short communications

Metabolism of 3α,7α,12α-trihydroxy-5β-cholestan-26-oic acid in normal subjects with an intact enterohepatic circulation

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Abstract The formation of cholic acid from 3α,7α,12α-trihydroxy-5β-cholestan-26-oic acid (THCA) was studied in two normal subjects. [^{3}H]THCA and [^{14}C]cholic acid were administered intravenously by simultaneous injection and the specific activities (percent injected amount/μmol) of [^{3}H]- and [^{14}C]cholic acid were measured in bile samples collected over a 5-day period. If the administered [^{3}H]-THCA is rapidly and completely metabolized into cholic acid, the areas under the specific activity curves of [^{3}H]- and [^{14}C]cholic acid should be identical. In these two subjects, the area under the [^{3}H]cholic acid specific activity decay curves was only 18.4% and 9.0% less than the area under the [^{14}C]cholic acid specific activity decay curves. Thus, there is rapid and nearly complete metabolism of intravenously administered [^{3}H]THCA into cholic acid.

Supplementary key words Cholic acid synthesis

Cholic acid is formed in the liver from cholesterol through a series of reactions that starts with changes in the steroid ring system leading to the formation of the intermediate, 3α,7α,12α-trihydroxy-5β-cholestane, before oxidation and shortening of the side chain. 3α,7α,12α-Trihydroxy-5α-cholestane that has undergone oxidation at position 26 of the side chain (1), is metabolized into cholic acid in bile fistula patients (2) and rats (3). Based on this evidence THCA is considered a natural precursor of cholic acid. However, the presence of a bile fistula may alter the normal metabolism of bile acid precursors. Therefore, we investigated the metabolism of THCA in normal subjects with an intact enterohepatic circulation. The study was carried out by administering [^{3}H]THCA and [^{14}C]cholic acid intravenously by simultaneous injection. If the injected THCA is rapidly and irreversibly converted into cholic acid, the specific activities of [^{3}H]- and [^{14}C]cholic acid (expressed as the percent of the injected amount/μmol of cholic acid) should be the same. Incomplete metabolism of [^{3}H]THCA into cholic acid would result in a lower specific activity of [^{3}H]cholic acid when compared to the specific activity of [^{14}C]cholic acid. Comparisons of the cholic acid specific activities were made using the areas under the specific activities decay curves of [^{3}H]- and [^{14}C]cholic acid.

METHODS

Labeled compounds

25-L-3α,7α,12α-Trihydroxy-5β-cholestan-26-oic acid (THCA) was isolated from the bile of the *Alligator mississippiensis* and identified by mass spectrometry (3). [2,4-^{3}H]THCA ([^{3}H]THCA) was prepared as described previously (3) and had a specific activity of 10.5 μCi/μmol. [24-^{14}C]Cholic acid, with a specific activity of 45.6 μCi/μmol, was purchased from New England Nuclear Corporation, Boston, MA. Each of these labeled compounds was shown to be greater than 98% pure by TLC.

Subjects

Two healthy male subjects, age 25 and 30, were studied in a clinical research center after informed consent was obtained. Neither of the subjects gave a

Abbreviations: THCA, 3α,7α,12α-trihydroxy-5β-cholestan-26-oic acid; TLC, thin-layer chromatography; GLC, gas–liquid chromatography.

TABLE 1. Areas under cholic acid specific activity decay curves

Subject	Area under Specific Activity Curves		% Difference of ^3H Area from ^{14}C Area
	^3H	^{14}C	
1	3.51 ± 0.34[a]	4.30 ± 0.33[a]	18.4
2	2.90 ± 0.24[a]	3.19 ± 0.24[a]	9.0

[a] ± Coefficient of variation.

history of liver or malabsorptive diseases and both were normal by physical examination. They were eating a normal diet prior to and during the investigation.

