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Research Article

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The results of this study demonstrate that 3α,7α-dihydroxy-5β-cholestan-26-oic acid is a metabolic product of cholesterol and is further metabolized, predominantly to chenodeoxycholic acid and to a minor extent to cholic acid in man.

The Formation and Metabolism of 3α,7α-Dihydroxy-5β-Cholestan-26-Oic Acid in Man

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ABSTRACT The formation and metabolism of a naturally occurring C_{27} bile acid, 3α,7α-dihydroxy-5β-cholestan-26-oic acid, was studied in patients with T-tube bile fistulas. C-26-cholesterol-^{14}C was shown to be converted to this C_{27} bile acid. After synthesis and labeling with tritium, 3α,7α-dihydroxy-5β-cholestan-26-oic acid was efficiently metabolized to chenodeoxycholic acid. After oral and i.v. administration there was conversion of about 80% of the administered amount to chenodeoxycholic acid. A small amount, less than 2% of the administered radioactivity, was converted to cholic acid. The remainder of the radioactivity was excreted in two unidentified peaks of radioactivity.

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INTRODUCTION

The C_{24} primary bile acids formed from cholesterol in man are cholic acid (3α,7α,12α-trihydroxy-5β-cholanoic acid) and chenodeoxycholic acid (3α,7α-dihydroxy-5β-cholanoic acid). The production of cholic acid from cholesterol involves the intermediary formation of the C_{27} trihydroxy bile acid, 3α,7α,12α-trihydroxy-5β-cholestan-26-oic acid (THCA) (1).[1] The conversion of this compound to cholic acid is thought to take place through the mechanism of β-oxidation with removal of the terminal C_3 fragment of the side chain as propionic acid (2).

A C_{27} dihydroxy bile acid, 3α,7α-dihydroxy-5β-cholestan-26-oic acid (DHCA) has also been isolated from human bile (3). The present study demonstrates that this C_{27} dihydroxy bile acid is formed from cholesterol and is converted to C_{24} primary bile acids in man.

METHODS

Methyl 3α,7α-dihydroxy-5β-cholestan-26-oate (methyl DHCA) was synthesized by electrolytic coupling of chenodeoxycholic acid and the monomethyl ester of methyl succinic acid by the method of Bridgewater (4). The physical properties of the synthetic C_{27} bile acid used in these experiments have been described previously and were identical with DHCA isolated from alligator and human bile (3).

Tritium labeling of DHCA was done using the Wilzbach method (5). Purification of the tritiated product was carried out using Celite column partition chromatography (6). After chromatography, repeated crystallizations demonstrated a constant SA of 5.9 mCi/μmole. The mass spectra of this crystalline DHCA-^{3}H was identical with the spectra of unlabeled DHCA. Radiopurity was also demonstrated by adding 10 mg of unlabeled DHCA to a small portion of the tritiated material, and this mixture was rechromatographed on Celite. Single coincident peaks were obtained for the radioactivity and the titration of DHCA. Before usage the radioactive compound was rechromatographed, and portions of the radioactivity from the three peak tubes administered to patient volunteers after informed consent had been obtained.

C-26-cholesterol-^{14}C with a SA of 45 μCi/μmole was purchased from New England Nuclear Corp., Boston, Mass. Chenodeoxycholic acid was purchased from Sigma Chemical Co., St. Louis, Mo., and cholic acid from Eastman Organic Chemicals, Rochester, N. Y. Methylation of bile acids was done using diazomethane. All weights were taken after drying for 24 hr in an evacuated desiccator over phosphorus pentoxide. Melting points are uncorrected. All solvents were reagent grade or distilled before use. Petroleum ether was distilled from 60–70°C. Radioactivity was measured in a scintillation counter using Biosolv 3 Solubilizer and Fluoralloy TLA counting mixture (Beckman Instruments, Inc., Fullerton, Calif.). Titration of bile acids was done using 0.01 N NaOH and phenol red as indicator.

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[1] *Abbreviations used in this paper:* DHCA, 3α,7α-dihydroxy-5β-cholestan-26-oic acid; THCA, 3α,7α,12α-trihydroxy-5β-cholestan-26-oic acid.

