Sex differences in gallbladder bile acid composition and hepatic steroid 12α-hydroxylase activity in hamsters[1]

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Abstract The gallbladder bile acid composition and the activity of the hepatic steroid 12α-hydroxylase were determined in male and female hamsters. Cholic acid, chenodeoxycholic acid, and deoxycholic acid were the major bile acids in both sexes; in addition, 7-ketodeoxycholic acid and lithocholic acid were present. A sex-linked difference in the ratio of cholic acid (plus its metabolites) to chenodeoxycholic acid (plus its metabolite) was observed. The ratio was 1.93 ± 0.39 in males and 2.74 ± 0.54 in females. Another sex-linked difference was found in the activity of the 12α-hydroxylase. The extent of the 12α-hydroxylation of 7α-hydroxycholest-4-en-3-one to yield 7α,12α-dihydroxycholest-4-en-3-one was about two times greater in the microsomal suspension obtained from the liver of female hamsters than in that of male hamsters. A positive correlation between the 12α-hydroxylase activity and the ratio of cholic acid/chenodeoxycholic acid was also observed. These results strongly support the proposal that the activity of the 12α-hydroxylase is the major factor in determining the relative proportion of cholic acid and chenodeoxycholic acid formed from cholesterol in the liver.—**Kuroki, S., S. Muramoto, T. Kuramoto, and T. Hoshita.** Sex differences in gallbladder bile acid composition and hepatic steroid 12α-hydroxylase activity in hamsters. *J. Lipid Res.* 1983. **24:** 1543–1549.

Supplementary key words 12α-hydroxylation • 7α-hydroxycholest-4-en-3-one • 7α,12α-dihydroxycholest-4-en-3-one • cholic acid to chenodeoxycholic acid ratio

The primary bile acids formed from cholesterol in the human liver are cholic acid and chenodeoxycholic acid (1). It has recently been shown that chenodeoxycholic acid dissolves cholesterol gallstones, whereas cholic acid has no cholelitholytic action (2). These facts suggest that the ratio of cholic acid to chenodeoxycholic acid in bile may play an important role in cholesterol gallstone formation. The biosynthesis of the two primary bile acids is believed to proceed via the intermediates 7α-hydroxycholesterol and 7α-hydroxycholest-4-en-3-one (3). 7α-Hydroxycholest-4-en-3-one is the last intermediate common to both cholic acid and chenodeoxycholic acid. It can either be 12α-hydroxylated, in which case cholic acid would be formed, or it can be 26-hydroxylated (or its reduction to 5β-cholestane-3α,7α-diol followed by 26-hydroxylation) and proceed to form chenodeoxycholic acid (1). Thus, the steroid 12α-hydroxylase which catalyzes the 12α-hydroxylation of 7α-hydroxycholest-4-en-3-one may have an important regulatory function in determining the ratio between cholic acid and chenodeoxycholic acid formed from cholesterol.

In order to investigate this possible connection between the 12α-hydroxylase activity and the gallbladder bile acid composition, the hamster appears to be a more suitable animal model than the rat, the most frequently used experimental animal for studies of bile acid metabolism. The rat does not have a gallbladder, and diversion of bile by cannulation of the bile duct may cause major changes in the types and amounts of bile acids synthesized in the liver owing to removal of feedback control mechanisms. On the other hand, the hamster has a gallbladder and the individual bile acid composition is similar to that of humans. However, the 12α-hydroxylase system in hamster liver has been little studied. In the present investigation, the activity of the hepatic 12α-hydroxylase and the gallbladder bile acid composition were determined in intact male and female hamsters.

EXPERIMENTAL PROCEDURES

General

Melting points were determined with a Kofler hot-stage apparatus and are uncorrected. Infrared (IR) spectra were taken on a JASCO IRA-1 spectrometer as KBr discs. Nuclear magnetic resonance (NMR) spectra were mea-

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sured at 90 MHz on a Hitachi R-40 spectrometer. "The usual work-up" refers to dilution with water, extraction with ether, washing with water to neutrality, drying over anhydrous Na_2SO_4, filtration, and evaporation under a reduced pressure.

