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On the Metabolism of 3α,12α-Dihydroxy-5α-cholanoic Acid in the Bile Fistula Rat

Bile Acids and Steroids 151

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After intraperitoneal or intracecal administration of tritium labeled 3α,12α-dihydroxy-5α-cholanoic acid to bile fistula rats about 30 % of the isotope was excreted in the first 24 h portion of bile. By means of reversed phase partition chromatography and subsequent thin layer chromatography the main labeled compounds excreted were shown to be 3α,12α-dihydroxy-5α-cholanoic acid and 3α,7α,12α-trihydroxy-5α-cholanoic acid.

From bile of a number of lower teleostean fish, snakes of the genus *Bitis*, frogs and certain birds, *e.g.* penguins, Haslewood and Kazuno and their collaborators [1-5] have isolated bile alcohols and bile acids having the *trans* configuration of the junction of rings A and B (5α-hydrogen). Haslewood has demonstrated the presence of 3α,7α,12α-trihydroxy-5α-cholanoic acid in the bile of a seal, which, however, feeds on penguins.[6] In a recent communication [7] the isolation of 3α,12α-dihydroxy-5α-cholanoic acid form rabbit feces was described demonstrating that the presence of 5α-cholanoic acids is not limited to the above-mentioned lower species. This acid was also present in rabbit bile and was apparently identical with the β-lagodeoxycholic acid isolated from rabbit bile in 1936 by Kishi.[8] In a preliminary report,[9] Sjövall and collaborators have described the presence of small amounts of 3α,7α,12α-trihydroxy--5α-cholanoic acid in human feces.

The mechanism of formation of 5α-cholanoic acids has not been completely elucidated. Karavolas and Elliott [11] have found that parenterally administered ^{14}C labeled 5α-cholestan-3β-ol is converted to 3α,7α,12α-trihydroxy-5α-cholanoic acid in the bile fistula rat. In the study by Danielsson *et al.*[7] it was shown that the 3α,12α-dihydroxy-5α-cholanoic acid from rabbit feces was a metabolite mainly of deoxycholic acid and it appeared probable that the transformation was carried out by intestinal micro-organisms.

The main bile acids in rat bile are cholic acid and chenodeoxycholic acids. During the enterohepatic circulation of bile these acids are transformed into

deoxycholic acid and lithocholic acid, respectively, which are the main bile acids excreted in feces. In addition, a number of less polar metabolites of deoxycholic and lithocholic acids occur in feces.[11] Recently, evidence has been obtained to indicate the presence of 3α,12α-dihydroxy-5α-cholanoic acid also in rat feces.[12]

As part of studies on the occurrence and metabolism of 5α-cholanoic acids, the metabolism of 3α,12α-dihydroxy-5α-cholanoic acid in the bile fistula rat has now been studied.

EXPERIMENTAL

3α, 12α-Dihydroxy-5α-cholanoic acid. This acid was prepared from methyl 12α-hydroxy-3-keto-5α-cholanoate as described by Danielsson *et al.*[7] About 10 mg of the acid was exposed to 2 C of tritium gas for three weeks at room temperature according to the method of Wilzbach [13] in the apparatus described by Bergström and Lindstedt.[14] The labeled material was dissolved in 1 M methanolic sodium hydroxide and the solution left overnight. After acidification the solution was extracted with ether, the ether extract washed with water until neutral and then evaporated to dryness under reduced pressure. The residue was chromatographed on a 4.5 g column of Hyflo Super-Cel with phase system F 1.[11] The main part of the radioactivity was eluted as a single peak at the position characteristic of 3α,12α-dihydroxy-5α-cholanoic acid. This material was shown to be homogeneous also in thin layer chromatography (*cf.* Fig. 1) and indistinguishable from

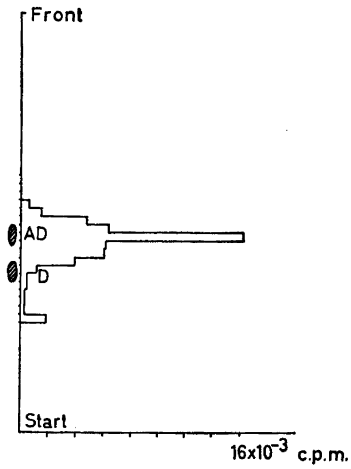

Fig.1. Thin layer chromatogram of 3α, 12α-dihydroxy-5α-cholanoic acid after exposure to tritium gas and purification on reversed phase chromatography. Reference compounds: 3α,12α-dihydroxy-5α-cholanoic acid (AD), and deoxycholic acid (D) Phase system S 12.

authentic 3α,12α-dihydroxy-5α-cholanoic acid. The specific activity of the tritium labeled 3α,12α-dihydroxy-5α-cholanoic acid was about 12 μC/mg.

