Metabolism of deoxycholic acid in bile fistula patients

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ABSTRACT Although it has been assumed that the secondary bile acid deoxycholic acid is not rehydroxylated by the human liver, little direct evidence is available to support this assumption. To investigate the metabolism of deoxycholic acid in man, deoxycholic acid-14C was given intravenously to two patients with complete external bile fistulas. After hydrolysis of the bile salts and chromatographic separation of bile acids, more than 94% of the radioactivity was found in deoxycholic acid and the remainder was scattered in several small unidentified peaks, none of which was cholic acid. Approximately 85% of deoxycholate was excreted as glycine conjugates and 13% as taurine conjugates in this experiment. No detectable sulfate esters were found. These results indicate that the metabolism of deoxycholic acid in man involves only the reconjugation with glycine and taurine without rehydroxylation to cholic acid or sulfation.

SUPPLEMENTARY KEY WORDS conjugation, sulfation

DEOXYCHOLIC ACID is one of the major bile acids derived from 7-dehydroxylation of cholic acid by bacterial enzymes in many animals, including man (1). When deoxycholic acid undergoes enterohepatic circulation in the rat, much of it is hydroxylated by the rat liver to either cholic acid (2) or 3α,6β,12α-trihydroxy-5β-cholanoic acid (3). The boid snakes hydroxylate deoxycholic acid at position 16 to give pyrocholic acid (4). The rabbit, on the other hand, does not rehydroxylate deoxycholic acid (5).

In man, deoxycholate ranges from 11% to 30% of the total bile salts present in duodenal aspirates (6, 7). It has been assumed that no appreciable rehydroxylation of deoxycholate takes place in the human liver; however, there is little direct evidence to support this assumption. We therefore administered sodium deoxycholate-24,14C to two patients with complete external bile fistulas to determine if this compound was converted to other products by the human liver.

METHODS AND RESULTS

Solvents and Compounds

All solvents were reagent grade or distilled just prior to use. Deoxycholic acid was purchased from Mann Research Chemicals, Orangeburg, N.Y., and recrystallized from acetone. The melting point was 174-177°C, reported 177°C (8). Taurolithocholate sulfate was received from Professor G. A. D. Haselwood, Guy's Hospital Medical School, London. Methylation of bile acids was done with diazomethane.

Labeled deoxycholic acid (3α,12α-dihydroxy-5β-chol-24,14C-oic acid) with a specific activity of 2.07 μCi/mg was purchased from Tracerlab, Waltham, Mass., and was purified by column partition chromatography on Celite; this gave a single peak of radioactivity. When a small amount of labeled material was added to unlabeled deoxycholic acid and chromatographed on Celite, single coincident peaks were obtained from the radioactivity and from the mass of deoxycholic as measured by titration. A constant specific activity was ob-
served when $2.58 \times 10^8$ dpm of $^{14}$C-labeled deoxycholic acid was added to 100 mg of unlabeled deoxycholic acid and recrystallized with acetone (2.09 $\times 10^8$ dpm/mg), ethyl acetate (2.18 $\times 10^8$ dpm/mg), and acetone–water (2.18 $\times 10^8$ dpm/mg). The specific activity of methyl deoxycholate prepared from the final acetone–water recrystallization was 2.31 $\times 10^3$ dpm/mg when recrystallized from acetone–water.

Additional evidence of radiopurity was obtained by chromatography of the $^{14}$C-labeled deoxycholic acid used in these studies on thin-layer chromatography. Iso-octane–ethyl acetate–acetic acid 5:5:1 (Eneroth S11, Ref. 9) was used, and after development the deoxycholic acid standard was located with $I_2$ vapor. The silica gel was divided into six sections and counted. All of the recovered radioactivity was in the section corresponding to the deoxycholic acid standard.

Approximately 10 $\mu$Ci of $^{14}$C-labeled deoxycholic acid was dissolved in a small volume of ethanol (0.5 ml) and slowly injected into two patients by an intravenous saline drip.

Patients

The first patient was a 69-yr-old man who had a T tube placed in his common bile duct at the time of cholecystectomy for cholecystitis. The second patient was a 63-yr-old female who had a T tube placed in her common bile duct to relieve an obstruction due to a carcinoma of the head of the pancreas. Both patients were studied approximately 10 days after surgery; they were eating a regular diet. The first patient had normal liver function tests (bilirubin, alkaline phosphatase), and the second patient had evidence of abnormal liver function with a serum bilirubin of 2.3 mg per 100 ml/4.7 mg per 100 ml (direct/total) and a serum alkaline phosphatase of 69 K.A. units, at the time of the study. The bile fistulas were virtually complete, as judged by the absence of the secondary bile salts in the fistula bile and excretion of less than 0.5% of the administered radioactivity in the feces collected over a 6-day period after the start of the study. Presumably, in the first patient a complete biliary diversion was produced by the siphoning action of the bile in the T tube.