Materials

7α-Hydroxycholest-4-en-3-one. 5β-Cholestane-3α,7α-diol was prepared by electrolytic coupling of chenodeoxycholic acid and isovaleric acid as previously described (4). The melting point was 83–84°C (reported mp 84–86°C (4)). A solution of 5β-cholestane-3α,7α-diol (6 g) in 65 ml of acetic anhydride, 40 ml of benzene, and 65 ml of pyridine was heated on a water bath for 2 hr. After the usual work-up, the product was recrystallized from methanol–water to give crystals (5.9 g) of 3α,7α-diacetoxy-5β-cholestane, mp 128°C. The IR spectrum showed acetate absorption (1730, 1250, 1050 cm^{-1}), but no hydroxyl absorption. To the stirred solution of the acetate (5.8 g) in methanol (175 ml) at 20°C, a solution of K_2CO_3 (1.4 g) in aqueous methanol (7 ml) was added and the stirring was continued for 4 hr. After the usual work-up, the product was chromatographed on a column of silica gel (300 g, Merck). Elution with a 4:1 mixture of benzene–ethyl acetate gave 7α-acetoxy-5β-cholestan-3α-ol (4.1 g) as a low melting solid, which could not be crystallized. The IR spectrum indicated a hydroxyl band at 3450 cm^{-1} in addition to acetate bands at 1730, 1250, and 1050 cm^{-1}. NMR (δ ppm): 0.68 (s, 3H, 18-CH_3), 0.86 (s, 3H, 19-CH_3), 0.99 (d, J = 6 Hz, 3H, 21-CH_3), 2.00 (s, 3H, 7-$OCOCH_3$), 3.75 (m, 1H, 3β-H), 5.11 (m, 1H, 7β-H). Oxidation of the acetoxy-cholestanol (4 g) with Jones reagent (5) at room temperature yielded a gummy residue (100% yield) which was refluxed with 200 ml of 5% methanolic KOH for 2 hr. After the usual work-up, the product was recrystallized from n-hexane and then from acetone–water to give crystals (1.9 g) of 7α-hydroxy-5β-cholestan-3-one, mp 127–127.5°C (reported mp 121–122°C (6)). 7α-Hydroxy-5β-cholestan-3-one (200 mg) was dissolved in 50 ml of 96% ethanol and treated with 150 mg of selenium dioxide at 80°C for 18 hr (7). After the usual work-up, crystallizations from acetone–water afforded 69 mg of 7α-hydroxycholest-4-en-3-one, mp 183.5–184.5°C (reported mp 181–182°C (7), and 183–184°C (3)).

Tritium-labeled 7α-hydroxycholest-4-en-3-one. 7α-Hydroxy-5β-cholestan-3-one was labeled with tritium by exposure to 15 curies of tritium gas (New England Nuclear, England) according to the procedure of Wilzbach (8). The tritium-labeled 7α-hydroxy-5β-cholestan-3-one (sp act 0.64 mCi/mg) was treated with selenium dioxide as described above. After the usual work-up and purification with preparative thin-layer chromatography (silica gel G, Merck) using a solvent system of benzene–ethyl acetate 3:1 (v/v), the labeled 7α-hydroxycholest-4-en-3-one was obtained (sp act 49 μCi/mg). The radiochemical purity of the substrate was more than 95% as determined by high performance liquid chromatographic analysis.

5β-Cholestane-3α,7α,12α-triol, 7α,12α-dihydroxy-5β-cholestan-3-one, and 7α,12α-dihydroxycholest-4-en-3-one. These compounds were prepared as previously described (9). Melting points: 188.5°C (reported mp 187–188°C (9)) for 5β-cholestane-3α,7α,12α-triol; 209–210°C (reported mp 209–210°C (9)) for 7α,12α-dihydroxy-5β-cholestan-3-one; 227–228°C (reported mp 228–229°C (9)) for 7α,12α-dihydroxycholest-4-en-3-one.