Animal experiments. Bile fistula rats were prepared in the usual manner using male rats of the Sprague-Dawley strain weighing about 250 g. Tritium labeled 3α,12α-dihydroxy-5α-cholanoic acid, 2—4 μC, was administered intraperitonelly 12 h after operation or intracecally during operation.

Bile was collected in ethanol in 24 h portions, filtered and concentrated. Hydrolysis was performed with 1 M potassium hydroxide in 50 % aqueous ethanol at 120° for 8 h in a closed steel bomb. The free bile acids were extracted with ether from the acidified saponification mixture and analyzed by the chromatographic procedures described below.

Chromatographic procedures. The extracts of hydrolyzed bile were chromatographed with phase system F 1. The effluent was titrated with 0.02 M methanolic potassium hydrox-

ide and assayed for radioactivity in a gas-flow counter. The fractions containing the dihydroxycholanoic acids were combined and analyzed by thin layer chromatography as described below. The fractions containing the trihydroxycholanoic acids were combined and rechromatographed with phase system C 1.[11] As soon as the indicator had been eluted the column was washed with 100 ml ethanol. The ethanol eluate was evaporated to dryness and the residue dissolved in 1 ml of methanol and methylated with diazomethane. The solvent was removed in a stream of nitrogen and the residue analyzed by thin layer chromatography.

Thin layer chromatography. Silica Gel G (Merck, A. G., Darmstadt, West-Germany) and glass plates (0.4 × 20 × 20 cm) were used. The plates were kept in a desiccator until used. The phase systems used were those described by Eneroth.[15] System S 6 was used for the trihydroxycholanoic acid methyl esters and system S 12 for the dihydroxcholanoic acids. The samples to be chromatographed were dissolved in acetone and 50—100 μl were applied on the plate in a 5—10 cm long band, containing not more than 5 mg of material and not less than 20 000 cpm. Reference compounds were applied on the plate as single spots on each side of the biological sample. After development the areas of the chromatoplates containing the standard mixtures were sprayed with sulphuric acid and developed on a hot plate. The other areas of the chromatoplates were divided into 3 mm wide bands, which were scraped off with a soft spatula and extracted on a sintered glass filter funnel, porosity grade 4, with 3 × 1.5 ml of a 2 % solution of acetic acid in ether. The fractions were assayed for radioactivity in a gas-flow counter.

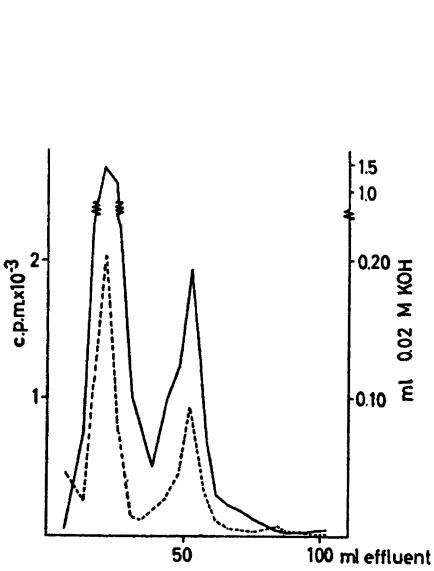

Fig. 2. Chromatography of the first 24 h portion of hydrolyzed bile after intraperitoneal administration of tritium labeled 3α, 12α-dihydroxy-5α-cholanoic acid. Column: 4.5 g Hydrophobic SuperCel. Phase system F 1.

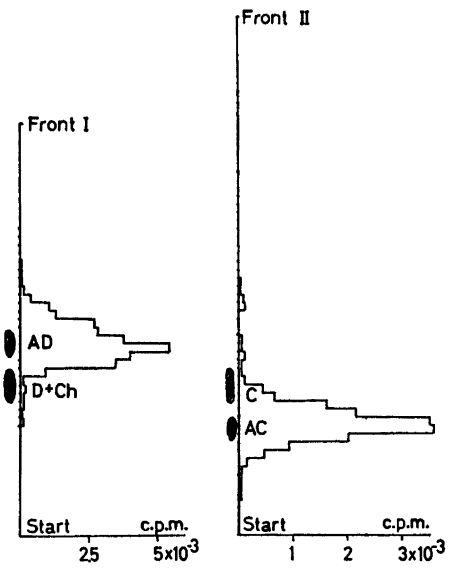

Fig. 3. Thin layer chromatography of the dihydroxycholanoic acid fraction (I) and trihydroxycholanoic acid fraction after methylation (II). Phase systems S 12 and S 6, respectively. Reference compounds: 3α,12α-dihydroxy-5α-cholanoic acid (AD); deoxycholic acid (D); chenodeoxycholic acid (Ch); methyl 3α,7α,12α-trihydroxy--5α-cholanoate (AC); methyl cholate (C).

