Metabolism of 3α,7α-dihydroxy-5β-cholestanoic acid by rat liver in vivo and in vitro

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Abstract The metabolism of 3α,7α-dihydroxy-5β-cholestanoic acid was studied in bile fistula rats and in preparations from rat liver homogenates. In the bile fistula rat, the main products were chenodeoxycholic acid, α-muricholic acid, and β-muricholic acid. Only small amounts of cholic acid were formed. Incubations of 3α,7α-dihydroxy-5β-cholestanoic acid with microsomes and NADPH yielded as the main product 3α,6β,7α-trihydroxy-5β-cholestanoic acid. The formation of small amounts of 3α,7α,12α-trihydroxy-5β-cholestanoic acid was shown. The major product in incubations of 3α,7α-dihydroxy-5β-cholestanoic acid with microsomes and the 100,000 *g* supernatant fluid fortified with ATP was identified as 3α,7α,24ξ-trihydroxy-5β-cholestanoic acid. This compound was converted into chenodeoxycholic acid and its metabolites in the bile fistula rat.—**Gustafsson, J.** Metabolism of 3α,7α-dihydroxy-5β-cholestanoic acid by rat liver in vivo and in vitro. *J. Lipid Res.* 1979. **20:** 265–270.

Supplementary key words bile acid synthesis

In the major pathways for bile acid biosynthesis in the rat, 5β-cholestane-3α,7α-diol and 5β-cholestane-3α,7α,12α-triol are regarded as main substrates for ω-hydroxylation, the initial side chain hydroxylation (1). Further oxidation of the ω-hydroxylated C_{27}-steroids yields 3α,7α-dihydroxy-5β-cholestanoic acid and 3α,7α,12α-trihydroxy-5β-cholestanoic acid (1). 3α,7α,12α-Trihydroxy-5β-cholestanoic acid is converted efficiently into cholic acid both in vivo and in vitro (2, 3). The mechanism is probably a β-oxidation process since in vitro 3α,7α,12α-trihydroxy-5β-cholestanoic acid is 24-hydroxylated and the 24-hydroxylated derivative is converted into cholic acid (4). The metabolism of 3α,7α-dihydroxy-5β-cholestanoic acid has not been studied in the rat. It has been assumed that the same mechanism for side chain cleavage occurs as is proposed to occur with 3α,7α,12α-trihydroxy-5β-cholestanoic acid (5). Also, it is not known if 3α,7α-dihydroxy-5β-cholestanoic acid can be converted into 3α,7α,12α-trihydroxy-5β-cholestanoic acid and cholic acid.

Early experiments in vivo indicated that introduction of a 26-hydroxyl group into a C_{27}-5β-steroid almost prevents 12α-hydroxylation (6). Recent investigations by Elliott and associates (7–12) showed that a number of 26-oxygenated steroids in the 5α-series, including 3α,7α-dihydroxy-5α-cholestanoic acid, are 12α-hydroxylated both in vivo and in vitro.

In view of these findings it appeared important to establish the metabolism of 3α,7α-dihydroxy-5β-cholestanoic acid in the rat.

EXPERIMENTAL PROCEDURE

Materials

3α,7α-Dihydroxy-5β-[3β,7β-$^3H_2$]cholestanoic acid (sp act 12 μCi/μmol) was prepared by reduction with tritium-labeled sodium borohydride (Radiochemical Centre, Amersham, England) of a mixture of 3α-hydroxy-7-oxo- and 7α-hydroxy-3-oxo-5β-cholestanoic acids obtained by oxidation of 3α,7α-dihydroxy-5β-cholestanoic acid with *N*-bromosuccinimide (4). The reaction mixture from oxidation with *N*-bromosuccinimide was subjected to thin-layer chromatography with system S11 (13) and the zone corresponding to the R_f values expected of 3α-hydroxy-7-oxo- and 7α-hydroxy-3-oxo-5β-cholestanoic acids was eluted. The material was first reduced with tritium-labeled sodium borohydride of high specific radioactivity followed by complete reduction with unlabeled sodium borohydride. The labeled 3α,7α-dihydroxy-5β-cholestanoic acid was purified by repeated thin-layer chromatography with solvent system S11 (13). The radiopurity of the material was more than 99% as judged by thin-layer chromatography and radio–gas–liquid chromatography of the trimethylsilyl ether