Animals

Six female and six male Golden Syrian hamsters, 7 weeks old with a mean weight of 96 g, were purchased from Shizuoka Laboratory Animal Center (Shizuoka, Japan). They were acclimated to the vivarium for 2 weeks with the temperature at 25°C and light periods from 6 AM to 6 PM. The animals had free access to water and Standard Powder Chow FM (Oriental Yeast Co., Ltd., Tokyo, Japan), which contains 0.6 mg of cholesterol per gram of food as determined by gas–liquid chromatography. The animals were killed between 10 and 11 AM. Under ether anesthesia, the abdomen and the chest were opened and blood was aspirated from the heart. The gallbladder was removed and the bile was stored at −20°C before being processed. The liver was then excised, rinsed with ice-cold homogenizing solution (see below for composition), chilled on ice, and weighed.

Measurement of microsomal 12α-hydroxylase activity

Two-gram portions of liver were homogenized in 8 ml of 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 tightly fitting pestle (10). 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 the 20,000 *g* supernatant fluid at 100,000 *g* for 1 hr. The microsomal pellet was suspended in 4 ml of the homogenizing medium and 1 ml of the suspension was used in a standard assay. A small aliquot was used for protein determination according to Lowry et al. (11).

The enzymatic conversion of 7α-hydroxycholest-4-en-3-one to 7α,12α-dihydroxycholest-4-en-3-one was assayed as follows. A solution of 50 nmol of tritium-labeled 7α-hydroxycholest-4-en-3-one in 10 μl of acetone was added to an incubation mixture containing, in a volume of 2.0 ml, potassium phosphate buffer, pH 7.4, 0.191 mmol; $MgCl_2$, 10 μmol; NADPH, 3 μmol; and 1.0 ml of the microsomal suspension (10). After 10 min of preincubation, incubations were started by addition of the sub-

strate, conducted at 37°C in air for 10 min, and terminated by addition of 20 ml of $CHCl_3$–methanol 2:1 (v/v) (12). The organic layer was evaporated under reduced pressure and the dried residue was dissolved in ethyl acetate. An aliquot was applied as a line to a thin-layer plate coated with 0.25 mm of silica gel G. Unlabeled reference compounds (5β-cholestane-3α,7α,12α-triol, 7α,12α-dihydroxy-5β-cholestan-3-one, 7α,12α-dihydroxycholest-4-en-3-one, and 7α-hydroxycholest-4-en-3-one) were also applied as spots at either sides of the line. The developing solvent was benzene–ethyl acetate 3:7 (v/v). Positions of the reference samples were established by exposure of the plates to iodine vapor. Pertinent bands were scraped off into scintillation vials and the steroids were extracted with 1 ml of methanol. Ten ml of scintillation cocktail, containing 4 g of 2,5-diphenyloxazol and 0.1 g of 2,2'-p-phenylenbis-(5-phenyloxazol) in 1.0 liter of toluene, was added and radioactivity was measured in a Packard Tri-Carb scintillation spectrometer. Suitable corrections were made for background and quenching.

Analysis of gallbladder bile acids

The gallbladder bile was deproteinized with 10 ml of ethanol. An aliquot of the ethanol solution was mixed with 2 ml of 2 N KOH. The solution in a Teflon tube was heated at 130°C for 3 hr. The hydrolyzate was acidified with dilute HCl, and extracted with ethyl acetate. After the usual work-up, the bile acids obtained were methylated with diazomethane, and then silylated with dimethylethylsilylimidazole (13). The resulting methyl ester–dimethylethylsilyl (DMES) ether derivatives were analyzed by gas–liquid chromatography using a glass column packed with 3% QF-1 on 100–120 mesh Gas-Chrom Q. Column temperature was 230°C. Relative retention times of methyl ester–DMES ether derivatives of bile acids were as follows (deoxycholic acid, 1.00): cholesterol, 0.45; lithocholic acid, 0.77; chenodeoxycholic acid, 1.09; ursodeoxycholic acid, 1.22; cholic acid, 1.38; 7-ketodeoxycholic acid, 3.83.

Ratio of glycine and taurine conjugated bile acids (G/T ratio) in the gallbladder bile was determined as described previously (14).