RESULTS

Intraperitoneal administration. After intraperitoneal administration of 3α,12α-dihydroxy-5α-cholanoic acid about 30 % of the administered isotope was excreted in the first 24 h portion of bile. Fig. 2 shows a chromatogram of such a portion of bile after hydrolysis. The radioactivity was distributed in two main peaks appearing within the trihydroxycholanoic acid fraction (15—30 ml of effluent) and the dihydroxycholanoic acid fraction (40—60 ml of effluent). Thin layer chromatographic analysis of these fractions (*cf.* Fig.3) revealed that the radioactivity in the trihydroxycholanoic acid fraction (after methylation) consisted of a single compound with chromatographic properties as those of methyl 3α,7α,12α-trihydroxy-5α-cholanoate. The only labeled compound present in the dihydroxycholanoic acid fraction was 3α, 12α-dihydroxy-5α-cholanoic acid.

Intracecal administration. After intracecal administration of 3α, 12α-dihydroxy-5α-cholanoic acid also about 30 % of the isotope was excreted in the first 24 h portion of bile. Fig. 4 shows a chromatogram of such a portion after hydrolysis. The radioactivity was eluted with the trihydroxycholanoic as well as the dihydroxycholanoic acid fractions. Thin layer chromatography of these fractions (*cf.* Fig. 5) demonstrated that the labeled compounds excreted were 3α,7α,12α-trihydroxy-5α-cholanoic acid and 3α,12α-dihydroxy-5α-cholanoic acid, respectively.

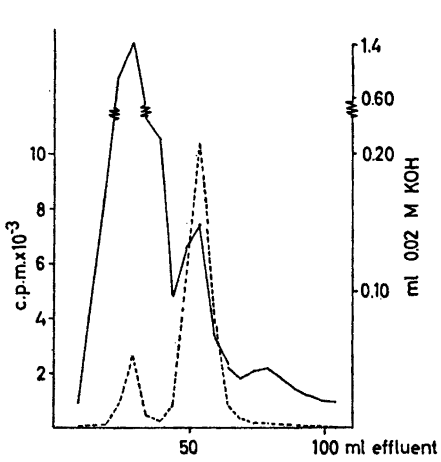

Fig. 4. Chromatography of the first 24 h portion of hydrolyzed bile after intracecal administration of tritium labeled 3α,12α-dihydroxy-5α-cholanoic acid. Column: 4.5 g Hydrophobic SuperCel. Phase system F1.

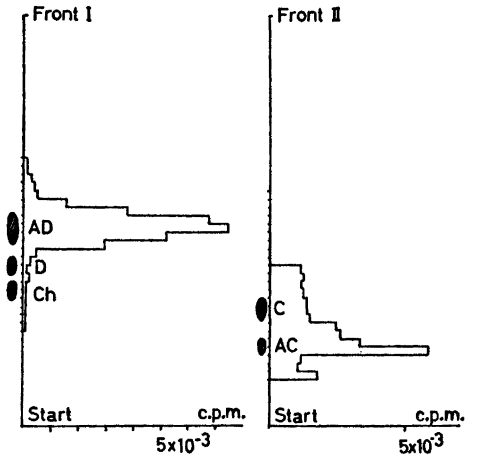

Fig. 5. Thin layer chromatography of the dihydroxycholanoic acid fraction (I) and trihydroxycholanoic acid fraction after methylation (II). Reference compounds as in Fig. 3. Phase systems S 12 and S 6, respectively.

DISCUSSION

The results obtained in the present investigation indicate that no enzyme systems occur in rat liver or in its intestinal microorganisms capable of transforming 3α,12α-dihydroxy-5α-cholanoic acid into an acid with the 5β-configuration. The reverse reaction formation of 3α,12α-dihydroxy-5α-cholanoic acid from deoxycholic acid, has been observed in the rabbit [6] and in the rat [12] and it has been suggested that this reaction, probably carried out by intestinal microorganisms, is the major one in the formation of 3α, 12α-dihydroxy-5α-cholanoic acid in these species.

The present experiments have shown that 3α,12α-dihydroxy-5α-cholanoic acid is hydroxylated in the C-7 position yielding 3α,7α,12α-trihydroxy-5α-cholanoic acid. This reaction is probably carried out by an enzyme system in the liver, but it is not known if this system is the same as that catalyzing the 7α-hydroxylation of deoxycholic acid.[16]

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