ACTA CHEMICA SCANDINAVICA 13 (1959) 236—240

The Metabolism of 7-Ketolithocholic Acid-24-^{14}C in the Rat *

Bile Acids and Steroids 70

BENGT SAMUELSSON**

Department of Physiological Chemistry, University of Lund, Lund, Sweden

The metabolism of intraperitoneally administered 7-ketolithocholic acid-24-^{14}C has been studied in bile fistula rats. This acid is partly transformed into 3α,6β,7β-trihydroxycholanic acid (Acid I) and 3α,7β-dihydroxycholanic acid (ursodeoxycholic acid). The results are discussed in relation to the metabolism of chenodeoxycholic acid.

Studies of the metabolism of chenodeoxycholic acid in the rat liver have shown that this acid is transformed into two more polar acids [1], which have recently been identified by Doisy *et al.* as 3α, 6β, 7β-trihydroxycholanic acid (Acid I) and 3α,6β,7α-trihydroxycholanic acid (Acid II) [2,3]. In the former metabolite an inversion of the original 7α-hydroxylgroup has occurred. Experiments with 7β-tritiochenodeoxycholic acid have shown that the 7β-hydroxy-acid is formed with loss of the tritium labell [4], and it was thus of interest to study the metabolism of carboxylabelled 7-ketolithocholic acid in the rat liver. Similar studies have been reported by Doisy *et al.*[5], who found that 7-ketolithocholic acid, intragastrically administered to rats, was converted into chenodeoxycholic acid, ursodeoxycholic acid, Acid I and Acid II.

EXPERIMENTAL

7-Ketolithocholic acid-24-^{14}C. 66.9 mg of methyl chenodeoxycholate-24-^{14}C[6] were treated with ethylchlorocarbonate in dioxane and pyridine [7], and the 3α-cathylate oxidized with 26 mg of sodium dichromate in aqueous acetic acid for 16 h at 25°C. After saponification the product was chromatographed with phase system F[9], yielding 50.5 mg of 7-ketolithocholic acid — 24—^{14}C. Specific activity 1.85 μC/mg. M.p. 200—202°.

* A preliminary report of this work was read at the meeting of the Danish Biochemica Society in Copenhagen, June 1957 [11].

** Present address: Department of Chemistry, Karolinska Institutet, Stockholm 60, Sweden.

Table 1. Recrystallizations of the radioactive material contained in peak A in the chromatogram shown in Fig. 2.

Inactive bile acid added	Crystallizing solvent	Weight mg	c.p.m. per mg
3α,6β,7β-Trihydroxy-cholanic acid (Acid I)		50	410
	Acetone/light petroleum	40	415
	Methanol/water	31	390
	Acetone	23	395
	Methanol/ethyl acetate	19	410
	Acetone/light petroleum	12	400

0.8 mg of this acid was neutralized with sodium hydroxide and injected intraperitoneally in 0.9 % sodium chloride solution into each of two 200—250 g white male rats of the institute stock on which the common bile duct had been cannulated 24 h before the administration. The rats had free access to white bread and oats and 0.9 % sodium chloride solution. The bile was collected, hydrolyzed, extracted with ether and chromatographed on hydrophobic supercel as described earlier [8,9]. The following systems were used.

System	Moving phase	Stationary phase
C[8].	150 ml of methanol	15 ml of *iso*octanol
	150 ml of distilled water	15 ml of chloroform
F[9].	165 ml of methanol	45 ml of chloroform
	135 ml of distilled water	5 ml of heptane

4 ml of the stationary phase were used per 4.5 g hydrophobic supercel. All chromatograms were run at a constant temperature of +23°C.

Reference substances used for identification of the metabolites

3α,6β,7β-Trihydroxycholanic acid (Acid I) was isolated from rat bile after hydrolysis and chromatography with phase system C. 0.084 g was obtained from the bile of 10 rats, collected during the first 12 h after the bile duct cannulation, at which time the concentration of this acid is maximal. M. p. 226—227°, $[\alpha]_D^{23} = +62° \pm 2°$ (*c* 0.51, methanol). The m. p. was not depressed by synthetic Acid I, prepared through hydroxylation of methyl-3α-acetoxy-Δ^6-cholenate with osmium tetroxide [3].