Cholesterol determinations

Liver lipids were extracted with chloroform–methanol 2:1 (v/v). Serum was deproteinized with 10 ml of methanol. The liver extract and an aliquot of the methanol solution were hydrolyzed with 1 N methanolic KOH and subjected to the usual work-up. The cholesterol concentration was determined as trimethylsilyl ether derivative by gas–liquid chromatography using a glass column packed with 3% OV-17 on 80–100 mesh Gas-Chrom Q (15). Column temperature was 270°C. 5α-Cholestane was used as an internal standard.

High performance liquid chromatography

The instrument used was Shimadzu 830 liquid chromatograph equipped with a single-wavelength (254 nm) UV detector. A reversed phase column, TSK GEL LS-410 (Toyo Soda Ltd., Japan), 30 cm × 4 mm i.d., particle size 5 μm, was used for analyzing the purity of the substrate and catalytic products of the substrate. The column was operated at ambient temperature and the eluting solvent was methanol–water 94:6 (v/v). Flow rate was 0.7 ml/min. Eluted radioactivity was measured using a liquid scintillation spectrometer.

Statistical methods

Results are expressed as mean ± SD. Group means were compared by paired Student's t test. Bivariate regression analyses were performed by the method of least squares.

RESULTS

Steroid 12α-hydroxylase

The rate of the conversion of 7α-hydroxycholest-4-en-3-one to 7α,12α-dihydroxycholest-4-en-3-one by liver microsomes of both male and female hamsters was almost linear with time during the first 18 min (**Fig. 1**). A 10-min incubation period was adopted as representing the initial reaction rate. The effects of substrate concentration, enzyme concentration, and NADPH concentration on the rate of the reaction are shown in **Fig. 2, Fig. 3,**

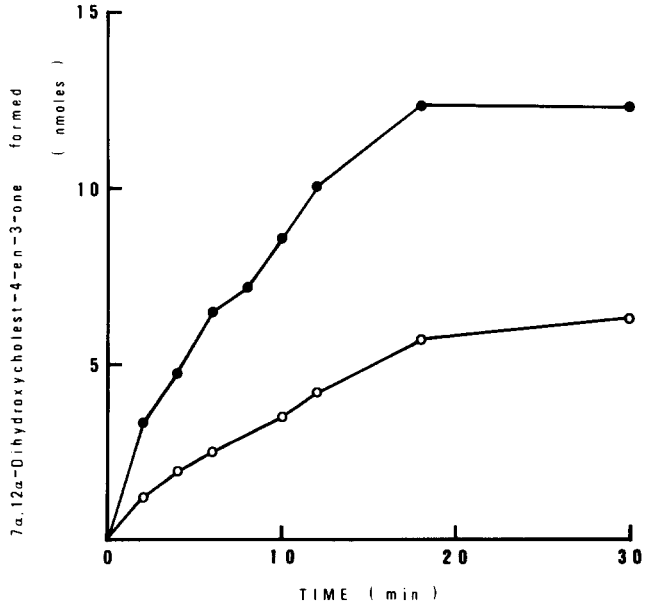

Fig. 1. Time course of 12α-hydroxylation of 7α-hydroxycholest-4-en-3-one in male (O) and female (●) hamsters. Standard assay conditions, except that incubation time was varied from 2 to 30 min.