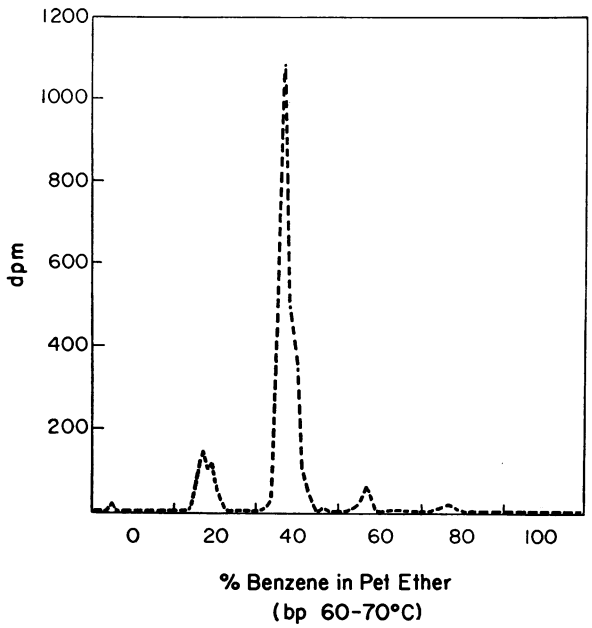

FIGURE 1 Chromatographic separation of radioactivity in bile after administration of DHCA-^{3}H. The bile was collected during the first 12 hr after administration, hydrolyzed, and chromatographed on Celite.

Patients and initial chromatographic separation of bile acids. Three patients, who had T-tubes placed in their common bile ducts at the time of cholecystectomy for cholelithiasis, were studied 10–14 days after surgery when they were eating a regular diet and when their liver function tests had returned to normal. Bile collections were made in ethanol for periods of 2–8 hr and stored at 3°C. To determine if DHCA is formed from cholesterol, one patient was given 23 μCi of C-26-cholesterol-^{14}C dissolved in 1.5 ml of absolute ethanol and injected slowly through an intravenous saline drip. The metabolic fate of DHCA was studied in two other patients, one of which received 3 μCi intravenously (patient A), and the other patient received 10 μCi orally (patient B) as the sodium salt.

Initial separation of the bile acids in the bile collected from these patients after hydrolysis was done using Celite column partition chromatography as described previously (3, 6).

TABLE I

Specific Activity of the Material in the 40% Benzene Fraction Recrystallized from Ethyl Acetate

Recrystallization number	Specific activity	
	Patient A	Patient B
	dpm/mg	
1	13,247	14,837
2	13,087	14,674
3	11,730	14,751
4	13,000	14,108

RESULTS

Formation of DHCA from cholesterol. Bile was collected for 4 days from the patient who received C-26-cholesterol-^{14}C intravenously. After hydrolysis and initial chromatographic separation of the bile acids, the fraction known to contain small amounts of DHCA (3) was rechromatographed on a smaller Celite column and counted for radioactivity. A small peak of radioactivity representing less than 1% of the administered ^{14}C was present in this fraction which, when added to 15 mg of unlabeled synthetic DHCA and rechromatographed on Celite, gave single coincident peaks for radioactivity and titratable acid. The material in this peak was combined and recrystallized, using ethyl acetate–petroleum ether (1:2 v/v), to a constant specific activity (50.2, 50.7, and 51.2 dpm/mg).

Metabolism of DHCA to C_{24} bile acids. There was prompt biliary excretion of radioactivity with excretion of approximately 85% of the injected radioactivity from patient A in the first 12 hr and about 90% excretion in the first 3 days indicating a virtually complete bile fistula. Patient B excreted about 70% of the orally administered DHCA-^{3}H in the first 12 hr. Approximately 300 ml of bile was collected from each patient during this time period. The bile salts in this quantity of bile were hydrolyzed and subjected to Celite column partition chromatography, and the eluent was counted for radioactivity. Similar elution patterns were obtained for both patients. The elution pattern for patient B is shown in Fig. 1 and demonstrates that approximately 80% of the radioactivity was eluted with the 40% benzene fraction, and the remainder was divided into three smaller peaks. These were located in the 20%, 60%, and 80% benzene fractions. This paper deals only with the identification of the radioactive material in the 40% and 80% benzene fractions. The identity of the two other peaks has not been investigated except that the material

