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Formation of Lithocholic Acid from Chenodeoxycholic Acid in the Rat

Bile Acids and Steroids 103

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Chenodeoxycholic acid-24-^{14}C has been injected into the cecum of bile fistula rats. 50–60 % of the isotope was absorbed. Lithocholic acid comprised more than 50 % of the labelled fecal acids but only traces were found in the bile. At least eight other microbial transformation products were present. One of these was probably 3-ketocholanic acid.

Cholic and chenodeoxycholic acids are the primary bile acids formed from cholesterol in the rat liver (*cf.* Ref.[1]). Chenodeoxycholic acid is further tranformed by liver enzymes into 3α, 6β, 7α- and 3α, 6β, 7β-trihydroxycholanic acids and to a small extent into 7-ketolithocholic and ursodeoxycholic (3α, 7β-diol) acids [2–4].

During the enterohepatic circulation, the bile acids are attacked by microbial enzymes in the cecum and colon and the metabolites formed are partly absorbed [5]. By this mechanism deoxycholic acid is formed from cholic acid. The aim of the present investigation was to find out whether a similar removal of the 7-α-hydroxyl group from chenodeoxycholic acid takes place during the enterohepatic circulation. If this were so, labelled lithocholic acid would be found in the bile and feces of rats which had received chenodeoxycholic acid-24-^{14}C. The presence of lithocholic acid in the bile of different animals has been repeatedly shown (*cf.* Sobotka [6]).

EXPERIMENTAL

Chenodeoxycholic acid-24-^{14}C (10 μC per mg) was prepared according to Bergström *et al.*[7]

Animal experiments. Bile duct-cannulated white rats weighing 200–300 g were used [8]. 0.5 mg of labelled sodium chenodeoxycholate in 0.2 ml of saline was injected into the cecal content [5]. Bile and feces were collected for two days.

Analysis of bile and feces. Bile acids were extracted from feces by refluxing three times for 2 h with 80 % aqueous ethanol. After evaporation to dryness the bile acids were extracted with ether from an acidified water solution. The bile was hydrolyzed with N NaOH in a closed steel tube for 6 h at 120° and the bile acids were extracted as described above.

The bile acids were separated with reversed phase column chromatography [9,10] using the following solvent systems:

Phase system	Moving phase (ml)	Stationary phase (ml)
C 1	Methanol-water 150:150	Chloroform-*iso*octanol 15:15
F 1	Methanol-water 165:135	Chloroform-heptane 45:5
F 2	Methanol-water 180:120	Chloroform-heptane 45:5
F 3	Methanol-water 195:105	Chloroform-heptane 25:25

4 ml of stationary phase were supported on 4.5 g of hydrophobic Supercel. The fractions collected from the columns were titrated and isotope determined on aliquots. For the sake of comparison, the effluent volumes of the figures have been corrected to correspond to a 4.5 g column.

RESULTS

After intracecal injection of labelled sodium chenodeoxycholate 40—50 % of the isotope was excreted in the feces. The remaining labelled products were absorbed and excreted in the bile.

Separation of labelled bile acids in feces. The ether extract of the feces was first chromatographed with phase system F 2. The labelled products appearing before lithocholic acid and those remaining in the stationary phase were rechromatographed with phase systems F 1 and F 3, respectively. The front band from the chromatography with phase system F 1 was re-run with phase system C 1.

As shown in Fig. 1 only a few per cent of the chenodeoxycholic acid was unchanged (Fig. 1., Phase system F 1, 45—65 ml). At least nine different metabolites were separated. Most of these were more hydrophobic than chenodeoxycholic acid. More than half of the isotope appeared at the site of lithocholic acid added as carrier (Fig. 1., Phase system F 1, 70 — 100 ml). The identity of the radioactive compound with lithocholic acid was established by isotope dilution. Rechromatography of the metabolites more hydrophobic than lithocholic acid (Fig. 1., Phase system F 3), together with 3-ketocholanic acid, showed that the radioactivity of the fourth band (100—150 ml) coincided with the titration peak of the carrier.

Since *E. coli* oxidizes chenodeoxycholic acid to 7-ketolithocholic acid *in vitro* we expected to find this acid in the feces [11]. A radioactive peak was present in the chromatogram very close to 7-ketolithocholic acid added as carrier (Fig. 1., Phase system F 1, A). Rechromatography showed that only a small fraction of isotope was eluted together with the carrier acid while the main peak appeared immediately afterwards.

The small amount of metabolites more hydrophilic than chenodeoxycholic acid was separated with phase system C 1 (Fig. 1). One band of activity appeared before, and another one just after, cholic acid added as carrier.