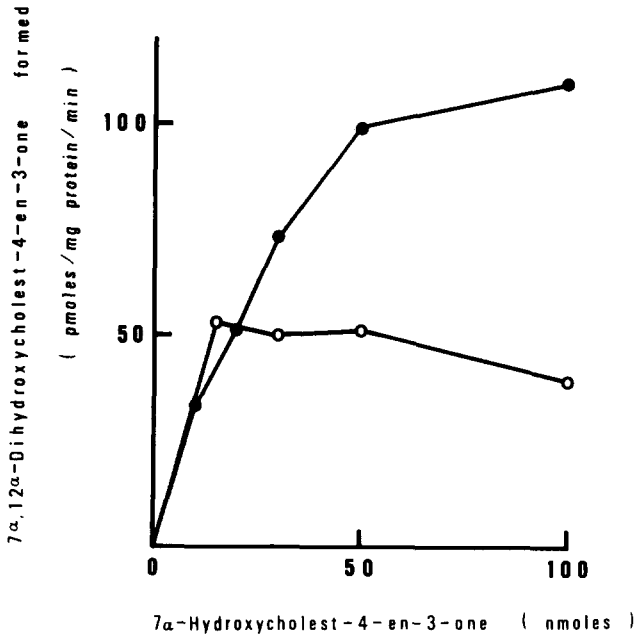

Fig. 2. Effect of substrate concentration on 12α-hydroxylation of 7α-hydroxycholest-4-en-3-one in male (○) and female (●) hamsters. Standard assay conditions, with substrate concentrations from 10 to 100 nmol in 2.0 ml of incubation mixture.

and **Fig. 4,** respectively. From these results, 50 nmol of substrate concentration, 1.0 ml of the microsomal suspension, and 3 μmol of NADPH in 2.0 ml of the incubation mixture were chosen as standard assay conditions.

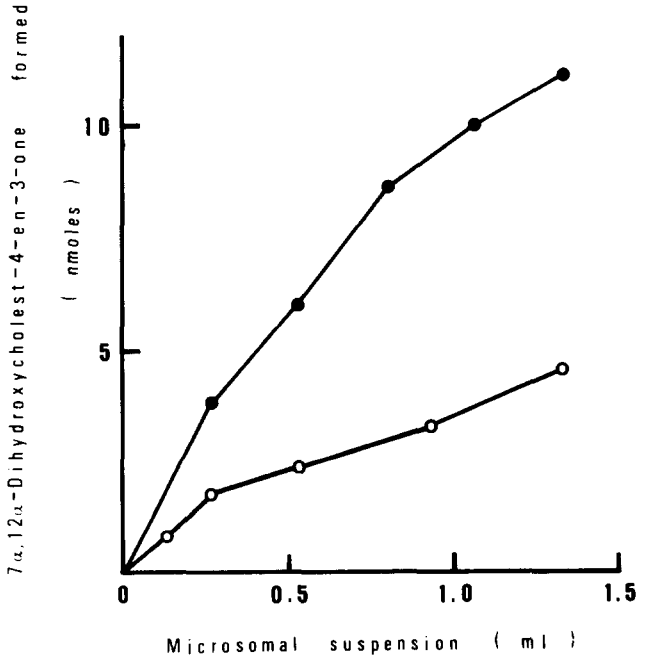

Fig. 3. Dependence of hepatic 12α-hydroxylase activity on the amount of the microsomal suspension in male (○) and female (●) hamsters. Standard assay conditions, with volume of microsomal suspension varied over indicated ranges in 2.0 ml of incubation mixture.

Microsomes obtained from male hamsters also catalyzed the reaction, though the rate of conversion was less than that obtained from females (Figs. 1–4).

An area of silica gel corresponding to the R_f value of 7α,12α-dihydroxycholest-4-en-3-one on the thin-layer plate was extracted with methanol. The material was rechromatographed by reversed-phase high performance liquid chromatography; there was observed a single peak of radioactivity with an absorption peak at 254 nm of 7α,12α-dihydroxycholest-4-en-3-one which was added as a carrier. Under the standard assay conditions, reaction products other than 7α,12α-dihydroxycholest-4-en-3-one were barely detected. Analysis of reaction products at a longer incubation time (i.e., 30 min) by high performance liquid chromatography revealed the presence of 5β-cholestane-3α,7α,12α-triol (about 5%), while no metabolites more polar than 5β-cholestane-3α,7α,12α-triol (for example, 3α,7α,12α-trihydroxy-5β-cholestanoic acid and cholic acid) were detected.

Under the standard assay conditions, the mean enzyme activity was 48.5 ± 8.9 pmol/mg protein per min in the male and 99.8 ± 8.6 pmol/mg protein per min in the female (**Table 1**). The difference between male and female hamsters was statistically significant ($P < 0.0005$).