Abbreviations: The following systematic names are given to bile acids referred to by trivial names: cholic acid, 3α,7α,12α-trihydroxy-5β-cholanoic acid; α-muricholic acid, 3α,6β,7α-trihydroxy-5β-cholanoic acid; β-muricholic acid, 3α,6β,7β-trihydroxy-5β-cholanoic acid; chenodeoxycholic acid, 3α,7α-dihydroxy-5β-cholanoic acid.

of the methyl ester. The position of the label was determined with 3α-hydroxysteroid dehydrogenase and 7α-hydroxysteroid dehydrogenase. Of the total tritium label, 80% was located in the 3-position and 20% in the 7-position. It is worth pointing out that, under the conditions employed, oxidation of milligram amounts of 3α,7α-dihydroxy-5β-cholestanoic acid with *N*-bromosuccinimide oxidation occurs not only in the 7- but also in the 3-position. The preponderance of label in the 3-position might be due to a more rapid reduction of this position than of the 7-position with the trace amounts of tritium-labeled sodium borohydride used in the first phase of the reduction.

Unlabeled 3α,7α-dihydroxy-5β-cholestanoic acid was isolated from a sample of bile of a *Caiman* species. The isolation procedure included hydrolysis for 10 hr at 110°C in 1 M NaOH, extraction with ethyl acetate after acidification, and purification by reversed-phase partition chromatography, system F1 (14) and thin-layer chromatography, system S11 (13). Attempts to crystallize the compound failed. 3α,7α-Dihydroxy-5β-cholestanoic acid isolated in this way showed the same R_f value as the acid prepared by Briggs (15) when subjected to thin-layer chromatography using benzene–acetic acid 80:20 (v/v) for the free acid and benzene–ethyl acetate 50:50 (v/v) for its methyl ester. The identity of the compound was further confirmed by combined gas–liquid chromatography–mass spectrometry of the trimethylsilyl ether of the methyl ester. It was assumed that 3α,7α-dihydroxy-5β-cholestanoic acid isolated in this way consisted mainly of the 25-*S* isomer (L-form) since 3α,7α,12α-trihydroxy-5β-cholestanoic acid isolated from the same sample of bile was shown to consist mainly of the 25-*S* form (4). Thin-layer chromatographic methods are now available to separate the 25-*S* and 25-*R* forms of 5β-cholestane-3α,7α,26-triol (16).[1] After reduction of the methyl ester of 3α,7α-dihydroxy-5β-cholestanoic acid with lithium aluminum hydride, the material was chromatographed on alumina-coated thin-layer plates with chloroform–acetone–methanol 35:25:3.5 (v/v/v). After two developments, about 80% of the material had an R_f value corresponding to that of the 25-*S* form of 5β-cholestane-3α,7α,26-triol and the remainder had an R_f value corresponding to that of the 25-*R* form of the triol. It is known that hydrolysis during the preparation of C_{27} bile acids may affect the configuration at C-25 (17, 18). This might explain the occurrence of the 25-*R* form of 3α,7α-dihydroxy-5β-cholestanoic acid.

[1] Shefer, S., and E. H. Mosbach. Personal communication.

Cofactors and enzymes

Cofactors and 7α-hydroxysteroid dehydrogenase were obtained from Sigma Chemical Co. (St. Louis, MO). 3α-Hydroxysteroid dehydrogenase was obtained from Nyegaard & Co. (Oslo, Norway).

Methods

Male Sprague–Dawley rats weighing about 200 g were used. Bile duct cannulation was performed as described previously (19). The labeled compounds were administered intraperitoneally 48 or 72 hr after cannulation in an emulsion of a 1% (w/v) solution of serum albumin in 10% (v/v) aqueous ethanol. Bile was collected for two 24-hr periods. The bile samples were hydrolyzed in 2 M NaOH at 110°C for 10 hr. The hydrolysates were acidified and extracted with ethyl acetate. The residues of the ethyl acetate extracts were subjected to reversed phase partition chromatography using phase system F1 (14). In most instances, the material in the fractions corresponding to the trihydroxy acids was rechromatographed in phase system C1 (14). In some instances, the residues of the ethyl acetate extracts were methylated with diazomethane and subjected to thin-layer chromatography in acetone–benzene 2:3 (v/v), (20) using methyl cholate, methyl chenodeoxycholate, and methyl lithocholate as external standards. Liver homogenates, 20% (w/v), were prepared in 0.1 M Tris-Cl buffer, pH 7.4 (21). The microsomal fraction and the 100,000 *g* supernatant fluid were prepared as described previously (21).

