

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- ^{14}C 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 $3\alpha,6\beta,12\alpha$ -trihydroxy-5 β -cholanoic acid (3). The boid snakes hydroxylate deoxycholic acid at position 16 to give pythocholic 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- $^{24}\text{-}^{14}\text{C}$

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). Tauroolithocholate 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 ($3\alpha,12\alpha$ -dihydroxy-5 β -cholanoic acid) with a specific activity of 2.07 $\mu\text{Ci}/\mu\text{mole}$ 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 acid as measured by titration. A constant specific activity was ob-

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Systematic names of bile acids referred to in the text by their trivial names are as follows: deoxycholic acid, $3\alpha,7\alpha$ -dihydroxy-5 β -cholanoic acid; cholic acid, $3\alpha,7\alpha,12\alpha$ -trihydroxy-5 β -cholanoic acid; pythocholic acid, $3\alpha,12\alpha,16\alpha$ -trihydroxy-5 β -cholanoic acid; tauroolithocholate sulfate, 3α -hydroxy-5 β -cholanoic acid-taurine-3-sulfate; glycodeoxycholic acid, $3\alpha,12\alpha$ -dihydroxy-5 β -cholanoic acid-glycine; taurodeoxycholic acid, $3\alpha,12\alpha$ -dihydroxy-5 β -cholanoic acid-taurine.

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served when 2.58×10^6 dpm of ^{14}C -labeled deoxycholic acid was added to 100 mg of unlabeled deoxycholic acid and recrystallized with acetone (2.09×10^3 dpm/mg), ethyl acetate (2.18×10^3 dpm/mg), and acetone-water (2.18×10^3 dpm/mg). The specific activity of methyl deoxycholate prepared from the final acetone-water recrystallization was 2.31×10^3 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. Isooctane-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 μCi 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 1 RECOVERY OF DEOXYCHOLIC ACID- ^{14}C

Patient	dpm Given	% Recovered in Bile, First 24 hr	% Recovered in 6 days
1	2.5×10^7	72.3	77.6
2	1.67×10^7	71.7	83.0

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 $60\text{--}70^\circ\text{C}$) 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-

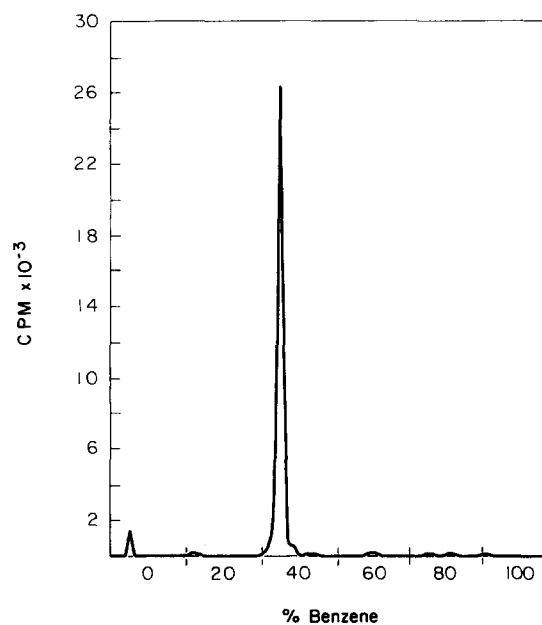


FIG. 1. Chromatographic distribution of radioactivity in bile after intravenous administration of ^{14}C -labeled deoxycholic acid. Celite column: stationary phase, 70% aqueous acetic acid; moving phase, increasing concentrations of benzene in petroleum ether. 5-ml fractions were collected.