Bile acid composition in gallbladder bile

The gallbladder bile acid composition and G/T ratio in male and female hamsters are shown in Table 1. The major gallbladder bile acids in both sexes were cholic acid, chenodeoxycholic acid, and deoxycholic acid. Lith-

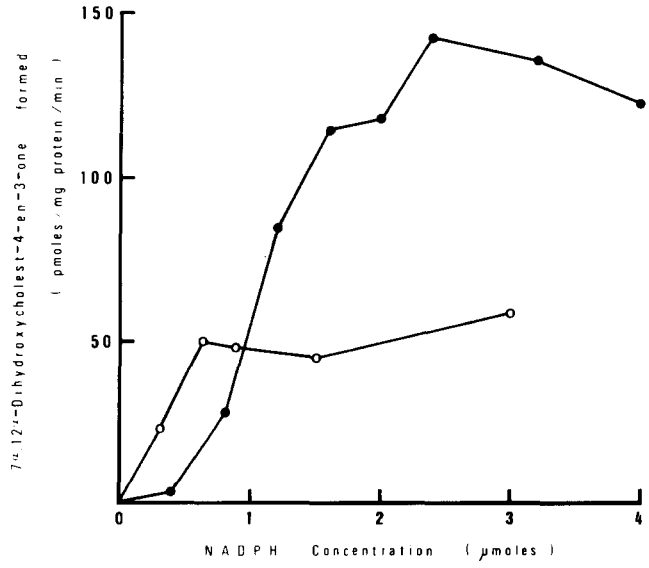

Fig. 4. Effect of NADPH concentration on 12α-hydroxylation of 7α-hydroxycholest-4-en-3-one in male (○) and female (●) hamsters. Standard assay conditions, with NADPH concentration varied over indicated ranges in 2.0 ml of incubation mixture.

TABLE 1. Hepatic microsomal steroid 12α-hydroxylase activity, bile acid composition, and serum and liver cholesterol concentrations in male and female hamsters

	Male	Female	P
12α-Hydroxylase activity			
pmol/g liver per min	518 ± 140[a]	1039 ± 100	<0.0005
pmol/mg protein per min	48.5 ± 8.9	99.8 ± 8.6	<0.0005
Bile acid composition (%)			
Cholic acid	55.5 ± 2.9	62.2 ± 5.8	<0.025
Chenodeoxycholic acid	33.0 ± 4.7	24.9 ± 3.6	<0.005
Deoxycholic acid	7.3 ± 2.8	7.0 ± 2.7	NS[b]
Lithocholic acid	1.6 ± 1.2	2.3 ± 1.3	NS
7-Ketodeoxycholic acid	2.6 ± 0.5	3.6 ± 1.0	<0.05
Cholic acid/chenodeoxycholic acid	1.72 ± 0.33	2.56 ± 0.54	<0.005
Cholic acid plus its metabolites/chenodeoxycholic acid plus its metabolite[c]	1.93 ± 0.39	2.74 ± 0.54	<0.01
G/T ratio	2.13 ± 0.61	2.06 ± 1.15	NS
Serum cholesterol (mg/dl)	80.6 ± 21.7	69.9 ± 10.1	NS
Liver cholesterol (mg/g liver)	7.00 ± 1.91	6.45 ± 0.43	NS

[a] Each value is expressed as mean ± SD.
[b] NS, statistically not significant.
[c] Cholic acid plus its metabolites/chenodeoxycholic acid plus its metabolite: (cholic acid + deoxycholic acid + 7-ketodeoxycholic acid)/(chenodeoxycholic acid + lithocholic acid).

ocholic 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 amounts of cholic acid ($P < 0.025$) and 7-ketodeoxycholic acid ($P < 0.05$) were higher in female than in male hamsters, while that of chenodeoxycholic acid ($P < 0.005$) was lower in females than in males. The sex difference may be further appreciated from the cholic acid/chenodeoxycholic acid ratios. The ratios were 1.72 ± 0.33 in males and 2.56 ± 0.54 in females ($P < 0.005$). As shown in **Fig. 5,** the ratio between cholic acid (plus its metabolites, i.e., deoxycholic acid and 7-ketodeoxycholic acid) and chenodeoxycholic acid (plus its metabolite, i.e., lithocholic acid) correlated well ($r = 0.82$, $P < 0.001$, n = 12) with the activity of the steroid 12α-hydroxylase.