At 8 AM of day 0, each subject swallowed a double lumen tube which was positioned in the duodenum. A liquid formula containing 40% of the calories as fat (4) was infused for the next 7 hr through the orifice of the distal tube located 10 cm from the orifice of the proximal tube. Two hours after starting the infusion each subject received an intravenous injection of 6 μCi of [^{3}H]THCA and 6 μCi of [^{14}C]cholic acid dissolved in 2 ml of sterile absolute ethanol. Bile samples (2–3 ml) were collected hourly for the next 5 hours after which the tube was removed and the subjects given a regular diet. Additional bile samples were obtained after gall bladder contraction at 24, 48, 72, and 96 hr after the injection.

Analytical methods

The specific activities of [^{3}H]- and [^{14}C]cholic acid in the bile samples were measured as follows. An aliquot of bile containing approximately 2 mg of bile salts was hydrolyzed in 2 N NaOH at 130°C for 3 hr. After acidification and extraction, the cholic acid was isolated by TLC (Silica Gel G 0.025 mm., Brinkman Instruments, Westbury, NY) using isooctane–ethyl acetate–acetic acid 5:5:1 (5) as the developing solvent. The cholic acid fraction, identified with iodine vapor, was scraped off and eluted with methanol. The 3α-hydroxy steroid dehydrogenase method (6) was used to assay the mass of cholic acid. The cholic acid fraction, isolated as described above, was shown to be pure by GLC of its methyl ester triacetate derivative (7) on 0.5% QF-1 on Gas Chrom Q. The following temperatures were used: column, 240°C; flash heater, 245°C; detector bath, 270°C.

Radioactivity was measured in a scintillation counter (Beckman Model LS-250, Fullerton, CA) using a Fluorally TLA counting mixture containing butyl PBD and PBBD (Beckman Instruments). Quenching was estimated by adding internal standards of [^{3}H]- and [^{14}C]toluene to the counting vials. Correction for the spillover of the radioactivity from [^{14}C]cholic acid into the ^{3}H channel was made by calculating the percentage of the [^{14}C]toluene radioactivity found in the ^{3}H channel.

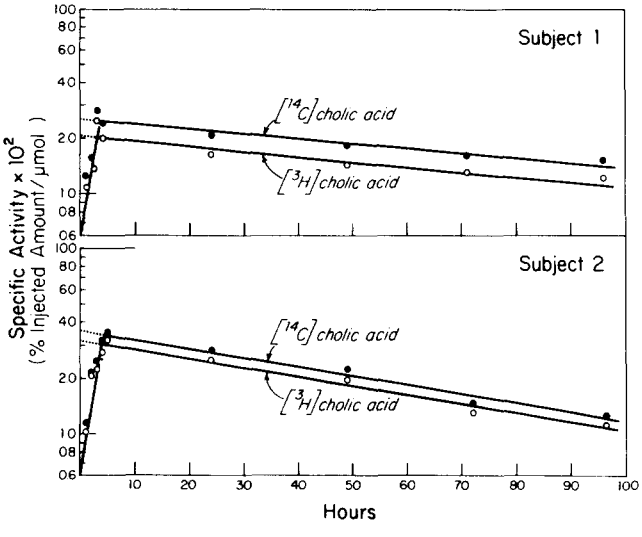

Fig. 1. Specific activity decay curves of [^{3}H[- and [^{14}C]cholic acid. The points indicate the experimental data and the lines indicate computer simulation.

Calculations and computer analysis

Previous studies have shown that the kinetics of bile acid metabolism in normal subjects following a single injection of a labeled bile acid can be described by the equation $A = Be^{-\alpha t}$ (8). In this equation A is specific activity of the bile acid at time t; B is specific activity at the time of injection (t_0) assuming complete instantaneous mixing; e is base of the natural logarithm, and α is the slope of the specific activity decay curve. In this study we compared the areas under the specific activity decay curves of [^{3}H]- and [^{14}C]cholic acid and, in order for these comparisons to be meaningful, the specific activities of cholic acid were expressed as percent of the injected amount of the isotope/μmol of cholic acid. The parameters, B and α, of the equation $A = Be^{-\alpha t}$ were obtained by computer analysis using the methods of least squares

