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Formation of Lithocholic Acid from Ursodeoxycholic Acid in the Rat

Bile Acids and Steroids 116

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Carboxy labelled ursodeoxycholic acid has been synthesized and its metabolism has been studied after intracecal injection into bile duct cannulated rats. The 7β-hydroxyl group of ursodeoxycholic acid is effectively eliminated by microorganisms in the intestine forming lithocholic acid. β-Muricholic acid (3α,6β,7β-trihydroxycholanic acid), chenodeoxycholic acid, and lithocholic acid were identified among the labelled metabolites excreted in the bile.

Cholic and chenodeoxycholic acids are the primary bile acids formed from cholesterol in the rat liver [1]. Chenodeoxycholic acid is metabolized to α-muricholic acid (3α,6β,7α-trihydroxycholanic acid) which is further converted into β-muricholic acid (3α,6β,7β-trihydroxycholanic acid) by an epimerization involving an oxidation-reduction at C7 [2-4]. Two minor metabolites of chenodeoxycholic acid have been isolated, *viz.* 7-ketolithocholic acid (3α-hydroxy-7-ketocholanic acid) and ursodeoxycholic acid (3α,7β-dihydroxycholanic acid). The latter acid is formed by reduction of 7-ketolithocholic acid which yields predominantly the β-epimer [1]. The 7-ketolithocholic acid may be furnished by dehydrogenation of chenodeoxycholic acid either in the liver [4] or in the intestine by microorganisms [5]. In the rat liver ursodeoxycholic acid is hydroxylated in 6β-position to β-muricholic acid and is in addition transformed into chenodeoxycholic acid and an unidentified metabolite [11].

During the enterohepatic circulation of bile acids containing hydroxyl groups in 7α-position, *viz.* cholic, chenodeoxycholic and hyocholic acids, the hydroxyl is eliminated with formation of deoxycholic, lithocholic and hyodeoxycholic acids, respectively [1]. The possibility of eliminating a 7β-hydroxyl group was demonstrated by the transformation of 3α,7β,12α-trihydroxycholanic acid into deoxycholic acid in the intestine [6]. The aim of the present investigation was to find out whether a similar dehydroxylation takes place during the enterohepatic circulation of ursodeoxycholic acid.

EXPERIMENTAL

Ursodeoxycholic acid-24-^{14}C. 7-Ketolithocholic acid-24-^{14}C (m.p. 200—201°) was prepared by oxidation of chenodeoxycholic acid as described earlier [7]. To a solution of 25 mg of this acid in 5 ml of anhydrous propanol was added 0.075 g of sodium. The solution was refluxed for 3 h, diluted with water, acidified and extracted with ether. The residue obtained after evaporation was chromatographed with solvent system F1 which separates ursodeoxycholic acid from chenodeoxycholic acid. Crystallization from ethyl acetate/hexane yeilded 14 mg of ursodeoxycholic acid-24-^{14}C, m.p. 200—201° [8]. Specific actity: 0.55 μC/mg.

Animal experiments. The sodium salt of ursodeoxycholic acid-24-^{14}C (2.0 mg) in 0.9 % sodium chloride solution was injected into the cecal content of three rats (Sprague-Dawley strain, 200—250 g) immediately after cannulation of the bile duct. Bile and feces were collected for two days.

Analysis of feces and bile. Feces were refluxed three times with 80 % aqueous ethanol. After evaporation to dryness the residue was extracted with ether from an acidified water solution.

The bile, collected in ethanol, was filtered, evaporated and hydrolyzed with 2 N NaOH in a closed steel tube for 8 h at 120°.

The free bile acids were separated with reversed phase partition chromatography using the following solvent systems [9,10].

Solvent system	Moving phase (ml)	Stationary phase (ml)
C	Methanol-water 150:150	Chloroform-isooctanol 15:15
F1	Methanol-water 165:135	Chloroform-heptane 45:5
F2	Methanol-water 180:120	Chloroform-heptane 45:5

Four ml of the stationary phase were supported on 4.5 g of hydrophobic Super-Cel. The fractions collected from the columns were titrated with 0.02 N NaOH and the radioactivity was determined by counting of an aliquot in an infinitely thin layer on aluminium planchets. A gas flow counter, Frieseke-Hoepfner FH 51, was used.

