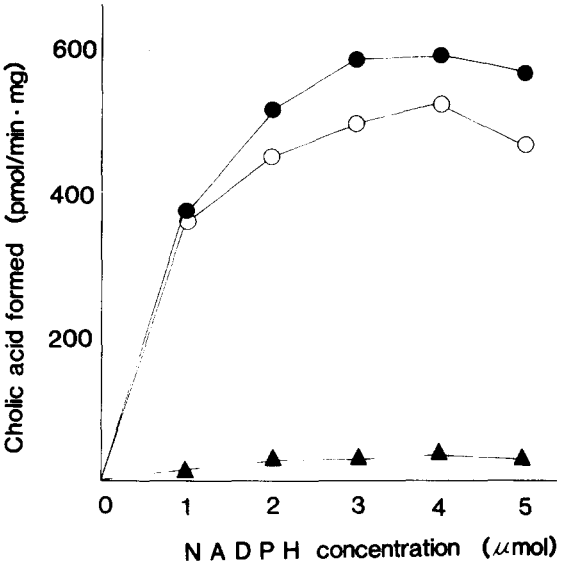

Fig. 5. Effect of NADPH concentration on 7α-hydroxylations of free deoxycholate (▲), glycodeoxycholate (○), and taurodeoxycholate (●). Standard assay conditions, with NADPH concentration varied over indicated ranges in 2.5 ml of incubation mixture.

DISCUSSION

The 7α-hydroxylation of deoxycholic acid was extensively studied in the rat (8, 16–19) and these studies dealt mainly with 7α-hydroxylation of taurodeoxycholic acid. To our knowledge, there were no studies concerning the 7α-hydroxylation of glycodeoxycholic acid which was thought unimportant in rats, because the bile acids are almost all conjugated with taurine in this species (32). In contrast, bile acids in hamsters are conjugated with glycine more than with taurine (20). In the present study it was shown that free and conjugated deoxycholates were 7α-hydroxylated by the hamster liver microsomes. The present results confirmed the previous findings in vivo in the hamster, in which labeled unconjugated deoxycholic acid administered either intravenously or intraduodenally was recovered in fistula bile as conjugated cholic acid as well as conjugated deoxycholic acid (3). In order to compare substrate specificity of 7α-hydroxylation of deoxycholates, we determined optimal conditions for measuring activities of 7α-hydroxylase for free and conjugated deoxycholates in the hamster. This is the first study in which the activities of 7α-hydroxylase of free and conjugated deoxycholates were assayed simultaneously in vitro. It was shown that the 7α-hydroxylase activities for conjugated deoxycholates were always higher than those for free deoxycholate in the hamster. The substrate specificity was also studied in the rat: free deoxycholate was less efficiently metabolized and glycodeoxycholate as well as taurodeoxycholate was an efficient substrate. These results support our hypothesis and that of Bergström and Gloor (8) that administered deoxycholic acid may be 7α-hydroxylated after conjugation with glycine or taurine in vivo (3).

The rates of 7α-hydroxylation of conjugated deoxycholates were almost the same under varying incubation conditions. 7α-Hydroxylase activity of glycodeoxycholate and that of taurodeoxycholate are well correlated both in

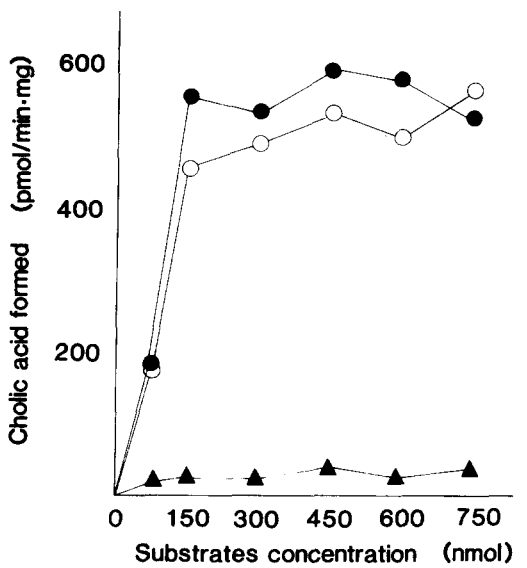

Fig. 4. Effect of substrate concentration on 7α-hydroxylations of free deoxycholate (▲), glycodeoxycholate (○), and taurodeoxycholate (●). Standard assay conditions, with substrate concentrations from 75 to 750 nmol in 2.5 ml of incubation mixture.

