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On the Metabolism of Ursodeoxycholic Acid in the Rat

Bile Acids and Steroids 84

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The metabolism of intraperitoneally administered tritium labelled ursodeoxycholic acid has been studied in bile fistula rats. This acid is partly converted into 3α,6β,7β-trihydroxycholanic acid (Acid I), chenodeoxycholic acid and one unidentified metabolite.

Ursodeoxycholic acid (3α,7β-dihydroxycholanic acid) has been isolated from certain bear species, the coypu [1] and recently also from the rat [2] and man [3]. In the two last-mentioned species it occurs in very small amounts. Studies of the metabolism of chenodeoxycholic acid in the rat have shown that together with the two principal metabolites [4,5], 3α,6β,7β-(Acid I) and 3α,6β,7α- (Acid II) trihydroxycholanic acid, small amounts of 7-ketolithocholic acid [6] and ursodeoxycholic acid [2,6] are formed. Furthermore it has been shown that 7-ketolithocholic acid is mainly transformed into ursodeoxycholic acid and Acid I in the rat liver [2,7]. In order to get more information about the course of the metabolism of chenodeoxycholic acid, tritium labelled ursodeoxycholic acid has been injected intraperitoneally into bile fistula rats, and the labelled products excreted in the bile have been separated by chromatography.

EXPERIMENTAL

Tritium labelled ursodeoxycholic acid. Ursodeoxycholic acid was prepared by the procedure of Samuelsson [6], (M. p. 201–202°). 10 mg of this acid was exposed to tritium gas (2C, 200 mm Hg, 95.5 % pure) for 6 days at room temperature according to the method of Wilzbach [8]. The tritium labelled product was diluted with 25 mg of inactive ursodeoxycholic acid and chromatographed with phase system F[9]. The titration and activity peaks coincided.

Crystallization from ethylacetate/light petroleum yielded 24 mg of ursodeoxycholic acid (M. p. 201–202°), specific activity; 40 × 10^6 c.p.m/mg when counted in a Tracerlab flow counter or approximately 70 μC/mg. A sample of this acid was diluted with inactive ursodeoxycholic acid and recrystallized from aqueous acetic acid, ethylacetate/light petroleum and aqueous methanol. The specific activity remained constant.

3α,6β,7β-Trihydroxycholanic acid (Acid I) was prepared according to the method of Doisy *et al.*[5] (M. p. 226–227°).

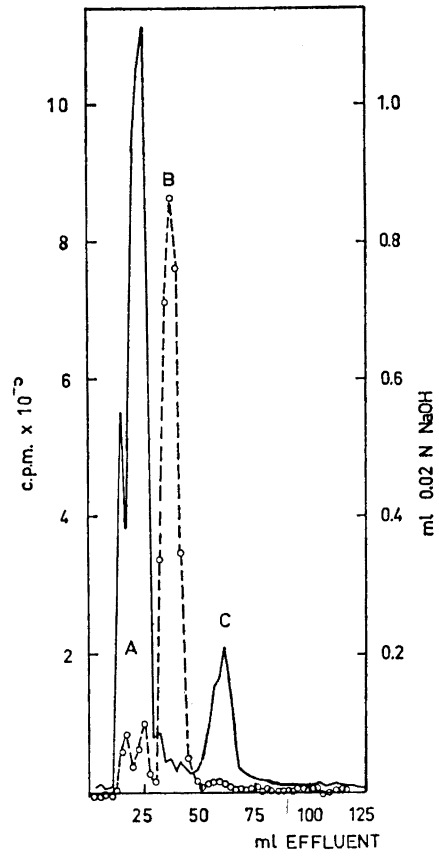

Fig. 1. Chromatographic separation of acids from hydrolyzed bile, excreted during 24 h following intraperitoneal administration of 0.3 mg of tritium labelled ursodeoxycholic acid. Column: 4.5 g hydrophobic Supercel. Phase system: Type F, see page 971. Solid line: Titration values. Broken line: Radioactivity.

Animal experiments. Bile fistulas were made on white male rats of the institute stock weighing about 200 g. The rats had free access to white bread and oats and 0.9 % sodium chloride solution. The bile was collected in ethanol. 0.3 mg of the tritium labelled ursodeoxycholic acid was neutralized with sodium hydroxide and injected intraperitoneally in 0.9 % sodium chloride solution into each of three rats 12 h after the bile fistula had been made. The bile was collected for 24 h. The conjugated bile acids were hydrolyzed in 1.5 N NaOH for 6 h at 120° in a sealed tube. The free bile acids were extracted with ether after acidification with hydrochloric acid.