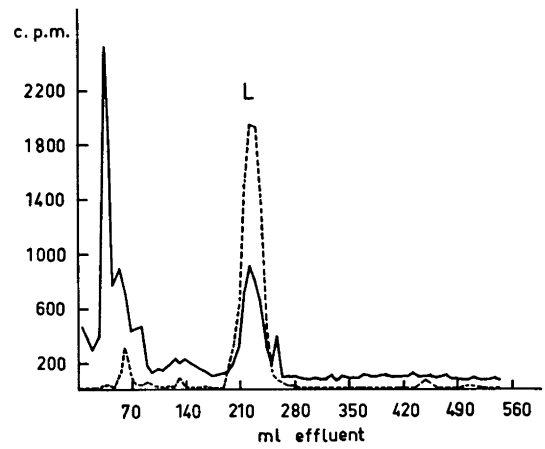

Fig. 1. Chromatographic separation of labelled products in feces after intracecal injection of ursodeoxycholic acid-24-^{14}C into a bile duct cannulated rat. Solvent system F2. Column: 13.5 g. Inactive lithocholic acid (6 mg) added as carrier (L).

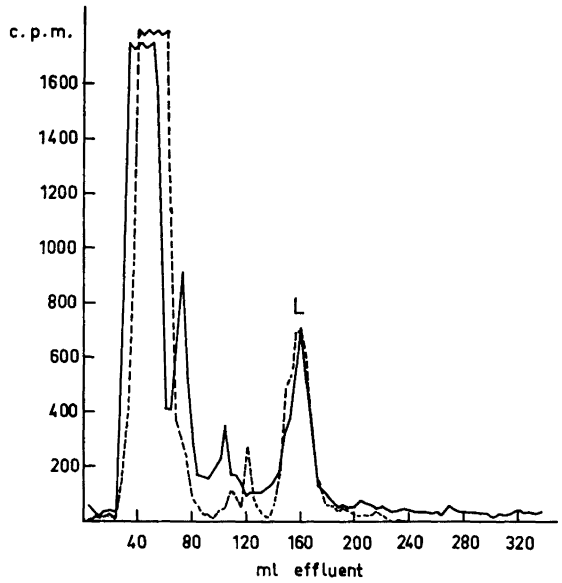

Fig. 2. Chromatographic separation of labelled products excreted in bile after intracecal injection of ursodeoxycholic acid-24-^{14}C into a bile duct cannulated rat. Solvent system: F2. Column: 9 g. Inactive lithocholic acid (5 mg) added as carrier (L).

RESULTS

The sodium salt of ursodeoxycholic acid was injected into the cecal content of bile duct cannulated rats. Of the administered radioactive material

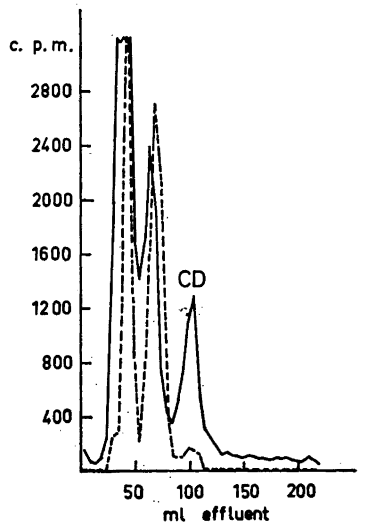

Fig. 3. Rechromatography of 25—85 ml effluent of the chromatography shown in Fig. 2. Solvent system: F1. Column: 9 g. Chenodeoxycholic acid (CD).

about 28—40 % appeared in feces within 36 h whereas the remaining part was absorbed from the intestine and excreted in the bile.