Incubations with 3α,7α-dihydroxy-5β-[3β,7β-$^3H_2$]-cholestanoic acid, 10 or 50 μg, were performed in 0.1 M Tris-Cl buffer, pH 7.4, with 1.5 ml of microsomal fraction and NADPH, 3 μmol (22) or with 1 ml of microsomal fraction, 1.5 ml of 100,000 *g* supernatant fluid and 7 μmol of ATP (4). The protein concentration (22) in the microsomal fraction was about 5 mg/ml and in the 100,000 *g* supernatant fluid about 20 mg/ml. The substrate was added to the incubation mixtures dissolved in 25 μl of methanol. Incubation temperature was 37°C and time 40 min. The incubations were terminated by the addition of 5 ml of 95% (v/v) aqueous ethanol. The reaction mixtures were diluted with water, acidified, and extracted twice with ethyl acetate. The residues of the ethyl acetate extracts were subjected to thin-layer chromatography using solvent system S5 (13). In some cases, the residues of the ethyl acetate extracts were methylated with diazomethane and subjected to thin-layer chromatography with the solvent system acetone–benzene, 2:3 (v/v) (20). The extent of conversion was calculated from

measurements of the peak area of the radioactivity tracings obtained by scanning with a thin-layer scanner (Berthold, Karlsruhe, Germany).

In some instances, material from reversed-phase partition chromatography or thin-layer chromatography was analyzed by radio–gas–liquid chromatography after methylation and trimethylsilylation. The instrument was a Barber–Colman 5000 instrument equipped with a 1.5% SE-30 or 3% QF-1 column. Combined gas–liquid chromatography–mass spectrometry was performed with the LKB 9000 instrument equipped with a 1.5% SE-30 column.

RESULTS

Metabolism of 3α,7α-dihydroxy-5β-[3β,7β-$^3H_2$]-cholestanoic acid in the bile fistula rat

When injected intraperitoneally into a bile fistula rat, 3α,7α-dihydroxy-5β-cholestanoic acid was excreted rapidly in bile as bile acids. About 50% of injected dose (8 μCi) was excreted during the first 24 hr and about 10% during the following 24 hr. Fractionation of the saponified bile by reversed-phase partition chromatography showed that about 50% of the radioactivity was in the trihydroxy bile acid fraction and the remainder in the dihydroxy bile acid fraction (**Fig. 1**). The radioactivity in the dihydroxy bile acid fraction coincided with the titration peak of chenodeoxycholic acid. The identity of the radioactive material with chenodeoxycholic acid was further established by thin-layer chromatography of the methyl ester with methyl chenodeoxycholate as external standard. Very little of the substrate (less than 2%) was recovered. It was clearly shown that the substrate did not coincide with chenodeoxycholic acid in the reversed partition chromatography. The trihydroxy bile acid fraction was rechromatographed with phase system C1. The radioactivity was found to be distributed mainly in two peaks before and after cholic acid (**Fig. 2**). Thin-layer chromatography [acetone–benzene 2:3 (v/v)] of methylated material from the two peaks gave radioactivity peaks with R_f values corresponding to those of α- and β-muricholic acids (20). The identity of the material in the second peak with β-muricholic acid was further confirmed by gas–liquid chromatography–mass spectrometry of the trimethylsilyl ether. The mass spectrum was the same as that described earlier for β-muricholic acid (23). As can be seen from Figs. 1 and 2, the specific radioactivity of chenodeoxycholic acid was somewhat higher than that of α-muricholic acid. This could be expected from a precursor–product relationship between the two acids. **Table 1** shows data on crystallization to constant specific radioactivity of the fractions in the titration peak of cholic acid. As can be seen less than 15% of the radioactivity in these fractions could be accounted for as cholic acid. Thus, less than 10% of the total radioactivity in the first 24-hr portion of bile could be cholic acid.