Chromatographic separations. The free bile acids were chromatographed on hydrophobic Supercel as described by Bergström, Sjövall and Norman [9,10]. The following solvent systems were used.

System	Moving phase	ml	Stationary phase	ml
F[9]	Methanol-water	165:35	Chloroform-heptane	45:5
C[10]	Methanol-water	150:150	Chloroform-*iso*octanol	15:15

4 ml of the stationary phase was supported on 4.5 g of hydrophobic Supercel. Each fraction from the chromatography was titrated with 0.02 N NaOH and an aliquot plated in an "infinitely thin" layer on an aluminium planchet which was counted in a Tracerlab flow counter.

Table 1. Recrystallizations of radioactive bands isolated by chromatography from the bile after administration of tritium labelled ursodeoxycholic acid to bile fistula rats.

Sample	Inactive bile acid added	Crystallizing solvent	Weight mg	*c.p.m.* / mg
Chenodeoxycholic acid band. Fig. 1.	Chenodeoxycholic acid		80	2 100
		Ethylacetate-light petroleum	62	1 980
		Acetic acid-water	43	1 920
		Ethanol-water	28	2 020
		Ethylacetate-light petroleum	17	1 930
3*α*, 6*β*, 7*β*-Trihydroxycholanic acid (Acid I) band. (Fig. 2)	3*α*, 6*β*, 7*β*-Trihydroxycholanic acid		60	9 600
		Ethylacetate	53	9 850
		Acetone-water	42	10 050
		Acetone-light petroleum	34	9 900
		Methanol-water	21	9 750

RESULTS

Tritium labelled ursodeoxycholic acid was administered intraperitoneally into three bile fistula rats. 70—82 % of the administered ^3H was recovered in the bile collected during 24 h following the injection. The saponified bile from each animal was chromatographed with phase system F. With this system ursodeoxycholic acid (peak at 40 ml effluent) chenodeoxycholic acid (60 ml) and more hydrophobic bile acids separate from cholic acid and other more polar bile acids which appear with the front. In Fig. 1 is shown a chromatogram of the hydrolyzed bile from one rat (R I). Most of the activity is eluted at the place of ursodeoxycholic acid (B), but about 10 % of the activity appears almost with the front as an incompletely separated double peak (A). A small but significant amount of the activity was found to coincide with the titration peak, caused by chenodeoxycholic acid present in the bile (C). Less than one per cent of the activity remained in the stationary phase. The material in the first band (A) was rechromatographed with phase system C, suitable for the separation of trihydroxycholanic acids (Fig. 2). The activity then appears as two separate peaks, one at 40—50 ml and one at the place of Acid I (120—160 ml), immediately after the titration peak of inactive cholic acid.

Identification of the labelled products separated by chromatography. Unchanged ursodeoxycholic acid (Fig. 1, peak B) was identified by recrystallizations from four different solvent systems after rechromatography with unlabelled ursodeoxycholic acid with phase system F. The labelled material eluted at the place of chenodeoxycholic acid was rechromatographed with phase system F and identified by isotope dilution (Table 1). The radioactive material eluted immediately after cholic acid (Fig. 2) was diluted with inactive Acid I and identified by isotope dilution after chromatography with phase system C.

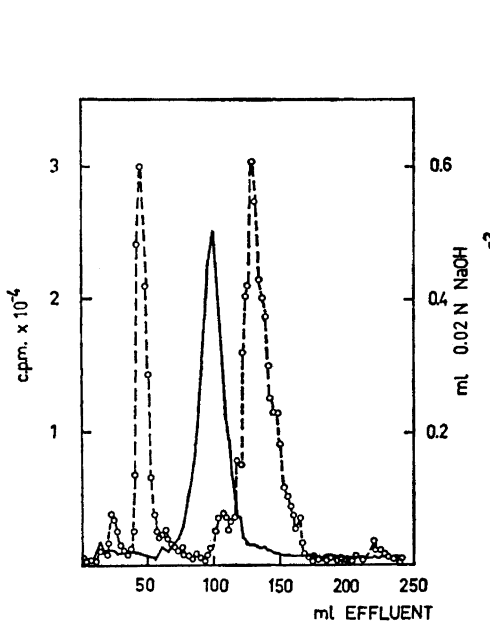

Fig. 2. Chromatographic separation of peak A in the chromatogram shown in Fig. 1. Column: 4.5 g hydrophobic Supercel. Phase system: Type C, see page 971. Solid line: Titration values. Broken line: Radioactivity.