The G/T ratios for males and females were not significantly different (Table 1).

Serum and liver cholesterol concentrations

Similar concentrations of cholesterol per dl of serum and per gram of liver were found in male and female hamsters (Table 1).

DISCUSSION

Steroid 12α-hydroxylase is one of the microsomal mixed function oxidases and catalyzes cholest-5-en-3β,7α-diol, 7α-hydroxycholest-4-en-3-one, 5β-cholestane-3α,7α-diol, and some cholestanols of 5α-configuration to their 12α-hydroxylated compounds (12, 16). In the biosynthesis of the two primary bile acids, the most effective substrate of the enzyme is considered to be 7α-hydroxycholest-4-en-3-one (17). In order to investigate the enzyme activity

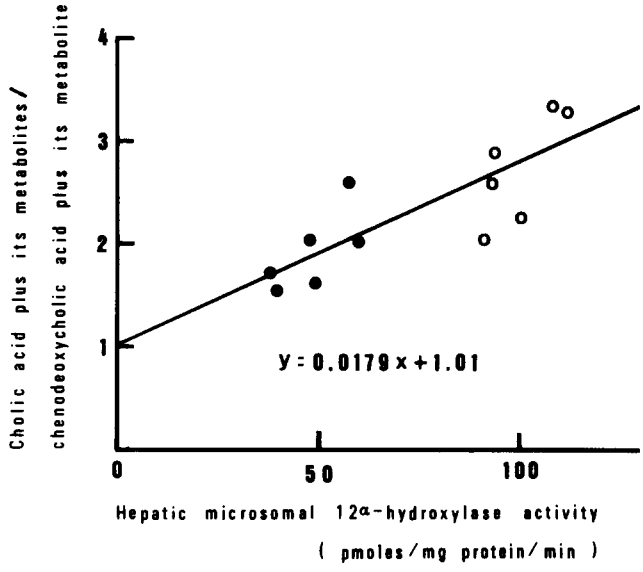

Fig. 5. Correlation between the 12α-hydroxylase activity and the ratio of cholic acid (plus its metabolites) to chenodeoxycholic acid (plus its metabolite). Standard assay conditions were used for determining the 12α-hydroxylase activity in male (●) and female (○) hamsters. Cholic acid plus its metabolites means (cholic acid + deoxycholic acid + 7-ketodeoxycholic acid) and chenodeoxycholic acid plus its metabolite means (chenodeoxycholic acid + lithocholic acid). Positive correlation was found between the 12α-hydroxylase activity and the ratio ($r = 0.82$, $P < 0.001$, n = 12).

under various physiological and pathological conditions, we synthesized the substrate and determined conditions to assay the enzyme activity in hamsters. Under standard assay conditions, liver microsomes of hamsters catalyzed the substrate to 7α,12α-dihydroxycholest-4-en-3-one just as rat and rabbit microsomes (12, 16). Since catalytic products of the substrate other than 7α,12α-dihydroxycholest-4-en-3-one were barely detected, the hamster is considered to be a more suitable animal for assay of the enzyme activity than the rat (12).

The data in Table 1 confirm and extend the results of previous investigations (18, 19) that showed that cholic acid, chenodeoxycholic acid, and deoxycholic acid are the major bile acids in gallbladder bile of hamsters in both sexes. Ginsberg, Duane, and Flock (18) found that gallbladder bile acids in male hamsters consist of cholic acid (59%), chenodeoxycholic acid (36%), and deoxycholic acid (5%). The present data on the bile acid composition of male hamsters is both qualitatively and quantitatively consistent with their findings. Pearlman et al. (19) reported that the gallbladder bile from female hamsters had the following composition: cholic acid, 46.0%; chenodeoxycholic acid, 20.9%; deoxycholic acid, 28.2%. Although a marked difference was noted in the ratio of deoxycholic acid to cholic acid between their female hamsters and ours, this may be of little importance and may be due to diet which altered the intestinal microflora. Deoxycholic acid is a secondary bile acid formed from cholic acid by the action of intestinal microorganisms during enterohepatic circulation, and the ratio of deoxycholic acid to cholic acid is relatively easily varied by qualitative and quantitative differences in microbial population and strains. It should be mentioned that there is no difference in the ratio of cholic acid (plus its metabolites, i.e., deoxycholic acid and 7-ketodeoxycholic acid) to chenodeoxycholic acid (plus its metabolite, i.e., lithocholic acid) between the previous and present data.