TABLE 1. 7α-Hydroxylase activities of free and conjugated deoxycholates and bile acid composition in control and phenobarbital-treated hamsters

	Control	Treated	P
Body weight (g)	124 ± 6	126 ± 8	NS
Liver weight (g)	3.9 ± 0.3	5.5 ± 0.6	<0.01
Microsomal protein (mg/g liver)	13.4 ± 0.9	21.1 ± 3.4	<0.01
7α-Hydroxylase activity[a] of			
Deoxycholate	60 ± 20	42 ± 41	NS
Glycodeoxycholate	720 ± 132	447 ± 165	<0.01
Taurodeoxycholate	640 ± 160	401 ± 147	<0.01
Bile acid composition (%)			
Cholic acid	64.9 ± 5.2	47.9 ± 5.2	<0.01
Chenodeoxycholic acid	24.0 ± 4.4	38.1 ± 4.3	<0.01
Deoxycholic acid	3.8 ± 0.9	4.5 ± 2.0	NS
Lithocholic acid	3.9 ± 0.9	7.5 ± 1.9	<0.01
7-Ketodeoxycholic acid	3.4 ± 0.6	2.0 ± 0.4	<0.01
Ratio of cholic acid to deoxycholic acid	18.0 ± 4.2	13.0 ± 6.6	NS

Each value is expressed as mean ± SD; NS, statistically not significant.
[a]Activity of hydroxylase expressed as pmol/mg protein per min.

control and phenobarbital-treated hamsters. In rats, the activity of glycodeoxycholate 7α-hydroxylase was also as high as that of taurodeoxycholate and both activities were well correlated, although very little glycine-conjugated bile acids are present in rat bile (32). These results suggested the possibility that the same enzyme may well be catalyzing the 7α-hydroxylation of both glycodeoxycholate and taurodeoxycholate in both species, although we cannot confirm this without further purification of the enzyme(s).

Phenobarbital was once proposed as a potential therapeutic agent for cholelithiasis because of the salutary effect in decreasing the lithogenicity of biliary lipid composition (33, 34). Some of the hydroxylations in bile acid biosynthesis and metabolism were reported to be stimulated by prior treatment with phenobarbital (16–19). The increase in hydroxylase activity correlated with increased amounts of cytochrome P-450 and NADPH-cytochrome P-450 reductase in rat liver microsomes (19). Einarsson and Johansson (16, 17) had reported that the activity of taurodeoxycholate 7α-hydroxylase was stimulated by the administration of phenobarbital in the rat. This was confirmed in the present study in which the activity of this enzyme was increased by phenobarbital treatment in the rat. However, in the hamster, 7α-hydroxylation of glyco- and taurodeoxycholate was inhibited by phenobarbital treatment (Table 1). There seems to be a species difference in the regulation of the enzyme following the treatment with phenobarbital: most probably, specific cytochrome P-450 enzyme involved in the bile acid 7α-hydroxylation may have decreased relative to other types of induced microsomal enzymes in hamsters. Biliary bile acid composition was altered by phenobarbital treatment in the hamster. The ratio of cholic acid to deoxycholic acid was considered to depend mainly on microbial 7α-dehydroxylation of cholic acid and hepatic 7α-hydroxylation of deoxycholic acid during the enterohepatic circulation. If activity of 7α-hydroxylation of deoxycholate is inhibited after phenobarbital treatment, conversion of deoxycholate to cholate might be decreased and the ratio of cholic acid to deoxycholic acid would be expected to decrease. Our results, that the ratio of cholic acid to deoxycholic acid was decreased by about 30% in gallbladder bile by phenobarbital treatment, may reflect decreased deoxycholate 7α-hydroxylase activity, although the change in cholate to deoxycholate ratio was not statistically significant. The proportion of cholic acid to chenodeoxycholic