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 iso-octane-ethyl acetate-acetic acid 5:5:1 as solvent, Ene-roth 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 3.16×10^4 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

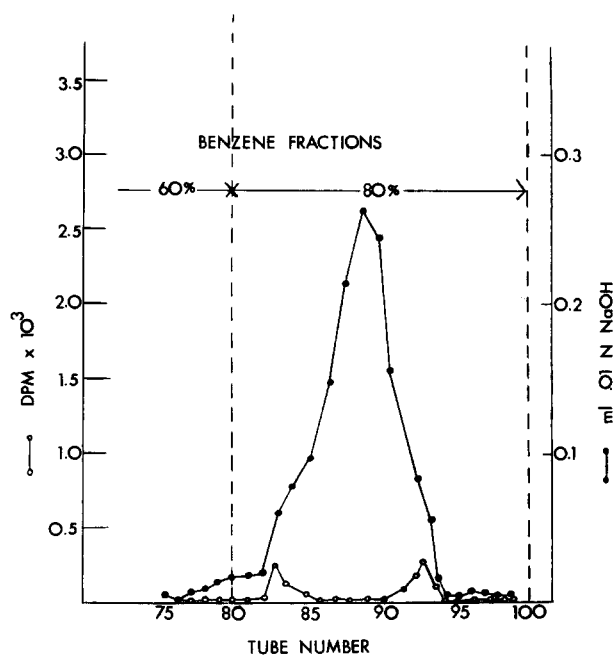


FIG. 2. Chromatography of the 80% benzene fraction of Fig. 1 (cholic acid). Titration, closed circles; radioactivity, open circles.

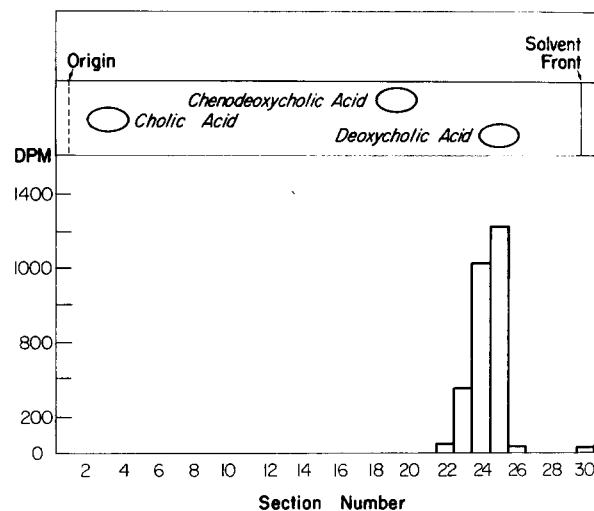


FIG. 3. Paper chromatography of an aliquot of the radioactivity found in the 40% benzene fraction of Fig. 1, along with standards: chenodeoxycholic, cholic, and deoxycholic acids. The developing solvent was isopropyl ether-petroleum ether 20:80. After development and identification of the standards, the paper was cut into 30 sections and assayed for radioactivity.

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)

TABLE 2 SPECIFIC ACTIVITY OF RECRYSTALLIZED DEOXYCHOLIC ACID

Number	Solvent	Weight	Specific Activity
		mg	dpm/mg
1	Acetone	49.0	282.0
2	Ethyl acetate	31.5	278.5
3	Methyl deoxycholate Acetone-water	10.8	288.6

50:9.25:0.75 as the developing solvent (14). This solvent system separates the more polar sulfate conjugates from the less polar unsulfated conjugates of lithocholate. Presumably, the sulfate conjugates of deoxycholate would be even more polar than the sulfates of lithocholate. Taurolithocholate sulfate and the taurine and glycine conjugates of deoxycholate were used as standards. The plate was developed and counted as described above. All radioactivity was found in the area of glycine and taurine conjugates of deoxycholate, and no appreciable radioactivity was in the more polar zones where sulfated esters of deoxycholate would have appeared.

DISCUSSION

This study provides direct evidence that the human liver is not able to rehydroxylate deoxycholate to any significant extent. More than 94% of the administered radioactivity was excreted as conjugated deoxycholate. Lower forms of animals, such as the rat, can add hydroxyl groups at positions 6 or 7 of the sterol and excrete these in the bile as secondary bile salts. The human liver has less ability to rehydroxylate secondary bile acids. As shown in Fig. 1, there were three or four small peaks that were more polar than deoxycholate. Two of these peaks were located in the 80% benzene fraction where cholic acid was eluted from this column, but neither was cholic acid, as shown in Fig. 2.