Table 2. Recrystallizations of the radioactive material contained in peak B in the chromatogram shown in Fig. 1.

Inactive bile acid added	Crystallizing solvent	Weight mg	c.p.m. per mg
Ursodeoxycholic acid		60	690
	Acetic acid/water	50	710
	Ethyl acetate/light petroleum	44	730
	Acetone/water	35	700
	Methanol/water	22	725
	Ethyl acetate/light petroleum	14	705

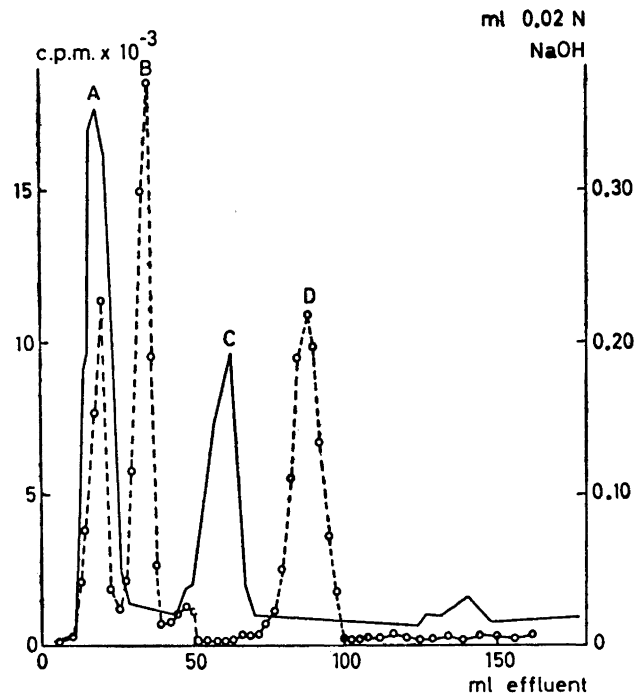

Fig. 1. Chromatography of acids from hydrolyzed bile, excreted during 24 h following intraperitoneal administration of 0.8 mg of 7-ketolithocholic acid —24—^{14}C. Column: 4.5 g hydrophobic supercel. Phases: Type F, see page 237. Solid line: Titration values. Broken line: Radioactivity.

Ursodeoxycholic acid was prepared through reduction of 7-ketolithocholic acid with sodium in *n*-propanol and chromatography with phase system F[10]. M. p. 201—202°, $[\alpha]_D^{23} = +56° \pm 2°$ (*c* 0.50, methanol).

RESULTS AND DISCUSSION

The carboxylabelled 7-ketolithocholic acid was injected intraperitoneally into two rats with bile fistulas and the bile was collected daily. The administered ^{14}C was almost completely recovered in the bile within the first 24 h. A chromatographic separation of the hydrolyzed bile with phase system F is shown in Fig. 1. The front peak (A) was rechromatographed with phase system C, Fig. 2. The activity is then eluted immediately after the band of inactive cholic acid. The radioactive material (105—140 ml) was diluted with inactive Acid I and the identity established by recrystallizations from different solvents and determination of the specific activity of the material from each recrystallization. (Table 1). About 2—3 % of the total radioactivity was eluted at about 50 ml, but the amount of this material was insufficient for identifica-

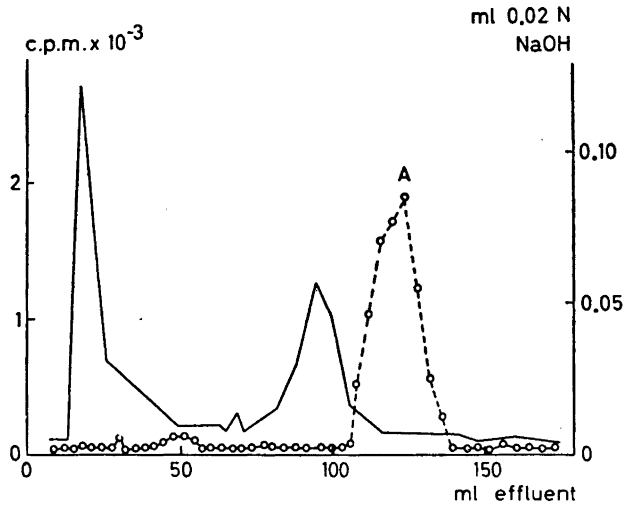

Fig. 2. Chromatography of peak A in the chromatogram shown in Fig. 1. Column: 4.5 g hydrophobic supercel. Phases: Type C, see page 237. Solid line: Titration values. Broken line: Radioactivity.