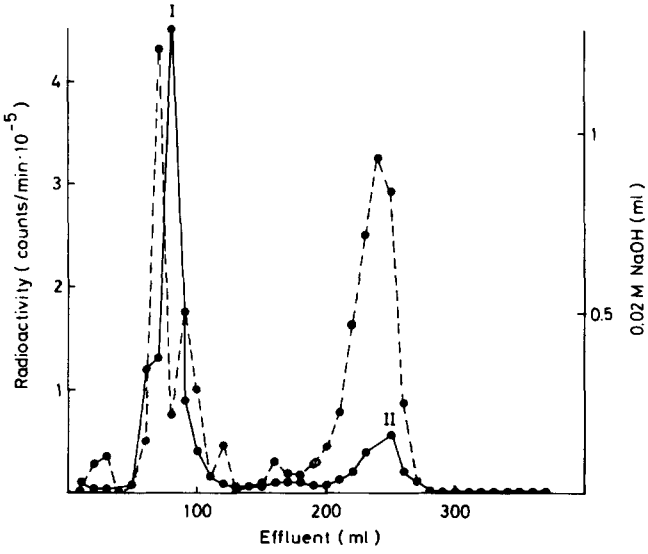

Fig. 1. Chromatogram of first 24-hr portion of hydrolyzed bile from a bile fistula rat injected with 3α,7α-dihydroxy-5β-[3β,7β-$^3H_2$]cholestanoic acid. Column, 4.5 g of hydrophobic Hyflo Super-Cel; phase system F1. ——, Titration values; – – –, radioactivity. Peak I corresponds to mass of trihydroxy fraction (cholic acid + muricholic acid) and peak II corresponds to mass of dihydroxy fraction (chenodeoxycholic acid).

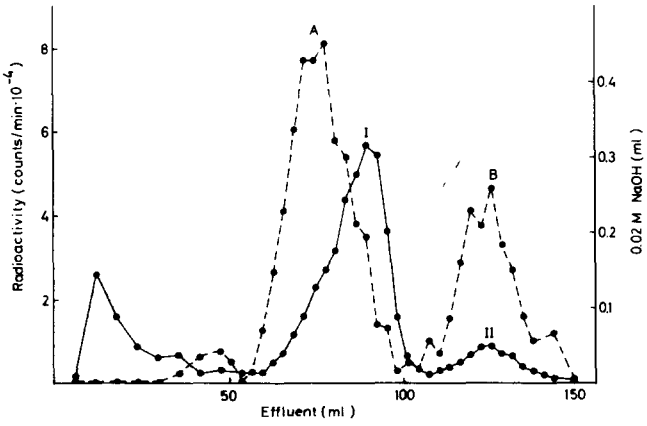

Fig. 2. Chromatogram of trihydroxy acid fractions from chromatogram shown in Fig. 1. Column, 4.5 g of hydrophobic Hyflo Super-Cel; phase system C1. Symbols as in Fig. 1. Peak I corresponds to mass of cholic and α-muricholic acids and peak II corresponds to mass of β-muricholic acid. Peak A corresponds to radioactivity of α-muricholic acid and peak B corresponds to radioactivity of β-muricholic acid.

TABLE 1.[a]

Solvent	No. of Crystallizations	Weight	Specific Activity
		mg	*cpm* × 10^{-3} *per mg*
None	0	67.9	7.60
Methanol–water	1	56.4	3.42
Methanol–water	2	45.4	1.83
Methanol–water	3	34.6	1.62
Methanol–water	4	30.4	1.26
Methanol–water	5	19.6	1.17

[a] Fractions corresponding to cholic acid (cf. Fig. 2) from a bile fistula rat injected with 3α,7α-dihydroxy-5β-[3β,7β-$^3H_2$]cholestanoic acid were mixed with unlabeled cholic acid and crystallized.