Previous work has shown that the other major secondary bile acid in man, lithocholic acid, is also not rehydroxylated by the human liver (15). Conjugated lithocholate, however, can undergo esterification with sulfate at position 3, and approximately 50% of the lithocholate in human gallbladder bile has been demonstrated to be present as the sulfate ester (14). The possibility that conjugated deoxycholate can also undergo sulfation has not previously been investigated in man. The present study suggests that no appreciable sulfation has taken place.

Some patients with cirrhosis of the liver have been found to excrete very little deoxycholate in their bile

(6, 16). One of the suggested explanations for this finding is a rapid rehydroxylation of deoxycholate to cholate in the liver (16). If this phenomenon exists, it must be peculiar to the cirrhotic liver.

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REFERENCES

1. Norman, A., and M. S. Shorb. 1962. In vitro formation of deoxycholic and lithocholic acid by human intestinal microorganisms. *Proc. Soc. Exp. Biol. Med.* **10**: 552-555.
2. Norman, A., and J. Sjövall. 1958. On the transformation and enterohepatic circulation of cholic acid in the rat. *J. Biol. Chem.* **233**: 872-885.
3. Ratliff, R. L., J. T. Matschiner, E. A. Doisy, Jr., S. L. Hsia, S. A. Thayer, W. H. Elliott, and E. A. Doisy. 1959. A new metabolite of deoxycholic acid. *J. Biol. Chem.* **234**: 3133-3136.
4. Haselwood, G. A. D., and V. M. Wootton. 1951. Comparative studies of bile salts. Pythocholic acid. *Biochem. J.* **49**: 67-71.
5. Ekdahl, P.-H., and J. Sjövall. 1955. Metabolism of deoxycholic acid in the rabbit. *Acta Physiol. Scand.* **34**: 287-294.
6. Vlahcevic, Z. R., I. Buhac, C. C. Bell, Jr., and L. Swell. 1970. Abnormal metabolism of secondary bile acids in patients with cirrhosis. *Gut.* **11**: 420-422.
7. Sjövall, J. 1960. Bile acids in man under normal and pathological conditions. *Clin. Chim. Acta.* **5**: 33-41.
8. Fieser, L. F., and M. Fieser. 1959. *In Steroids*. Reinhold, New York. 54.
9. Eneroth, P. 1963. Thin-layer chromatography of bile acids. *J. Lipid Res.* **4**: 11-16.
10. Sjövall, J. 1954. Separation of conjugated and free bile acids by paper chromatography. *Acta Chem. Scand.* **8**: 339-345.
11. Carey, J. B., Jr., and H. S. Bloch. 1954. The rapid identification of bile acids with an antimony trichloride color reagent. *J. Lab. Clin. Med.* **44**: 486-489.
12. Bruusgaard, A. 1970. Quantitative determination of the major 3-hydroxy bile acids in biological material after thin-layer chromatographic separation. *Clin. Chim. Acta.* **28**: 495-504.
13. Hofmann, A. F. 1962. Thin-layer adsorption chromatography of free and conjugated bile acids on silicic acid. *J. Lipid Res.* **3**: 127-128.
14. Palmer, R. H. 1967. The formation of bile acid sulfates: a new pathway of bile acid metabolism in humans. *Proc. Nat. Acad. Sci. U.S.A.* **58**: 1047-1050.
15. Carey, J. B. Jr., and G. Williams. 1963. Metabolism of lithocholic acid in bile fistula patients. *J. Clin. Invest.* **42**: 450-455.
16. Turnberg, L. A., and G. Grahame. 1970. Bile salt secretion in cirrhosis of the liver. *Gut.* **11**: 126-133.