TABLE II

Specific Activity of the Recrystallized Material from the 80% Benzene Fraction

Solvent	Specific activity	
	Patient A	Patient B
	dpm/mg	
Ethyl acetate	85.3	113.8
Ethyl acetate	82.2	91.2
Ethyl acetate	76.1	83.6
Ethyl acetate	68.9	85.9
Ethyl acetate	—	84.9
Acetone	65.3	83.9
Acetone	68.9	83.5
Acetone	73.3	—

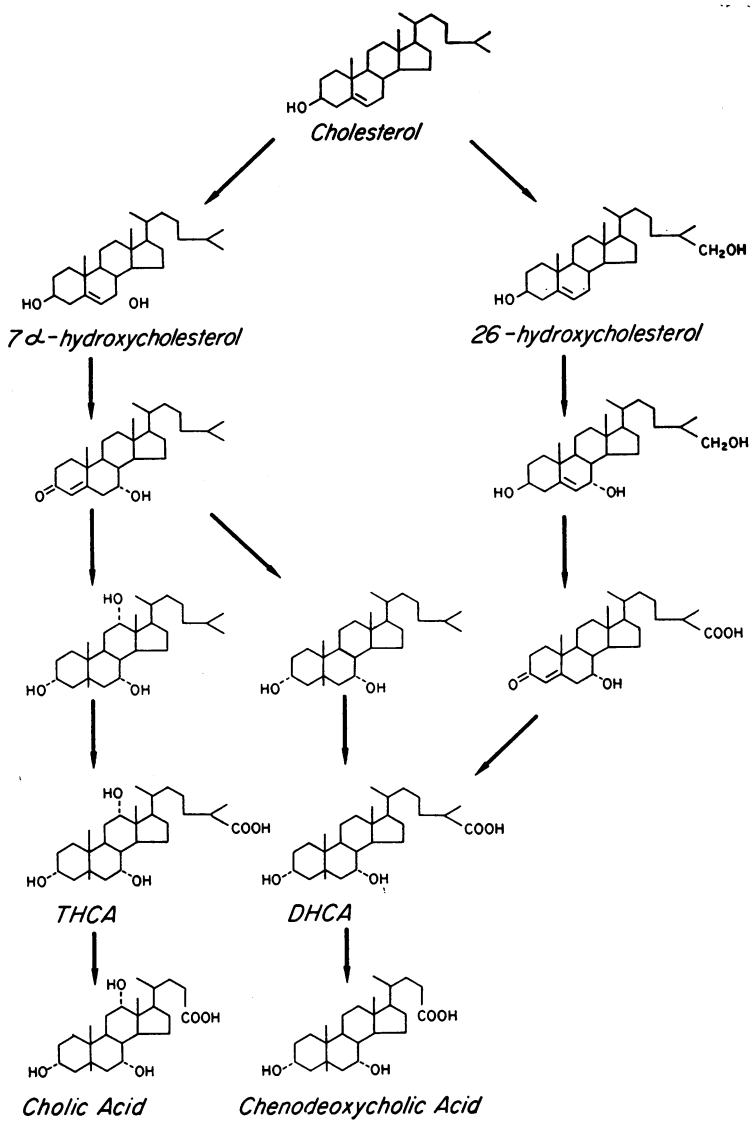

FIGURE 2 Proposed pathways of primary bile acid formation through 7α-hydroxycholesterol (left) and 26-hydroxycholesterol (right). Only some of the possible intermediates present in these two pathways are shown.

in the 20% benzene fraction was found to have a mobility slightly more polar than unlabeled synthetic DHCA on Celite partition chromatography.