Metabolism of 3α,7α-dihydroxy-5β-[3β,7β-$^3H_2$]-cholestanoic acid in vitro

Reversed phase partition chromatography with phase system F1 of incubations of 3α,7α-dihydroxy-5β-cholestanoic acid (10 or 50 μg) with the microsomal fraction and NADPH showed conversion of about 50 and 10%, respectively, into a compound with the chromatographic mobility of a trihydroxy acid. By thin-layer chromatography of methylated material with acetone–benzene 2:3 (v/v), the product had an R_f value of 0.22. The methyl ester of the starting material, 3α,7α-dihydroxy-5β-cholestanoic acid, had an R_f value of 0.52. The ratio between the two R_f values was 0.42. According to Shefer et al. (20) the ratio between R_f values of methyl α-muricholate and methyl chenodeoxycholate in the same solvent system is 0.43. The results indicate that the compound was 3α,6β,7α-trihydroxy-5β-cholestanoic acid. The mass spectrum of the trimethylsilyl ether of the methyl ester showed prominent peaks at *m/e* 665 (M − 15), 590 (M − 90), 500 (M − 2 × 90), 410 (M − 3 × 90), 343, and 253. Since the mass spectrum of the product was similar to that of 3α,7α,12α-trihydroxy-5β-cholestanoic acid, the material was reduced with lithium aluminum hydride and compared with 5β-cholestane-3α,7α,12α,26-tetrol by thin-layer chromatography. The major peak was somewhat more polar than 5β-cholestane-3α,7α,12α,26-tetrol. Thus, the original product of the incubation was not identical with 3α,7α,12α-trihydroxy-5β-cholestanoic acid. In some experiments, a radioactive peak corresponding to 5β-cholestane-3α,7α,12α,26-tetrol was detected after reduction. This material never accounted for more than 20% of the total radioactivity in the product zone. The tentatively identified 3α,6β,7α-trihydroxy-5β-cholestanoic acid was administered to a bile fistula rat. About 80% of the radioactivity appeared in the first 24-hr portion of bile. After saponification and extraction, it was shown by reversed-phase partition chromatography in phase system F1 that almost all

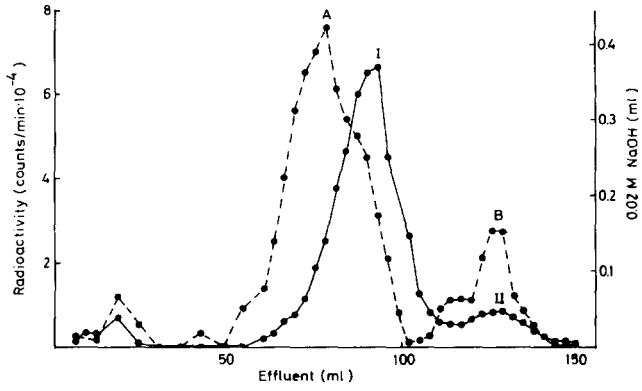

Fig. 3. Chromatogram of trihydroxy acid fractions of first 24-hr portion of hydrolyzed bile from bile fistula rat treated with injection of material from incubations of 3α,7α-dihydroxy-5β-[3β, 7β-$^3H_2$]cholestanoic acid with microsomal fraction and NADPH. Column, 4.5 g of hydrophobic Hyflo Super-Cel; phase system C1. Symbols as in Fig. 1. The notations of the peaks are the same as in Fig. 2.

of the radioactivity was present in the trihydroxy bile acid fraction. Rechromatography of this fraction in phase system C1 showed that about 80% had the chromatographic mobility of α-muricholic acid (**Fig. 3**). The remainder had the mobility of β-muricholic acid. Crystallization to constant specific radioactivity (**Table 2**) of the fractions corresponding to the titration peak of cholic acid showed that less than 15% of the radioactivity could be accounted for as cholic acid. It can be concluded that the major product in incubations of 3α,7α-dihydroxy-5β-cholestanoic acid with the microsomal fraction fortified with NADPH is 3α,6β,7α-trihydroxy-5β-cholestanoic acid.

Thin-layer chromatography and reversed phase partition chromatography of extracts from incubations of 3α,7α-dihydroxy-5β-cholestanoic acid with the microsomal fraction in combination with the 100,000 *g* supernatant fluid and ATP showed that small amounts of 3α,6β,7α-trihydroxy-5β-cholestanoic acid were formed. The main product representing

TABLE 2.[a]

Solvent	No. of Crystallizations	Weight	Specific Activity
		mg	*cpm* × 10^{-3} *per mg*
None	0	68.0	4.32
Methanol–water	1	63.3	2.85
Methanol–water	2	52.5	1.84
Methanol–water	3	51.2	1.33
Methanol–water	4	47.1	0.73
Methanol–water	5	40.9	0.59

[a] Fractions corresponding to cholic acid (cf. Fig. 3) from bile fistula rats injected with material from incubations of 3α,7α-dihydroxy-5β-[3β,7β-$^3H_2$]cholestanoic acid with a liver microsomal fraction and NADPH were mixed with unlabeled cholic acid and recrystallized.