tion. With phase system C, Acid II (65—85 ml) is eluted before and Acid I (105—140 ml) after cholic acid (85—105 ml). The second peak (B) in the chromatogram shown in Fig. 1 was diluted with inactive ursodeoxycholic acid and recrystallized from different solvents. There was no depression of the specific activity (Table 2). Peak C in the chromatogram in Fig. 1 represents inactive chenodeoxycholic acid, normally present in the bile, and peak D consists of unchanged 7-ketolithocholic acid-24-^{14}C. This compound was also identified through isotope dilution. The composition of the labelled products was calculated from determinations of the total activity of the material and the ^{14}C contained in peak A, B and D. The results from two rats are shown in Table 3.

The ketogroup of 7-ketolithocholic acid is thus reduced in the liver to a 7β-hydroxylgroup and any formation of the 7α-epimer could not be demon-

Table 3. Percentage composition of the labelled products excreted in the bile during 24 hours after intraperitoneal administration of 7-ketolithocholic acid-24-^{14}C to two rats with bile fistulas.

Compound	Per cent		
	Rat I	Rat II	Average
3α,6β,7β-Trihydroxycholanic acid	24.2	29.3	26.8
Ursodeoxycholic acid	38.1	33.4	35.8
7-Ketolithocholic acid	32.8	33.7	33.3
Total	95.1	96.4	95.9

strated. The pathway for the formation of 3α,6β,7β-trihydroxycholanic acid (Acid I) may either be an initial hydroxylation of 7-ketolithocholic acid in the 6β-position and a later reduction of the 7-ketone, or a 6-hydroxylation of ursodeoxycholic acid. The facts that 7-ketolithocholic acid and ursodeoxycholic acid are only formed in small amounts in the metabolism of chenodeoxycholic acid, and that they are only partly metabolized, when injected intraperitoneally into a bile fistula rat [4], suggest that these acids constitute only a minor pathway in the formation of Acid I from chenodeoxycholic acid. Furthermore, evidence for the direct conversion of Acid II into Acid I will be presented in a subsequent publication [4].

This work is part of an investigation supported by the *Medical Faculty of the University of Lund* and by a research grant (H 2842) to Prof. S. Bergström from the *National Institutes of Health, United States Public Health Service.*

REFERENCES

1. Bergström, S. and Sjövall, J. *Acta Chem. Scand.* **8** (1954) 611.
2. Hsia, S. L., Matschiner, J. T., Mahowald, T. A., Elliott, W. H., Doisy, E. A., Jr., Thayer, S. A. and Doisy, E. A. *J. Biol. Chem.* **226** (1957) 667.
3. Hsia, S. L., Matschiner, J. T., Mahowald, T. A., Elliott, W. H., Doisy, E. A., Jr., Thayer, S. A. and Doisy, E. A. *J. Biol. Chem.* **230** (1958) 573.
4. Samuelsson, B. *To be published in Acta Chem. Scand.*
5. Mahowald, T. A., Mao Wu Yin, Matschiner, J. T., Hsia, S. L., Doisy, E. A., Jr., Elliott, W. H. and Doisy, E. A. *J. Biol. Chem.* **230** (1958) 581.
6. Bergström, S., Rottenberg, M. and Voltz, J. *Acta Chem. Scand.* **7** (1953) 481.
7. Fieser, L. F., Herz, J. E., Klohs, M. W., Romero, M. A. and Utne, T. *J. Am. Chem. Soc.* **74** (1952) 3309.
8. Bergström, S. and Norman, A. *Proc. Soc. Exptl. Biol. Med.* **83** (1953) 71.
9. Bergström, S. and Sjövall, J. *Acta Chem. Scand.* **5** (1951) 1267.
10. Samuelsson, B. *To be published in Acta Chem. Scand.*
11. Bergström, S., Lindstedt, S., Samuelsson, B. and Corey, E. J. *Acta Chem. Scand.* **11** (1957) 1096.

Received October 27, 1958.