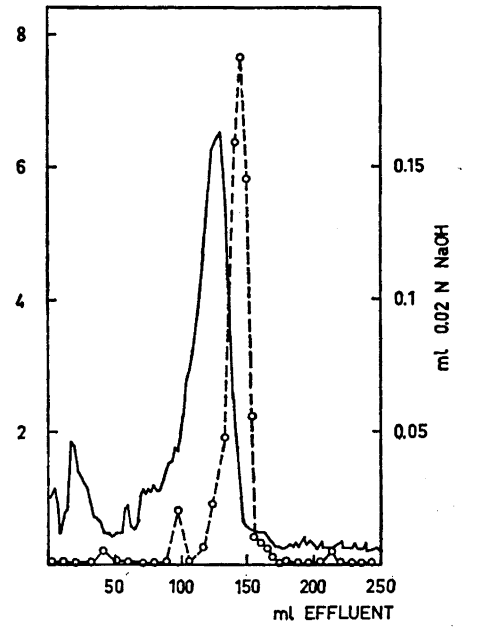

Fig. 3. Chromatographic separation of the reaction product after chromic acid oxidation of Metabolite III and 25 mg of inactive cholic acid. Column: 4.5 g hydrophobic Supercel. Phase system: Type C, see page 971. Solid line: Titration values. Broken line: Radioactivity.

The appearence of the radioactive band at about 50 ml (metabolite III) with phase system C (Fig. 2) is characteristic of a trihydroxycholanic acid or a dihydroxymonoketocholanic acid. As it was chromatographically identical with another 7β-hydroxylated bile acid (3α,7β,12α-trihydroxycholanic acid) with this phase system [6], it was, together with inactive cholic acid, oxidized with chromic acid under conditions [11] known to give dehydrocholic acid from 3,7,12-trihydroxycholanic acids. The chromatography of the reaction product with phase system C is shown in Fig. 3. The radioactivity is eluted immediately after the titration peak of inactive dehydrocholic acid. This result also excludes that metabolite III is a 3,6,7-trihydroxycholanic acid as chromic acid oxidation of these acids under the conditions used results in the formation of the more hydrophilic 3-keto-6,7-secocholanic acid-6,7-dioic acid [12,13], which appears with the front with phase system C. No further attempts were made to identify metabolite III. The percentage composition of the labelled products excreted in the bile is given in Table 2.

Table 2. Percentage composition of the labelled products excreted in the bile during 24 h following intraperitoneal injection of tritium labelled ursodeoxycholic acid into three rats with bile fistulas.

Compound	Per cent		
	R I	R II	R III
3*α*, 6*β*, 7*β*-Trihydroxycholanic acid	7.4	3.9	6.1
Chenodeoxycholic acid	1.5	0.6	1.4
Metabolite III	2.7	1.9	3.1
Ursodeoxycholic acid	79.7	91.5	85.3
Total	91.3	97.9	95.9

DISCUSSION

The results show that ursodeoxycholic acid is only slightly transformed in the rat liver. Its principal metabolite consists of 3*α*,6*β*,7*β*-trihydroxycholanic acid (Acid I), *i.e.* a 6*β*-hydroxyl group is introduced.

However, ursodeoxycholic acid seems only to be a minor intermediate in the formation of Acid I from chenodeoxycholic acid, as it is formed in very small amounts from chenodeoxycholic acid [2,6]. The demonstration of the direct transformation of Acid II into Acid I also points in this direction [6]. Another more polar bile acid was also isolated, but the structure of this metabolite has not been determined. A metabolite with the same elution rate as this acid was also found [7] in the bile after intraperitoneal administration of 7-ketolithocholic acid-24-^{14}C[7].

A small amount (0.6—1.5 %) of the recovered avtivity was identified as chenodeoxycholic acid. This acid is probably formed by dehydrogenation of ursodeoxycholic acid to 7-ketolithocholic acid which is then reduced to chenodeoxycholic acid.

Ursodeoxycholic acid present in normal rat bile may probably be formed by two routes. The direct formation of ursodeoxycholic acid from chenodeoxycholic acid in the liver has been demonstrated [2,6], and furthermore *in vitro* experiments have shown that chenodeoxycholic acid is dehydrogenated by different strains of *E. coli* to 7-ketolithocholic acid [14], which is readily reduced in the liver to ursodeoxycholic acid.

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