80% of total products formed was slightly less polar than 3α,6β,7α-trihydroxy-5β-cholestanoic acid. The overall conversion of 3α,7α-dihydroxy-5β-cholestanoic acid was about 40% in incubations with 10 μg and about 10% in incubations with 50 μg. Omission of ATP inhibited almost completely the formation of the less polar product. The product gave one peak when subjected to radio–gas–liquid chromatography after methylation and trimethylsilylation. The ratio between the retention times of the unknown material and 3α,7α-dihydroxy-5β-cholestanoic acid was equal to the ratio between the retention times of 3α,7α,12α,24-tetrahydroxy-5β-cholestanoic acid and 3α,7α,12α-trihydroxy-5β-cholestanoic acid. Gas–liquid chromatography–mass spectrometry supported the contention that the unknown material was 3α,7α,24ξ-trihydroxy-5β-cholestanoic acid. Prominent peaks were *m/e* 665 (M − 15), 500 (M − 2 × 90), 410 (M − 3 × 90), 345, and 255 (cf. ref. 4). The intensities of the peaks were similar to those of the corresponding peaks of the trimethylsilyl ether of the methyl ester of 3α,7α,12α,24ξ-tetrahydroxy-5β-cholestanoic acid. When the compound was injected into a bile fistula rat, about 50% of the radioactivity appeared in the first 24-hr portion of bile. Reversed-phase partition chromatography of hydrolyzed bile showed that about 35% of the radioactivity was present in the trihydroxy bile acid fraction and the remaining 65% in the dihydroxy bile acid fraction. The material in the dihydroxy bile acid fraction was identified as chenodeoxycholic acid by thin-layer chromatography. Rechromatography of the trihydroxy bile acid fraction with phase system C1 showed that the radioactivity was distributed in two peaks with the mobilities of α- and β-muricholic acids.

DISCUSSION

3α,7α-Dihydroxy-5β-cholestanoic acid and 3α,7α,12α-trihydroxy-5β-cholestanoic acid, which are predominant bile acids in several lower species such as alligators and crocodiles (17), appear to be important intermediates in chenodeoxycholic and cholic acid biosynthesis in mammals (1). Based on experiments with 3α,7α,12α-trihydroxy-5β-cholestanoic acid, the conversion of the C_{27}-acids into C_{24}-acids probably occurs by a β-oxidation process involving 24-hydroxylation and formation of a 24-keto acid (4). Early experiments in vivo indicate a high degree of specificity of the 12α-hydroxylase activity since insertion of an ω-hydroxyl group into a C_{27}-steroid hinders 12α-hydroxylation (6). This assumption is based on the findings that several different 26-hydroxysteroids, i.e., 5-cholestene-3β,26-diol, 5-cholestene-3β,7α,26-triol, and 5β-cholestane-3α,7α,26-triol, when administered to bile fistula rats, are converted mainly into chenodeoxycholic acid and its metabolites (6). In vivo experiments by Bergström and Sjövall (24) showed that chenodeoxycholic acid is not transformed into cholic acid in the rat. Recent experiments by Yousef and Fisher (25), using isolated perfused rat liver, showed a small capacity for synthesis of cholic acid from chenodeoxycholic acid. Elliott et al. (7–12) have shown that some 26-oxygenated C_{27}-steroids of the 5α-series as well as 3α,7α-dihydroxy-5α-cholestanoic acid and allochenodeoxycholic acid are efficiently 12α-hydroxylated in vitro. In vivo, allochenodeoxycholic acid is converted only to a small extent into allocholic acid (9, 26). The present results show that, in contrast to the corresponding 5α-acid, there is a very limited 12α-hydroxylation of 3α,7α-dihydroxy-5β-cholestanoic acid in rat liver both with regard to the formation of 3α,7α,12α-trihydroxy-5β-cholestanoic acid in vitro and to the formation of cholic acid in vivo in the rat with a bile fistula. In this case, the metabolisms in the rat and in man appear to be the same. Hanson (27) has found that in human subjects with a bile fistula 3α,7α-dihydroxy-5β-cholestanoic acid is excreted predominantly as chenodeoxycholic acid.

