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The Formation of Bile Acids from 7 α-Hydroxycholesterol in the Rat *

Bile Acids and Steroids 47

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Tritium labelled 7α-hydroxycholesterol has been prepared. It has been injected intraperitoneally into bile fistula rats and cholic and chenodeoxycholic acid identified as acid metabolities. The mechanism of the degradation of cholesterol is discussed.

In the autoxidation of cholesterol the two epimeric 7-hydroxycholesterols are formed [2,3]. They have also been reported to occur in small amounts in the unsaponifiable fraction in various organ lipids [4-7]. The possibility of their formation by autoxidation during the isolation procedure has, however, not been rigorously excluded.

It has been suggested that a 7-hydroxycholesterol may appear as an intermediate in the *in vivo* degradation of cholesterol [2,8].

In this work the formation of bile acids from 7α-hydroxycholesterol is demonstrated.

EXPERIMENTAL

7α-Hydroxycholesterol was prepared essentially according to Henbest *et al.* [9,10]. 2 g of sodium formiate dissolved in 20 ml of formic acid was added to a solution of 2 g of 7α-bromocholesterylbenzoate in 20 ml of ether. After 24 h water was added and the steroid extracted with ether. The material was dissolved in 2 ml of benzene and 15 ml of methanol added. After 8 h at room temperature 1.35 g of crystals melting at 115—144° were obtained. This material was brought onto a 20 g alumina column and eluted with increasing concentration of benzene in light petroleum. The material eluted with 0—30 % of benzene in light petroleum was crystallized from benzene/methanol and yielded 0.91 g of 7α-formoxycholesterolbenzoate m. p. 150—152°. Saponification with alcoholic potassium hydroxide gave 7α-hydroxycholesterol m. p. 150°.

Tritium-labelled 7α-hydroxycholesterol. Attempts to label 7α-hydroxycholesterol by platinum catalyzed exchange in tritiated water/acetic acid or tritiated water-ethanol/

* For a discussion of the steric configuration at the 7-position see Ref.[1]. A preliminary report of this work was read at the meeting of the Norwegian Biochemical Society, June 1956 [20].

sodium hydroxide were not successful and led to extensive destruction. The labelled 7α-hydroxycholesterol was therefore synthesized by the above procedure from tritium-labelled cholesterol prepared according to Bloch [11]. To ensure the absence of radioactive contaminants it was chromatographed according to Danielsson [12] on hydrophobic kieselguhr with 50 % (v/v) aqueous *iso*propanol as moving phase and 20 % (v/v) chloroform in heptane as stationary phase. The tritium labelled 7α-hydroxycholesterol had a specific activity of 4.3×10^5 c.p.m. when counted in an "infinitely thin" layer in a gas-flow counter.

Chromatographic separations of the bile acids were performed according to Sjövall and Norman [13,14]. The following two systems were used:

Phase system	moving phase	stationary phase
C(Ref.[14])	methanol/water 50/50	*iso*octanol/chloroform 1/1
F(Ref.[13])	methanol/water 55/10	chloroform/heptane 9/1

Each fraction was titrated with 0.02 N sodium hydroxide and an aliquot plated on a copper planchet for counting in a gas-flow counter. Accurate determinations of specific activity were performed by the gas-phase counting technique of Glascock [15].

RESULTS

2—3 mg of tritium-labelled 7α-hydroxycholesterol was dissolved in 0.2 ml of ethanol and brought into a colloidal suspension by the addition of 2 ml of 0.9 % sodium chloride containing 2 mg of sodium oleate. It was injected intraperitoneally into white rats with a bile fistula, and the bile collected.

After hydrolysis in 2 N sodium hydroxide in a sealed glass tube at 120° for 8 h the bile acids were extracted and isolated by chromatography with phase system C.

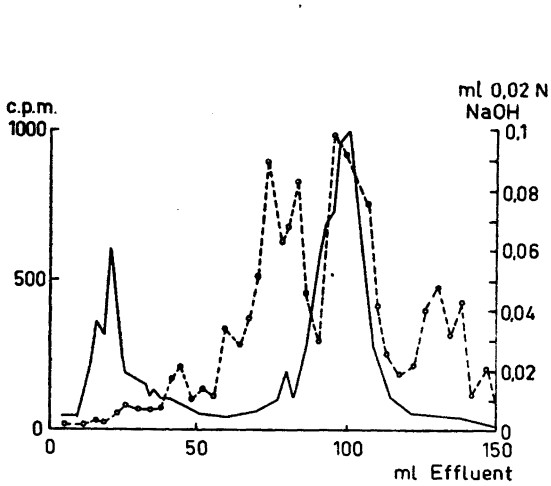

Fig. 1. Chromatogram of hydrolyzed bile after intraperitoneal injection of tritium-labelled 7α-hydroxycholesterol. Phase system C. Broken line: isotope determination. Solid line: titration values.