This is the first study in which the bile acids in hamster bile have been analyzed in animals of both sexes under similar experimental conditions. A sex-linked difference was found in the relative amounts of chenodeoxycholic acid and in the ratio of cholic acid (plus its metabolites) to chenodeoxycholic acid (plus its metabolite). These differences could be explained by the fact that there was another sex-linked difference in the activity of the hepatic 12α-hydroxylase. The extent of the conversion of 7α-hydroxycholest-4-en-3-one to 7α,12α-dihydroxycholest-4-en-3-one was about two times greater in the microsomal suspension obtained from the liver of female hamsters than in that of male hamsters. The higher activity of the 12α-hydroxylase in the females than in the males would result in the increased production of 7α,12α-dihydroxycholest-4-en-3-one and consequent increase in the formation of cholic acid at the expense of chenodeoxycholic acid.

A sex difference in the ratio of cholic acid plus its metabolites to chenodeoxycholic acid plus its metabolite has been found in rats: the ratio is 1.90 in female rats and 1.35 in male rats (20). This is in contrast to the findings of Einarsson (12) who reported that the extent of the 12α-hydroxylation of 7α-hydroxycholest-4-en-3-one was about 50% higher in male rats than in female rats. The discrepancy that the activity of the 12α-hydroxylase is higher, yet the production of cholic acid is lower, in male rats than in female rats cannot be explained. Possibly the bile acid composition in rat bile obtained by cannulation of the bile duct is not a reflection of the enzyme activity of the liver, which is obtained from the intact rats.

In most mammalian species, cholic acid and chenodeoxycholic acid occur in proportions that are relatively species-specific. The relative 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 (21). For example, guinea pig bile contains only traces of cholic acid. The major bile acid is chenodeoxycholic acid and the 12α-hydroxylase level is quite low (22). Rabbits have an extremely high 12α-hydroxylase level (23) and hence produce cholic acid almost exclusively as a primary bile acid (24). On the other hand, in a rare inherited disease, cerebrotendinous xanthomatosis, liver mitochondria obtained from a patient were completely devoid of 26-hydroxylase activity, and the primary bile acid was mainly cholic acid (25). In addition, there are alternative pathways of chenodeoxycholic acid biogenesis in which the side-chain cleavage precedes all of the transformations of the cholesterol nucleus to form 3β-hydroxychol-5-enoic acid (26), or after 7α-hydroxylation of cholesterol to form 3β,7α-dihydroxychol-5-enoic acid (27), finally producing chenodeoxycholic acid. In such cases, 12α-hydroxylation might not occur, and hence, little cholic acid is formed. However, the relationship between 12α-hydroxylase activity and biliary bile acid composition in guinea pig and rabbit suggests that the activity of the hepatic 12α-hydroxylase plays a major role in determining the relative proportions of cholic acid and chenodeoxycholic acid synthesized in liver of animals whose ability for side-chain cleavage is not affected.

The present study demonstrates the positive correlation (Fig. 5) between the 12α-hydroxylase activity and the ratio of cholic acid (plus its metabolites) to chenodeoxycholic acid (plus its metabolite). Because of differences in bile acid kinetics such as intestinal absorption (28),

hepatic uptake (29), and pool sizes, the percent composition of biliary bile acids does not always represent the synthetic rate of the two primary bile acids. However, this result as well as the above-mentioned findings concerning the sex differences again emphasizes the central role of the 12α-hydroxylase system in regulation of the ratio of the two primary bile acids.

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