As with lithocholic acid and chenodeoxycholic acid (28), the microsomal fraction of rat liver catalyzes an efficient 6β-hydroxylation of 3α,7α-dihydroxy-5β-cholestanoic acid. Studies by Samuelsson (29) have shown that during the conversion of 7β-^{3}H-labeled chenodeoxycholic acid into β-muricholic acid there is a complete loss of the label. This would mean that in the present study the extent of formation of β-muricholic acid from 3α,7α-dihydroxy-5β-cholestanoic acid is underestimated by about 20%, since 20% of the label in the C_{27}-acid was located in the 7β-position.

The mechanism of conversion of 3α,7α-dihydroxy-5β-cholestanoic acid into chenodeoxycholic acid appears to be the same as that of 3α,7α,12α-trihydroxy-5β-cholestanoic acid. Thus, 3α,7α,24ξ-trihydroxy-5β-cholestanoic acid was identified as the main product of incubations with the microsomal–cytoplasmic fractions and ATP (4) and, as with 3α,7α,12α-trihydroxy-5β-cholestanoic acid, ATP was necessary.

There is evidence indicating moderate to high and opposite stereospecificity of the microsomal and mitochondrial 26-hydroxylase systems in rat liver (30–32). The quantitative roles of the two systems have not been established. Early experiments by Bridgwater and Lindstedt (2) showed that 3α,7α,12α-trihydroxy-5β-cholestanoic acid was converted efficiently into cholic acid regardless of the configuration at C-25. Corresponding experiments have not been performed with 3α,7α-dihydroxy-5β-cholestanoic acid, although methods are available for the synthesis of 25-*S*- and 25-*R*-3α,7α-dihydroxy-5β-

cholestanoic acid (15). The aim of the present study was not to study the importance of the configuration at C-25; however, it is obvious that the used isomer of 3α,7α-dihydroxy-5β-cholestanoic acid is converted efficiently into chenodeoxycholic acid and its metabolites. It seems reasonable to assume that the same situation prevails in vivo for 3α,7α-dihydroxy-5β-cholestanoic acid as for 3α,7α,12α-trihydroxy-5β-cholestanoic acid.

In conclusion, the results of the present investigation provide experimental evidence for the assumptions made concerning the metabolism of 3α,7α-dihydroxy-5β-cholestanoic acid in the rat.