Separation of labelled bile acids in bile. As shown in Fig. 2 chromatographic analysis of the bile revealed the presence of at least eight radioactive com-

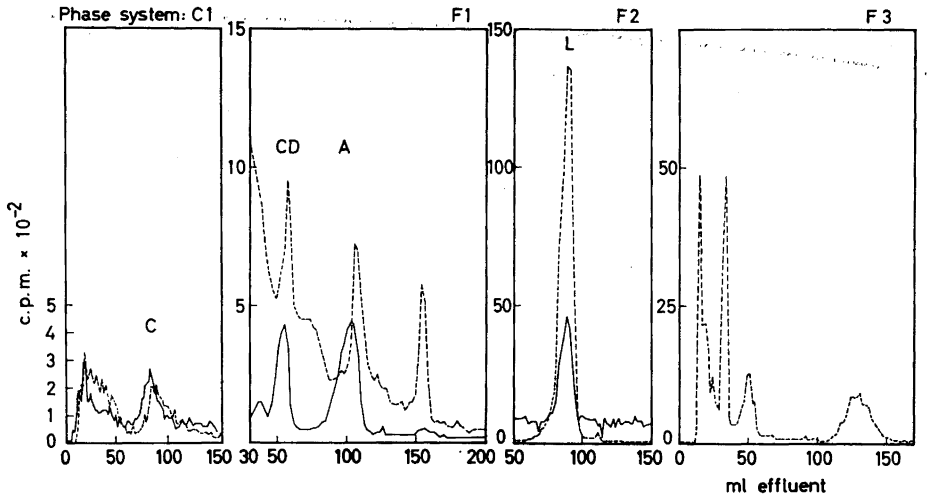

Fig. 1. Chromatographic separations of labelled products in feces after the injection of chenodeoxycholic acid-24-^{14}C into the cecum of a bile fistula rat. Reference substances: cholic (C), chenodeoxycholic (CD), 7-ketolithocholic (A), and lithocholic (L) acids. Solid line: Titration. Broken line: Radioactivity.

pounds. Apart from unchanged chenodeoxycholic acid (Fig. 2., Phase system F 1, 40—60 ml) radioactive peaks appeared at the sites of 3α, 6β, 7α- and 3α, 6β, 7β- trihydrocycholanic acids (Fig. 2., Phase system C 1, 60—80 ml and 100—140 ml, respectively). The metabolite eluted before chenodeoxycholic

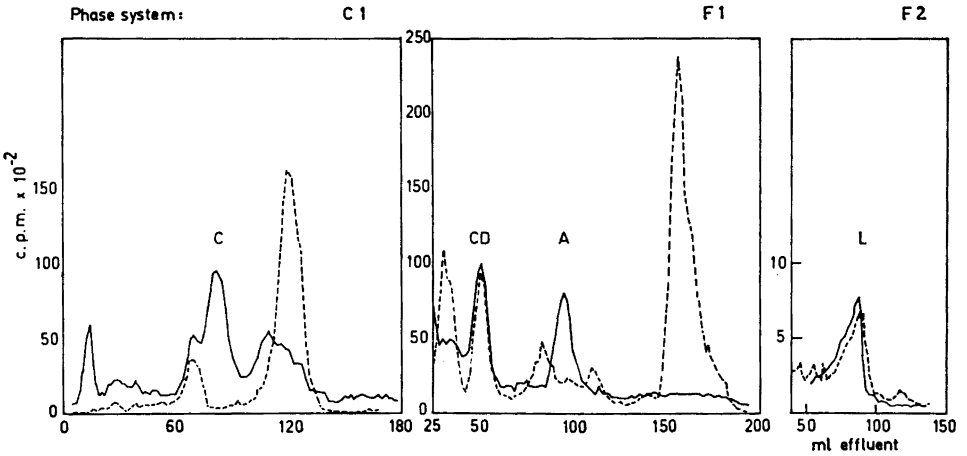

Fig. 2. Chromatographic separations of the labelled products in the bile of the same rat as used for the experiment shown in Fig. 1. The extract was first chromatographed with phase system F 1. The activity in the front band and that remaining in the stationary phase were rechromatographed with phase systems C 1 and F 2, respectively. For reference substances see Fig. 1.

acid (Fig. 2., Phase system F 1, 25—40 ml) was rechromatographed with ursodeoxycholic acid and the radioactivity and the titration peak coincided.

The metabolites described above are also present in the bile after intraperitoneal injection of chenodeoxycholic acid. The other compounds separated are thus microbial transformation products and/or liver metabolites of these substances. Except for lithocholic acid present in very small amounts, the nature of these compounds is unknown.

DISCUSSION

According to Siperstein *et al.*[12] lithocholic acid might be an intermediate in the formation of cholic and chenodeoxycholic acids from cholesterol. This would explain the presence of lithocholic acid in the bile. Their hypothesis has not been generally accepted (*cf.* Ref.[1]). In the present study we have shown that the 7α-hydroxylgroup of chenodeoxycholic acid can be removed by microorganisms in the large intestine. The lithocholic acid thus formed can be absorbed and appears in the bile. At the present time the microbial biogenesis seems to us to be the most plausible mechanism for the formation of lithocholic acid.

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