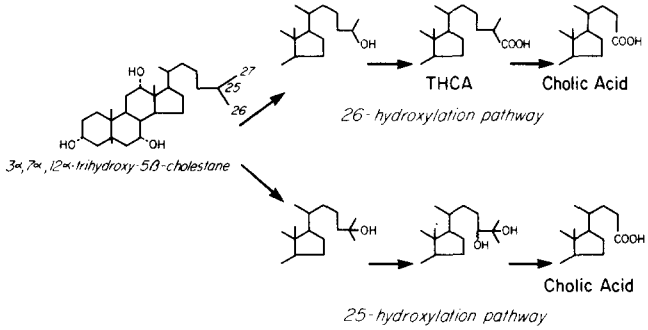

Fig. 2. Pathways of side chain oxidation of 3α,7α,12α-trihydroxy-5β-cholestane into cholic acid.

(9) to calculate the best fit of the data to the linear equation $\ln A = \ln B - \alpha t$. The coefficient of variation of the specific activity decay curves was determined by computer analysis as described previously (10). The area under the specific activity decay curve from t_0 to a point where the specific activities are negligible is given by the equation: Area = B/α.

RESULTS

Fig. 1 shows the specific activities of [^{3}H]- and [^{14}C]cholic acid measured in bile samples from the two normal subjects following the simultaneous injection of [^{3}H]THCA and [^{14}C]cholic acid. The specific activities rose quickly and peaked within 3–5 hr after injection of the isotopes. The slopes of the [^{3}H]- and [^{14}C]cholic acid specific activity decay curves were nearly identical; 0.57 day^{-1} and 0.59 day^{-1} for subject 1 and 0.112 day^{-1} and 0.110 day^{-1} for subject 2, respectively.

The areas under the specific activity decay curve were calculated from the formula: area = B/α (see Calculations) which gave the area from the time of injection, assuming instantaneous mixing of the injected isotopes with the cholic acid pool, to a point where the specific activities were negligible. The small triangular area above the data points and below the extrapolated line during the first 4 hr was subtracted to give the final area measurement shown in **Table 1**. The area under the [^{3}H]cholic acid specific activity decay curve was less than the area under the [^{14}C]-cholic acid specific activity decay curve in these subjects by 18.4% and 9.0%, respectively.

DISCUSSION

The metabolism of 3α,7α,12α-trihydroxy-5β-cholestane into cholic acid is postulated to take place either via a pathway involving oxidation at position 25 or 26 of the side chain. (See **Fig. 2**). Both THCA (10) (26-hydroxylation pathway) and 3α,7α,12α,25-tetrahydroxy-5β-cholestane (11, 12) (25-hydroxylation pathway) have been isolated from human bile and are metabolized into cholic acid in normal individuals (2, 11, 13). The conversion of THCA into cholic acid has also been demonstrated using human liver homogenates (14). Thus, at this time, the natural pathway of side chain oxidation of 3α,7α,12α-trihydroxy-5β-cholestane may occur via either (or both) pathway.

The present study was carried out to determine the percentage of an intravenous injection of THCA that is metabolized into cholic acid in normal subjects with an intact enterohepatic circulation. [^{3}H]THCA and [^{14}C]cholic acid were administered intravenously and the specific activities of [^{3}H]- and [^{14}C]cholic acid were measured in the bile over a 5-day period. To ensure mixing of the injected isotopes with the cholic acid pool, the gallbladder was contracted by an intraduodenal infusion of fat (4) 2 hr before and 5 hr after injection of the isotopes. Fig. 1 shows that during the first 5 hr after the injection, the rate of increase of the specific activity of [^{3}H]cholic acid was identical to the rate of increase of [^{14}C]cholic acid. Therefore, the metabolism of THCA into cholic acid was rapid. If all of the injected THCA was converted into cholic acid, the areas under the specific activity decay curves of [^{3}H]- and [^{14}C]cholic acid would have been identical. In the two subjects studied the area under the [^{3}H]cholic acid specific activity decay curves was less than the area under the [^{14}C]cholic acid specific activity decay curves by only 18.4% and 9.0%, respectively, indicating that between 81% and 91% of the injected THCA was metabolized into cholic acid. The remainder may have been excreted in the bile as conjugated THCA. Previously, we administered the [^{3}H]THCA used in the present study to a bile fistula rat and recovered 97% of the label as cholic acid (3). Thus the differences in the areas and the cholic acid specific activity decay curves in this investigation were probably not due to the loss of the ^{3}H label from the THCA molecule.