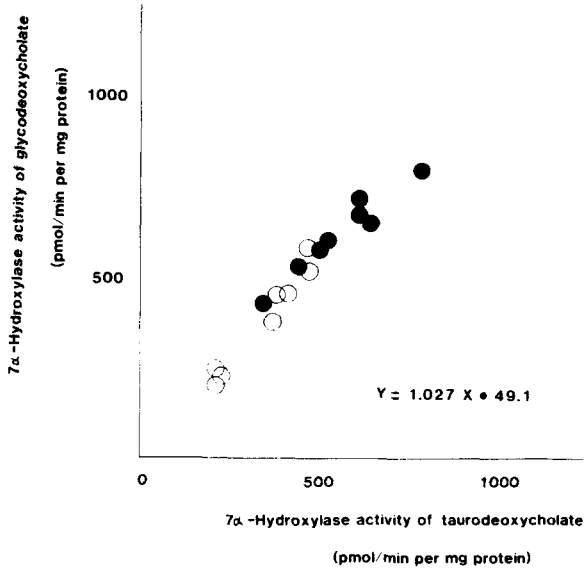

Fig. 6. Correlation between the 7α-hydroxylase activity of glycodeoxycholate and that of taurodeoxycholate in control group (●) and phenobarbital-treated group (○). Positive correlation was found ($n = 16$, $r = 0.98$, $P<0.01$).

acid was decreased by phenobarbital treatment. This is considered to be due to the change of the synthetic ratio of primary bile acids. In most mammalian species, cholic acid and chenodeoxycholic acid occur in proportions that are relatively species-specific. The ratio of cholic acid to chenodeoxycholic acid synthesized in the liver is thought to be determined by *a*) 12α-hydroxylase activity and *b*) activities of mitochondrial and/or microsomal 26- and/or 25-hydroxylases, i.e., enzymes that catalyze the initial step of side-chain degradation (35). In rats, it was reported that the activity of steroid 26-hydroxylase was not influenced (36, 37) and that of 12α-hydroxylase was inhibited (16, 38) by administration of phenobarbital. Our results suggested that phenobarbital also inhibited the activity of 12α-hydroxylase resulting in lower cholic to chenodeoxycholic acid ratio in the hamster, although the effect of phenobarbital on those enzymes has been scarce ly studied in this species.

Biliary concentration of deoxycholic acid differs among species, varying from 0 to 10% of bile acids in rats (32), mice (9), guinea pigs (39), prairie dogs (10), and hamsters (20), 15 to 30% in dogs (40) and humans (41), and greater than 90% in rabbits (42). Species capable of converting deoxycholic acid to cholic acid have lower proportions of deoxycholic acid in bile. Cohen et al. (10) demonstrated that the low concentration of deoxycholic acid in prairie dog bile was due to active 7α-hydroxylase in the liver by using perfused prairie dog liver. On the other hand, the high proportion of deoxycholic acid in the rabbit bile is explained by the efficient microbial 7α-dehydroxylation of cholic acid and by the inability of the 7α-hydroxylation of deoxycholic acid (43). Thus, 7α-hydroxylase activity for deoxycholic acid is thought to be one of the major factors that determine the bile acid composition. It may be considered a self-protecting mechansim. Animals with a low concentration of deoxycholic acid in their bile are unable to tolerate deoxycholic acid and thus have 7α-hydroxylase activity to decrease the concentration. Haslewood (44) suggested that the dog and man are indifferent to deoxycholic acid whereas the rat and the mouse do not tolerate it. Several experimental studies in dogs, rats, and rabbits support this hypothesis (45–47). We reported that deoxycholic acid produced severe acute toxicity whether infused intravenously or intraduodenally in the hamster (3). Several observations suggested that the cytotoxicity of bile acids decreased as the number of hydroxy groups on the steroid nucleus increased (4, 5). The 7α-hydroxylation of deoxycholic acid may thus be a detoxification mechanism in the hamster liver.

In summary, we demonstrated that the hamster could rehydroxylate deoxycholate to cholate. Glycodeoxycholate and taurodeoxycholate were obviously better substrates for the enzyme than unconjugated deoxycholate and the enzyme activity was inhibited significantly by phenobarbital treatment. A highly significant correlation was observed between the 7α-hydroxylation of glyco- and taurodeoxycholate both in the control and the phenobarbital-treated animals. Therefore, we conclude that deoxycholic acid is 7α-hydroxylated after amidation in vivo and the same enzyme may catalyze the 7α-hydroxylation of both glycodeoxycholate and taurodeoxycholate. 7α-Hydroxylation of deoxycholate is likely to be one of the mechanisms involved in detoxifying the harmful secondary bile acid in the hamster.

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