Collection and Analysis of Bile

Complete bile collections were obtained at intervals ranging from 2 to 12 hr; the bile was mixed with an equal volume of ethanol and stored at 5°C. Radioactivity was determined by counting in a scintillation counter using Biosolv 3 Solubilizer and Fluorally TLA Counting Mixture (Beckman Instruments, Inc., Fullerton, Calif.). The amount of quenching was estimated after the addition of an internal standard of $^{14}$C-labeled toluene to the counting vials containing the bile.

<table>
<thead>
<tr>
<th>Patient</th>
<th>dpm Given</th>
<th>% Recovered in Bile, First 24 hr</th>
<th>% Recovered in 6 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$2.5 \times 10^7$</td>
<td>72.3</td>
<td>77.6</td>
</tr>
<tr>
<td>2</td>
<td>$1.67 \times 10^7$</td>
<td>71.7</td>
<td>83.0</td>
</tr>
</tbody>
</table>

As shown in Table 1, approximately 72% of the total radioactivity recovered in bile was excreted in the first 24 hr. Hydrolysis and chromatographic separation of the bile acids were carried out as follows. Aliquots from the fractions of bile collected during the first 24 hr were combined and evaporated to near dryness on a steam bath under a nitrogen stream. The residue was hydrolyzed with 4.5 N NaOH in a steel bomb at 130°C for 24 hr. The hydrolysate was diluted with water, adjusted to pH 1 with concentrated HCl, and extracted three times with equal volumes of diethyl ether. The evaporated ether extract was chromatographed on Celite using 70% aqueous acetic acid (v/v) as the stationary phase and increasing concentrations of benzene in petroleum ether (bp 60–70°C) as the moving phase. Radioactivity in each fraction was measured, and similar results were obtained in both patients. The distribution of radioactivity in the eluate of this column is shown for the first patient in Fig. 1. The small peak of radioactivity in the 0% benzene fraction appeared with the solvent front and probably represents radioactivity which did not equili-
brate with the stationary phase. Over 94% of the radioactivity placed on the column was eluted in a single peak in the 40% benzene fraction; this is the dihydroxy acid zone. The remainder of the radioactivity was distributed as very small peaks, none of which contained more than 1% of the radioactivity placed on the column.

The endogenous cholic acid in the 80% benzene fraction was titrated with 0.01 N NaOH and compared with the small amounts of radioactivity found in the fraction (Fig. 2). The small peaks of radioactivity did not appear to migrate with the mass of cholic acid.

**Identification of Deoxycholic Acid**

From the Celite column (Fig. 1), aliquots taken from the three tubes of peak radioactivity found in the 40% benzene fraction were chromatographed on Whatman No. 3 paper using isopropyl ether–petroleum ether (bp 60–70°C) 20:80 as solvent (10). Unlabeled deoxycholic, cholic, and chenodeoxycholic acids were used as standards. Bile acids were identified using ultraviolet light after wetting the paper with antimony trichloride (11). The paper was cut into 30 sections, each of which was placed in a vial with scintillation fluid and counted. Over 93% of the radioactivity placed on the paper was accounted for. All radioactivity migrated with deoxycholic acid, as shown in Fig. 3.

A second aliquot was taken from the same tubes, and thin-layer chromatography was carried out using isooctane–ethyl acetate–acetic acid 5:5:1 as solvent, Eneroth S11 (9). Three developments were done in the same direction to separate deoxycholic acid from chenodeoxycholic acid (12). This technique gave final \( R_f \) values of 0.64 and 0.74 for chenodeoxycholic acid and deoxycholic acid, respectively. After the last development, the silica gel was cut into sections and the section corresponding to the deoxycholic acid standard was eluted with methanol. Unlabeled deoxycholic acid (100.0 mg) was added to the eluate which contained \( 3.16 \times 10^4 \) dpm. The acid was recrystallized with different solvents (Table 2) and the specific activity remained constant. The specific activity of methyl deoxycholate prepared from the material recovered from the ethyl acetate recrystallization was unchanged from the specific activity of deoxycholic acid.

**Conjugation of Deoxycholic Acid**

An aliquot of unhydrolyzed bile from the first patient was placed on a silica gel G plate, which was developed in isoamyl acetate–propionic acid–N-propanol–water 4:3:2:1 (v/v/v/v), Hofmann System II (13). Glyco- and taurodeoxycholate were used as standards. After visualization of the spots with iodine vapor, the silica gel from each of six approximately equal zones was scraped off and heated at 60°C for 60 min in 4 ml of methanol. The samples were counted in a scintillation counter after addition of 12 ml of counting fluid. Of the recovered radioactivity, 85% was found in the glycodeoxycholate zone, and 13.3% in the taurodeoxycholate zone.

In order to determine whether or not \(^{14}C\)-labeled deoxycholate had undergone sulfation, a reaction known to occur with lithocholate (14), another aliquot of unhydrolyzed bile was chromatographed on thin-layer plates using butanol–Tris buffer–propionic acid (pH 3)
and approximately 50% of the lithocholate in bile fistula patients. 450-455.


REFERENCES


HANSON AND WILLIAMS Metabolism of Deoxycholic Acid in Man 691