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REFERENCES

1. Danielsson, H. 1960. On the oxidation of 3α,7α,12α-trihydroxy-coprostane by mouse and rat liver homogenates. *Acta Chem. Scand.* **14:** 348–352.
2. Carey, J. B., Jr. 1964. Conversion of cholesterol to trihydroxycoprostanic acid and cholic acid in man. *J. Clin. Invest.* **43:** 1443–1448.
3. Hanson, R. F., J. N. Isenberg, G. C. Williams, D. Hachey, P. Szczepanik, P. D. Klein, and H. L. Sharp. 1975. The metabolism of 3α,7α,12α-trihydroxy-5β-cholestan-26-oic acid in two siblings with cholestasis due to intrahepatic bile duct anomalies. *J. Clin. Invest.* **56:** 577–587.
4. Grundy, S. M. 1975. Effects of polyunsaturated fats

on lipid metabolism in patients with hypertriglyceridemia. *J. Clin. Invest.* **55:** 269–282.
5. Eneroth, P. 1967. Thin-layer chromatography of bile acids. *J. Lipid Res.* **4:** 11–16.
6. Iwata, T., and K. Yamasaki. 1964. Enzymatic determination and thin-layer chromatography of bile acids in blood. *J. Biochem. (Tokyo)* **56:** 424–431.
7. Roovers, J. E., E. Evrard, and H. Vanderhaeghe. 1968. An improved method for measuring human blood bile acids. *Clin. Chim. Acta.* **19:** 449–457.
8. Lindstedt, S. 1957. The turnover of cholic acid in man. *Acta Physiol. Scand.* **40:** 1–9.
9. Bevington, P. R. 1969. Least-squares fit to an arbitrary function *In* Data Reduction and Error Analysis for the Physical Sciences, McGraw-Hill, New York. 204.
10. Hanson, R. F., P. A. Szczepanik, P. D. Klein, E. A. Johnson, and G. C. Williams. 1976. Formation of bile acids in man: Metabolism of 7α-hydroxy-4-cholesten-3-one in normal subjects with an intact enterohepatic circulation. *Biochim. Biophys. Acta.* **431:** 335–346.
11. Carey, J., Jr., and G. A. D. Haslewood. 1963. Crystallization of trihydroxycoprostanic acid from human bile. *J. Biol. Chem.* **238:** PC 855–856.
12. Setoguchi, T., G. Salen, G. S. Tint, and E. H. Mosbach. 1974. A biochemical abnormality in cerebrotendinous xanthomatosis: impairment of bile acid biosynthesis associated with incomplete degradation of the cholesterol side chain. *J. Clin. Invest.* **53:** 1393–1401.
13. Salen, G., S. Shefer, T. Setoguchi, and E. H. Mosbach. 1975. Bile alcohol metabolism in man: conversion of 5β-cholestane-3α,7α,12α,25-tetrol to cholic acid. *J. Clin. Invest.* **56:** 226–231.
14. Hanson, R. F., H. L. Sharp, and G. C. Williams. 1976. The metabolism of 3α,7α,12α-trihydroxy-5β-cholestan-26-oic acid into cholic acid: an enzyme assay using homogenates of human liver. *J. Lipid Res.* **17:** 294–297.