The material in the first one-half of the 40% benzene fraction from patient A was rechromatographed on a smaller Celite column, the eluent was counted for radioactivity, and the bile acids were measured by titration. Single coincident peaks were found for the radioactivity and titration curves. This same fraction from both patients was subjected to repeated recrystallizations, and the specific activities of each recrystallization are constant as shown in Table I. The mass and infrared spectra of the methyl ester of this acid were identical with the spectra obtained from authentic methyl chenodeoxycholate. The melting point of these crystals was 142–143°C, which was also identical with that reported for chenodeoxycholic acid (7).

To identify the small peak of radioactivity in the 80% benzene fraction, the material in this fraction from both patients was recrystallized first with ethyl acetate and then subsequently with acetone (Table II). After the first two recrystallizations the specific activity remained constant. The melting point and the mass and infrared spectra of this crystalline material were identical with

those of authentic cholic acid. Additional evidence that cholic acid was labeled with tritium was obtained by chromatography of 15 mg of cholic acid obtained from the final acetone recrystallization, on Celite. The mobility of the radioactivity peak and mass of cholic acid as measured by titration were identical.

The production of cholic acid and chenodeoxycholic acid during the 12 hr period of study determined as trihydroxy (cholic acid) and dihydroxy (chenodeoxycholic acid) bile acids (8) was found to be 164 mg and 68 mg, respectively, for patient A, and 1205 mg and 620 mg, respectively, for patient B. In these experiments approximately 80% of the DHCA administered was converted to C_{24} primary bile acids. Of this amount the percentage of DHCA converted to cholic acid calculated using the specific activities of the C_{24} bile acids found in these experiments was only 1.3% (patient A) and 1.5% (patient B).

DISCUSSION

DHCA is one of the predominant bile acids in alligator bile (9) and is present in low concentration in human bile (3). This study provides direct evidence in man that this compound is synthesized from cholesterol and is rapidly and efficiently metabolized to chenodeoxycholic acid by the human liver. DHCA thus appears to be an intermediate in C_{24} bile acid synthesis in man. The Wilzbach procedure (5), used in these studies to label DHCA, randomly substitutes the hydrogens with tritium. The label located on the terminal C_3 fragment of the side chain was then lost during metabolism, and therefore the 80% conversion of DHCA to chenodeoxycholic acid found in these experiments represents a minimum figure.

In addition to the conversion of DHCA to C_{24} primary bile acids, two other peaks of radioactivity were found after hydrolysis and chromatographic separation of the bile. One of these peaks had a chromatographic mobility slightly more polar than DHCA. These two peaks have not been further characterized; however, one could speculate that these may be intermediates formed in β-oxidation of DHCA to chenodeoxycholic acid.

The endogenous pool size of DHCA is not known; however, it is unlikely that the amount of the precursor given to these patients (less than 1.7 nmoles) would have significantly increased the size of the DHCA pool.

The conversion of cholesterol to primary bile acids in man is thought to involve initial hydroxylation to form cholest-5-en-3β,7α-diol (7α-hydroxycholesterol) followed by sequential changes in the ring structure and then oxidation of the side chain (10) (Fig. 2). An alternate pathway of primary bile acid formation with initial side chain oxidation of cholesterol to cholest-5-en-3β, 26-diol (26-hydroxycholesterol) has also been proposed (11–13). As shown in Fig. 2, DHCA could be an intermediate in either pathway. However, the present study would suggest that the human liver is capable of metabolizing only a small amount of DHCA to cholic acid. Therefore, if 26-hydroxycholesterol is a quantitatively important intermediate in cholic acid synthesis, 12α-hydroxylation of compounds with partial oxidation of the side chain would have to occur at a step before the formation of DHCA.

However, data indicating synthetic pathways of bile acids, obtained by administering a proposed bile acid precursor extrahepatically, have to be interpreted with caution. As Danielsson has pointed out (14), the enzymes involved in the formation of bile acids are probably arranged in an orderly fashion such that substrates in bile acid formation are passed along a chain of enzymes. Therefore, when a proposed bile acid precursor is administered extrahepatically, the quantitative relationships of the products formed may be different than the products formed from *de novo* synthesis of the precursor.

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