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REFERENCES

1. Danielsson, H. 1973. Mechanisms of bile acid biosynthesis. *In* The Bile Acids, vol. 2. P. P. Nair and D. Kritchevsky, editors. Plenum Press, New York, N.Y. 1–32, 305–306.
2. Bridgwater, R. J., and S. Lindstedt. 1957. On the metabolism of 3α,7α,12α-trihydroxycoprostanic acid in the rat. *Acta Chem. Scand.* **11:** 409–413.
3. Bergström, S., R. J. Bridgwater, and U. Gloor. 1957. On the conversion of 3α,7α,12α-trihydroxycoprostanic acid to cholic acid in rat liver homogenates. *Acta Chem. Scand.* **11:** 836–838.
4. Gustafsson, J. 1975. Biosynthesis of cholic acid in rat liver: 24-Hydroxylation of 3α,7α,12α-trihydroxy-5β-cholestanoic acid. *J. Biol. Chem.* **230:** 8243–8247.
5. Hanson, R. F., and J. M. Pries. 1977. Synthesis and enterohepatic circulation of bile salts. *Gastroenterology.* **73:** 611–618.
6. Danielsson, H. 1963. Present status of research on catabolism and excretion of cholesterol. *Adv. Lipid Res.* **1:** 335–385.
7. Mui, M. M., and W. H. Elliott. 1975. Comparison of 12α-hydroxylation of oxygenated 5α-cholestanes and allochenodeoxycholate with rat liver microsomes. *Biochemistry.* **14:** 2712–2717.
8. Noll, B. W., E. A. Doisy, Jr., and W. H. Elliott. 1973. Bile acids XXXIX. Metabolism of 5α-cholestane-3β,26-diol and 5α-cholestane-3β,7α,26-triol in the rat with a bile fistula. *J. Lipid Res.* **14:** 391–399.
9. Mui, M. M., and W. H. Elliott. 1971. Bile acids XXXII. Allocholic acid, a metabolite of allochenodeoxycholic acid in bile fistula rats. *J. Biol. Chem.* **246:** 302–304.
10. Ali, S. S., and W. H. Elliott. 1976. Bile acids LI. Formation of 12α-hydroxyl derivatives and companions from 5α-sterols by rabbit liver microsomes. *J. Lipid Res.* **17:** 386–392.
11. Blaskiewicz, R. J., G. J. O'Neil, Jr., and W. H. Elliott. 1974. Bile acids XLI. Hepatic microsomal 12α-hydroxylation of allochenodeoxycholate to allocholate. *Proc. Soc. Exp. Biol. Med.* **146:** 92–95.
12. Ali, S. S., and W. H. Elliott. 1975. Bile acids. XLVI: 12α-Hydroxylation of precursors of allo bile acids by rabbit liver microsomes. *Biochim. Biophys. Acta.* **409:** 249–257.
13. Eneroth, P. 1963. Thin-layer chromatography of bile acids. *J. Lipid Res.* **4:** 11–16.
14. Norman, A., and J. Sjövall. 1958. On the transformation and enterohepatic circulation of cholic acid in the rat. *J. Biol. Chem.* **233:** 872–885.
15. Briggs, T. 1970. Partial synthesis of 25 D- and 25-L-cholestanoic acids from some common bile acids. *J. Org. Chem.* **35:** 1431–1433.
16. Dayal, B., A. K. Batta, S. Shefer, G. S. Tint, G. Salen, and E. H. Mosbach. 1978. Preparation of 24(R)- and 24(S)-5β-cholestane-3α,7α,24-triols and 25(R)- and 25(S)-5β-cholestane-3α,7α,26-triols by a hydroboration procedure. *J. Lipid Res.* **19:** 191–196.
17. Haslewood, G. A. D. 1967. Bile Salts. Methuen, London.
18. Mendelsohn, D., and L. Mendelsohn. 1969. The catabolism of cholesterol in vitro. Formation of 3α,7α,12α-trihydroxy-5β-cholestanoic acid from cholesterol by rat liver. *Biochem. J.* **114:** 1–3.
19. Fisher, B., and H. M. Vars. 1951. A method of collecting bile in rats; normal values on rat bile. *Am. J. Med. Sci.* **222:** 116.
20. Shefer, S., S. Hauser, J. Bekersky, and E. H. Mosbach. 1969. Feedback regulation of bile acid biosynthesis in the rat. *J. Lipid Res.* **10:** 646–655.
21. Björkhem, I., and J. Gustafsson. 1973. ω-Hydroxylation of steroid side-chain in biosynthesis of bile acids. *Eur. J. Biochem.* **36:** 201–212.
22. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
23. Sjövall, J., P. Eneroth, and R. Ryhage. 1971. *In* The Bile Acids, vol. 1. P. P. Nair and D. Kritchevsky, editors. Plenum Press. New York. 209–248.
24. Bergström, S., and J. Sjövall. 1954. Occurrence and metabolism of chenodeoxycholic acid in the rat. *Acta Chem. Scand.* **8:** 611–616.
25. Yousef, I. M., and M. M. Fisher. 1975. Bile acid metabolism in mammals: IX. Conversion of chenodeoxycholic acid to cholic acid by isolated perfused rat liver. *Lipids.* **10:** 571–573.
26. Kallner, A. 1967. Metabolism of allocholic acid, allochenodeoxycholic acid and allolithocholic acid in the rat. *Ark. Kemi.* **26:** 567–576.
27. Hanson, R. F. 1971. The formation and metabolism of 3α,7α-dihydroxy-5β-cholestan-26-oic acid in man. *J. Clin. Invest.* **50:** 2051–2055.
28. Björkhem, I., and H. Danielsson. 1974. Hydroxylations in biosynthesis and metabolism of bile acids. *Mol. Cell. Biochem.* **4:** 79–95.
29. Samuelsson, B. 1959. On the metabolism of chenodeoxycholic acid in the rat. *Acta Chem. Scand.* **13:** 976–983.
30. Berséus, O. 1965. On the stereospecificity of 26-hydroxylation of cholesterol. *Acta Chem. Scand.* **19:** 325–328.
31. Gustafsson, J., and S. Sjöstedt. 1978. On the stereospecificity of microsomal "26"-hydroxylation in bile acid biosynthesis. *J. Biol. Chem.* **253:** 199–201.
32. Mosbach, E. H., S. Shefer, and G. Salen. 1978. Side-chain hydroxylations in bile acid biosynthesis: chenodeoxycholic acid. *In* Biological Effects of Bile Acids. W. Gerok, G. Paumgartner, and A. Stiehl, editors. MTP Press, Lancaster, England. In press.