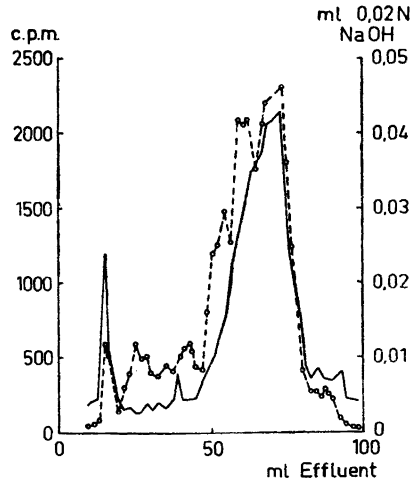

Fig. 2. Chromatogram of dihydroxy-acids after intraperitoneal administration of tritium-labelled 7α-hydroxycholesterol. Phase system F. Broken line: isotope determination. Solid line: titration values.

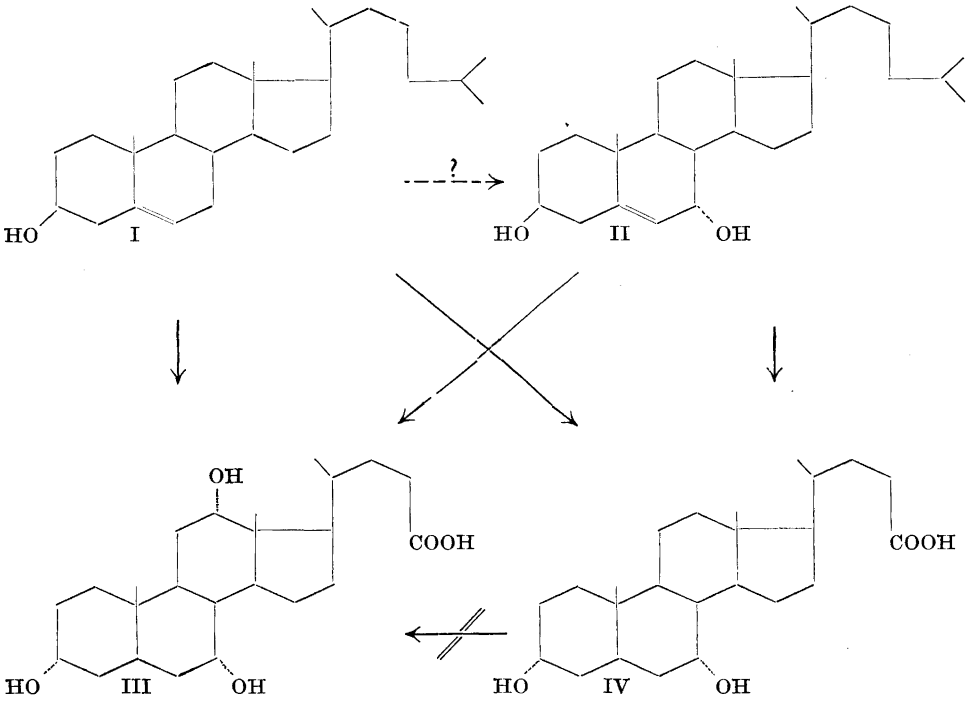

Fig. 3.

Fig. 1 shows a chromatogram where the titration peak of cholic acid appears at 80—120 ml. At the same position a peak of radioactivity is seen. This material was collected, rerun with the same chromatographic system, diluted with carrier cholic acid and recrystallized from different solvents. From each crystallization material was taken for determination of the specific activity by the gas-phase counting method [15]. It remained constant throughout five recrystallizations (EtOH, EtOAc, acetone/water) and the identity of the material with cholic acid seems established.

The material remaining in the column was eluted and rechromatographed on system F suitable for the separation of dihydroxycholanic acids. The chromatogram which is seen in Fig. 2 shows a titration peak at 50—80 ml caused by chenodeoxycholic acid in the bile. The identity of the labelled compound eluted at this position with chenodeoxycholic acid was established by recrystallization with carrier and determination of specific activity as for cholic acid.

In Fig. 1 labelled material is eluted before and after the cholic acid peak. When labelled chenodeoxycholic acid is administered to rats labelled metabolites appear at these locations [16,21] and in this experiment they probably also represent metabolites of the chenodeoxycholic acid.

DISCUSSION

In the rat cholesterol (I) gives rise to cholic acid (III) and chenodeoxycholic acid (IV) [16,17]. Work by Bergström and Sjövall [16] has, however, established that cholic acid is not formed by a 12α-hydroxylation of chenodeoxycholic acid. This type of reaction has been shown to take place when 3α,7α-dihydroxycoprostane is administered leading to the formation of cholic acid as well as of chenodeoxycholic acid [18]. This has brought further support to the view that in the formation of bile acids from cholesterol hydroxylations in the steroid nucleus precede the total degradation of the side chain [19].

Although the *in vivo* formation of 7α-hydroxycholesterol (II) from cholesterol has never been demonstrated, the results suggest the possibility that one of the primary steps in the degradation of cholesterol in the rat is a 7-hydroxylation.

A summary of some of the reactions studied in the rat is given in Fig. 3.

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