Separation of labelled bile acids in feces. The radioactive compounds excreted in feces were first separated by chromatography with solvent system F2 together with unlabelled lithocholic acid (Fig. 1). The main radioactive peak (200—300 ml) coincided with the peak of the carrier. The identification of labelled lithocholic acid was established by isotope dilution. A minor band of radioactivity appeared at the place of unchanged ursodeoxycholic acid, which was confirmed by rechromatography together with the unlabelled acid with solvent system F1. From 6 to 14 % of the chromatographed radioactive material remained in the column. The nature of these compounds was not further investigated.

Separation of labelled bile acids in bile. The bile acids excreted in the bile were separated after hydrolysis by chromatography with solvent system F2 together with unlabelled lithocholic acid (Fig. 2). A part of the radioactive material coincided with the titration peak of lithocholic acid (140—175 ml). Isotope dilution established the identity. About 3—5 % of the chromatographed radioactivity remained in the stationary phase. The radioactive compounds, which were eluted immediately before (100—130 ml) lithocholic acid were chromatographically similar to 7-ketolithocholic acid but rechromatography with carrier acid showed that they were eluted later. No further attempts were made to determine their structures.

Separation of the more hydrophilic compounds was carried out with solvent system F1 (Fig. 3). In this system chenodeoxycholic acid present in rat bile

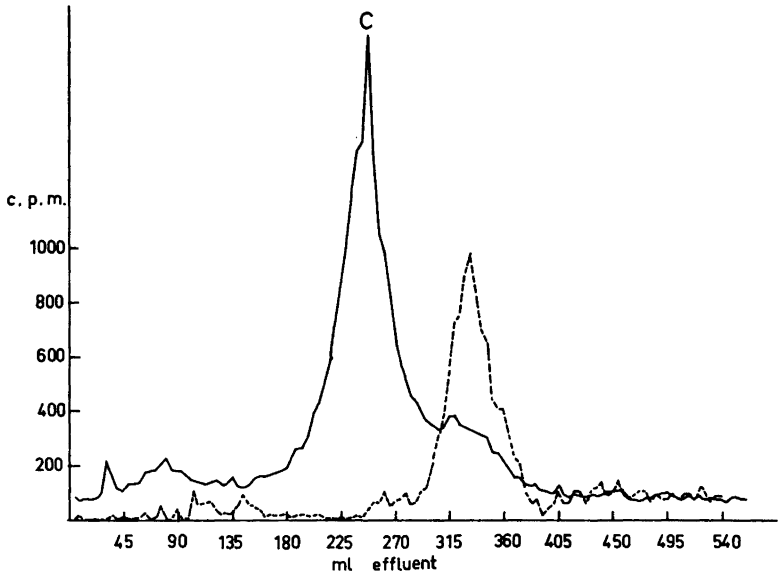

Fig. 4. Rechromatography of 25—50 ml effluent of the chromatography shown in Fig. 3. Solvent system: C. Column: 9 g. Cholic acid (C).

appears at 90—110 ml effluent. A small fraction of the isotope appeared at this place. The radioactive compound eluted immediately before this peak was identified as ursodeoxycholic acid.

The radioactive material in the front peak was chromatographed with solvent system C (Fig. 4). The labelled compound now appeared at the place of β-muricholic acid (3α,6β,7β-trihydroxycholanic acid) immediately after the inactive cholic acid present in the bile. The identity was established by isotope dilution.

DISCUSSION

The results show that the 7β-hydroxyl group of ursodeoxycholic acid is eliminated very efficiently by microorganisms in the intestine forming lithocholic acid. A striking feature is the almost complete absence of other metabolites, which are numerous when chenodeoxycholic acid is administered.

The formation of lithocholic acid from ursodeoxycholic acid is a reaction analogous to the transformation of 3α,7β,12α-trihydroxycholanic acid into deoxycholic acid. As in this case nothing is known about the mechanism of the elimination.

The radioactive compounds found in the bile consist of ursodeoxycholic acid and lithocholic acid absorbed from the intestine together with their liver metabolites, *e.g.* chenodeoxycholic acid and β-muricholic acid.

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